PhD
CONFERENCE

CHATEAU TŘEŠŤ
Conference Centre of the CAS
Dr. Richtra 234
589 01 Třešť

May 6 – 8
2024

PhD Conference of the Institute of Experimental Medicine,
Czech Academy of Sciences.

Contents

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Dear colleagues,

After a long break, it is a great honour to organise the next edition of the PhD conference of the IEM CAS and introduce you to our talented students, who will present their exciting research projects and other achievements. As the Director, I consider the support of the young generation of scientists to be essential. At the same time, I am very proud that we have excellent and experienced supervisors who help our students throughout their challenging studies.

Every year, we can see an increasing interest from applicants to study and work at the IEM CAS. We received 169 applications from candidates from 35 countries worldwide during this year's PhD recruitment. Although we are a relatively small institution compared to other institutes, we are fully competitive. I refer in particular to the prestigious HR Excellence in Research Award, which is a huge benefit for our institute, not only in terms of employee care but also in terms of transparency, prestige and the highest standards. We continuously strive to improve and offer you a better working environment and ongoing professional development.

I would like to wish you all the best in enjoying this event in this beautiful location, making important contacts with your colleagues across departments, and finding opportunities for possible collaboration because teamwork is the basis for every research institution's success.

Thank you for your participation, and I look forward to the exciting presentations and our informal discussions.

Miroslava Anděrová
Director of the IEM CAS

PhD students represent the future of science.
We appreciate your support of the young generation of scientists.
Thank you

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We look forward to seeing you and will be available for any questions you may have, prior to or during the event.

Programme

MONDAY 6. 5. 2024

9.00	Departure from IEM CAS
11.00	Expected arrival to Chateau Třešť
11.00 – 12.00	**Registration & Early Check-in** (if possible)
12.00 – 13.30	**Lunch**
13.30 – 13.45	**Opening Speech – Miroslava Anděrová & IAB**
13.45 – 15.30	**Oral Presentations – Session 1** *Chairpersons: Ján Kriška & Zuzana Heřmanová*
Knotek T.	Astrocyte-like subpopulation of NG2 glia shares features with neural progenitor cells
Marchetti V.	TRPV4 channels in NG2 glia affect the formation of glial scar after cerebral ischemia
Heřmanová Z.	The role of astrocyte TRPV4 channels in ischemic injury and cerebral edema development
Filipi T.	Are cortical glia affected by ALS-like pathology in the SOD1(G93A) mouse model?
Vaňátko O.	Horizontal transfer of mitochondria in a mouse model of glioblastoma
Jirát-Ziółkowska N.	Responsive Polymers for Dual 31P/19F-Magnetic Resonance: A Way to Detect Reactive Oxygen Species in vivo
Melichar A.	Tinnitus-Related Changes in GABAB Receptor Expression in the Mouse Dorsal Cochlear Nucleus
15.30 – 16.15	**Coffee Break & Check-in**
16.15 – 18.00	**Oral Presentations – Session 2** *Chairpersons: Viktorie Ročková & Klára Vokáčová*
Žižková R.	Development of the bone in vitro model
Šebová E.	In vitro assessment of osteogenic capacity across various calcium phosphate ceramics
Voltrová B.	Nanostructured Ti-36Nb-6Ta alloy as a promising material for orthopaedic applications
Ročková V.	In vitro intestinal tissue model for early toxicological screening
Šišková A.	Biomarkers indicating the transition from colon epithelium to adenoma
Balounová K.	Investigating mitochondrial DNA copy number variations and telomere characteristics in the transition from colorectal adenomas to early cancer stages
Tomášová K.	Telomere Length as a Predictor of Therapy Response and Survival in Patients Diagnosed with Ovarian Carcinoma
18.00 – 19.00	**Dinner**
19.00 – 0.00	**Free Time Activities**

TUESDAY 7. 5. 2024

8.00 – 9.00	**Breakfast**
9.00 – 10.15	**Oral Presentations – Session 3** *Chairpersons: Tereza Červená & Kateřina Hoňková*
Dudko N.	Genomic stability of mammalian oocytes and somatic cells
Mondal S.	Spatial sequestration of Xrn1 requires halted glycolysis and is conserved from yeast to human
Červená T.	Toxicity Assessment of Light-Duty Vehicle Emissions Using Air-Liquid Interface in Real Driving Emission Methodology
Šímová Z.	Transcriptome changes in humans acutely exposed to nanoparticles during grinding of dental nanocomposites
Palacká K.	Interplay between mesenchymal stem cells and degenerated retina
10.15 – 11.00	**Coffee Break**
11.00 – 12.00	**Oral Presentations – Session 4** *Chairpersons: Petra Zahumenská & Marharyta Kolcheva*
Netolický J.	Independent regulation of early trafficking of NMDA receptors by ligand-binding domains of the GluN1 and GluN2A subunits
Zahumenská P.	Impact of Disulfide Bonds on Early Trafficking and Functional Properties of NMDA Receptors
Langore E.	Spatial and temporal dynamics of NMDA Receptors and their role in spine and synaptic alterations during excitotoxicity and Alzheimer's disease
Misiachna A.	Developing a potent open-channel blocker for GluN1/GluN2A NMDA receptors with enhanced Membrane-to-channel inhibition
12.00 – 13.15	**Lunch**
13.15 – 15.00	**Oral Presentations – Session 5** *Chairpersons: Barbora Smejkalová & Olena Rohulska*
Smejkalová B.	Shaping the glial scar: The effect of Drebrin knockout on spinal cord injury repair
Štěpánková K.	4-MU-mediated degradation of CSPGs leads to functional recovery, reduction of pathology and modulation of immune reaction after chronic SCI
Šintáková K.	The role of small extracellular vesicles in the regeneration of nervous tissue
Cimpean A.	Autophagy in axonal growth and regeneration of adult sensory neurons
Arzhanov I.	The role of miR-20a in the nervous tissue injury
Vavřinová E.	Metabolic and secretome profile of multipotent mesenchymal stromal cells spheroids
Havelková J.	Towards the development of 3D modelling system for glioblastoma research and drug testing

15.00 – 15.45	**Coffee Break**
15.45 – 18.45	**VIP Speakers**
Petr Baldrian	How to succeed in a grant competition: the Czech Science Foundation (GAČR) and the others
Ondřej Slabý	Which projects can succeed in the Czech Health Research Council (AZV ČR), and how are they evaluated?
Pavla Hubálková	Science Communication
Josef Horák	An Unexpected Journey: Is the NCI Experience Worth it?
Jiří Růžička	From technology to startup. From startup toward investment
Josef Uskoba	Protein interactions in complex matrices. Use of Flow induced dispersion analysis for binding characterization.
19.00 – 0.00	**Grandeur Gourmet Evening & Discoteque**

WEDNESDAY 8. 5. 2024

8.00 – 9.00	**Breakfast & Check-out**
9.00 – 10.30	**Poster Session**
10.30 – 11.00	**Coffee Break**
11.00 – 11.30	**Photoshoot**
11.30 – 12.00	**Awards**
12.00 – 13.00	**Lunch**
13.00	Departure from Třešť

VIP Speakers

Petr Baldrian
President, Czech Science Foundation

Ondřej Slabý
President, Czech Health Research Council

Pavla Hubálková
Scientist, populariser and author
of the book Science Communication

Josef Horák
PostDoc, National Cancer Institute,
Functional Genomics Department

Jiří Růžička
Project Manager, i&i Prague Bio-Innovation Centre

Josef Uskoba
Product Manager, BioTech a.s.

Session 1

Monday 6. 5. 2024

13.45 – 15.30

Department

Cellular Neurophysiology
Auditory Neuroscience

Astrocyte-like subpopulation of NG2 glia shares features with neural progenitor cells

Knotek, T. (1); Janeckova, L. (2); Kriska, J. (1); Hermanova, Z. (1); Kirdajova, D. (1); Kubovciak, J. (3); Berkova, L. (2); Tureckova, J. (1); Camacho Garcia, S. (1); Galuskova, K. (2); Kolar, M. (3); Korinek, V. (2); Anderova, M. (1)

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Glia expressing NG2 antigen (NG2 glia) are predominantly known as oligodendrocyte precursors. However, their differentiation potential is not limited to the oligodendroglial lineage after focal cerebral ischemia (FCI): recent findings describe the appearance of astrocyte-like cells derived from NG2 glia after injury. Nevertheless, the role of NG2 glia differentiation after FCI remains unresolved; therefore, we employed transgenic mice with tamoxifen-inducible Cre-mediated recombination for lineage tracing of NG2 glia, single-cell RNA sequencing (scRNA seq), immunohistochemical staining, RT-qPCR, and patch-clamp technique on NG2-expressing cells in healthy tissue and in tissue affected by FCI. Single-cell RNA sequencing data revealed that NG2 glia form five distinct subpopulations: a basal subpopulation with typical NG2 glia gene expression, a subpopulation with a mitotic or migratory activity, quiescent NG2 glia, subpopulation of cells differentiating into oligodendrocytes, and the recently described astrocyte-like subpopulation. Contrary to previous observations, all of these subpopulations were present in both healthy and ischemic tissue. Interestingly, the astrocyte-like subpopulation shared properties with neural progenitor cells. In addition, the expression of genes linked to neurogenesis was detected by scRNA seq and confirmed by RT-qPCR and immunohistochemical staining. This expression changed at different time points after FCI. Furthermore, 28 days after the stroke, we observed NG2 expressing cells positive for NeuN, a marker of mature neurons. Together, these data provide new insight into progenitor-like properties of NG2 glia.

