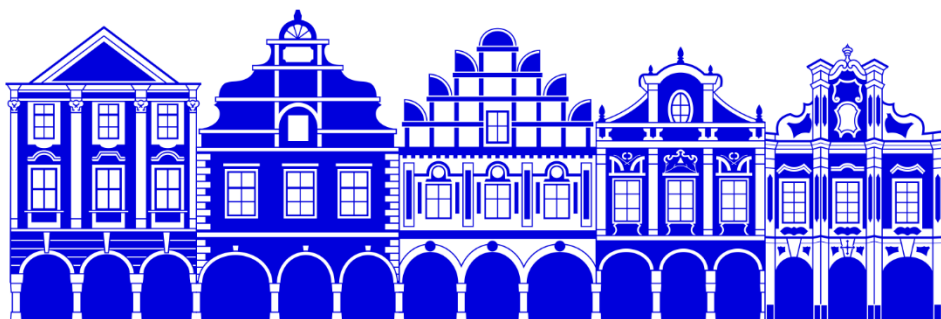


**MUNI
MED**

**Masaryk University, Faculty of Medicine
Department of Anatomy**

Programme and Abstracts



MORPHOLOGY 2024 CONFERENCE BOOK

55th International Congress of Czech Anatomical Society

60th Lojda Symposium on Histochemistry

**MASARYK
UNIVERSITY
PRESS**

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For faster orientation and movement in the document, you can use [links](#) (some blue headings, texts and blue texts in the footer).

MUNI Department
of Anatomy
MED

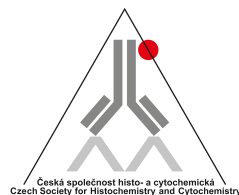
MORPHOLOGY 2024
CONFERENCE BOOK

55th International Congress of Czech Anatomical Society
60th Lojda Symposium on Histochemistry

PROGRAMME AND ABSTRACTS

Masaryk University Press

Brno 2024



CONTENT

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Czech Anatomical Society

Czech Society for Histochemistry and Cytochemistry

Masaryk University, Faculty of Medicine, Department of Anatomy

MORPHOLOGY 2024

55th International Congress of Czech Anatomical Society

60th Lojda Symposium on Histochemistry

Under the Auspices of

prof. MUDr. Martin Repko, Ph.D., Dean of the Faculty of Medicine, Masaryk University

prof. MUDr. Martin Bareš, Ph.D., Rector of Masaryk University

Scientific Board

Marek Joukal, Petr Dubový, Karel Smetana, Ondřej Naňka, Jaroslav Mokrý, Václav Bába, David Kachlík

Organizing Committee

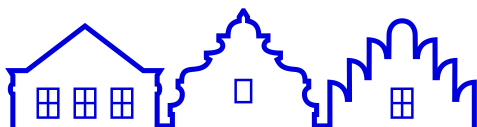
Marek Joukal, Petr Dubový, Klaudia Hašanová, Alemeh Zamani, Karolína Bretová, Jitka Skládaná

Topics

Neurosciences, Molecular and Cell Biology, Developmental Morphology, Histology, Clinical Anatomy, Anthropology, Teaching and Anatomical Terminology

September 8–10, 2024

Telč, Czech Republic



MORPHOLOGY 2024

GENERAL INFORMATION

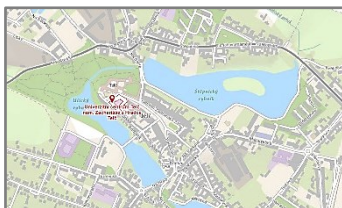
Venue

University Centre Telč, Masaryk University, Náměstí Zachariáše z Hradce 2
588 56 Telč, Czech Republic

For detailed information see the websites:

<https://www.uct.muni.cz/>

Map of Telč



Contacts

e-mail: morphology@med.muni.cz, web page: <https://morphology.med.muni.cz>

Registration and Information Desk

Ground level of the University Centre Telč, Masaryk University, hallway.
Náměstí Zachariáše z Hradce 2, 588 56 Telč

Office Hours:

Sunday, September 8, 2024 15:00–17:00

Monday, September 9, 2024 08:00–17:00

Tuesday, September 10, 2024 08:00–12:00

Language

English

Oral Presentations

Invited lectures are scheduled for 30 minutes, regular lectures are scheduled for 10 minutes presentation followed by 5 minutes of discussion. Windows PC with USB port are installed in lecture halls. Presentation software is PowerPoint 365. Videos can be presented only through PowerPoint presentation. Speakers are kindly asked to check their presentations in the lecture hall before beginning of each session.

Poster Presentation

The recommended size of poster panel is A0 (84.1 cm width × 118.9 cm height). Maximal size is 85 cm (width) × 139 cm (height). All posters will be displayed from Monday to Tuesday in hallway (ground level). Tapes and clips will be available. Posters will be presented during poster session, on Monday, September 9. Authors are asked to be present at posters during the poster session.

Sponsors Exhibition

The exhibitions are located at the ground level and open throughout the meeting.

Exhibitors:

All gene, s.r.o.

Bombus, s.r.o.

Cheirón, a.s.

Animalab, s.r.o.

Dr. Max, s.r.o.

Mattoni 1873, a.s.

Bamed, s.r.o.

Elsevier

Megabooks CZ, s.r.o.

BioRad, s.r.o.

Grada Publishing, a.s.

Trigon plus, s.r.o.

Welcome Reception

will be held immediately after the Opening ceremony on Sunday, September 8, at the University Centre Telč in the lecture **hall A**. It will include buffet and welcome drink free of charge. Other drinks are available for purchase.

Social Evening

Social Evening will take place at Panský Dvůr Telč, brewery, Slavatovská 86, Telč on Monday, September 9 from 19:30. Please buy a ticket at the registration desk (price 800,- CZK).

Coffee breaks

Refreshments will be served free of charge.

Lunch

Lunches will be served in the dining room, pre-paid by attendant (price 190,- CZK).

City Transportation

All key locations in the city of Telč are easily accessible on foot.

Czech Anatomical Society – member of the Council of Scientific Societies of the Czech Republic

Secretary office: Institute of Anatomy, First Faculty of Medicine, Charles University, U Nemocnice 3, CZ 128 00 Prague 2

phone: +420-224 965 780, fax: +420-224 965 770, e-mail: anat@lf1.cuni.cz

<http://cas.lf1.cuni.cz>

Czech Society for Histo – and Cytochemistry – member of the Council of Scientific Societies of the Czech Republic

Secretary office: Šimkova 870, 500 38 Hradec Králové

phone: +420-495 816 294, fax: +420-495 816 376, e-mail: mokry@lfhk.cuni.cz

<http://www.cshc.cz>

Czech Anatomical Society

Czech Society for Histochemistry and Cytochemistry

Masaryk University, Faculty of Medicine, Department of Anatomy

MORPHOLOGY 2024

55th International Congress of Czech Anatomical Society

60th Lojda Symposium on Histochemistry

PROGRAMME

- SUNDAY
- MONDAY
- TUESDAY

September 8–10, 2024

Telč, Czech Republic

SUNDAY, SEPTEMBER 8, 2024

17:00–19:00 Opening Ceremony

Chairs: Ondřej Naňka, Ingrid Hodorová, Jaroslav Mokrý, Marek Joukal

Words of Welcome

- President of CAS: doc. MUDr. Ondřej Naňka, Ph.D.
- President of SAS: prof. MUDr. Ingrid Hodorová, Ph.D.
- President of CSHC: prof. MUDr. Jaroslav Mokrý, Ph.D.

Winners' Lectures

Chairs: Naňka O., Mokrý J.

Czech Anatomical Society – Best PhD thesis Award – Winner's Lecture

Fojtík Petr

Syndesmotoc lesion in fracture-dislocations of the ankle - Maisonneuve fracture

Czech Society for Histo- and Cytochemistry & BARIA Award – Winner's Lecture

Bretová Karolína

Subpial astrocytes reactivity in the medial prefrontal cortex in the experimental neuropathic pain model

Koubová Kateřina

PTEN and soluble epoxide hydrolase in intestinal cell differentiation

Annual Prize of the Czech Anatomical Society (Bochdalek Prize of CAS)

Musilová Zuzana

Use of free radial forearm and pronator quadratus muscle flap: Anatomical study and clinical application

Khadanovich Anhelina

The communication patterns between the lateral antebrachial cutaneous nerve and the superficial branch of the radial nerve

On the 60th Anniversary of the Lojda Symposium on Histochemistry

Dubový Petr

The memory of Professor Zdeněk Lojda, a pioneer of Czechoslovak histochemistry and a prominent person in Czech science

19:00 Music performance

19:00–20:30 Welcome evening

MONDAY, SEPTEMBER 9, 2024

8:00–8:30 Lecture Hall A – Invited Lecture

Chair: Dubový P.

Kmieć Z., Kiežun J. (Med. Univ. Gdańsk, Poland)

The galaninergic system in the progression of colorectal cancer: the role of immunohistochemistry

8:40–10:00 Lecture Hall A – Molecular and Cell Biology L5

Chairs: Adamkov M., Smetana K., Jr.

Smetana K., Jr., Lacina I., Raška M., Malý P.

Targeting the IL-6 receptor alpha chain to manipulate the cancer microenvironment

Cígerová V., Adamkov M., Grendár M.

SATB2 as a good prognostic marker for colorectal cancer

Krajňáková B., Mešťanová V., Hurta Csizmár S., Výbohá D., Adamkov M.

The role of PRMT5 and DSG2 in precancerous lesions of the uterine cervix.

Mešťanová V., Krajňáková B., Kúdela E., Kalman M., Hurta Csizmár S., Adamkov M.

Prognostic role of E2 & E4 proteins in progression of the cervical lesions

Lacková L., Samec M., Mazuráková A., Dvorská D., Braný D., Kajo K., Adamkov M., Kubatka P.

Epigenetic modulation via *Salvia officinalis* L. in chemopreventive model of breast carcinoma

8:40–10:00 Lecture Hall B – Experimental Morphology

Chairs: Faghani M., Hampl M.

Faghani M., Alijani M., Mohammadghasemi F., Esmaeili-bandboni A.

Beneficial effects of quercetin on mouse epididymal sperm parameters induced by nicotine

Mohammadghasemi F., Daeihamed M., Fallah Kochakam N., Taghizadeh Valdi A., Faghani M., Farzanegan A.

Tocopherol alleviates oxidative stress and increases androgen receptors in mice testicular tissues under forced swimming in warm water

Čimborová K., Pelková V., Kotasová H., Bohačiaková D., Sedláková V., Hampl A.

From decellularization to proteomics: exploring the lung extracellular matrix across species

Hامل M., Jandová N., Novaková M., Romanová A., Kavková M., Parobková V., Kompaniková P., Čada Š., Zikmund T., Kohoutek J., Buchtová M.

CDK13 mouse mutants as useful models of the CHDFIDD disorder

Pavlíková Z., Zahradníček O., Jelínek Michaelidesová A., Šrámek J., Davidková M., Hovoráková M.

Potential protective interaction of hyperthermia and hydrocortisone addition on fibroblasts proliferation

10:00–10:30 Coffee Break

10:30–11:00 Lecture Hall A – Invited Lecture

Chair: Joukal M.

Brassett Cecilia (Univ. Cambridge, UK)

From Pupil to Professional: The role of anatomy in the journey of a medical student in Cambridge

11:10–12:25 Lecture Hall A – Clinical Anatomy

Chairs: Hodorová I., Joukal M.

Vrzgula M., Josayová L., Lovásová K., Hodorová I.

Ultrasound-guided treatment of upper arm muscle spasticity: an anatomical and radiological study

Musilová Z., Joukal M.

Vascular supply of the dorsal portion of the wrist joint capsule and dorsal capsulotomy

Kohut P., Mezera V., Goals W., Zajíc P., Beneš M., Kachlík D.

Anatomical relationships and variations of the lateral femoral cutaneous nerve to surrounding structures during minimally invasive total hip arthroplasty via the anterior approach

Malakhov S., Shmarhalov A., El Falougy H., Lukáčiková P., Polák Š.

Canals of the zygomatic bone: a morphometric study on dry adult human skulls

Vecanová J., Hvizdošová N., Hodorová I.

Case of the persistence of the female processus vaginalis (canal of Nuck) and review of literature

11:10–12:25 Lecture Hall B – Neurosciences I

Chairs: Dubový P., Kubíčková L.

Dubový P., Rokoský Z., Rábová A.