Tomáš Knotek / tomas.knotek@iem.cas.cz

TRPV4 channels in NG2 glia affect the formation of glial scar after cerebral ischemia

Marchetti, V. (1); Hermanova, Z. (1); Ziolkowska, N. (1,2); Jirak, D. (1,2); Chmelova, M. (3); Sucha, P. (3); Vargova, L. (1,3); Anderova, M. (1)

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Cerebral ischemic injury is a potentially fatal condition accompanied by the development of brain edema and the formation of glial scar. In this pathology, glial Transient Receptor Potential Vanilloid 4 (TRPV4) channels are suspected to play a crucial role and affect the outcome of the injury. Global deletion of TRPV4 showed increased size of the ischemic lesion (measured by magnetic resonance imaging – MRI) together with altered parameters of the extracellular space measured by real-time iontophoresis. However, these results did not show which type of TRPV4-expressing glia is responsible for these changes. One of the possible candidates are NG2 cells: a dynamic population of glia displaying a strong proliferation and differentiation potential under both physiological and pathological conditions. Notably, we already proved that after ischemia, NG2 cells shift towards a more astrocyte-like gene expression profile, indicating their contribution to glia scar formation. Therefore, we decided to utilize NG2 glia-specific TRPV4 knockouts (cTrpv4-/-) to further elucidate the role of TRPV4 channels in post-ischemic NG2 cells. MRI analysis did not show any differences in the volume of ischemic lesions after middle cerebral artery occlusion (MCAO) between the cTrpv4-/- and controls. However, subsequent staining for Iba1 (a marker of microglia) and GFAP (a marker of reactive astrocytes and astrocyte-like NG2 cells) revealed an increase in the area of the gliosis around the lesion after MCAO. These results indicate that even though TRPV4 channels in NG2 glia do not affect the development and extent of brain edema, they play a role in the formation of a glia scar and thus affect the outcome of the ischemic brain injury.

Valeria Marchetti / valeria.marchetti@iem.cas.cz

The role of astrocyte TRPV4 channels in ischemic injury and cerebral edema development

Hermanova, Z. (1); Tureckova, J. (1); Sirotova, N. (1); Kriska, J. (1); Jirak, D. (1,2); Pulpanova, D. (2); Ruzickova, A. (1); Knotek, T. (1); Valihrach, L. (1,3); Kubista, M. (3); Anderova, M. (1)

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Astrocyte Transient receptor potential vanilloid 4 (TRPV4) channels are suspected to be the key players in cellular volume regulation, and therefore may affect the development and severity of cerebral edema during ischemia. In our project, we examined astrocyte swelling/volume regulation in mice with TRPV4 deletion in response to ischemic conditions, to determine how the deletion of these channels can affect ischemic brain injury. We employed several models of ischemia or ischemia-related pathological conditions and observed their effect on astrocytes in the cortex of TRPV4 knock-out mice. Magnetic resonance imaging (MRI) showed a larger volume of the damaged tissue 1, 3 and 7 days after permanent middle cerebral artery occlusion (pMCAO) in TRPV4 knock-out mice, compared to controls (Ctrl). Furthermore, quantification of astrocyte volume changes during oxygen-glucose deprivation (OGD) revealed two distinct subpopulations of astrocytes with low and high volumetric responses (LRA and HRA). Their analyses showed that mainly HRA are affected by the global deletion of TRPV4 channels (Trpv4-/-). In addition, gene expression analysis revealed reduced expression of the ion transporters KCC1 and ClC2 as well as the receptors GABAB and NMDA in Trpv4-/- mice. Next, we generated astrocyte-specific TRPV4 knock-outs (cTrpv4-/-) to further specify the role of these channels in astrocyte volume regulation and cerebral edema development. The OGD exposure revealed decreased swelling of cTrpv4-/- astrocytes. Moreover, western blot analysis showed a decreased level of Aquaporin 4 channels in cTrpv4-/- 3 days after pMCAO, compared to Ctrl, which corresponds with the bigger cerebral edema observed during MRI analysis. Taken together, we have shown that deletion of TRPV4 channels not only affects their specific functions in astrocytes, but also the expression of other proteins, which may modulate the ischemic cascade and thus influence the final impact of ischemia.

Zuzana Heřmanová / zuzana.hermanova@iem.cas.cz

Are cortical glia affected by als-like pathology in the SOD1(G93A) mouse model?

Filipi, T. (1); Matusova, Z. (2); Abaffy, P. (2); Vanatko, O. (1); Tureckova, J. (1); Valihrach, L. (1,2); Anderova, M. (1)

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(2) Institute of Biotechnology, Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic

Amyotrophic lateral sclerosis (ALS) is a motor neuron (MN) disease characterized by progressive muscle atrophy that eventually causes respiratory failure and death. Patients suffer from MN degeneration in the motor cortex, brainstem and spinal cord, accompanied by pathological changes in glial cells. The glial role in ALS has been extensively studied, mainly in animal models, especially the SOD1(G93A), which has led to several proposed hypotheses for underlying pathological mechanisms. However, the majority of information we have comes from the spinal cord and some from the brainstem, but the data describing cortical glia are sparse and, what is more, contradictory. We thus aimed for a comprehensive study of glia in the cortex of SOD1(G93A) using single-cell RNA sequencing (scRNA-seq) and immunohistochemistry. We inspected astrocytes, microglia, and oligodendrocytes in four stages of the disease, respecting the factor of sex. Our results suggest only minimal changes in glia throughout the disease progression, regardless of sex. Pseudobulk and single-cell analyses revealed subtle disease-related transcriptional alterations at the end-stage in microglia and oligodendrocytes, which we supported by immunohistochemical analysis. Therefore, our data favor the hypothesis that the SOD1(G93A) mouse cortex does not fully recapitulate the disease in patients, and we recommend the use of a different model for future studies of cortical ALS pathology.

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Horizontal transfer of mitochondria in a mouse model of glioblastoma

Vanatko, O. (1,2); Kubiskova M. (1,4); Brisudova, P. (3,4); Nahacka, Z. (3); Zobalova, R. (3); Truksa, J. (3); Kapaj, K. (5); Foltynova, A. (1,2); Ziolkowska, N. (1,6); Jirak, D. (1,6); Neuzil J. (3,4,7,8); Anderova, M. (1)

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Glioblastoma is a malignant type of tumor characterized by fast progression, extreme recurrence rate, and poor prognosis. A recently described feature in tumors is the horizontal transfer of mitochondria (HTM) – a process of exchange of mitochondria between tumor cells and the surrounding non-malignant cells. HTM restores cellular respiration and proliferation, as these processes are negatively affected by the accumulation of damage to mitochondrial DNA in tumor cells. Molecular mechanisms and the involvement of individual brain cell types in HTM remain largely unexplored. This study aims to confirm the validity of our experimental model and elucidate whether and to which extent specific brain cell types (astrocytes, oligodendrocytes, microglia, NG2 glia, pericytes) participate in HTM in a mouse model of glioblastoma. To explore the cellular origin of mitochondria in HTM in glioblastoma, we developed and evaluated mouse glioblastoma cell lines devoid of mtDNA (GL261 rho0) expressing either cytoplasmic GFP or mito::mKate2. MtDNA sequencing, oxygraph, IHC, and western blot were used to validate the GL261-rho0 cell lines. To determine the donors of mitochondria in HTM, different mouse cre strains are crossed with the MitoTag reporter strain, resulting in the expression of EGFP targeted into the outer mitochondrial membrane, and orthotopically implanted with mito::mKate2-expressing rho0 cells. Live-cell imaging, immunohistochemistry, FACS, and in vivo imaging are used for the determination of mitochondrial donors. Rho0 tumors show delayed onset of tumor formation compared to parental cells. MtDNA qPCR analysis shows mtDNA is absent in cultured rho0 cells but is present in tumor-derived rho0 cells; this is further supported by the expression of mtDNA-encoded genes in tumor-derived rho0 cells. Analysis of mtDNA polymorphisms shows mtDNA isolated from rho0 cells is of host origin. Immunohistochemical staining shows the presence of Mtco1 (mtDNA-coded) in rho0 tumors. Preliminary results from immunohistochemistry suggest astrocytes as possible mitochondrial donors.

Ondřej Vaňátko / ondrej.vanatko@iem.cas.cz

Responsive polymers for dual 31P/19F-magnetic resonance: a way to detect reactive oxygen species in vivo

Jirát-Ziółkowska, N. (1,2,3); Sulková, K. (2); Kracíkovác, L. (4,5); Androvič, L. (4); Havliček,D. (1,2); Laga,R. (4); Jirák, D. (1,2,3)

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Biocompatible metal-free contrast agents (CAs) have emerged as a promising alternative to commercially utilized CAs in magnetic resonance imaging (MRI). In this investigation, a dual 31P/19F-MR probe comprised of hydrophilic phospho-/fluoropolymers was developed and evaluated. The incorporation of a biologically rare thiophosphoester (P = S) group into the probe structure resulted in a distinctive chemical shift (Δδ ~ 59 ppm) in phosphorus MR (31P-MR), effectively mitigating phosphorous biological background signal challenges. Furthermore, in the presence of reactive oxygen species (ROS), the P = S group underwent oxidation, inducing a detectable chemical shift in 31P-MR. This responsiveness to physiological conditions enables the localization and assessment of ROS, particularly abundant in pathological conditions such as inflammation and cancer. To facilitate the quantification of the phosphorus MR signal as an efficient ROS sensor in vivo, a bioinert trifluoromethyl group (CF3) was integrated into the probe structure. This addition serves as a precise „hotspot" for fluorine MRI (19F-MRI), enabling in vivo localization and serving as an on-site reference. Through both in vitro (cells, 31P-MR) and in vivo (mice tumor model, 31P/19F-MR) testing, the high specificity reaching 100% and responsiveness of this dual MR probe were demonstrated. These findings underscore the potential of the polymer as a sensitive ROS sensor and a background-free dual MR-traceable tool in cancer research.