Priming and signal transduction of delta opioid receptors in dorsal root ganglia neurons in a mouse experimental model of neuropathic pain - utilizing immunohistochemical detection to elucidate intracellular processes

Kubíčková L., Bahavan B., Karafiátová E., Leung V., Joukal M.

The Expression and Cellular Distribution of DOR in Two Different Animal Models During the Orofacial Neuropathic Pain

Voleská E., EmamiAref P., Kubíčková L., Joukal M., Zamani A.

Neuropathic pain and immunoreactive dynamics: Investigating the influence of paclitaxel in brain ventricles

EmamiAref P., Kubíčková L., Bakhshinejad B., Dubový P., Joukal M., Zamani A.

The reaction of choroid plexus to chemotherapy

Solár P., Šerý O., Vojtíšek T., Krajsa J., Srník M., Dziedzinská R., Králík P., Kessler M., Dubový P., Joukal A., Balcar V. J., Joukal M.

The Blood-Cerebrospinal Fluid Barrier as a Potential Entry Site for the SARS-CoV-2 Virus

12:15–13:15 Lunch

13:15–13:30 Congress Photo

13:30–15:00 Lecture Hall A – Developmental Morphology

Chairs: Tonar Z., Buchtová M.

Zavhorodnia I., Samec M., Hešková G., Straka L., Janík M., Výbohová D.

Morphological characteristics of developmental anomalies of the thyroid gland: a prospective autopsy-based study

Burianová A., Čížková K., Tauber Z., Koubová K.

In vitro model of trophoblast

Olbertová K., Hrčkulák D., Jesionek W., Kříž V., Hrubá E., Ešner M., Kořínek V., Buchtová M.

Lgr5: an old player with a potentially new role in the mesenchymal cells during development

Mihalik J., Čurgali K., Petrášová D., Hodorová I.

Antioxidant enzymes SOD1 and 2 in rat embryos and female genital organs during preimplantation period of pregnancy

Tonar Z., Vaňková L., Malečková A., Křížková V.

Single Best Answer (SBA) questions for formative and summative assessment in histology and embryology classes

Křížková V., Vaňková L., Bufka J.

Embryology applied (not only) in Pediatrics

13:30–15:30 Lecture Hall B – Symposium on Quality of Teaching Anatomy

15:00–16:00 Coffee Break – Poster Session

16:00–17:00 Lecture Hall A – Oral Morphology

Chairs: Křivánek J., Šedý J.

Šedý J., Kachlík D., Žižka R.

Ligaments of the temporomandibular joint: new classification

Žižka R., Šedý J., Tauber Z.

A histological observation of human immature permanent tooth after failed regenerative endodontic treatment and subsequent root canal treatment

Gonzalez Lopez M., Azam M., Kavková M., Lavický J., Henys P., Sadílek V., Buchtová M., Vořechovský M., Křivánek J.

How is the renewal of ever-growing teeth controlled? Integrating the concepts of regeneration and repair

Steklíková K., Dalecká L., Kubovciak J., Corneloup C., Pantalacci S., Hovořáková M.

Novel genes involved in the first lower mouse molar enamel knot establishment and its early maturation

16:00–17:15 Lecture Hall B – Clinical Morphology

Chairs: Tauber Z., Mokřý J.

Mokřý J., Čížková D., Reims H. M., Sullivan G.J., Almaas R.

Light microscopy structure of liver parenchyma in Aagenaes syndrome patients

Čížková D., Bezrouk A., Mokřý J., Sullivan G.J., Reims H. M., Almaas R.

Liver ultrastructure in paediatric patients with Aagenaes syndrome

Kolesová H., Neffeová K., Zábrodská E., Olejníčková V., Mašek J., Sedmera D.

Jagged1 disrupted signaling in conditional deletion mouse model and single nucleotide variant of patient-based mouse model causes severe cardiac defects – morphology and physiology.

Makovický P., Tůmová E., Makovická M., Volek Z.

The influence of feed restrictions on small bowel parameters on an experimental rabbit model

Tauber Z., Burianová A., Koubová K., Mrštík M., Jirkovská M., Čížková K.

The interplay of inflammation and placenta in maternal diabetes: insights into Hofbauer cell expression patterns

17:15–17:45 Meetings of society committees of the CAS and CSHC

17:45–18:15 Plenary meetings of the CAS and CSHC

19:30 Social evening

TUESDAY, SEPTEMBER 10, 2024

9:00–10:30 Lecture Hall A – Neurosciences II.

Chairs: Zamani A., Kubíčková L.

Cesnáriková S., Sedmík J., Fojtík P., Amruz Černá K., Pospíšilová V., Váňová T., Bohačiaková D.

Unraveling Alzheimer's Disease Mechanisms Using 3D Cerebral Organoids Derived from Induced Pluripotent Stem Cells

Jeřábková K., Orviská P., Zareravasan A., Dianat M., Joukal A., EmamiAref P., Brázda V., Zamani A.

Investigate the Role of Choroid Plexus in Alzheimer's Disease

Orviská P., EmamiAref P., Jeřábková K., Brázda V., Zamani A.

Tau phosphorylation and neuroinflammatory response to A β in choroid plexus cells

Báľentová S., Kalenská D., Borbélyová V., Filová B., Muriň P., Kecskés I.

Ionizing radiation-induced histological and functional changes in the brain under experimental conditions

Zamani A., Kocourková K., Kadlečková M., Mikulka F., Minaříková R., Smolka P., Rábová A., EmamiAref P., Kubíčková L., Hašanová K., Joukal M., Minařík A.

In vitro study of materials for functional replacements in nerve tissue regeneration

Báčová T., Dvořáková V., Vilímek M., Kachlík D., Báča V., Horák Z.

Changes in the loading of anatomical structures of the intact eye and the eye implanted with an artificial intraocular lens during blunt impact in a biomechanical model

9:00–10:20 Lecture Hall B – Anatomical Terminology

Chairs: Musil V., Naňka O.

Naňka O., Bartoniček J.

Terminology of the growing long bone: A historical study

Musil V., Modrý M., Stingl J., Kachlík D.

Evolution of the term naming the 8th cranial nerve

Stingl J., Riedlová J., Mrzilková J., Musil V.

Finis coronat opus

Požížková K., Račanská M.

Anatomical Nomenclature from a Linguistic Perspective: a Data-Driven Approach

Šimon František

Slovak and Czech anatomical terms with prefixes pred- / před-, i. e. in front of, and za-, i. e. behind.

Sponsor Lecture

Krupa Michal (Elsevier Health)

Anatomy Academy: Empowering Education for In-depth Understanding

10:30–11:00 Coffee Break

11:00–12:00 Lecture Hall A – Varia

Chairs: Račanská M.

Korim F., Kuricová M., Ivašková S., Eberlová L.

Incidence of duplication of the caudal vena cava in companion animals

Shbat A., Moraes C., Malá-Zedníková P., Beránek M., Kvasilová A., Klepáček I.

Face approximations of the Czech Countess Sidonia von Schlick

Čížková Kateřina

New ImageJ/Fiji script for assessment of nuclear receptor translocation

12:00–12:15 Closing ceremony

12:15 Lunch

Czech Anatomical Society

Czech Society for Histochemistry and Cytochemistry

Masaryk University, Faculty of Medicine, Department of Anatomy

MORPHOLOGY 2024

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POSTERS

- MOLECULAR AND CELL BIOLOGY
- NEUROSCIENCES
- SKELETON AND MUSCLES
- HEART AND VASA
- CLINICAL ANATOMY
- HISTOLOGY, EMBRYOLOGY
AND REGENERATION
- VARIA

September 8–10, 2024

Telč, Czech Republic

MOLECULAR AND CELL BIOLOGY

- poster 1** **Houdek M., Szotkowská T., Buchtová M., Kolísková P.**
Use of the CAM method in chicken embryos for the study of human tumors
- poster 2** **Kolísková P., Moldovan Putnová B., Hurník P., Štembírek J., Buchtová M.**
The role of primary cilia in the progression of oral squamous cell carcinoma (OSCC)
- poster 3** **Cakmakci R. C., Acimovic I., Jan L., Portakal T., Eid M., Vlažný J., Moravčík P., Kala Z., Vaňhara P.**
Investigating the role of endoplasmic reticulum stress and unfolded protein response in patient derived pancreatic adenocarcinoma cells
- poster 4** **Acimovic I., Vašíčková K., Vaškovicová N., Vodinská M., Eid M., Moravčík P., Vlažný J., Kala Z., Vaňhara P.**
Expansion and morphological characterization of patient-derived pancreatic ductal adenocarcinoma cells cultured in planar conditions and three-dimensional spheroids
- poster 5** **Pavliuk-Karachevtseva A., Benetinová Z., Bohuš P., Mihalik J., Hodorová I.**
Role of GPx5 in human colorectal adenocarcinoma
- poster 6** **Vrzgula M., Mihalik J., Briškárová V., Hodorová I.**
Immunohistochemical Detection of GPx4 in Pancreatic Ductal Adenocarcinoma Cells: A Pilot Study Investigating its Presence in Well-Differentiated Tumors
- poster 7** **Kreheřová A., Čurgali K., Mihalik J., Hodorová I.**
Glutathione peroxidase 6 in female genital organs of rat
- poster 8** **Mlejnek Petr**
Lysosomal-mediated drug resistance - Fact or illusion? (the role of pH gradients)
- poster 9** **Hudáková P., Korim F., Almášiová V.**
Malignant melanoma in 15 years old Hungarian Warmblood

NEUROSCIENCES

- poster 10** [Havránek T.](#), [Bogyová E.](#), [El Falougy H.](#), [Polák S.](#), [Báčová Z.](#), [Bakoš J.](#)
SHANK3 Deficiency Affects Neurite Length and Branching in Dopaminergic Neurons In Vitro
- poster 11** [Yakushko O.](#), [Cendelin J.](#), [Jelínková D.](#), [Kolinko Y.](#)
Investigating microglial roles in cerebellar degeneration of lurcher mice
- poster 12** [Kovalská M.](#), [Fábryová M.](#), [Hnilicová P.](#), [Kalenská D.](#), [Lehotský J.](#)
Effects of a High-Methionine Diet on the Cerebellum of Female Rats
- poster 13** [Kovács L. Á.](#), [Golgol A.](#)
Intermediate early genes' products and their age-dependent changes in prefrontal cortex in acute and chronic stress rat models
- poster 14** [Macková L.](#), [Báilentová S.](#), [Kalenská D.](#), [Murín P.](#), [Kecskés I.](#)
The effect of ionizing radiation on the population of glial cells in the neurogenic regions of the brain
- poster 15** [Čavka M.](#), [Zanze Beader M.](#), [Esclapez M.](#), [Petanjek Z.](#), [Hladník A.](#)
Massive migration of prospective telencephalic GABAergic neurons through the marginal zone in monkey and human fetuses
- poster 16** [Rábová A.](#), [Dubový P.](#), [Kubičková L.](#), [Joukal M.](#)
Cellular Distribution and Semi-Quantitative Changes of Delta Opioid Receptors in The Spinal Dorsal Horn of a Mouse Spared Nerve Injury Model
- poster 17** [Kročka E.](#), [Kubičková L.](#), [Joukal M.](#)
Diabetes Mellitus and Cellular Changes in the Choroid Plexus
- poster 18** [Abna Z.](#), [Moghadasi A. N.](#)
A case of Fahr's disease misdiagnosed with multiple sclerosis
- poster 19** [Plešingrová K.](#), [Raška J.](#), [Satková M.](#), [Fojtík P.](#), [Pospíšilová V.](#), [Sedmík J.](#), [Cesnáriková S.](#), [Amruz Černá K.](#), [Bártová S.](#), [Hříbková H.](#), [Bohačiaková D.](#)
Studying the pathological SORLA variant and its involvement in Alzheimer's disease development
- poster 20** [Satková M.](#), [Sedmík J.](#), [Raška J.](#), [Plešingrová K.](#), [Fojtík P.](#), [Bohačiaková D.](#)
Using human pluripotent stem cell-based glial models to study Alzheimer's disease

- poster 21** **Mečava M., Fojtík P., Vajrychová M., Raška J., Plešingrová K., Sedmík J., Kadaková S., Pospíšilová V., Váňová T., Soukup O., Bohačiaková D.**
Insights into changes in endosomal proteom in Alzheimer's disease using SORL1-deficient cortical neurons as a model
- poster 22** **Petanjek T. L., Blažević A., Prkačin M. V., Grgurević L., Jalšovec D., Hladnik A.**
Importance of variations in anterior divisions of the brachial plexus
- poster 23** **Bučan J., Holečková B.**
Diagnosis of selected hereditary eye diseases in dogs by PCR