Natalia Ziólkowska / natalia.ziolkowska@iem.cas.cz

Tinnitus-related changes in GABAB receptor expression in the mouse dorsal cochlear nucleus

Melichar, A. (1); Hrušková, B. (1); Rybalko, N. (1); Suchánková, Š. (1); Janáček, J. (2); Tureček, R. (1)

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Tinnitus, phantom auditory perception, is one of the most common audiological disorders. However, the mechanisms underlying this condition remain unclear. It has previously been shown that tinnitus is accompanied by increased excitability and hyperactivity of neurons in the dorsal cochlear nucleus (DCN), the first auditory processing station in the brainstem. GABAB receptors (GABABRs) are metabotropic receptors for the major inhibitory transmitter GABA and serve as important regulators of excitability in the nervous system, including the auditory pathway. In this study, we investigated changes in the expression of GABABRs in the DCN of mice with tinnitus and the link between GABABR-associated KCTD12 proteins and audiological conditions. Tinnitus was induced by acoustic trauma or deletion of the KCTD12 proteins, and its presence was assessed by behavioral methods based on gap pre-pulse inhibition of the acoustic startle reflex. The distribution of the GABABR was analyzed by quantitative immunohistochemistry and its functional properties were investigated using the patch-clamp technique. We found that GABABRs are highly expressed throughout the DCN with maximum density in the molecular layer and show a high degree of colocalization with KCTD12 proteins. Quantitative analysis of GABABR labeling in different cell types of the DCN in mice with tinnitus showed a reduction in the receptor density in KCTD12-positive inhibitory interneurons, cartwheel cells, but not in KCTD12-negative projection neurons, fusiform cells. The differential reduction in GABABR expression in DCN neurons was confirmed by analysis of their pharmacological responses recorded using the patch-clamp technique in live brain stem slices. Based on our data, we proposed a plausible model of the pathophysiological consequences of impaired GABABR function for the activity of neural circuits in the DCN. Our results suggest that changes affecting GABABR signaling may be part of the processes of maladaptive plasticity that lead to tinnitus.

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Session 2

Monday 6. 5. 2024

16.15 – 18.00

Department

Tissue Engineering
Molecular Biology of Cancer

Development of the bone in vitro model

Žižková, R. (1, 2); Klusáček Rampichová, M. (1); Hedvičáková, V. (1); Kuželová Košťáková, E. (2); Litvinec, A. (1), Lukáš, D. (2); Filová, E. (1)

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Current testing of bone substitutes usually involves in vitro and then in vivo studies. In vitro testing using monocultures of osteoblasts is insufficient as it lacks the complexity of the bone tissue. The development of an in vitro model consisting of more cell types present in the bone would have provided detailed information about the tested bone substitute. Thus, a more complex model would help to reduce the number of animals used to test potential bone substitutes. Physiological bone remodeling involves the degradation of damaged tissue by osteoclasts and the formation of new tissue by osteoblasts. Therefore, we used a co-culture of rat osteoblasts and osteoclasts derived from peripheral blood mononuclear cells. All the conditions e.g., culture medium, supplements, number of seeded cells, and the ratio between the cell types, needed to be optimized. Different parameters such as metabolic activity, proliferation, and expression of markers specific to osteoblasts or osteoclasts were analyzed. We succeeded in developing a balanced co-culture and wanted to verify that it fulfills its purpose. Therefore, we prepared nanofibrous membranes from poly-ε-caprolactone with 0, 10, or 20% hydroxyapatite, which is physiologically present in bone. Membranes were prepared by alternating current electrospinning, which created thicker fibers, or direct current electrospinning, which created finer fibers. Osteoblast and osteoclast activity on the different membranes was examined. We really observed different behavior of both cell types corresponding to different properties of the substitute. Finer fibers, achieved by the direct current electrospinning, supported the activity of osteoblasts and osteoclasts, which means better substitute integration in the body. We also found out that the used concentration of hydroxyapatite was not sufficient to increase osteoblast activity and new bone formation, so a higher concentration of hydroxyapatite in the bone substitute is needed to further improve the substitute integration and bone healing.

Acknowledgment: Project was supported by CZ.02.01.01/00/22_008/0004562.

Radmila Žižková / radmila.zizkova@iem.cas.cz

In vitro assessment of osteogenic capacity across various calcium phosphate ceramics

Šebová, E. (1); Oliver-Urrutia, C. (2); Hedvičáková, V. (1); Hefka Blahnová, V. (1); Žižková, R. (1); Wagner, A. (1); Dvořák, K. (3); Čelko, L. (2); Filová, E. (1); Montufar, E. B. (2)

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Background and purpose: This study examines the impact of various calcium phosphate ceramics (CaPC) on bone healing. Three distinct materials – hydroxyapatite (HA), beta-tricalcium phosphate (β-TCP), and calcium-deficient hydroxyapatite (CD-HA) – were subjected to in vitro testing to evaluate their osteogenic properties. CaPCs are widely recognized for their effectiveness as bone substitutes due to their remarkable biocompatibility, bioactivity, and ability to stimulate bone growth.

Methods: Osteoblastic cell line SaOS-2 was seeded onto discs, and their metabolic activity was assessed using MTS assay on days (D)1, 3, 7, and 14. Confocal microscopy, with talin staining, was employed to visualize cell adhesion to materials after 24-hours. Gene expression of osteogenic markers (D1, D7, D14) and alkaline phosphatase activity (D1, D7) were analyzed via qPCR and enzymatic assays, respectively.

Results: On D3 and D7, cells seeded on β-TCP exhibited higher metabolic activity ($p < 0.05$) compared to those on HA and CD-HA. Notably, early osteogenic marker gene expressions RUNX2 (D1), ALP (D7), and Col I (D7) were significantly elevated in the cells cultured on HA. In contrast, OCN gene expression was lowest on D14 in these cells. ALP activity was lowest on D1 in cells on β-TCP, with no differences observed on D7.

Conclusions: Our findings suggest that HA promotes the expression of early osteogenic markers, whereas β-TCP enhances metabolic activity but demonstrates weaker ALP activity. Overall, our results confirm the potential of CaPC to enhance bone healing.

Financial support: Ministry of Education, Youth and Sports of the Czech Republic – program IMPROVE V (CZ.02.01.01/00/22_010/0002552); European Regional Development Fund – Project Excellence in Regenerative Medicine" No. CZ.02.01.01/00/22_008/0004562; European Union's H2020 Research and Innovation Staff Exchange programme, project IP OSTEO "Induced pluripotent stem cell for bone and cartilage defects" under grant agreement 824007.

Eva Šebová / eva.sebova@iem.cas.cz

Nanostructured Ti-36Nb-6Ta alloy as a promising material for orthopaedic applications

Voltrova, B. (1, 2); Jarolimova, P. (3); Hybasek, J. (3); Hefka Blahnova, V. (1,4); Sepitka, J. (5); Sovková V. (1,4); Matejka, R. (6); Daniel, M. (5); Fojt, J. (3); Filova, E. (1,4)

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The formation of nanostructures on titanium implant surfaces is a promising strategy to enhance cell adhesion and osteointegration, which are crucial for future application in bone regeneration. The aim of the study was to evaluate the impact of a nanostructured surface created on novel β-titanium alloy, Ti-36Nb-6Ta, on the growth, adhesion and differentiation of human mesenchymal stem cells (hMSC). Anodic oxidation was used to fabricate nanotubes on Ti-36Nb-6Ta with average diameters of 18, 36 and 46 nm. Morphology, hydrophilicity and mechanical properties of the nanotube layers were characterized. The biocompatibility and osteogenic potential of the nanostructured Ti-36Nb-6Ta were established using cell adhesion, metabolic activity, and proliferation assays. The differentiation potential of the samples was investigated using PCR and specific staining of osteogenic markers collagen type I and osteocalcin. Early adhesion was evaluated using SEM, confocal microscopy and specific staining of adhesive molecules (vinculin, talin). It was shown that the nanotubes lowered the elastic modulus close to that of bone and significantly increased the wettability of all the nanostructured samples, compared with the untreated reference. The presence of nanotubes positively influenced the cell adhesion on the surface, improved ALP activity, synthesis of type I collagen and osteocalcin expression, but diminished early hMSC proliferation. Specifically, Ti-36Nb-6Ta treated with potential 20V with average nanotube diameters of 36 nm showed superior properties for future bone applications. In conclusion the nanostructured surface of β-titanium alloys implants can provide a biologically active interface between the implant and bone tissue with great clinical potential.

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In vitro intestinal tissue model for early toxicological screening

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In the realm of toxicological testing, efforts have been made to explore the alternatives to animal models aligning with the „replace, reduce, and refine" principles. Addressing this, we employed a co-culture of Caco2 and HT29 intestinal cell lines cultured on a nanofibrous membrane to emulate the natural physiology of the intestinal tissue. Initially, we characterized this model through monocultures and co-cultures of varying Caco2:HT29 ratios, evaluating alkaline phosphatase activity (ALP assay) and mucus production (alcian staining) over 21 days. Results revealed significant ALP activity differences, with mucus production in the co-culture, representing small intestine functionality. Subsequently, we transitioned to a 3D culture method using nanofibrous scaffolds to mimic in vivo intestinal architecture. When cultured on the scaffolds, the confluency of the model was reached 7 days post-seeding (ICC/IF staining) and the ratio of Caco2:HT29 was preserved as monitored for 21 days (ALP activity, qPCR). Building upon this foundation, we tested the impact of silver zinc oxide (ZnO) nanoparticles (NPs) and benzo[a]pyrene (BaP) on the engineered intestinal barrier integrity (LDH assay, qPCR of tight junctions) and cell viability (MTS assay). Caco2 and HT29 co-cultures seeded on nanofibers were exposed to previously identified NP and BaP concentrations. When co-cultured, the model exhibited greater resilience to the exposure, suggesting that monoculture may be artificially sensitive. The interplay between Caco2 and HT29 cells, along with the presence of mucus in the co-culture, enhances our model's relevance when responding to xenobiotics and underscores the necessity for advanced tissue models in in vitro toxicological research. Further ahead, we propose incorporating additional cell types, such as immune cells, to further enhance the physiological relevance of our model, potentially extending its utility as an intestinal disease model.

This work was supported by the Czech Ministry of Industry and Trade, project number FV40437 and by EU's H2020 MSCA number 823981.