SKELETON AND MUSCLES

- poster 24** **Korim F., Vdoviaková K., Ivašková S., Krešáková L., Vrzgula M., Giretová M., Medvecký L.**
Radiologic evaluation of the calcium phosphate-based material degradation in pig animal model
- poster 25** **Krešáková, L., Vdoviaková, K., Humeník, F., Ivašková, S., Korim, F., Medvecký L.**
Morphological evaluation of the efficacy of biocement with the addition of phytic acid/phytase in the therapy of osteochondral defects
- poster 26** **Ivašková S., Krešáková L., Vdoviaková K., Korim F., Humeník F., Medvecký L.**
Regeneration of bone defects in sheep animal model with calcium phosphate enriched with calcium sulfate hemihydrate
- poster 27** **Šimaiová V., Teleky J., Demčišáková Z., Hurníková, J.**
Evaluation of bone tissue regeneration in the area of the mandible using a hydroxyapatite bone plate.
- poster 28** **Teleky J., Šimaiová V., Demčišáková Z., Hurníková J.**
Temporomandibular disc in pig - the best animal model for the human?
- poster 29** **Pračková I., Vargová L., Páral V., Vymazalová K.**
Muscles of the forearm and hand in the Anubis baboon (*Papio anubis*) – anatomical case report
- poster 30** **Morávek A., Henyš P., Kuchař M., Harnádková K., Velemínská J., Prstková K.**
Bone mineral density as a sex determination feature
- poster 31** **Harnádková K., Hřebcová E., Měšťák J., Dupej J., Morávek A., Velemínská J.**
Manifestations of facial directional asymmetry in young adulthood

HEART AND VASA

- poster 32** **Artykov T., Čížková D., Žurmanová J. M., Galatík F., Šilhavý J., Pravenec M., Mokřý J.**
Differences in nestin expression in the heart of young and aging spontaneously hypertensive rats
- poster 33** **Kvasilová A., Neradilová C., Sanková B., Olejníčková V., Neffeová K., Sedmera D.**
Atrial myocardial architecture: Is development of the pectinate muscles homologous to development of the ventricular trabeculae?
- poster 34** **Lexová M., Bílková L., Naňka O., Sedmera D.**
Morphology of the left and right atrial auricle in the human
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ABSTRACTS

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FROM PUPIL TO PROFESSIONAL: THE ROLE OF ANATOMY IN THE JOURNEY OF A MEDICAL STUDENT IN CAMBRIDGE

Brassett Cecilia

Magdalene College, University of Cambridge, United Kingdom

cb457@cam.ac.uk

This talk documents the journey that a student would take from being interested in applying to study medicine in Cambridge and, after having been admitted onto the medical course, the important role that anatomy plays in both the preclinical and clinical stages of their studies. I will begin by explaining the history of the University of Cambridge and the unique features of the collegiate system, especially with regard to where and how teaching is conducted for the medical course. I will then focus on the individual journey of one particular student whose journey would be relatively typical of all the students here. This will be presented as a three-act play with a prologue, which introduces the concept of access events, where we encourage young people from all backgrounds who have the academic potential and enthusiasm to succeed at university to apply. The role of anatomy will then take centre stage, highlighting two key themes: the development of professionalism in our medical students and management of their mental welfare. We will follow the student up to qualification as a medical doctor, with an update of their current aspirations as the epilogue.

THE GALANINERGIC SYSTEM IN THE PROGRESSION OF COLORECTAL CANCER: THE ROLE OF IMMUNOHISTOCHEMISTRY

Kmieć Z.^{1,2}, Kieżun J.²

¹ Department of Histology, Medical University of Gdańsk, Poland

² Department of Human Histology and Embryology, Faculty of Medicine, Collegium Medicum, University of Warmia and Mazury, Olsztyn, Poland

zbigniew.kmiec@gumed.edu.pl

The incidence of colorectal cancer (CRC) has been increasing in the patients younger than 50 years and the evidence suggests, apart from molecular changes in tumor cells, an involvement of the central and autonomous nervous systems including the enteric nervous system (ENS) has been implicated in the progression of neoplasm. Our preliminary investigations suggested different immunoexpression of some ENS neurotransmitters depending on the distance from the CRC tumor. Therefore, we decided to investigate the serum concentration of galanin (Gal) and its levels in the surgically separated compartments of colon wall: mucosa with submucosa and muscularis externa.

We applied RT-qPCR, ELISA, immunohistochemistry (IHC), immunofluorescence and histomorphometry to study surgically separated compartments of colon wall: mucosa with submucosa and muscularis externa as well as paraffin embedded sections of CRC tissue and non-cancerous large intestine wall. By using IHC we determined localization of the three known types of galanin receptors (GalRs) in the tumor tissue and the wall of tumor-adjacent and distant parts of the large intestine.

The Gal content in the homogenates of myenteric plexuses located in the proximity of the tumor was higher than in the CRC tissue and colon wall distant to the tumor. However, the mean area of Gal-immunoreactive (-Ir) neurons in myenteric plexuses close to the tumor mass was by half lower than in the distant muscularis. This discrepancy between biochemical and morphological findings suggested that the proximity of CRC tissue could induce a compensatory increase of the Gal production in the neurons of myenteric plexuses. We found that the intensity of the GalR1 and GalR3 immunostaining in the CRC cells was clearly higher than in the epithelial cells of the tumor-adjacent mucosa, whereas that of GalR2 was similar. The relative immunoreactivity of GalR3 (tumor cells vs epithelial cells), but not GalR1 and GalR2, was associated with shorter overall survival of patients with CRC and correlated with the larger tumor size and its metastases to regional lymph nodes. Moreover, relative immunoreactivity of GalR1 (GalR1-Ir in submucosal plexuses close to CRC tissue vs plexuses distant from the tumor) correlated with the overall survival of CRC patients and with the higher tumor grading. Such relationships were not found when the immunoreactivities of GalR2 and GalR3 were assessed.

In conclusion, our studies showed that Gal and GalRs might be involved in the progression of the CRC. However, the ways by which this neuropeptide and its

receptors act during CRC development cannot be explained solely on the basis of morphological studies. Nevertheless, the *in vitro* data concerning the action of Gal on gastrointestinal cancer cell lines as well as studies of human GI cancers provide a framework to consider possible mechanisms of interactions between CRC cells and Gal and its receptors.

The studies were supported by the statutory grants of the School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Poland.

CHANGES IN THE LOADING OF ANATOMICAL STRUCTURES OF THE INTACT EYE AND THE EYE IMPLANTED WITH AN ARTIFICIAL INTRAOCULAR LENS DURING BLUNT IMPACT IN A BIOMECHANICAL MODEL

Báčová T., Dvořáková V., Vilímeck M., Kachlík D., Báča V., Horák Z.

Department of Health Care Studies and Department of Technical Studies, College of Polytechnics Jihlava, the Czech Republic

azerets@centrum.cz

The loading of anatomical structures in the human body can also be modelled biomechanically as a function of changes in position, changes in activity, as well as changes induced by trauma during the traumatic event or during and after healing. Biomechanical modelling is already a routine tool in healthcare to predict the load on different types of implants. In our case, we modelled the distribution of tissue loading in the optical system of the eye under physiological conditions and after implantation of an artificial intraocular lens, and subsequently during a blunt impact on the eyeball.

The source data were obtained from a literature search and from our own observations. The Finite Element Method (FEM) analysis was performed as a numerical simulation using the Abaqus program. Simulations on mathematical models showed the loading of individual structures of the eye under physiological conditions and during a high-intensity blunt impact. They showed the structures at risk and the locus minoris resistentiae both for damage in the physiological state and for altered conditions after surgery.

Numerical FEM analyses of the physiological eye model and the eye model with implanted IOL were performed. The results of the analyses show that the dynamic loading of the eye after IOL implantation leads to a significantly higher load on the biological tissues compared to the physiological eye model. In our IOL model we see a large load on the cornea during impact, whereas in the physiological eye model the overload is distributed over the hinge apparatus of the human lens.

The modelling showed a complete difference in the stability of the optical system before and after surgery, which should be taken into account in the care of the injured eye.

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IONIZING RADIATION-INDUCED HISTOLOGICAL AND FUNCTIONAL CHANGES IN THE BRAIN UNDER EXPERIMENTAL CONDITIONS

Bálintová S.¹, Kalenská D.², Borbélyová V.³, Filová B.⁴, Muriň P.⁵, Kecskés I.⁵

- ¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ² Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ³ Institute of Biomolecular Medicine, Faculty of Medicine, Comenius University in Bratislava, Bratislava, the Slovak Republic
- ⁴ Department of Histology, Medical Faculty, Comenius University in Bratislava, Bratislava, the Slovak Republic
- ⁵ Department of Radiotherapy and Oncology, Martin University Hospital, Martin, the Slovak Republic

sona.balentova@uniba.sk

Fractionated whole-brain irradiation (fWBI) has been a mainstay in the radiotherapy of multiple brain metastases for many decades. However, with increasing median survival rates, post-therapeutic changes in neurocognitive functions and quality of life have gained more importance. As the age of patients rises, the risk of late radiation-induced changes manifesting in the form of structural and functional deficits increases. In this study, we investigated the delayed effect of fWBI using two approaches: histological changes in selected brain regions and functional, cognitive patterns targeting hippocampus-dependent memory.

Adult male Wistar rats (4 months old) received fWBI with a total dose of 32 Gy administered in 4 fractions (dose 8 Gy per fraction) once a week on the same day for 4 consecutive weeks. Three months after fWBI, the animals were subjected to behavioral testing to examine cognitive changes. After being sacrificed five months following fWBI, histopathological changes in two brain neurogenic regions were investigated: the hippocampal dentate gyrus (DG) and the rostral migratory stream (RMS).

Although histological image analysis of brain slides is still in progress, the results indicate a well-preserved population of immature neurons within the RMS and stem/progenitor cells in the RMS and DG. Similarly, preliminary results of behavioral testing show some changes in spatial orientation and long-term memory three months after fWBI.

We can tentatively state that fWBI led to subtle histological changes in neurogenic areas that correspond to cognitive deficits that are more severe from a prognostic point of view. For definitive conclusions, it is necessary to correlate the data with other results of histological analysis, metabolomics, and volumetry.

This work was supported by a grant from the Ministry of Education, Slovak Republic (VEGA) No. 1/0131/22.

SUBPIAL ASTROCYTES REACTIVITY IN THE MEDIAL PREFRONTAL CORTEX IN THE EXPERIMENTAL NEUROPATHIC PAIN MODEL

Bretová K., Svobodová V., Dubový P.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

karolina.bretova@med.muni.cz

Subpial astrocytes are a specific subpopulation of astrocytes at the surface of the brain, called “glia limitans superficialis” (GLS). GLS forms an interface between the brain parenchyma and the cerebrospinal fluid (CSF) in the subpial space and, in rodents consists of astrocyte bodies intermingled with their cytoplasmic processes. Many studies have observed astrocyte reactivity in the medial prefrontal cortex (mPFC) parenchyma induced by a peripheral nerve injury, while the response of GLS astrocytes is still not fully understood.

In our study, we focused on the reactivity of rat subpial astrocytes in response to sciatic nerve compression (SNC) over different time periods (postoperative day (POD) 1, 3, 7, 14, and 21). Changes in subpial astrocyte reactivity were examined using immunofluorescence (IF) intensities of glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), NFκBp65, connexin-43 (Cx43) and aquaporin-4 (AQP4) after sham operation and SNC.

Our results showed increased intensities of GFAP-IF, and longer extensions of cytoplasmic processes into lamina I. On the other hand, subpial astrocytes displayed a significant decrease in GS-IF and NFκB-IF in response to sham and SNC operation compared with naïve control rats. The integrated intensity of Cx43-IF was significantly increased in subpial astrocytes of sham- and SNC-operated rats during all survival periods compared with those of naïve animals. The mean intensities of AQP4-IF were significantly decreased in GLS profiles after sham and SNC operations at POD3, POD7, and POD14 compared with naïve controls.

These results suggest that both peripheral nerve injury and peripheral tissue damage may induce significant changes in subpial astrocyte reactivity, illustrated by changes of GFAP, GS and NFκB-IF intensities. Further, our data suggest increased communication of subpial astrocytes via Cx43 gap junctions and impaired transport of water and solutes between the CSF in the subpial space and the cortical parenchyma through transiently reduced AQP4 protein levels.

This work was supported by MUNI/A/1331/2021 and MUNI/A/1563/2023.

LGR5: AN OLD PLAYER WITH A POTENTIALLY NEW ROLE IN THE MESENCHYMAL CELLS DURING DEVELOPMENT

Olbertová K.¹, Hrčkulák D.², Jesionek W.³, Kříž V.², Hrubá E.¹, Ešner M.³, Kořínek V.², Buchtová M.^{1,4}

- ¹ Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Brno, the Czech Republic
- ² Laboratory of Cell and Developmental Biology, Institute of Molecular Genetics, Praha, the Czech Republic
- ³ Central European Institute of Technology, Masaryk University, Brno, the Czech Republic
- ⁴ Department of Experimental Biology, Faculty of Natural Sciences, Masaryk University, Brno, the Czech Republic

buchtova@iach.cz

Leucine-rich repeat containing G-protein-coupled receptor 5 (LGR5) is a WNT pathway member, which has been recognized as a stem cell marker in numerous tissues.

Here, we used Lgr5-EGFP-CreERT2 mice to analyze the distribution of LGR5-positive cells during craniofacial structures development.

LGR5 expressing cells were surprisingly located mostly in the mesenchyme adjacent to epithelial craniofacial structures undergoing folding such as palatal shelves, nasopharyngeal duct, lingual groove or vomeronasal organ. No overlap of LGR5 signal with other stem cell marker was observed in analyzed craniofacial structures and expression pattern of LGR5 and SOX2 was rather complementary to each other. To further follow the fate of LGR5-positive cells, we performed their lineage tracing using an inducible Cre knock-in allele in combination with Rosa26-tdTomato reporter mice. The slight expansion of LGR5-positive cells was found around vomeronasal organ, in the nasal cavity and around epithelium in the lingual groove, however, the most of LGR5-positive cells remained in their original location supporting their signaling function to adjacent epithelium rather than their role as stem cells for craniofacial area. Moreover, LGR5 knockout mice displayed distinct defects in LGR5-positive areas especially nasopharyngeal duct reduction, and the alteration of palatal shelves shape, abnormal epithelial folding in the lingual groove area and the disruption of salivary gland development with atypical gland duct localization and number. In disrupted areas, downregulation of WNT signaling was observed.

LGR5-positive cells in the craniofacial area represent very specific population of mesenchymal cells, which is characteristically adjacent to the epithelium undergoing folding or groove formation. Our results indicate possible novel role of LGR5 in regulation of morphogenetic processes during the formation of complex epithelial structures in the craniofacial areas, which is not related to stem cell properties as was previously defined for the intestine.

This work was supported by the Czech Science Foundation (19-01205S, 22-02794S).

IN VITRO MODEL OF TROPHOBLAST

Burianová A., Čížková K., Tauber Z., Koubová K.

Department of Histology and Embryology, Faculty of Medicine and Dentistry Palacky University in Olomouc, the Czech Republic

adela@burian.eu

During the development of the human placenta, the trophoblast gives rise to the cytotrophoblast, which further differentiates along either the villous or the extravillous trophoblast pathway. In the villous pathway, cytotrophoblast cells fuse to form an overlying multinucleated syncytiotrophoblast. In the case of extravillous trophoblast, the cytotrophoblast cells invade into the decidua and attach the placenta to the uterine wall and remodel the spiral arteries. Trophoblast-derived choriocarcinoma cell lines BeWo and JEG3 are commonly used as in vitro models of trophoblast. The BeWo cell line shares morphological and biochemical characteristics of villous trophoblasts, including syncytial fusion and hormone secretion. In contrast to BeWo cells, the JEG-3 cell line is non-fusogenic and it is considered as extravillous trophoblast. Alterations in the trophoblast have a major impact on placental maintenance and integrity. Chorioamnionitis is an inflammation of the chorionic and amniotic membranes most frequently caused by a bacterial infection. It increases the risk of preterm labour and is also associated with adverse neonatal outcomes.

BeWo and JEG-3 cell lines, In-Cell ELISA

We established appropriate differentiation cell culture conditions and induced an inflammatory response to resemble chorioamnionitis, one of the most common pregnancy complications.

The established cell line models can be used to study the role of arachidonic acid metabolizing enzymes.

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UNRAVELING ALZHEIMER'S DISEASE MECHANISMS USING 3D CEREBRAL ORGANOIDS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

Cesnáríková S.¹, Sedmík J.¹, Fojtík P.^{1,2}, Amruz Černá K.¹, Pospíšilová V.¹, Váňová T.^{1,2}, Bohačiaková D.^{1,2}

¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

² International Clinical Research Center (ICRC), St. Anne's University Hospital, Brno, the Czech Republic

sona.cesnarikova@med.muni.cz

Alzheimer's disease (AD) is associated with amyloid plaques, neurofibrillary tangles, and neuronal degeneration and remains incurable despite many therapeutic efforts. Induced pluripotent stem cells (iPSCs) are crucial for studying human neurodevelopment and diseases like AD. These cells have been used to create 3D cerebral organoids (COs) that mimic AD in vitro, helping to clarify the disease's molecular mechanisms. Our project uses these unique in vitro 3D stem cell models to investigate AD development. Our data provide further evidence that developmental disorders and altered neurogenesis could contribute significantly to the development of a familial form of AD (fAD).

Three patient-specific iPSC lines and three control iPSC lines were derived from fAD patients and corresponding sex-, age-, and APOE status-matched non-demented controls (NDC). iPSCs were generated from primary human fibroblasts reprogrammed with Yamanaka factors (OSKM) using the Epi5 Reprogramming Kit. Pluripotency was confirmed by the expression of pluripotency factors and their ability to differentiate into all three germ layers in vitro. All cell lines are registered at the Human Pluripotent Stem Cell Registry. Cerebral organoids were generated following the Lancaster (2014) protocol with slight modifications. Samples were collected at specific timepoints and analyzed using western blot, qRT-PCR, immunohistochemistry, bulk mRNA sequencing, and single-cell sequencing.

Our preliminary data, from analysing mature organoids (60, 85, 110, and 130 days old) revealed AD-COs' ability to mimic AD pathology. Single-cell sequencing of 60-day-old COs showed premature neural differentiation in AD-COs, suggesting altered early development. These changes manifest in COs' organization, structure, and different activity of key signaling pathways.

The observed alterations in AD organoids extend to the molecular level, with different activities in key signaling pathways. Understanding these differences is crucial for following the early development of Alzheimer's disease. Examining these changes in AD organoids provides valuable insights into the mechanisms underlying the disease's progression.

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SATB2 AS A GOOD PROGNOSTIC MARKER FOR COLORECTAL CANCER

Cígerová V.¹, Adamkov M.¹, Grendár M.²

- ¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ² Department of Bioinformatics, Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic

veronika.cigerova@uniba.sk

Colorectal cancer (CRC) is the third most common malignancy diagnosed in both men and women and the fourth leading cause of cancer-related deaths worldwide. Special AT-rich sequence-binding protein 2 (SATB2), a nuclear matrix-associated transcription factor and epigenetic regulator, was identified as a highly tissue-type-specific protein being predominantly expressed in glandular cells of the lower gastrointestinal tract.

The aim of this study was to evaluate the immunohistochemical expression of SATB2 in 111 cases of CRC and its association with clinico-morphological parameters, microsatellite instability (MSI) status, pro-inflammatory enzyme cyclooxygenase-2 (COX-2) and hypoxia-inducible factor 1 α (HIF-1 α).

We observed nuclear staining with predominantly strong immunoreaction intensity (67.6%) and percentage of SATB-2 positive cells in more than 50% of cells (87.4%). The statistically significant associations were recorded between high SATB2 expression and low grade, negative lymph nodes and negative vascular invasion. Statistical analysis confirmed a significant correlation between SATB2 expression and microsatellite stability, tendency to correlate with COX-2 and no significant correlation with HIF-1 α .

High expression of SATB2 in CRC is a marker of good prognosis and may represent a potential therapeutic target.

The study was supported by grant VEGA 1/0129/16 and UK/98/2016.

FROM DECELLULARIZATION TO PROTEOMICS: EXPLORING THE LUNG EXTRACELLULAR MATRIX ACROSS SPECIES

Čimborová Katarína¹, Pelková Vendula¹, Kotasová Hana^{1,2}, Bohačiaková Dáša^{1,2}, Sedláková Veronika¹, Hampl Aleš^{1,2}

¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

² St.Anne's University Hospital, International Clinical Research Center, Brno, the Czech Republic

katarina.cimborova@med.muni.cz

The extracellular matrix (ECM) is a fundamental yet often overlooked component of tissue architecture. Far beyond being a passive structural scaffold, the ECM serves as a dynamic regulator of cellular behaviors and tissue homeostasis through intricate biochemical and biomechanical signals. Its continuous remodeling enables adaptation to environmental changes, while dysregulation of ECM dynamics can lead to serious conditions such as fibrosis and cancer. This study employs a decellularization technique to isolate the ECM from lung tissues of mouse, pig, and human origins, followed by comprehensive proteomic analysis. Our goal is to uncover both conserved and divergent ECM components across species, thereby validating the utility of these animal models for human health research and advancing strategies in pulmonary tissue.

Decellularization was achieved through a series of washes with hypotonic buffers, 0.2% SDS, DNase, and MilliQ water. The effectiveness of this approach was assessed using histological and immunofluorescence staining, electron microscopy, and DNA quantification. LC-MS/MS (liquid chromatography-tandem mass spectrometry) analyses were then employed for a thorough proteomic comparison.

The decellularization protocol effectively removed cellular and nuclear materials, preserving ECM structure, as validated by multiple assays. Proteomic analysis revealed conserved ECM proteins across mouse, pig, and human lung tissues, highlighting fundamental components. Species-specific differences were identified, reflecting unique adaptations and functional specialisations.

The optimised decellularization method successfully isolated lung ECM while preserving its integrity. Comparative proteomic analysis underscored conserved ECM components across species and revealed significant species-specific variations. These findings enhance understanding of the lung ECM matrisome, providing insights for tissue engineering, regenerative medicine, and therapeutic strategies for ECM-related diseases. The study validates animal models in respiratory research, bridging preclinical studies and human health applications.

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LIVER ULTRASTRUCTURE IN PAEDIATRIC PATIENTS WITH AAGENAES SYNDROME

Čížková D.¹, Bezrouk A.², Mokrý J.¹, Sullivan G. J.³, Reims H. M.⁴, Almaas R.³

¹ Department of Histology and Embryology, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové, the Czech Republic

² Department of Medical Biophysics, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové, the Czech Republic

³ Department of Paediatric Research, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway

⁴ Department of Pathology, Oslo University Hospital, Oslo, Norway

cizkova@lfhk.cuni.cz

Aagenaes syndrome/lymphedema cholestasis syndrome 1 represents a very rare inherited disease that manifests in newborns, with the main symptoms including cholestasis, hyperbilirubinemia and increased concentration of bile acids in blood. In majority of patients cholestasis disappears during childhood, but in puberty lymphedema can occur, most pronounced in the legs. Neonatal liver biopsies show paucity of bile ducts, cholestasis, giant cell transformation and variable inflammation.

In this study we examined hepatic bioptic samples from 8 patients with Aagenaes syndrome in the transmission electron microscope to define ultrastructural features of the liver affected by this disease.

The liver tissue from seven patients with Aagenaes syndrome aged up to four months at biopsy showed signs of giant cell hepatitis and intrahepatic cholestasis. Numerous hepatocytes and multinucleated giant cells contained biliary material and focal cytoplasmic degenerative or necrotic changes were also observed. The characteristics of bile canaliculi corresponded to bile retention. They were dilated or deformed, with reduced or lost microvilli and contained varying amounts of biliary material of different appearance and electron density. The cholestatic bile canaliculi were located between two adjacent liver cells or among several hepatocytes arranged in structures called rosettes. Disappearance of plasmalemma between two adjacent liver cells and rare mitoses of hepatocytes were noticed thus fusion represented a more probable mechanism of giant cell formation in infant patient with Aagenaes syndrome. In the liver sample from the 2-year-old patient only very rare giant cells, minimal amounts of intracellular and intracanalicular bile material and normal-appearing bile canaliculi were observed.

This study, the results of which have been published in *J Hepatol* 2023 Oct;79(4):945-54, extended our knowledge of the liver ultrastructure in patients with Aagenaes syndrome.