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Biomarkers indicating the transition from colon epithelium to adenoma

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The precancerous stages of colorectal cancer (CRC) represent an unexplored area of research in the identification of new markers mapping the transition from healthy epithelium to adenoma and subsequently to adenocarcinoma. The aim of the study was to describe new signs of this process. A whole transcriptome analysis (RNA-seq) was performed for 16 fresh frozen colorectal adenomas (CA) and matched adjacent mucosa. Candidate gene expression was validated in an independent group of 20 fresh frozen paired CA and adjacent mucosa as well as eight independent public datasets. In addition, genome-wide methylation profiles were determined in 5 adenoma pairs and confirmed by pyrosequencing. Functional analysis was performed in vitro. The AOM-DSS–induced tumorigenesis mouse model was used as an in vivo model. Candidate protein levels were investigated by immunohistochemical staining of CA and the early stages (pT1) of CRC. A total of 1,917 differentially expressed genes and 148,191 differentially methylated CpG sites were observed in CA compared to adjacent mucosa. Based on the transcriptome data and relevance to CRC, the upregulated genes in adenomas TACSTD2, MMP7, MMP1, CLDN2, CLDN1, and ETV4 were selected for further validations. Hypomethylation of the TACSTD2 regulatory region was found in the adenoma tissue of 5 patients. In a validation group of 20 patients, the CA displayed a hypomethylated TACSTD2 promoter and correspondingly an increased TACSTD2 expression compared to the adjacent mucosa. The role of TACSTD2 overexpression in CA development was further confirmed in an in vitro transformation model of Human Colonic Epithelial Cells (HCEC). We found elevated levels of TACSTD2 protein TROP2 in human adenomas and pT1 tumors, as well as in low grade CA from the AOM-DSS mouse model, while TROP2 was mostly absent in the unaffected adjacent mucosa.

The work was supported by the Czech Science Foundation (GACR) (grant no. 22-05942S) and the Programme Exceles LX22NPO5102.

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Investigating mitochondrial DNA copy number variations and telomere characteristics in the transition from colorectal adenomas to early cancer stages

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Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide, which emphasizes the importance of finding new biomarkers for its early detection, ideally in the form of its non-malignant precursors, adenomas. Therefore, our focus was on the potential involvement of telomeres and mitochondrial DNA in the development of adenomas and early CRC stages, specifically UICC TNM Stage 1 and TNM Stage 2. The current study hypothesizes that the crosstalk between telomeres and mitochondrial DNA could serve as potential biomarkers for the formation of adenomas or even for the first stages of CRC. This study was carried out on 145 adenoma patients and 95 early-stage CRC patients (TNM1, N = 47; TNM 2, N = 48). Telomeres were characterized by telomere length (TL) and the gene expression of telomerase reverse transcriptase (TERT), the catalytic subunit of the enzyme telomerase, which is involved in maintaining telomeric ends. For mitochondrial DNA, mitochondrial DNA copy number (mtDNA-CN) and the gene expression of mitochondrial transcription factor A (TFAM), a protein involved in determining the abundance of the mitochondrial genome, were assessed. Results showed a significant shortening of TL in adenomas ($p = 5.64e-14$) and early CRC stages, TNM1 ($p = 3.49e-05$) and TNM2 ($p = 2.29e-04$), in comparison to their adjacent mucosa. This trend continued in TERT expression with higher expression detected in adjacent mucosa compared to adenomas ($p = 1.72e-06$) and TNM1 ($p = 3.66e-05$), while TNM2 had shown no difference. Although distinct TFAM expression patterns were evident across all three groups: adenomas ($p = 0.001$), TNM1 ($p = 0.009$), and TNM2 ($p = 8.85e-04$), an elevation in relative mtDNA-CN was solely detected in adenomas compared to adjacent mucosa ($p = 2.54e-07$), where it correlated with relative TL ($p = 0.0038$). These findings offer a deeper understanding of the relationship between telomeres and mtDNA in the early CRC progression.

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Telomere length as a predictor of therapy response and survival in patients diagnosed with ovarian carcinoma

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Background and Purpose: Impaired telomere length (TL) maintenance in ovarian tissue may play a pivotal role in epithelial ovarian carcinoma (OvC) onset. TL in either target or surrogate tissue (blood) is currently being investigated as a predictor in anti-OvC therapy or as a biomarker of the disease progression, respectively. There is presently an urgent need for an appropriate approach to chemotherapy response prediction. Methods: We performed a monochrome multiplex qPCR measurement of TL in peripheral blood and tumor tissues of 209 OvC patients. Methylation status and gene expression of shelterin complex and telomerase catalytic subunit (hTERT) were determined within tumor tissues by High-Throughput DNA methylation profiling and RNA sequencing (RNA-Seq) analysis, respectively. Results and Conclusions: The patients sensitive to cancer treatment (n = 46) had shorter telomeres in peripheral blood cells compared to those treatment-resistant (n = 93; P = 0.037). Cancer tissue TL lower than the median predisposed to better overall survival (P = 0.002). Gene expression of TPP1, which recruits telomerase to telomeres, was positively associated with TL in tumor tissue (P = 0.026). TL measured in peripheral blood cells could serve as a marker of platinum therapy response in OvC patients. Additionally, TL determined in tumor tissue provides information on OvC patients' overall survival.

The study was financed by the project NPO (LX22NPO05102), the Czech Health Research Council (NU21-03-00145), and the Czech Science Foundation (21-27902S).

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Session 3

Tuesday 7. 5. 2024

9.00 – 10.30

Department

Cell Nucleus Plasticity
Developmental Biology
Functional Organization of Membranes
Toxicology and Molecular Epidemiology

Genomic stability of mammalian oocytes and somatic cells

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Mutations constantly arise in all mammalian cells under the influence of various endogenous and exogenous factors, but their rates vary greatly between different cell types. Mammalian oocytes and early embryos are known to have substantially lower levels of de novo mutations in direct comparison with somatic cells. The most likely explanations for this include a higher amount of repair proteins, the specific features of the chromatin landscape, restrained replication in fully-grown oocytes, and low tolerance to specific DNA lesions, but the exact reasons remain unknown. Although the tolerance of oocytes to double-strand breaks has been studied relatively well, not much is known about the tolerance to single-strand breaks and other DNA insults, which are a substantial source of de novo mutations. Our preliminary results from immunofluorescence show that oocytes and early embryos indeed have high levels of several DNA repair proteins involved in single-stand break repair. Concurrently, with the exception of Xrcc1 and Pcna, the protein levels of Base excision repair (BER) components do not correlate directly with mRNA levels. This indicates that maternal BER proteins, rather than mRNAs, are involved in protecting the newly formed embryo and its genome. Overall however, according to our current quantitative western blot results, zygotes do not have higher levels of BER components, except for XRCC1, when compared to somatic cells, suggesting that the abundance of repair machinery is not the most essential contributor to embryonic genomic stability. We hypothesize that since early embryos have more “open” chromatin than differentiated cells, this might be the key parameter allowing for more efficient DNA repair. We hope that our current and subsequent results will improve the current understanding of what factors are critical in maintaining the high genomic stability of oocytes and early embryos.

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Spatial sequestration of Xrn1 requires halted glycolysis and is conserved from yeast to human

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The evolutionarily conserved 5'-3' exoribonuclease Xrn1 represents a key factor for the dominant mRNA degradation pathway in eukaryotes. In the dendrites of hippocampal neurons in mice and rats, post-synaptic Xrn1 aggregates (SX-bodies) appeared when protein synthesis at the synapse was globally attenuated by N-methyl-D-aspartate (NMDA) stimulation, and dissolved after enhancing global proteo-synthesis through the activation of metabotropic glutamate receptors. Similarly, in yeast, Xrn1 was sequestered at the plasma membrane in response to the actual supply of fermentable carbon sources. In addition, in this yeast model, the enzyme aggregation at the plasma membrane was correlated with the inhibition of its exoribonuclease activity. We found that independent of the actual growth phase, exposure of yeast cells to a non-specific glycolytic inhibitor leads to irreversible aggregation of Xrn1 at the eisosomes, the protein complexes sculpting a specialized microdomain in the yeast plasma membrane. This led us to the conclusion that the trigger for Xrn1 release from inhibitory sequestration at the eisosomes arises during glucose utilization. Differential localization of C-terminally truncated versions of Xrn1 suggested possible involvement of the SH3-like domain of the protein in eisosome binding. When we expressed human Xrn1 ortholog in yeast, it reversibly bound to the eisosomes under glucose depleted condition, analogous to the native yeast Xrn1. This documents that the described mechanism of metabolically induced spatial sequestration of the enzyme is evolutionarily conserved from yeast to humans.

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Toxicity assessment of light-duty vehicle emissions using air-liquid interface in real driving emission methodology

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The adoption of energy-efficient engines, cleaner fuels, and eco-friendly technologies in transportation represents a pivotal strategy in lowering the impacts associated with transportation. In our study, we utilized a methodology to transfer Real Driving Emission (RDE) conditions to a laboratory setting. The objective was to compare the toxicity of exhaust fumes on complex human cell models (MucilAirTM, Epithelix Sàrl, Switzerland). Five tested cars were driven using a chassis dynamometer facility through a 72-minute lasting RDE simulating cycle (RDEsim) in a climatic chamber under controlled temperature (23°C and −9°C). MucilAirTM cell inserts were exposed using a patented in-house built air-liquid interface exposure system for direct exposure. Each set of cells (5 sets corresponding to tested cars) was exposed to diluted exhaust fumes from RDEsim twice within 24 h and 24 h after second exposure the cells were harvested. Cell culture medium was collected at T0 (before exposure), T24 (24 h after first exposure) and T48 (24 h after second exposure) and Transepithelial electrical resistance (TEER) was measured at T0 and T48. Cell cytotoxicity measured in the medium using lactate dehydrogenase (LDH) assay was overall low (cell survival after 48 hours was > 90%) but we observed higher toxicity in the samples exposed at −9°C. TEER confirmed the results from LDH and the cellular integrity remained intact following exposure; however, noteworthy alterations were observed when comparing exposures at 23°C and −9°C. In summary, while overall cell cytotoxicity remained low, increased toxicity was observed in samples exposed to exhaust fumes produced at −9°C, and notable temperature-dependent changes in TEER were measured with opposing trends between exposures at 23°C and −9°C. These findings emphasize the temperature-dependent nature of exhaust emission effects on cellular health, with potential implications for environmental and human well-being. This work was supported by the European Union's Horizon Europe research and innovation programme under grant agreement No 101096133.

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Transcriptome changes in humans acutely exposed to nanoparticles during grinding of dental nanocomposites

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The rapid expansion of nanotechnologies brings many benefits, but also may negatively affect human health. Nanoparticles (NPs) are commonly used in commercial products including dental nanocomposites. These materials, developed as a highly aesthetic material for teeth reconstruction, may contain up to 75 wt. % of nanoparticles to match requirements on their long-term durability in the oral cavity. In this project we searched for the possible impact of acute inhalation exposure to NPs released during grinding dental nanocomposites (to simulate the exposure of dental personnel) on transcriptome changes in 24 female volunteers. Subjects were sampled twice per day, before (pre-shift) and after exposure (post-shift). Whole blood was used for RNA isolation, followed by RNA libraries preparation. Analyses of the differential mRNA and miRNA expression were performed. We detected large interindividual variability in gene expression changes after exposure. Among others, significant deregulation of DDIT4, PER1, and GPR124 or hsa-miR-15b-5p, hsa-miR-6861-3p, hsa-miR-548o-3p, and hsa-miR-3913-5p was observed when compared post- and pre-shift sampling. In summary, we observed a negative effect on the processes and biological pathways associated with increased oxidative stress, synthesis of eicosanoids, cells division, and pathogenesis of neurodegenerative disorders and carcinogenesis.

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

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Interplay between mesenchymal stem cells and degenerated retina

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Mesenchymal stem cells (MSCs) are widely studied as a potential treatment option for ocular diseases mainly due to their neuroprotective and regenerative properties. However, during the retinal degeneration, the activation of the inflammatory response represents one of the main causes of the development of pathology. Thus, regulation of immune reaction by MSCs could provide a promising solution for the treatment of retinal diseases. In a recent study, we induced chronical retinal degeneration in BALB/c mice by repeated intraperitoneal application of sodium iodate (NaIO3). Repeated application of NaIO3 caused increased infiltration of the retina with CD45+CD11b+ (microglia/macrophages) and CX3CR1+P2RY12+ (microglia) cells, increased local expression of genes for Iba-1 (activated microglia/macrophages), interleukin (IL)-1β and galectin-3 and decreased local expression of the gene for rhodopsin (photoreceptors). In vitro, cultivation of degenerated retinal explants in the presence of MSCs decreased the expression of genes for Iba-1, IL-1β and galectin-3 and increased the expression of the gene for rhodopsin in the retinal tissue. In vivo, MSCs were intravitreally injected 48 h before the last application of NaIO3 and retinas were isolated after an additional 48 h or on day 7. It was shown that MSCs (labeled with vital dye PKH-26) were still present in the retina on day 7 after application. Similarly, as in our in vitro experiment, the application of MSCs also decreased the expression of genes for Iba-1, IL-1β and galectin-3 in the retinal tissue. Moreover, the applied MSCs isolated from the degenerated retina increased the expression of genes for TGF-β, TSG-6 and IGF-1 and for retinal cell markers. These results suggest that MSCs modulate the microglia/macrophages activity and respond to the environment of the degenerated retina by increasing the expression of genes for immunomodulatory and growth factors.

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Session 4

Tuesday 7. 5. 2024

11.15 – 12.15

Department

Neurochemistry

Independent regulation of early trafficking of NMDA receptors by ligand-binding domains of the GluN1 and GluN2A subunits

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The essential role of N-methyl-D-aspartate receptors (NMDARs) in excitatory neurotransmission is underscored by numerous pathogenic variants in the GluN subunits, including those identified in their ligand-binding domains (LBDs). The prevailing hypothesis postulates that the endoplasmic reticulum (ER) quality control machinery verifies the agonist occupancy of NMDARs; however, whether it controls the structure of LBDs or the functionality of NMDARs is unknown. Using alanine substitutions combined with microscopy and electrophysiology, we found that the surface expression of GluN1/GluN2A receptors, the primary NMDAR subtype in the adult forebrain, correlates with EC50 values for glycine and L-glutamate. Interestingly, the co-expression of both GluN1 and GluN2A subunits with alanine substitutions led to an additive reduction in the surface number of GluN1/GluN2A receptors, as did the co-expression of both GluN1 and GluN2A subunits containing closed cleft conformation of LBDs. The synchronized ER release confirmed the altered regulation of early trafficking of GluN1/GluN2A receptors bearing alanine substitutions in the LBDs. Furthermore, the human versions of GluN1/GluN2A receptors containing pathogenic GluN1-S688Y, GluN1-S688P, GluN1-D732E, GluN2A-S511L, and GluN2A-T690M variants exhibited distinct surface expression compared to the corresponding alanine substitutions. Mutant cycles of GluN1-S688, GluN1-D732, GluN2A-S511, and GluN2A-T690 residues revealed, in most cases, a weak correlation between the surface expression of the mutant GluN1/GluN2A receptors and their EC50 values for glycine or L-glutamate. Consistently, molecular modeling and dynamics showed that the ER quality control machinery likely perceives structural changes of the LBDs but not the functionality of GluN1/GluN2A receptors.

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Impact of disulfide bonds on early trafficking and functional properties of NMDA receptors

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N-methyl-D-aspartate receptors (NMDARs) are pivotal ionotropic glutamate receptors in excitatory synaptic transmission across mammals. These heterotetrameric structures are composed of two obligatory GluN1 subunits paired with two GluN2 (GluN2A-GluN2D) and/or GluN3 (GluN3A-GluN3B) subunits. The biogenesis of NMDARs in the endoplasmic reticulum (ER) involves the enzymatic formation of disulfide bonds, crucial for the assembly and early trafficking of functional receptors to the cell surface. Through microscopy, we observed that altering the disulfide bonds in GluN1 by serine substitution significantly affected the surface expression of diheteromeric GluN1/GluN2A, GluN1/GluN2B, and GluN1/GluN3A NMDARs in HEK293 cells, following the order: wild-type (WT) > GluN1-C744S-C798S > GluN1-C79S-C308S > GluN1-C420S-C454S > GluN1-C436S-C455S. Electrophysiological studies linked the surface expression of GluN1/GluN2 receptors with disrupted disulfide bonds to EC50 values for L-glutamate (for GluN1/GluN2A and GluN1/GluN2B) and glycine (for GluN1/GluN2A), but not to open probability. Additionally, GluN1/GluN3A receptors with altered disulfide bonds showed glycine-induced responses, particularly enhanced after unmasking with CGP-78608, with GluN1-C744S-C798S/GluN3A receptors showing increased EC50 values for glycine. Employing ARIAD technology for synchronous NMDAR release from the ER highlighted that disulfide bond disruption in GluN1 affects early receptor trafficking, likely at the ER stage. A pathogenic variant, GluN1-C744Y, identified in databases, which disrupts a disulfide bond, showed reduced surface expression and altered functional properties similar to GluN1-C744S-C798S mutations when combined with GluN2A, GluN2B, and GluN3A subunits. Experiments in hippocampal neurons infected with YFP-GluN1-1a-C744Y exhibited increased NMDA-induced excitotoxic damage compared to WT YFP-GluN1-1a. This study elucidates the significant role of specific disulfide bonds in the GluN1 subunit on the early trafficking and functionality of NMDARs, highlighting their potential impact on receptor regulation and associated pathologies.

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Spatial and temporal dynamics of NMDA receptors and their role in spine and synaptic alterations during excitotoxicity and Alzheimer's disease

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N-methyl-D-aspartate receptors (NMDARs) are essential for transmitting excitatory signals in synapses and are crucial for learning and memory. Increased activity of NMDARs is associated with loss of dendritic spines and synaptic weakening and can also lead to excitotoxicity. Alzheimer's disease (AD) is marked by the buildup of amyloid-beta oligomers (Aβo) within the brain, with cognitive deficits in AD being associated with the loss of dendritic spines and synaptic connections. In our research, we explored the positioning and movement of NMDARs across excitatory synapses, employing direct stochastic optical reconstruction microscopy (3D-STORM) and universal point accumulation imaging nanoscale topography (uPAINT). Our study utilized hippocampal neurons from wild-type and genetically engineered mice with a conditional knockout of specific NMDAR subunits (GluN2A and GluN2B). Neurons were virally transduced to express GFP-tagged NMDAR subunits and a synaptic marker, either tdTomato-Homer1c or Cre-tdTomato-Homer1c. We analyzed the positioning and dynamics of the NMDARs by labeling the GFP-tagged receptors with anti-GFP nanobodies. Our results revealed distinct localization and dynamics among the NMDAR subtypes: GFP-GluN2A subunits predominantly localized in synaptic regions, while GFP-GluN2B subunits were more prevalent in the extrasynaptic areas, exhibiting quicker mobility. Conversely, GluN3A subunits were primarily found in extrasynaptic areas, with the shortest duration within synaptic regions and the fastest movement rates, even without GluN1/GluN2 NMDARs. We next measured neuronal death through an excitotoxicity assay evaluating changes in nuclear size as an apoptosis marker. Neurons expressing mCherry were studied and subjected to N-methyl-D-aspartate (NMDA) treatment, a specific agonist that triggers NMDAR activation and calcium influx. Our observations indicated that NMDAR activation alongside Aβo oligomers increased neuronal death. Future investigations will explore how Aβo modifies NMDAR functionality, localization, and mobility within dendritic spines and synapses and its consequences on synaptic transmission. This study aims to shed light on the significance of NMDARs in the pathology of Alzheimer's disease.