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NEW IMAGEJ/FIJI SCRIPT FOR ASSESSMENT OF NUCLEAR RECEPTOR TRANSLOCATION

Čížková Kateřina

Department of Histology and Embryology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, the Czech Republic

katerina.cizkova@upol.cz

Nuclear receptors are ligand-activated transcription factors that control a variety of cellular processes. Frequently, ligand binding occurs in the cytoplasm and the receptor is subsequently translocated to the nucleus. Their dynamic shuttle between cellular compartments thus regulates the cellular response. Its subcellular localisation can be monitored by immunostaining. Manual assessment is very time consuming and introduces a considerable degree of subjectivity and bias, hence the standardisation is needed.

20 micrographs of the SHSY5Y cell line, in which the nuclear receptor PPAR α was detected by DAB immunohistochemistry, were manually analysed by six observers. The agreement between them was evaluated by intraclass correlation coefficient. A script was also written in the Python programming language for the ImageJ/Fiji program to determine the representation of cells with nuclear positivity in a given field of view for both DAB- and fluorescence-stained samples.

Manual grading results were highly variable. Due to the low value of some intraclass correlation coefficients, the micrographs with the largest range were excluded from the set of micrographs. As in most cases the positive signal does not represent 100 % of the nuclear area, the data from the manual evaluation was also used to determine the optimal overlap of the positive signal with the nuclear area. The script determines the representation of nuclear positive cells in a given field of view and is applicable to both DAB and fluorescence stained specimens. It also allows semi-quantitative or quantitative measurement of signal intensity, depending on the detection method used. Its major advantage is that it evaluates only one specific nuclear localisation of the signal and the results are not influenced by the signal from the cytoplasm.

The script reduces subjectivity in the assessment of nuclear receptor translocation.

IGA_LF_2024_010.

PRIMING AND SIGNAL TRANSDUCTION OF DELTA OPIOID RECEPTORS IN DORSAL ROOT GANGLIA NEURONS IN A MOUSE EXPERIMENTAL MODEL OF NEUROPATHIC PAIN - UTILIZING IMMUNOHISTOCHEMICAL DETECTION TO ELUCIDATE INTRACELLULAR PROCESSES

Dubový P., Rokoský Z., Rábová A.

Department of Anatomy, Cellular and Molecular Research Group, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

pdubovy@med.muni.cz

Delta opioid receptor (DOR) agonists are promising for the peripheral treatment of neuropathic pain without serious adverse side effects. However, DOR is functionally inactive for antinociceptive signaling under basal conditions. Tissue damage or exposure to inflammatory mediators can convert DOR from a nonresponsive state to a functionally competent state. Based on our previous results, a nerve injury results in increased inflammatory mediators in the primary sensory neurons (PSNs).

We used mouse a spared nerve injury model with unilateral spared tibial nerve (SNIt) to study the intraneuronal localization of DOR protein in PSNs. We utilized double immunofluorescence staining for DOR with WGA, GRK2, b-arrestin2, EEA1, and Rab7 to visualize intraneuronal DOR trafficking and verify some hypotheses regarding its intraneuronal fate.

The results demonstrated that DOR immunofluorescence (-IF) is predominantly present in large-diameter PSNs of naïve mice, and SNIt resulted in increased DOR-IF in all types of PSNs. Double immunostaining confirmed the localization of DOR in the plasma membrane of PSNs together with GRK2, which was reduced by the SNIt. Naïve PSNs displayed b-arrestin2-IF concentrated in a ring at the superficial region of PSNs without colocalization with DOR-IF, which was also observed after SNIt. Increased DOR-IF was detected intraneuronally in early endosomes (EEA1+) following SNIt compared with naïve controls, but limited DOR-IF was present in late endosomes (Rab7+).

In conclusion, SNIt, as a mouse model of neuropathic pain, induced an increased level of DOR in the plasma membrane and predominantly in early endosomes of PSNs. Reduced colocalization of DOR with GRK2 may indicate the functional competence of DOR in the bodies of PSNs after SNIt.

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THE MEMORY OF PROFESSOR ZDENĚK LOJDA, A PIONEER OF CZECHOSLOVAK HISTOCHEMISTRY AND A PROMINENT FIGURE IN CZECH SCIENCE

Dubový Petr

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

pdubovy@med.muni.cz

Prof. MUDr. Zdeněk Lojda, DrSc., Dr. med. h.c., dr. jur. h.c. (1927-2004) was one of the founders and long-time chairman (until 2001) of the Czechoslovak (Czech) Society for Histochemistry and Cytochemistry. Prof. Z. Lojda was born on December 7, 1927, in Třebíč, a town located 35 km east of Telč.

He graduated from the Medical Faculty of Charles University in Prague and first worked as an assistant at the Institute of Embryology. He founded the Angiology Laboratory, which later became the Laboratory for Histochemistry. Prof. Lojda was engaged in enzyme histochemistry, a method for the in-situ detection of enzyme activity. Together with other notable pioneers such as Prof. Peter J. Stoward (1935-2021), and Prof. Reinhart Gossrau, they developed and standardized methods for the detection of enzyme activities in tissue sections. Prof. Z. Lojda began organizing the Annual Symposium of the Czechoslovak Society of Histochemistry and Cytochemistry, titled „Progress in Basic, Applied, and Diagnostic Histochemistry“. With the development of immunohistochemical detection, he always emphasized that with this method we detect the enzyme as a protein, but this does not mean that it is related to enzyme activity, which is significant for the interpretation of the obtained results.

Prof. Lojda was the recipient of several prestigious awards, including the Schleiden Medal of the Leopoldina Academy (1987), the Pioneer of Histochemistry IFSHC plaque (1988), the gold Purkyně plaque (1987), and the gold Jesenius plaque (1987). He was honored as an honorary Doctor of Medicine at the University of Halle (1990) and at the Christian University of Vienna (1993). Prof. Lojda was a member of the Executive Committee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC, 1975–2001). As vice-rector of Charles University (1990–1994), he delivered laudations in excellent Latin.

Considering the personality of prof. Lojda, we decided in 2004 to name our annual symposia of the Czech Society for Histochemistry and Cytochemistry the Lojda symposium.

There was no specific funding for this work.

THE REACTION OF CHOROID PLEXUS TO CHEMOTHERAPY

EmamiAref P.¹, Kubíčková L.¹, Bakhshinejad B.², Dubový P.¹, Joukal M.¹, Zamani A.¹

- ¹ Alemeh Zamani Research Group Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Parisaemamiaref@gmail.com

Paclitaxel, a widely used anti-cancer drug, stabilizes microtubules to hinder cell division but causes neuropathic pain, likely due to its effects on the immune system. This research explores how paclitaxel affects the choroid plexus (CP), forming the blood-cerebrospinal fluid barrier and entry sites for immune cells into the brain. We study CP macrophage-like Kolmer cells (KC) involved in CNS immune surveillance. Our previous research found paclitaxel increased activated and resident KC and proliferating cells. This study aims to further investigate the origins of these KC cells.

We administered four doses (2mg/kg) of paclitaxel or its vehicle to male Wistar rats. We conducted behavioral tests (thermal hyperalgesia and mechanical threshold) at 1, 7, 14, 21 days after the last injection. Immunohistochemical analysis was used to study the colocalization of ED1 (activated KC) and ED2 (resident KC) with Ki67 (proliferating cells). Additionally, we treated Z310 choroidal epithelial cells with 20nM paclitaxel and harvested them at 6, 24, 48, and 72 hours for Western blotting with Zonula occludens-1 (ZO1) and Occludin antibodies.

In vivo Behavioral tests confirmed neuropathic pain development after paclitaxel treatment, evidenced by reduced front paw force tolerance and increased hind paw force tolerance and temperature resistance compared to controls. We did not observe any colocalization of ED1/Ki67, nor ED2/Ki67, suggesting the aforementioned KC originated from peripheral immune cells. In vitro studies tested paclitaxel's effect on barrier integrity by altering tight junctions of choroidal epithelial cells. Occludin levels initially increased at 6 and 24 hour before declining at 48 and 72 hour, while ZO1 initially decreased at 6 hour, increased at 24 and 48 hour, and decreased again at 72 hour.

In conclusion, paclitaxel-induced neuropathic pain is associated with immune response changes and barrier integrity alterations in the CP. Targeting KC activation and related barrier disruptions could mitigate paclitaxel's side effects.

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BENEFICIAL EFFECTS OF QUERCETIN ON MOUSE EPIDIDYMAL SPERM PARAMETERS INDUCED BY NICOTINE

Faghani M., Alijani M., Mohammadghasemi F., Esmaeili-bandboni A.

Gilan University of Medical Sciences, Rasht, Iran

mfaghani2000@gmail.com

The reproductive system is negatively impacted by nicotine, which is one of the main components of cigarettes. The toxic effects of nicotine on sperm parameters in smokers can be reduced by using antioxidants, such as quercetin. This study was designed to examine the impact of quercetin as a phytoestrogen on the sperm and sex hormones of mice treated with nicotine.

A total of thirty-two adult male mice were separated into four groups, A-Control, B-nicotine, C-quercetin, and D-nicotine+quercetin. After five weeks, the sperm count, motility, and abnormalities in the epididymis were analyzed. ELISA was used to analyze estradiol (E2) and LH.

Nicotine had a significant reduction in LH when compared to quercetin ($p=0.001$). The co-treatment of quercetin and nicotine resulted in an increase in LH ($p=0.05$) when compared to nicotine. When compared to the control group, the nicotine-treated group had an insignificant increase in E2. In comparison to the nicotine group, quercetin had an anti-estrogenic effect and decreased E2 levels ($p=0.005$). Furthermore, E2 levels decreased ($p=0.001$) in the co-treated group when compared to nicotine. Nicotine had a significant impact on sperm count and motility when compared to control animals. The co-treated group showed an increase in sperm count ($p<0.05$). The percentage of sperms with normal morphology was lower in the nicotine treated group compared to the other groups ($p<0.05$). There was no difference in the percentage of sperm with abnormal heads between groups. The percentage of sperms with abnormal tails increased only by nicotine ($p=0.006$). Co-treatment with nicotine and quercetin could reduce the percentage of sperms with abnormal tails ($p=0.01$).

This study showed quercetin through its influence on LH has an anti-estrogenic effect, which may increase sperm count and decrease the percentage of abnormal tails. However, it requires molecular and further studies.

Guilan University of Medical Sciences.

SYNDESMOTIC LESION IN FRACTURE-DISLOCATIONS OF THE ANKLE - MAISONNEUVE FRACTURE

Fojtík Petr^{1,2}

¹ Department of Orthopedics, First Faculty of Medicine, Charles University and the Central Military Hospital, the Czech Republic

² Institute of Anatomy, First Faculty of Medicine, Charles University, the Czech Republic

petr.fojtik@uvn.cz

A Maisonneuve fracture (MF) is a fracture dislocation of the ankle caused by external rotational force. In the traditional concept it includes a subcapital fracture of the fibula, an injury to the tibiofibular syndesmosis and to the interosseous membrane, and it is considered as an unstable injury. The experimental (anatomical) goal of the dissertation work was a detailed study of the *incisura fibularis tibiae* (IFT) and a detailed description of the lateral surface of the distal fibula, where there is a prominent but still nameless edge that gives the distal fibula its typical triangular shape on a transverse section (workingly we called it lateral malleolar crest, LMC). Both of these structures are extremely important in the diagnosis and treatment of ankle fractures. The clinical part of the work included an overview of all the literature devoted to MF and further analysis of our group of patients with MF with a focus on the posterior malleolus (PM) fracture. In the experimental part of the work, two anatomical structures were described in detail, i.e. IFT and LMC. The deepest point of the IFT was located and related to the assessment of the distal fibula position in the IFT on CT scans. We were the first to describe LMC. Its shape and course are of fundamental importance for operative stabilization of the tibiofibular fork with syndesmial screws and for plate osteosynthesis of external ankle fractures.

The analysis of all available studies devoted to MF in the clinical part shows that MF is not perceived the same by all authors (differences in the stability of the fracture and the list of injured structures). CT therefore plays a fundamental role in diagnosis, which must also be performed pre and postoperatively. PM fracture occurs in 74% of MFs in our study. Its importance has so far been underestimated, even though it fundamentally violates the integrity of the IFT and destabilizes the tibiofibular fork.

CDK13 MOUSE MUTANTS AS USEFUL MODELS OF THE CHDFIDD DISORDER

Hampl M.^{1,2}, Jandova N.¹, Novakova M.³, Romanova A.¹, Kavkova M.⁴, Parobkova V.⁵, Kompanikova P.², Čada Š.², Zikmund T.⁵, Kohoutek J.², Buchtova M.^{1,2}

- ¹ Institute of Animal Physiology and Genetics, CAS, Brno, the Czech Republic
- ² Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, the Czech Republic
- ³ Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, the Czech Republic
- ⁴ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁵ Laboratory of Computed Tomography, CEITEC, Brno University of Technology, Brno, the Czech Republic

marekhampl12@gmail.com

Congenital heart defects, facial dysmorphic features and intellectual developmental disorder (CHDFIDD) is a rare human disease which mostly affects development of heart, facial structures, brain and limbs. To study this disease, we use Cdk13-deficient mouse models, which bear mutations in gene Cdk13 encoding one of the transcriptional kinases.

To study role of the Cdk13 in development of various organs and tissues, we developed Cdk13-deficient mouse models, which are embryonically lethal. To evaluate individual morphological and functional changes, we use routine histological staining, microCT technique and moreover high-throughput sequencing techniques (RNAsequencing) and classic methods to detect changes in gene (qPCR, RNAScope) and protein (WB, ICC, IHC) expression.

We discovered that Cdk13-deficiency in mouse models results in developmental defects in heart, brain including choroid plexus, kidney, liver, peripheral nerves and also in facial structures such as cleft lip, cleft palate and midfacial cleft and in limbs expressed as various morphological changes, namely oligodactyly, clinodactyly and polydactyly. At the cellular level, we detected that Cdk13-deficiency results in increased numbers of cellular protrusions and altered cellular migration.

Our Cdk13-deficient mouse models are useful tools to study developmental defects which relate to development of CHDFIDD disorder in human patients who bear CDK13 mutations and will be used for further functional experiments of this rare disease.

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NOVEL GENES INVOLVED IN THE FIRST LOWER MOUSE MOLAR ENAMEL KNOT ESTABLISHMENT AND ITS EARLY MATURATION

Steklíková K.^{1,2}, Dalecká L.^{1,2}, Kubovčiak J.³, Corneloup C.⁴, Pantalacci S.⁴, Hovořáková M.¹

- ¹ Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Department of Cell Biology, Faculty of Science, Charles University, Prague, the Czech Republic
- ³ Laboratory of Genomics and Bioinformatics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, the Czech Republic
- ⁴ Laboratoire de Biologie et Modélisation de la Cellule, Ecole Normale Supérieure de Lyon, Lyon, France

Maria.Hovorakova@lf1.cuni.cz

Development of dentition is commonly studied as a representative of the development of ectodermal derivatives. The tooth development is conducted by signalling centres (enamel knots = EKs) with specific gene expression profiles. Their establishment is preceded by activation of the posterior epithelial region of dental lamina. Many molecules and their strict control in time and space have been described as participating during tooth development, but concrete molecular factors that drive the tooth germ maturation processes are still uncovered.

In this study we documented an importance of precise signalling balance during tooth germ formation using in vitro culturing of the posterior part of first molar (M1) tooth germs with molecules over-activating or silencing Shh and Fgf pathways. To identify the potential key players in the M1 enamel knot establishment and early maturation, RNA sequencing of posterior parts of the mouse lower first molar germ was performed at embryonic day 13.5 (the key stage for primary EK formation) and after 16 or 24 hours in vitro, respectively.

The alteration of the balance led to disrupted signalling centre establishment followed by arrested first molar germ formation. RNAseq data revealed 8 genes potentially involved in M1 germ formation: *Alas2*, *Cdkn2b*, *Gja5*, *Gm13889*, *Hbb-y*, *Plekhd1*, *Slc4a1*, *Slc6a4* and 7 novel candidates related directly to EK establishment and its early maturation: *Mybcp1*, *Pianp*, *Prune2*, *Rgs8*, *Sema3b*, *Sptb* and *Tcerg1l*.

Results from experimentally altered signalling in posterior part of tooth germ have clearly documented the importance of precisely balanced levels of molecules driving the tooth development. *Cdkn2b*, *Gja5*, *Pianp*, *Prune2*, *Rgs8* and *Sema3b* represent promising new candidate molecules playing a role during the first molar germ formation and its enamel knot establishment and early maturation. The roles of these genes need to be further investigated.

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THE COMMUNICATION PATTERNS BETWEEN THE LATERAL ANTEBRACHIAL CUTANEOUS NERVE AND THE SUPERFICIAL BRANCH OF THE RADIAL NERVE

Khadanovich A.^{1,2}, Herma T.^{1,2}, Al-Redouan A.^{1,2}, Kaiser R.^{1,2,3}, Kachlik D.^{1,2,4}

- ¹ Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Center for Endoscopic, Surgical and Clinical Anatomy (CESKA), Second Faculty of Medicine, Charles University, Prague, the Czech Republic
- ³ Department of Neurosurgery and Neurooncology, First Faculty of Medicine, Charles University and Military University Hospital Prague, the Czech Republic
- ⁴ Department of Health Care, College of Polytechnics Jihlava, the Czech Republic

xodanovich0112@gmail.com

The superficial branch of the radial nerve (SBRN) and the lateral antebrachial cutaneous nerve (LACN) are sensory nerves coursing within the forearm in a close relationship. This high degree of overlap and eventual communication between the nerves is of great surgical importance. The aim of our study is to identify the communication pattern and overlap of the nerves, to localize the position of this communication in relation to a bony landmark, and to specify the most common communication patterns.

One hundred and two adult formalin-fixed cadaveric forearms from 51 cadavers were meticulously dissected. The SBRN, as well as the LACN, were identified. The morphometric parameters concerning these nerves were measured with a digital caliper.

We have described the primary (PCB) and secondary communications (SCB) between the SBRN and the LACN and their overlap patterns. One hundred and nine PCBs were found in 75 (73.53%) forearms of 44 (86.27%) cadavers and fourteen SCBs in eleven hands (10.78%) of eight cadavers (15.69%). The mean length and width of the PCBs were 17.12 mm (ranged from 2.33 to 82.96 mm) and 0.73 mm (ranged from 0.14 to 2.01 mm), respectively. The PCB was located proximally to the styloid process of the radius at an average distance of 29.91 mm (ranged from 4.15 to 97.61 mm). The most frequent branch of the SBRN involved in the communication was the third (66.97%). Due to the frequency and position of the PCB with the third branch of the SBRN, the danger zone was predicted.

The patterns of communicating branch arrangements appeared to be not just a rare phenomenon or variation, but rather a common situation highlighting clinical importance. Due to the close relationship and connection of these nerves, there is a high probability of simultaneous lesion.

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ANATOMICAL RELATIONSHIPS AND VARIATIONS OF THE LATERAL FEMORAL CUTANEOUS NERVE TO SURROUNDING STRUCTURES DURING MINIMALLY INVASIVE TOTAL HIP ARTHROPLASTY VIA THE ANTERIOR APPROACH

Kohut P.^{1,3}, Mezera V.², Golas W.^{3,4}, Zajíc P.^{3,4}, Beneš M.^{3,4}, Kachlík D.^{3,4}

- ¹ Klinik für Orthopädie und Unfallchirurgie, Diakomed Diakoniekrankenhaus Chemnitzer Land gGmbH, Hartmannsdorf, Deutschland
- ² Geriatric Center, Pardubice Hospital, Pardubice, the Czech Republic
- ³ Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, the Czech Republic
- ⁴ Center for Endoscopic, Surgical and Clinical Anatomy (CESKA), Second Faculty of Medicine, Charles University, Prague, the Czech Republic

petrlea@hotmail.com

The growing number of patients requires a minimally invasive surgical technique for total hip arthroplasty. Anterior minimally invasive surgery (AMIS) also has its specific complications. One of these is the injury to the N. cutaneus femoris lateralis (LFCN) with conditions of hypoesthesia, paresthesia or neuropathic pain.

Between 12/2021 and 12/2023 we dissected 13 cadavers. The bodies were not treated in any way. The position and course of the LFCN were measured on the lying body. The body was then positioned in the extension device using the AMIS technique at different stages of the operation.

The distance between the division of the LFCN, the passage of the gluteal branch and the passage of the femoral branch over the ventral edge of the tensor fasciae latae (TFL) from the ASIS was on average almost twice as long in our sample as in the study conducted on the formalin-fixed body. Subsequent positioning of the body did not result in any statistically significant changes in the course of the nerve.

27% of the cadavers showed the fan-type branching pattern. With this branching pattern and the recommended surgical approach, it is almost impossible not to injure the nerve. We support the hypothesis that, in contrast, injuries to the nerve with sartorius-type and posterior-type branching patterns with their dominant branches can lead to symptomatic difficulties, while injuries to smaller branches of the FAN type can be compensated by the surrounding branches. To determine the so-called danger zone, measurements on formalin-fixed bodies should not be accepted uncritically. The intraoperative positioning of the patient itself does not have a significant influence on the position of the LFCN. But its position differs significantly when examining LFCN in formalin-fixed cadavers compared to non-fixed cadavers. Further examinations on non-fixed cadavers may help to determine the location of the so-called danger zone.

There was no specific funding for this work.

JAGGED1 DISRUPTED SIGNALING IN CONDITIONAL DELETION MOUSE MODEL AND SINGLE NUCLEOTIDE VARIANT OF PATIENT-BASED MOUSE MODEL CAUSES SEVERE CARDIAC DEFECTS – MORPHOLOGY AND PHYSIOLOGY

Kolesová H.¹, Neffeová K.¹, Zábrodská E.¹, Olejníčková V.¹, Mašek J.², Sedmera D.¹

¹ Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, the Czech Republic

² Faculty of Science, Charles University, Prague, the Czech Republic

hana.kolesova@lf1.cuni.cz

Jagged 1 (Jag1) is known to play an important role in cardiac development, where partial deletion of Jag1 is causing severe congenital heart defects. In this study we are comparing embryonic and postnatal hearts with conditional deletion of Jag1 with hearts with single nucleotide variant in Jag1, which was prepared based on patient data.

We analyzed prenatal as well as postnatal hearts of Jag1 floxed, Islet1-cre mouse line and two lines with single nucleotide variant in Jag1. Morphology of hearts was analyzed on histological sections. Physiological functions were assessed using ultrasound in vivo imaging - Vevo and optical mapping.

We found that Jag1, Islet1-cre mouse line exhibits severe heart defects during embryonic development, with variable phenotype ranging from mild abnormalities to Tetralogy of Fallot – double outlet right ventricle, VSD, and valve defects. Surviving postnatal mice present with milder defects, especially valve defects and physiological abnormalities in ventricular activation and contraction. Patients-based mouse model with single nucleotide variant in postnatal stages exhibits valve defects and physiological abnormalities.

Our results show that Jag1 is an important player in heart development and its disruption is causing various congenital heart defects. The humanized mouse models help to understand etiology and pathogenesis of congenital heart disease.

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INCIDENCE OF DUPLICATION OF THE CAUDAL VENA CAVA IN COMPANION ANIMALS

Korim F.¹, Kuricová M.¹, Ivašková S.¹, Eberlová L.²

¹ University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic

² Faculty of Medicine in Pilsen - Charles University, the Czech Republic

filip.korim@student.uvlf.sk

Duplication of the caudal vena cava (dCVC) is a rare anomaly of the caudal vena cava development. There are many developmental theories of the caudal/inferior vena cava segments in both humans and animals during physiological and pathological conditions – dCVC. The occurrence dCVC is low and this anomaly is rare in veterinary medicine. There are few observation studies and sporadic case reports documenting presence of dCVC.

Based on a PubMed search and our own observations, we performed an analysis of the incidence of dCVC in companion animals.

Cats and dogs are rarely affected by this anomaly, which can affect any segment of the dCVC. In some cases, it is a clinically silent condition, but in some cases it is associated with portocaval anastomoses and a non-standard course of the ureters - retrocaval ureter.

It is necessary to point out that anomalies of the vascular system should be sufficiently taken into account in order to improve the diagnosis and therapy of companion animals.

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PTEN AND SOLUBLE EPOXIDE HYDROLASE IN INTESTINAL CELL DIFFERENTIATION

Koubová K., Čížková K., Burianová A., Tauber Z.