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Developing a potent open-channel blocker for GluN1/GluN2A NMDA receptors with enhanced membrane-to-channel inhibition

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N-methyl-D-aspartate receptors (NMDARs) are key ionotropic glutamate receptors implicated in the pathology of various mammalian central nervous system (CNS) disorders. Among the pharmacological interventions, memantine and ketamine are prominent open-channel blockers of NMDARs, approved for Alzheimer's disease treatment and as an anesthetic/antidepressant, respectively. Notably, memantine uniquely exhibits a low-affinity inhibition mechanism, accessing the binding site via a hydrophobic pathway from the plasma membrane, termed „membrane-to-channel inhibition" (MCI). Our research aimed to develop an innovative open-channel blocker targeting NMDARs endowed with novel inhibitory properties. In our study, whole-cell patch-clamp assays on HEK293 cells expressing GluN1/GluN2A receptors unveiled that our lead compound, K2060, delivered a robust voltage-dependent blockade, demonstrated an IC50 value roughly two-fold lower than memantine at a −60 mV membrane potential, and proved to be around 2.7 times more effective than memantine in the presence of 1 mM Mg2+. Further analysis using a standard MCI protocol—initiating with a 30-second exposure to 10 μM K2060 without L-glutamate, followed by L-glutamate-induced activation of GluN1/GluN2A receptors—revealed that K2060's IMCI/Icontrol ratios significantly outperformed memantine's, with ~0.8 for 1 μM K2060, ~0.5 for 10 μM K2060, and ~0.3 for 100 μM K2060, against ~0.5 for 100 μM memantine. Intriguingly, our findings indicated no differences in the IMCI/Icontrol ratio for 10 μM K2060 at GluN1/GluN2A-M630A and GluN1/GluN2A-M630W receptors, diverging from previous observations with memantine. Our experiments revealed that K2060 acts as a highly potent open-channel blocker of NMDARs, employing a distinct MCI mechanism compared to memantine.

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Session 5

Tuesday 7. 5. 2024

13.30 – 15.15

Department

Neuroregeneration

Shaping the glial scar: The effect of Drebrin knockout on spinal cord injury repair

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Spinal cord injury (SCI) leads to severe neurological functional impairment due to neuronal cell death, inflammation, and a failure in axonal regeneration. One of the major obstacles blocking axon growth into the injured parenchyma is the formation of a glia-dependent scar, which includes the induction of complex inflammatory responses, generation of inhibitory proteoglycans and invasion of pericytes and fibroblasts to create a lesion core surrounded by astrocytes. Following CNS injury, reactive astrocytes densely populate the borders of the injury site, become hypertrophic, and polarize their long processes into barrier-like palisades surrounding the lesion core. The actin-binding protein Drebrin (DBN), which is upregulated in reactive astrocytes in response to CNS damage, is essential for normal glial scar formation. We have compared the lesion development in wt and DBN-/- mice after SCI at different time points. In the absence of DBN, astrocytes fail to undergo the changes in polarity and cytoskeleton that are essential for the formation of the astrocyte limitans barriers at the site of injury. This led to slower formation of the glial scar, which was less compact, a bigger lesion core, higher accumulation of microglia in the lesion area and degeneration of neurons around the lesion. The impaired healing was associated with slower spontaneous regeneration, which we observed during behavioral testing. In summary, these results demonstrate the essential role of DBN for astrocyte reactivity and damage containment during SCI. DBN loss perturbs injury-induced scar formation and suppresses the maintenance of astrocyte reactivity, providing a causal link of DBN function during injury-induced reactive astrogliosis.

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4-MU-mediated degradation of CSPGs leads to functional recovery, reduction of pathology and modulation of immune reaction after chronic SCI

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After spinal cord injury (SCI), chondroitin sulfate proteoglycans (CSPGs) inhibit repair. CSPGs also form perineuronal nets (PNNs), a compact and specialized form of extracellular matrix (ECM) unique to the central nervous system (CNS). PNNs are involved in pathological conditions during recovery from CNS trauma, such as SCI. In this study, we investigate the consequences of systemic CSPG digestion on spinal cord repair in the chronic phase using 4-methylumbelliferone (4-MU), an inhibitor of hyaluronan (HA) synthesis. We show that adult rats treated with 4-MU after spinal cord contusion have a significant reduction in secondary injury pathology, with reduced cavitation and increased axon sprouting at 16 weeks post-injury compared to placebo-treated animals. To understand these effects, we examined inflammatory changes following 4-MU treatment. An increase in the M2 macrophage marker CD206 suggests that systemic CSPG digestion may alter the macrophage phenotype in favor of alternatively activated M2 macrophages. 4-MU also promoted remodeling of the specific ECM filling the cystic cavity. These effects of 4-MU treatment corresponded to improvements in sensorimotor function as assessed by behavioral tests. The improvement in function was confirmed by axonal tracing (DiD) and increased serotonergic innervation. Thus, we have demonstrated that oral treatment with 4-MU at a dose of 2 g/kg/day can modulate plasticity and secondary damage processes, leading to long-term improvement in functional outcome. At the same time, we provided novel mechanistic evidence that modulation of the macrophage phenotype may underlie these effects.

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The role of small extracellular vesicles in the regeneration of nervous tissue

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Spinal cord injury (SCI) is a devastating condition with a complicated pathology and long-term functional impairment with no effective treatment. A traumatic primary injury is followed by an inflammatory cascade of secondary injury. Demyelination, cell death and glial scar formation occur. These processes, combined with the limited regenerative capacity of the CNS, are the main reasons for the poor prognosis after SCI. Promising therapeutic approaches include the application of stem cells, which have been shown to have neuroprotective and immunomodulatory effects in SCI. The exact mechanism of their action has not yet been satisfactorily explained. Based on published works, it is suggested that the positive effect is mediated by small extracellular vesicles (sEVs) released by stem cells, mainly by miRNA contained therein. The aim of this work is to perform a comprehensive characterization of sEVs isolated from the culture media of neural (NSCs) and mesenchymal stem cells (MSCs), including detection of expressed exosomal markers and several neuroprotective miRNAs. To evaluate the therapeutic potential of sEVs on an in vitro SCI model, sEVs suspension was applied to the culture of primary neurons and spinal cord slices. This led to a decrease in the levels of proteins involved in pathophysiological and apoptotic processes compared to the injured tissue. When applied to an in vitro model, NSC-sEVs have shown a stronger neuroprotective effect compared to mesenchymal stem cell derived sEVs.

Supported by: GAUK 409222 and OP JAK CZ.02.01.01/00/22_008/0004562

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Autophagy in axonal growth and regeneration of adult sensory neurons

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Autophagy is an intracellular recycling pathway which encapsulates damaged organelles and harmful cellular components in vesicles known as autophagosomes, which then merge with lysosomes to form autolysosomes, where the cargo is degraded. This dynamic process, termed autophagy flux, is highly important in maintaining cellular homeostasis, particularly in post-mitotic cells like neurons. It also assumes a critical role in the response to injury, however, its precise involvement is more intricate than expected, with both beneficial and detrimental effects observed, and further studies are required to elucidate the interplay between injury, regeneration, and autophagy. To delve into this complexity, we examined autophagy flux during axonal growth and regeneration of adult dorsal root ganglia neurons with live-imaging microscopy using a transgenic mouse to distinguish between autophagosomes and autolysosomes. In uninjured conditions, axons can either grow or remain static. Our findings reveal that static axons exhibit a blockage in autophagy flux at the fusion step, leading to the accumulation of stationary autophagosomes at the axon's tip. Following mechanical injury, similar patterns emerge, with all injured axons displaying immobile autophagosomes accumulation. Within two hours post-injury, regenerating axons return to normal autophagic levels, static axons maintain fusion blockage with autophagosomes accumulation, while retracting axons show an increase in autophagic flux. Additionally, we treated the neurons with various chemical modulators of autophagy and examined their impact on growth and regeneration. Surprisingly, increasing autophagy enhances axonal growth and regeneration capacities, which appears to contradict the previous results wherein axon retraction correlated with increased autophagy flux. However, this data highlights that the condition of the axon (uninjured or injured) dictates whether autophagy plays a beneficial or detrimental role. Collectively, these findings offer a more comprehensive understanding of autophagy's mechanisms and its role in axonal injury and provide new opportunities to manipulate and overcome current limitations of neuronal regeneration.

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The role of miR-20a in the nervous tissue injury

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Spinal cord injury (SCI) represents a devastating condition characterized by neural cell death and disruption of conduction pathways, leading to loss of motor and sensory functions. SCI is accompanied by the dysregulation of genes and microRNAs (miRNAs), especially in the critical secondary phase. To elucidate the dynamic changes in mRNA/miRNA/protein expression post-SCI, we used an in vivo SCI model in Wistar rats. In this study, rats underwent SCI induction via balloon compression at the T10 vertebra level, with control and sham-operated groups for comparison. Animals were sacrificed at different time points (3 h, 12 h, 24 h, 3 d, 7 d) post-injury. To understand the role of miRNAs in the pathophysiology of SCI we did a multi-omic analysis, that includes mRNA and miRNA sequencing, proteomic analysis, deconvolution, and integrative analysis of mRNA-miRNA-protein profile. Principal component analysis (PCA) revealed distinct mRNA and miRNA expression profiles across experimental groups and time points. We identified approximately 5000 upregulated and 4500 downregulated genes, along with 164 upregulated and 151 downregulated miRNAs post-SCI. Using deconvolution algorithms, we characterized cell populations based on their gene expression patterns at different time points after injury. Integration of mRNA, miRNA, and proteomic data facilitated the construction of an interactome, providing insight into the complex molecular interactions underlying SCI. Notably, we observed significant upregulation of miR-20a expression persisting for at least one week post-trauma. MiR-20a was found to target numerous genes involved in neural tissue regeneration. Inhibition of miR-20a resulted in decreased expression of pro-apoptotic proteins (Cytochrome C, Bax, Caspase-3) and increased expression of anti-apoptotic proteins (Mcl-1, Bcl-Xl), attenuating neural cell death following injury. This comprehensive analysis offers valuable insights into the intricate molecular mechanisms driving SCI pathophysiology and paves the way for targeted therapeutic interventions aimed at mitigating neural tissue damage and promoting recovery.

Supported by GACR 18-21942S and NU21-08-00286 and OP JAK CZ.02.01.01/00/22_008/0004562.

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Metabolic and secretome profile of multipotent mesenchymal stromal cells spheroids

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Multipotent mesenchymal stromal cells (MSCs) are currently the focus of extensive research due to their therapeutic properties, such as multilineage differentiation capacity, immunomodulatory and paracrine activity. Compared to standard monolayer cultures, the 3D spheroid cultivation of MSCs may closely recapitulate the native microenvironment by facilitating cell-cell interactions. In this study, we compared the metabolic activity, metabolomic and lipidomic profile, secretome composition and gene expression of human adipose tissue-derived MSCs (AT-MSCs) in 2D and 3D spheroid culture conditions. To generate spheroids, AT-MSCs were cultured in ultra-low adhesive plates. Metabolic activity of 2D and 3D cultured cells was assessed by Alamar blue test and Seahorse XF analysis. The secretome composition was evaluated in conditioned medium by luminex-based 11-Plex Human ProcartaPlex assay. LC-MS metabolomics of cells was performed by extracting metabolites using a biphasic solvent system and detecting them in negative and positive electrospray ion mode using Thermo Q Exactive Plus instrumentation. We detected a significant increase in the content of EGF, FGF-2, HGF, PIGF, LIF, SCF, and VEGF-A in the conditioned medium produced by spheroid cultures compared to monolayer. The metabolic activity was significantly decreased in 3D spheroids. A comparative examination of metabolomics data acquired from spheroids and monolayer cultures showed decreased content of membrane-associated lipids (cholesterol, cholesterol ethers), with a simultaneous increase in the content of triglycerides in 3D cultured cells. Therefore, we show that the MSCs within 3D spheroids undergo metabolic adaptations, reorganization of intracellular lipid composition, and a simultaneous increase in paracrine activity, confirming the potential of using MSC spheroids in regenerative medicine applications.

The study was supported by Czech Science Foundation grant GAČR 22-31457S, Charles University Grant Agency grant GAUK 390722 and ExRegMed project No CZ.02.01.01/00/22_008/0004562, of the Ministry of Education, Youth and Sports, is co-funded by the European Union.

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Towards the development of 3D modelling system for glioblastoma research and drug testing

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Glioblastoma multiforme (GBM), a highly aggressive brain tumor, requires innovative therapies and advanced in vitro models resembling the tumor microenvironment (TME). Over the past decade, GBM modelling has shifted from monolayer cultures (MC) to more complex three-dimensional (3D) environments. In this study, we compared the response of 2D and 3D cultured GBM cells to several types of treatment and attempted to optimize the 3D model system to improve the TME resemblance. The 3D spheroid cultures of GBM cells (U87MG line) were established in ultra-low adhesive plates during 3–7 days. The effect of integrin binding nanoparticles (cRGD-NPs) was analyzed in 2D and 3D culture conditions. The 3D invasion of GBM cells was studied in Geltrex™. Cell outgrowth was assessed by image segmentation analysis in ImageJ. To improve the 3D culture conditions, we established the perfusion system and validated it using standard cytostatic treatment. We detected higher resistance of 3D GBM cells to cytostatic treatment compared to the monolayer cultures. The response of 2D and 3D cultured cells to cRGD-NPs was different. In monolayer cultures the addition of cRGD-NPs led to the detachment of cells, confirming the integrin binding, while no visible effect of cRGD-NPs was detected in 3D cultures. The established perfusion system enabled studying the 3D invasion of GBM under various treatments and in co-culture with other cell types. Co-culture of GBM spheroids with multipotent mesenchymal stromal cells or astrocytes altered the invasion behavior pattern. This work underscores the importance of 3D culture for understanding GBM therapy responses and provides an adaptable system for diverse experimental needs.

The study was supported by Czech Science Foundation grants GAČR 22-12483S, GAČR 22-31457S, and ExRegMed project No CZ.02.01.01/00/22_008/0004562, of the Ministry of Education, Youth and Sports, is co-funded by the European Union.

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Poster session

Wednesday 8. 5. 2024

9.00 – 10.30

DNA repair capacity in various tissue compartments of colorectal cancer in relation to senescence-associated secretory (SASP) phenotype expression profile

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Background: By permanently stopping the cell cycle, restricting proliferation, and blocking the spread of harmful genetic alterations, cellular senescence protects the tissue from the onset of cancer. Because of this, anticancer treatments frequently try to stop the growth of the disease by causing cell cycle arrest. The eventual therapeutic resistance and/or relapse that many patients experience is a major setback in overall patient outcome. Despite the initial benefits of this strategy in lowering the tumor burden, senescent cells can develop an altered cellular secretome, which is linked to local inflammation, extracellular changes, and increased growth factor activity, which may modulate the tumor microenvironment through regulation of the immune response, cancer cells gene expression and resistance to chemotherapy (Cht). In this research we hypothesized that the chemotherapy resistance is due to the defective DNA repair response induced by cancer senescent cells, which hinder the detection of Cht-induced DNA damage and prevent cancer cells eradication by apoptosis. Hence measuring the senescent gene s expression might be a good prognostic factor.

AIM: We aim, in our experiment, to compare the expression level of senescent cell related genes expression level in the tumor tissue vs mucosa, and to compare the level of expression with the clinical prognostic parameter.

Methodology: total RNA will be isolated and reverse transcribed, then for the signature of senescent cells (SASP), gene expression level will be measured. The ratio of the expression level of the senescent cells will be compared to the patients' clinical features and DNA repair capacity between the tumor and the adjacent mucosa. DNA repair capacity will be measured by comet assay. Our findings will be validated in vitro by inducing the senescent cells in DLD1 cell lines and measuring the capacity of these cells to repair the DNA.

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Age-related changes in the extracellular space diffusion parameters and extracellular matrix in mouse cerebellum

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Brain aging is associated with complex brain tissue changes, including atrophy/loss of brain volume, structural and functional changes of cells and alterations in volume and content of the extracellular space (ECS). We previously demonstrated that age-related changes in the ECS diffusion properties are attenuated in specific brain structures, that are rich on protective perineuronal nets (PNNs) formed by specific extracellular matrix molecules (ECM). In the current study, we assessed the age-related changes in the ECS diffusion parameters, evaluated potential anisotropic diffusion and determined the ECM alterations in cerebellar nuclei, the brain structures rich on the PNNs. The values of the ECS volume fraction α (α = extracellular volume/total tissue volume) and tortuosity (λ2 = free/apparent diffusion coefficient) were determined by the real-time iontophoretic method using ion-selective microelectrodes in 400 µm thick slices from young adult (3 – 6 months) and aged (12 – 18 months) mice. To reveal anisotropy, diffusion measurements were performed in three orthogonal axes. In addition, we performed an evaluation of the ECM composition by immunohistochemistry. As we did not reveal diffusion anisotropy either in young or aged animals, the data from different axis were pooled. In young mice, $\alpha = 0.19 \pm 0.02$ and $\lambda = 1.75 \pm 0.02$ (means ± SEM); we did not detect any significant changes in α or λ in aged animals. We detected Brevican/Bral2 co-localization in the PNNs of the cerebellar nuclei in both young and aged mice. However, Brevican immunostaining was increased in aged animals, which corresponded with the age-related changes in its expression or distribution. Our results confirmed that structures rich on Brevican/Bral2-based PNNs are protected against typical age-related changes in the α and λ. Moreover, the Brevican staining was higher during ageing, which could even increase the protective ability of the PNNs. This hypothesis will be tested on Brevican and Bral2 knock-outs.

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Reprogramming glial cells into functional neurons following brain ischemia

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Ischemia is a serious neurological condition and the third most common reason for death in the western world. In ischemia, the restriction of blood flow in the cerebral cortex disrupts ionic gradients and energy metabolism, which ultimately leads to neuronal death. Nevertheless, there is no effective treatment and hence, new therapeutic avenues are being considered, including neuronal reprogramming. Glial cells such as astrocytes and NG2 glia proliferate in response to ischemic injury, which makes them perfect candidates for replenishing neuronal loss by neuronal reprogramming. Moreover, there is evidence that certain NG2 glial subtypes express neuronal markers after ischemic injury in vivo, and therefore, we will commence our study by transfecting murine astrocytes and NG2 glia with modified mRNA encoding Ascl1, Sox2, Foxg1, Lhx6, and Dlx5 in vitro. We have already established neonatal murine astrocyte and NG2 glia cultures in our lab and tested the efficiency of GFP-labeled modified mRNA transfection. In the next step, we will transiently, but robustly, overexpress key transcription factors in the GABAergic neuronal lineage. In order to follow the changes in the expression of the transcription factors on the nucleic acid and protein levels, we will perform qPCR and Western blotting analyses at different time points after transfection. The identity of the newly formed GABAergic interneurons will be assessed with immunocytochemistry, morphological analysis, and functional analyses (patch-clamp technique). The results from our in vitro experiments will be also tested in vivo, in mice subjected to middle cerebral artery occlusion, a widely used model of focal cerebral ischemia. Finally, we will perform behavioral experiments, such as the cylinder test, to examine a possible motor improvement after neuronal reprogramming in ischemic mice.