Department of Histology and Embryology, Faculty of Medicine and Dentistry, Palacky University Olomouc, the Czech Republic

katerina.koubova@upol.cz

Intestinal epithelial differentiation is a highly organised process. It is influenced by a variety of signalling pathways and enzymes, such as the PI3K pathway and soluble epoxide hydrolase (sEH) from arachidonic acid metabolism. We investigated the changes in the expression of enzymes and lipid messenger from the PI3K pathway, including PTEN, during intestinal cell differentiation in vitro using HT-29 and Caco2 cells and compared them with immunohistochemical patterns of these proteins in human colon. To investigate the possible crosstalk between the PI3K pathway and sEH, we treated HT-29 and Caco2 cells with the sEH inhibitor TPPU. Administration of TPPU to differentiated cells decreased the expression of PTEN, thus reversing the change in its expression observed during cell differentiation. In addition, multiplex immunofluorescence staining confirmed the relationship between the expression of PTEN and villin, a marker of intestinal cell differentiation, ranging from a moderate correlation in undifferentiated cells to a very strong correlation in differentiated cells treated with TPPU. Furthermore, we confirm that PTEN and sEH mirrored their expression patterns in samples of prenatal and adult human intestine compared to tumours using immunohistochemical staining. Taken together, it appears that PTEN and sEH cooperate in the process of intestinal cell differentiation. A better understanding of the crosstalk between the PI3K pathway and sEH and its consequences for cell differentiation is highly desirable, as several sEH inhibitors are under clinical investigation for the treatment of various diseases.

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THE ROLE OF PRMT5 AND DSG2 IN PRECANCEROUS LESIONS OF THE UTERINE CERVIX

Krajňáková B.¹, Mešťanová V.¹, Hurta Csizmár S.², Výbohová D.², Adamkov M.¹

¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic

² Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic

klinerova2@uniba.sk

Precancerous cervical lesions are metaplastic changes of epithelial cells manifested mainly in the transformation zone. They can develop into the cervical cancer, one of the major public health problems affecting women in low-income and middle-income countries. Methylation of arginine is a key post-translational modification, which plays an important role in tumor progression. PRMT5 is one of the nine members of PRMT family, major regulators of arginine modification. It catalyzes the symmetrical dimethylation in large number of cancers, including cervical cancer. PRMT5 regulates several cellular processes such as proliferation, cell cycle and multiple signaling pathways. It's a part of the transcriptional-repressive complexes and affects the epithelial-mesenchymal transition. Currently, the transmembrane and cell adhesion protein DSG2 is also coming to the fore. Based on the latest scientific studies, DSG2 is involved in the progression of dysplastic changes and its expression seems to be dysregulated in tumor cells. Key words: immunohistochemistry, PRMT5, DSG2, squamous intraepithelial lesions.

In our retrospective study we used 150 archival formalin-fixed paraffin-embedded (FFPE) blocks from adult women, which were immunohistochemically processed. We focused on semi-quantitative evaluation of PRMT5 and DSG2 expression, their subcellular localization and immunoreaction intensity. The protein expression was correlated with each other as well as with the expression of p16 and Ki-67. Obtained results were statistically analyzed.

Our preliminary results identified the differences in monitored parameters between low-grade SIL (LSIL) and high- grade SIL (HSIL) groups. Cytoplasmic expression of PRMT5 decreased with disease progression in contrast with nuclear expression. χ^2 test confirmed statistically significant differences in DSG2 subcellular localization between LSIL and HSIL.

In our study, we tried to understand the role of PRMT5 and DSG2 in cellular processes involved in the tumorigenesis of cervical cancer and to evaluate these proteins as potential prognostic biomarkers during the histopathological assessment of cervical precancers.

Our work was supported by a Comenius University Grant (UK/20/2023).

HOW IS THE RENEWAL OF EVER-GROWING TEETH CONTROLLED? INTEGRATING THE CONCEPTS OF REGENERATION AND REPAIR

Gonzalez Lopez M.¹, Azam M.¹, Kavková M.¹, Lavický J.¹, Henys P.², Sadílek V.³, Buchtová M.⁴, Vořechovský M.³, [Křivánek J.](mailto:jan.krivanek@med.muni.cz)¹

- ¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² Institute of New Technologies and Applied Informatics, Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Liberec, the Czech Republic
- ³ Institute of Structural Mechanics, Faculty of Civil Engineering, Brno University of Technology, the Czech Republic
- ⁴ Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, the Czech Republic

jan.krivanek@med.muni.cz

Continuously growing mouse incisors, have for decades served as a model system for investigating the mechanisms of regeneration and studying the stem cell niche. It has been shown that the growth rate of these teeth does not always proceed at the same pace, but can be accelerated following injury. However, the dynamics of growth acceleration, the mechanisms responsible for damage detection and subsequent process of stem cell niche activation remains entirely unresolved. Here, we provide new insights into the dynamics of mouse incisor regeneration, both in healthy (non-damaged) tooth and after its injury.

To quantify incisor growth, we have invented a new method (BEE-ST: Bones and tEEth Spatio-Temporal growth monitoring approach) that allows to monitor the growth and healing dynamics of any hard tissue on micrometre scale in both space and time.

Subsequently, using an interdisciplinary approach involving mathematical modelling, single-cell RNA-sequencing and lineage tracing, we reveal new mechanisms responsible for the detection of injury and we provide new insights into the cellular and molecular mechanisms responsible for the acceleration of the growth.

These results give a better understanding of mechanisms controlling hard tissue growth, stem cell niche homeostasis and could also reveal the processes underlying stem cell niche activation in a more general perspective.

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EMBRYOLOGY APPLIED (NOT ONLY) IN PEDIATRICS

Křížková V.¹, Vaňková L.¹, Bufka J.²

¹ Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, the Czech Republic

² Department of Pediatrics, Teaching Hospital in Pilsen, the Czech Republic

vera.krizkova@lfp.cuni.cz

The knowledge of embryology has applications in clinical fields such as pediatrics (including neonatology), but its understanding is also useful in all other clinical disciplines.

Results/case studies In collaboration with the Pediatric Department of our Teaching Hospital, we met in recent years with developmental anomalies of the digestive system, such as cleft palate, macroglossia, esophageal atresia, and rectal atresia. These anomalies have serious clinical manifestations and can be life-threatening. A clinically well-detectable developmental anomaly of the sensory organs is iris coloboma. However, there are also hidden developmental anomalies, such as ciliopathy. Ciliopathies are hereditary diseases affecting the morphology and function of cilia. Dysfunction of genes encoding ciliary proteins underlies several diseases with broad phenotypic manifestations (such as Kartagener's syndrome). Additionally, knowledge of embryology can help explain some symptoms related to COVID-19 and the subsequent limited organ regeneration (not only in pediatric patients). A more detailed discussion of individual case studies will be included in the presentation.

An understanding of developmental embryological processes is crucial for comprehending the causes, manifestations, and correlations of various anomalies or symptoms in pediatrics, as well as for accurate differential diagnosis in (not only) pediatric patients. The classic saying holds there "Non scholae, sed vitae discimus."

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THE EXPRESSION AND CELLULAR DISTRIBUTION OF DOR IN TWO DIFFERENT ANIMAL MODELS DURING THE OROFACIAL NEUROPATHIC PAIN

Kubičková L., Bahavan B., Karafiátová E., Leung V., Joukal M.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

lucie.kubickova@med.muni.cz

Facial skeleton fractures are common injuries connected with the injury of the 2nd or the 3rd branch of the trigeminal nerve, which is manifested by anesthesia in the innervation area of the damaged branch, and the condition may progress to neuropathic pain. The incidence, risk factors, and early signs of this disability are not satisfactorily known. Opioid receptors (PORs) are widely distributed in the nervous system and play a pivotal role in analgesia. Growing evidence has shown the involvement of PORs in the modulation of orofacial pain.

We studied the role of DOR during orofacial neuropathic pain using two animal models. Rats were divided into 3 groups: a group of animals that underwent unilateral ligation of the infraorbital nerve and unilateral transection of the alveolar inferior nerve, a sham-operated groups, and a control group of naïve rats. After putting the animals under deep anesthesia, the left infraorbital nerve (ION) was ligated according to the modified approach of Vos et al. (1994). Two silk ligatures (6-0 Ethicon) were tied to compress the nerve. The inferior alveolar nerve (IAN) was operated on according to the model described in the publication Němec, 2020. The nerves of sham-operated rats were merely exposed without any lesions. The harvested tissues (gingival mucose, trigeminal ganglion, and trigeminal subnucleus caudalis) were processed using indirect immunohistochemical staining and immunostained sections were analyzed under an epifluorescence microscope.

We found the increased level of DOR in operated groups compared to naive and DOR immunofluorescence in the IANT model was higher than in the IONL model. DOR was detected not only in the neurons but also in the glial cells within the trigeminal pathway. This study provides unique data on DOR expression in two animal models through trigeminal pathways. The activation of DOR could produce a significant analgesic effect in orofacial pain without central side effects.

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EPIGENETIC MODULATION VIA *SALVIA OFFICINALIS* L. IN CHEMOPREVENTIVE MODEL OF BREAST CARCINOMA

Lacková L.¹, Samec M.², Mazuráková A.³, Dvorská D.⁴, Braný D.⁴, Kajo K.⁵, Adamkov M.¹, Kubatka P.¹

- ¹ Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, the Slovak Republic
- ² Department of Medical Biology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, the Slovak Republic
- ³ Department of Anatomy, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, the Slovak Republic
- ⁴ Biomedical Centre Martin, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, the Slovak Republic
- ⁵ Department of Pathology, St. Elisabeth Oncology Institute, Bratislava, the Slovak Republic

lenka.lackova@uniba.sk

Breast cancer is the most common cancer among women worldwide. Early and appropriately chosen prevention can significantly reduce the incidence of breast cancer. Chemoprevention is a special type of prevention characterized by the use of naturally occurring phytochemicals or synthetic drugs with ability to reduce the risk of cancer or delay its development or recurrence. This research focused on chemopreventive activities of *Salvia officinalis* L. altering epigenetic mechanisms in experimental models of breast carcinoma.

The anticancer effects of dietary administered *S. officinalis* in two concentrations: SAL 0.1 (1 g/kg of food) and SAL 1 (10 g/kg of food) were evaluated in chemically induced (N-methyl-N-nitrosourea) rat models of breast cancer. Control group without administration of *S. officinalis* was used for comparison. In obtained rat tumor tissues, we analyzed immunohistochemical and molecular changes of epigenetic mechanisms, including DNA methylation, posttranslational histone modifications, and miRNA expressions.

After administration of *S. officinalis*, the evaluation of epigenetic changes revealed a significant decrease in DNA methylation in promoter regions of tumor suppressor genes ATM and PTEN (PITX2, RASSF1, and TIMP3 promoters were not altered). *S. officinalis* also decreased the lysine methylations of H3K4m3 and increased lysine acetylation of H4K16ac levels in both treated groups (H3K9m3 and H4K20m3 were not altered). In the analysis of miRNA expression, *S. officinalis* reduced the relative levels of oncogenic miR-21 and tumor-suppressive miR-145 (miR-210, miR-22, miR-34a, and miR-155 were not altered).

The experimental data from our study demonstrate significant chemopreventive and anticancer effects of *S. officinalis* that altered epigenetic mechanisms in rodent model of breast carcinoma.

This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic under Contract No. VEGA 1/0045/23 and 1/0498/23.

THE INFLUENCE OF FEED RESTRICTIONS ON SMALL BOWEL PARAMETERS ON AN EXPERIMENTAL RABBIT MODEL

Makovický P.¹, Tůmová E.², Makovická M.¹, Volek Z.³

¹ Department of Histology and Embryology, Faculty of Medicine, Ostrava, the Czech Republic

² Department of Animal Science, Czech University of Life Sciences Prague, the Czech Republic

³ Institute of Animal Science, Uhřetěves, the Czech Republic

peter.makovicky@osu.cz

Even though restrictive nutrition is perceived to be a method possessing a potentially beneficial effect on the body, there remains a lack of studies clarifying its impact on the physiological and morphological levels. The objective of this study was, therefore, to weigh the animals (WA); measure the length of the small bowel (LSB); the height of the villi (HV) as well as the depth of the crypts (DC) after restrictive feeding on experimental rabbits.

The experiment consisted of a total of 180 Hyplus experimental rabbits. There were 60 rabbits in the first experiment, 72 in the second and 48 in the third. Each experiment was divided into three groups. The first group were subject to an unregulated intake (ADL); the second received 50g feed/day/rabbit (R1) with the third getting 65g feed/day/rabbit (R2). The first experiment started at day 35 with restrictions between days 42–49 with small intestine sampling on days 49, 56, 63, 70/5 rabbits. The second experiment started on day 32 with restriction between 35–42 days and withdrawals on 42, 49, 70 days/8 rabbits. The third experiment started at 25–day with restrictions between 32–39 days, with withdrawals at 39, 81 days/8 rabbits.