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Single-cell profiling reveals the role of Meis2 in transcriptional regulation of osteogenesis during craniofacial morphogenesis

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The vertebrate skull is composed of many skeletal elements originating from cranial neural crest cells (CNCCs). CNCCs arise from the neural plate border, migrate into multiple regions of the developing embryo, and differentiate into various cell types such as cartilage and bones, peripheral nerves and glia, melanocytes or connective tissues. The morphogenesis of the craniofacial region encompasses several processes from epithelial-mesenchymal induction and mesenchymal condensation to endochondral or intramembranous ossification. However, the molecular and genetic pathways regulating cranial cartilage shaping and growth during embryogenesis are poorly understood. Employing Wnt1-Cre2-induced Meis2 conditional knock-out (cKO) mice, we focus on the development of hyoid apparatus, the mandibular arch, palatal shelves and maxillary prominence. We present a mouse model in which several craniofacial bones are malformed due to improper molecular mechanisms controlling mesenchymal condensations. Meis2 cKO embryos exhibit ectopic loci of mesenchymal condensations, proliferation of cartilage and bone precursors resulting in increased ossification. These processes contribute to the phenotype seen in our model, displaying aberrant hyoid apparatus, cleft palate and underdeveloped tongue resembling severe human pathologies. Combining single-cell RNA sequencing and spatial transcriptomics we further concentrate on underpinning the Meis2-dependent gene regulatory network involved in mesenchymal condensation. A comparison of control and Meis2 cKO embryonic stages E12.5 and E13.5 reveals several mesenchymal cell types responsible for craniofacial formation. We have identified markers for cell type specification and positional cue in the developing viscerocranium. Furthermore, differences identified from scRNA-seq in cell type composition correlate with the Meis2 cKO phenotype. Transcriptomics data in Meis2 cKO show elevated expression of multiple genes involved primarily in ossification, cell-cell adhesion, and cell-extracellular matrix contact. Altogether, identification of the hitherto unknown factors controlling the growth and shape of cranial bones may potentially contribute to the mitigation or prevention of human craniofacial abnormalities.

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A novel combination of PI3Kδ and Chondroitinase ABC to promote axon regeneration following spinal cord injury

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Paralysis following a spinal cord injury (SCI) stem from the inability of adult mammalian central nervous system (CNS) axons to regenerate. Thus, stimulating neuronal regeneration and facilitating axon growth through extracellular matrix (ECM) is imperative to recover function post lesion. This can be achieved using gene therapy to deliver regeneration-associated genes to corticospinal neurons while simultaneously degrading growth inhibiting chondroitin sulphate proteoglycans (CSPGs) in ECM. PI3kinase δ, regulating the Akt/mTOR pathway, has shown significant relevance in axon regrowth programmes; data from rat models subjected to SCI and administered PI3Kδ show significant functional improvements in fine and gross motor function. Chondroitinase ABC (ChABC) is a bacterial enzyme that degrades the glycosaminoglycan side chains of CSPGs, and many studies have demonstrated its efficacy in SCI treatment. Here, we used a model of C4 dorsal column crush SCI and gene therapy to deliver a novel combination of PI3Kδ and ChABC to the brain and spinal cord respectively. The right motor cortex was injected at 4 sites at 1.5 mm depth, immediately followed by lentiviral injection at 2 sites adjacent to the SCI border (0.5 µl/site). Adult Lister Hooded rats received cortical injections of AAV1-hSYN-PIK3CD (2.17×10^{12} gc/ml) + hSYN-GFP (1×10^{12} gc/ml) at a ratio of 9:1, and intraspinal Lenti-PGK ChABC or Lenti-PGK-empty concurrently. Control rats received titre matched AAV1-hSYN-GFP and Lenti-PGK-ChABC or PGK empty to create four experimental groups. Animals were tested weekly for 16 weeks to assess grip strength, horizontal ladder and staircase performance. Here we demonstrate that PI3Kδ or ChABC administered alone significantly improves upper limb motor function. However, when administered in combination, although motor function is improved, the effect is not synergistic. Further immunohistochemical analysis of tissues will assess the combinations effect on regeneration of corticospinal tract neurons and CS-56 analysis to assess degradation of the glycosaminoglycans and ECM.

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Identification of molecular and epigenetic candidates elucidating therapy resistance of solid cancers

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Colorectal cancer (CRC) is the third most lethal cancer subtype in Europe. The number of patients diagnosed with CRC and genetically related solid malignancies is increasing. 5-Fluorouracil (5-FU) is the first-line chemotherapeutic for metastatic colon cancer. 5-FU or other therapeutics (e.g. oxaliplatin) combined with 5-FU are commonly used to treat gastrointestinal cancers with significantly good results and increased lifetime of the patients. On the other hand, the generation of resistance to 5-FU (or other drugs) by tumor is a common obstacle to achieving a better prognosis. In this project, we aimed to address the transcriptomic and epigenetic changes underlying the therapy resistance. DLD-1 cell line will be used as a parental cell. Cell lines resistant to 5-FU and regorafenib will be generated by treating cells with increasing concentrations of each drug. Previously generated oxaliplatin-resistant DLD-1 cell line (OXREZ DLD-1) will also be used. Molecular characterization of these cell lines such as proliferation, cytotoxicity, and colony formation behaviors of these resistant cells will be performed. The resistance of the cells will be confirmed by qPCR and western blot with corresponding genes. RNA sequencing and methylation array of the resistant cells will be performed in comparison to the parental cell line. The influence of the selected differentially expressed genes on resistance will be investigated by CRISPR technology. The results will be validated with poor responder patients to each drug. The results will help to understand the mechanisms behind the cancer progression among cancer patients in response to therapy resistance and provide a basis for preclinical data.

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Effect of mTOR signaling pathway modulation on astrocyte function in focal cerebral ischemia

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Stroke is one of the leading causes of death and disability worldwide, whereas available treatment options are very limited. One of the potential therapeutic approaches is the modulation of the mTOR (mammalian target of rapamycin) signaling pathway, which plays a protective role after ischemic brain injury by inhibiting autophagy and supporting neurogenesis and angiogenesis. However, since mTOR signaling is implicated in many other cellular processes, including metabolism, proliferation, cell growth, migration, and differentiation, its modulation can affect various cell types and their functionally distinct subpopulations after ischemia differently. We therefore aim to elucidate the role of the mTOR pathway specifically in astrocytes, the main homeostatic cell type involved in several key processes related to cerebral ischemia. We have already crossbred a mouse strain enabling conditionally inducible inactivation of Rptor (regulatory associated protein of MTOR, complex 1) gene with B6N.FVB-Tg(Aldh1l1-cre/ERT2)1Khakh/J mice to selectively inhibit mTOR signaling in astrocytes. The efficiency of our Cre-loxP system was verified with RT-qPCR in cells sorted by fluorescence-activated cell sorting. In these mice, focal cerebral ischemia will be induced by permanent middle cerebral artery occlusion (pMCAO) in further experiments. The effect of mTOR pathway inhibition will be studied using scRNAseq, immunohistochemistry, MRI, and patch-clamp technique, while the overall functional impact will be assessed with behavioral testing. We have already tested several behavioral methods on control mice to verify their relevance to our mouse model. Based on our preliminary results, three methods were selected: the balance beam walking test, the pasta handling test, and the pole test. The results from behavioral testing were compared with an immunohistochemical analysis of ischemic lesion size and localization, which suggested that these lesion parameters, caused by anatomical differences between individuals, affect the animals' performance in behavioral tests.

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The angiogenic properties of stem cells-derived extracellular vesicles

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Acute ischemic stroke (AIS) is one of the leading causes of disability worldwide. The ischemic brain undergoes complex pathophysiological changes resulting in secondary damage to brain tissue. One of the secondary effects of AIS is the disruption of the blood-brain barrier (BBB), which leads to hemorrhage, brain edema, and overall leakage of damaging molecules into the brain. Previous studies have shown that angiogenesis is a crucial part of neuroregeneration following ischemic stroke. Stem cells have been extensively studied in the past for their regenerative potential in the context of numerous pathophysiologies. The protective and regenerative effects of stem cells have been mainly attributed to their ability to create such an environment. Extracellular vesicles (EVs) have been hypothesized to mediate these changes. We used sucrose cushion ultracentrifugation to isolate EVs derived from neural precursors and mesenchymal stem cells. We then characterized these EVs based on their size and the presence of protein markers. To evaluate the angiogenic effect of stem cell-derived EVs, we conducted scratch assays, proliferation assays, tube formation assays, and migration assays on Human Aortic Endothelial Cells. Dynamic light scattering measurements showed that EVs are typically sized between 30 – 150 nm. Immunoblotting further demonstrated the presence of CD63, CD9, CD81, Alix, and TSG101. Angiogenic properties of EVs were analyzed. For further steps, we hypothesized that the regenerative effect of EVs is mediated through their miRNA content which regulates the Wnt/b-catenin signaling pathway.

Supported by GAUK 409222, AZV NU22-08-00124, and OP JAK CZ.02.01.01/00/22_008/0004562.

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Audiometrical examination in patients with mild cognitive impairment

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The aim of the study is to evaluate the results of complex audiometric examinations in elderly patients, and compare the results with patients with mild cognitive impairment and patients with normal cognitive functions.

In every patient, an examination of cognitive functions with the czech version of Adenbrook cognitive examination III (ACE III) and original czech brief test ALBA and PICNIR was carried out. Mild cognitive imparment was evaluated as an ACE III score less than 90 points (from total 100 points). We also completed a complex audiometric examination, including pure tone audiology, sentence recognition in babble noise on two different levels (babble poise at the same level as the sentences and the noise at a five decibel louder level than the sentences) and a chopper test – sentences were periodically gated with a given duty cycle (at 30%, 55% and 70% duty cycle).

In total we examined 8 patients with mild cognitive imparment and 27 healthy controls of the same age. There were no significant differences in the results of pure tone average. When comparing the results of recognition of sentences in babble noise there, were no significant differences when the level of the noise was louder than the level of the sentences, but the healthy controls showed significantly better results when the levels were the same. In recognition of the gated speech, they did significantly better in sentences with 70% duty cycle. In 55% duty cycle there were some observable but not significant differences. In 30% sentences both groups did the same.

The results prove differences in recognition of speech between healthy patients and patients with mild cognitive imparment. This finding opens many options for research of central auditory processing disorders, and of the relation between hearing impairment and cognitive functions.

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