In the first experiment, the implementation of ADL resulted in a significant deepening of DC in the R1 and the R2 groups, compared to the ADL group. In addition, there was a significant growth of HV and LSB in both R1 and R2 groups, but without clear differences at the end of the experiment. In the second experiment, the use of ADL resulted in a significant lengthening of HV and DC in, both, the R1 and R2 groups, compared with the ADL group, while WA and LSB showed lower values after the restriction and at the end of the experiment compared with the ADL group. In the third experiment, significantly higher values were measured in the LSB, HV and DC after the restriction and at the end of the experiment in the ADL group at the expense of the R1 and R2 groups. WA values were in favour of ADL after restriction, but at the end of the experiment they favoured the R1 and R2 groups.

Restrictive feeding has an effect on the morphology of the small intestine, but is sensitive to timing and periodization: used earlier, it has a negative effect, but when prolonged, positive changes occur at the level of the small intestine.

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CANALS OF THE ZYGOMATIC BONE: A MORPHOMETRIC STUDY ON DRY ADULT HUMAN SKULLS

Malakhov S.¹, Shmarhalov A.², El Falougy H.¹, Lukáčiková P.¹, Polák Š.¹

¹ Comenius University Bratislava, Bratislava, the Slovak Republic

² Avalon University School of Medicine, Willemstad, Curacao

stanislav.malakhov@mfed.uniba.sk

The anatomic course of the zygomaticofacial and zygomaticotemporal nerves through the zygomatic bone (ZB) canals can vary. This variation can increase the risk of damage and iatrogenic consequences during facial surgeries and local anesthesia. Our research aimed to establish the anatomical variations of the ZB canals due to the current lack of comprehensive data characterizing their features.

114 zygomatic bones from 57 adult human skulls were examined. The canals between zygomatico-orbital, zygomaticofacial, and zygomaticotemporal foramina (ZOF, ZFF, and ZTF respectively) were probed with endodontic files to identify their length, trajectory, and communications.

ZB contains several variational canals that transmit zygomaticofacial and zygomaticotemporal nerves. In total 241 canals have been found on 114 specimens. Canals connecting ZOF and ZFF were found in 30.7% of samples and had singular (66.2%), double (17.6%), bifid (13.5%), and convergent (2.7%) variations. The average length was 9.31 ± 1.82 mm and the range of 5.57-13.44 mm. Canals connecting ZOF and ZTF were present in 45.6% with singular (96.4%) and double (3.6%) variations. The length range was from 2.18 to 19.88 mm with 8.02 ± 3.34 mm on average. Canals originated at ZOF, bifurcated on its course, and terminated in ZFF and ZTF are found in 17.8%. Their average length was 9.56 ± 2.15 mm and a range of 4.28-14.48 mm. Canals communicated the ZFF and ZTF directly, were present in 2.5% of cases with an average length of 8.67 ± 3.8 mm and a range of 4.48-11.65 mm. Additionally, canals between the lateral and temporal surfaces of the marginal process of ZB were noticed in 3.4%. They had an average length of 2.88 ± 1.75 mm with a range of 0.43-5.51 mm. This study identified significant variability in zygomatic bone canals. This knowledge can improve surgical and aesthetic procedures by minimizing nerve damage risk.

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PROGNOSTIC ROLE OF E2 & E4 PROTEINS IN PROGRESSION OF THE CERVICAL LESIONS

Mešťanová V.¹, Krajňáková B.¹, Kúdela E.², Kalman M.³, Hurta Csizmár S.⁴, Adamkov M.¹

- ¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ² Department of Obstetrics and Gynaecology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin University Hospital, Martin, the Slovak Republic
- ³ Department of Pathological Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin University Hospital, Martin, the Slovak Republic
- ⁴ Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic

veronika.mestanova@uniba.sk

Human papillomavirus (HPV) represents a family of low-risk (LR) and high-risk (HR) variants affecting a wide range of changes in the uterine cervix. Due to their heterogeneity, the diagnostic process and treatment of cervical lesions become more challenging. The need for novel biomarkers elucidating the fate of HPV-associated cervical lesions might minimize the underestimated or overestimated diagnoses. Early E2 and E4 proteins perform essential roles in the HPV life cycle, with typical expression within the productive and transforming phases.

A presented preliminary prospective immunohistochemical study is performed on the FFPE cervical tissue samples obtained from the Department of Obstetrics and Gynaecology JFM CU, MUH. Cervical lesions are graded by a skilled pathologist, Ki67 and p16 markers are assessed within the routine diagnostic process in the Department of Pathological Anatomy JFM CU. The two extra slides were cut from the LSIL, HSIL, and SCCa lesions and immunohistochemically processed in the Department of Histology and Embryology JFM CU. Anti-HPV16 E2 (ab17185) and Anti-HPV16 E1/E4 (ab270738) antibodies were visualized by DAB chromogen in order to evaluate their distribution within the cervical epithelium.

Protein E4 was observed in the well-differentiated cells of suprabasal layers of cervical epithelium. E4 positivity declines with the level of dysplastic changes. In combination with p16, E4 has the potential to become an objective and reliable marker of differentiation between the metaplasia or inflammation and true HPV-associated precancerous lesions. On the other hand, E2 protein synthesis is bound to the basal layer in which the marker is involved in the viral DNA replication. Moreover, E6 and E7 proteins engaged in the continuous proliferative activity are E2-dependent. The distribution pattern of proteins in question in association with the disease progression reveals an inverse correlation.

Generally, transforming HPV infection loses the potential of differentiation and maintains the proliferation capacity, leading to the E2 expression. However, E4 as a marker of productive infection declines with the disease progression. A detailed and more precise diagnostic approach in the management of cervical lesions should be a priority of current research. Implementing significant biomarkers such as E2 and E4 proteins into the routine diagnostic process might benefit patients in terms of personalized medicine.

The study was supported by Grant UK/89/2019 and Grant UK/20/2023.

ANTIOXIDANT ENZYMES SOD1 AND 2 IN RAT EMBRYOS AND FEMALE GENITAL ORGANS DURING PREIMPLANTATION PERIOD OF PREGNANCY

Mihalik J.¹, Čurgali K.², Petrášová D.³, Hodorová I.¹

- ¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, the Slovak Republic
- ² Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, the Slovak Republic
- ³ Laboratory of Research Bio-models, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, the Slovak Republic

jozef.mihalik@upjs.sk

Superoxide dismutases (SODs) are enzymes, which form one of the first antioxidant barrier of the organism against reactive oxygen species (ROS). Surplus of ROS causes damage of proteins, lipids and DNA, and subsequently provokes origin of many diseases, including infertility.

Mature Sprague Dawley females were mated with the males of the same strain. The presence of vaginal plug was the proof of successful mating. Females were killed by overdosing of anesthetic on the first, third and fifth day of pregnancy (D1, D3, D5). Monitored enzymes in preimplantation embryos were detected by immunofluorescence staining, while in ovaries, oviducts, and uterine horns were detected by immunohistochemistry.

Both enzymes were present in rat preimplantation embryos from 1-cell stage till blastocyst. In the cytoplasm of blastomeres was SOD1 located diffusely, while SOD2 formed predominantly clusters. In ovary were both enzymes observed in granulosa-lutein cells of corpus luteum, and inside follicular fluid of tertiary follicles. In oviduct and uterus was positive cytoplasm of epithelial cells, and on D5 also fibroblasts of decidua. Moreover, SOD1 was detected as many granules in apical parts of epithelial cells of oviduct, uterus, and uterine glands during preimplantation period of pregnancy. SOD2 formed granules only in uterine glands.

The fact that both enzymes occur in embryos during the entire pre-implantation phase of pregnancy testifies to their great importance in protecting the new individual from oxidative stress. The same protective function SODs probably play in specific cells of the mother's genital organs. The fact that we found enzymes also in the apical parts of the epithelial cells in the form of granules may mean that these enzymes are secreted into the lumen of the oviduct and the uterus.

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TOCOPHEROL ALLEVIATES OXIDATIVE STRESS AND INCREASES ANDROGEN RECEPTORS IN MICE TESTICULAR TISSUES UNDER FORCED SWIMMING IN WARM WATER

Mohammadghasemi F.¹, Daeihamed M.², Fallah Kochakam N.³, Taghizadeh Valdi A.³, Faghani M.¹, Farzanegan A.⁴

- ¹ Cellular & Molecular Research Center, Department of Anatomy, School of Medicine, Guilan University of Medical sciences, Rasht, Iran
- ² Department of Pharmaceutics, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
- ³ School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
- ⁴ Department of Microbiology, Guilan University of Medical Science, Anzali International campus, Guilan, Iran

parsahistolab@gmail.com

The purpose of this study was to investigate the effect of tocopherol supplementation and swimming in warm water on the spermatogenesis, expression of androgen receptors (ARs), and oxidative stress markers in mouse testis.

Adult male mice were divided into 7 groups: A-control, B-solvent, C-vitamin E (VE), D-swimming at 23°C water, E-swimming at 23°C water and receiving VE, F-swimming at 35°C water, and G-swimming at 35°C water and receiving VE. Hormones were measured using ELISA. Spermatogenesis was examined through histopathology. The ARs were studied by immunohistochemistry. The testis oxidative stress markers were assessed using colorimetric assays. The distribution of VE in testis tissue was also evaluated using HPLC.

Swimming of mice at 23°C was safe for sperm development. Swimming at 35°C resulted in lower testosterone, LH, sperm parameters, sperm differentiation, AR expression, SOD activity, and increased MDA in the testis, compared to control ($P<0.05$). The above-mentioned parameters were significantly altered by VE, in comparison to the animals that swam in warm water at 35°C ($P<0.05$). The results of HPLC showed that tissue distribution of VE was significantly higher in group G, compared to group E ($p<0.05$).

Swimming in water at 23°C is conducive to sperm development, whereas swimming at 35°C impairs sperm production. In conclusion, VE counteracts the negative effects of swimming at 35°C water on sperm development by altering the hypothalamic-pituitary-gonadal axis, distribution of VE, AR expression, and lipid peroxidation in testicular tissue.

Guilan University of Medical Sciences Research Deputy.

LIGHT MICROSCOPY STRUCTURE OF LIVER PARENCHYMA IN AAGENAES SYNDROME PATIENTS

Mokry J.¹, Čížková D.¹, Reims H. M.², Sullivan G. J.³, Almaas R.³

¹ Department of Histology and Embryology, Charles University, Medical Faculty in Hradec Kralove, the Czech Republic

² Department of Pathology, Oslo University Hospital, Norway

³ Department of Pediatric Research, Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Norway

mokry@lfhk.cuni.cz

Aagenaes syndrome is a rare autosomal recessive disease characterized by neonatal cholestasis associated with lymphedema of the lower extremities (lymphedema cholestasis syndrome 1). Impaired development of lymphatic vessels affects the liver parenchyma and drainage of bile that results in hyperbilirubinemia and increased blood bile acid levels responsible for clinical symptoms.

Needle biopsies of the liver from 11 Aagenaes syndrome patients embedded in paraffin were cut in histological sections, stained for haematoxylin-eosin or immunostained for Abcb11, MRP2, UNC45A, cytokeratin 7 and 19, visualized with horseradish peroxidase and diaminobenzidine, and analysed with a light microscope. Control sections included intact human liver and samples of other disorders characterized by intrahepatic cholestasis incl. neonatal hepatitis, liver from patients with septo-optic-dysplasia/hypopituitarism, alpha1-antitrypsin deficiency, and progressive familial intrahepatic cholestasis type 2.

Haematoxylin-eosin staining identified signs of intrahepatic cholestasis and cells pigmentation, absence of interlobular ducts in portal spaces accompanied by inflammatory changes including immune cell infiltrates to fibrous transformation. Hepatic giant cells were observed in all patients with age 1.2-3.9 month but ratio of such cells, their sizes and number of cell nuclei differed. Liver of older patients (21 and 151 months) were characterized by absence of hepatic giant cells. Visualization of bile canaliculi with immunodetection of Abcb11 and MRP-2 proved loss of hepatocyte polarity, disintegration of bile canaliculi associated with flattened branching and positive cytoplasmic granules. At the edges of giant cells canaliculi were often straightened. Anti-CK7/19 immunostaining confirmed a ductular reaction while anti-UNC45A staining revealed activation of hepatic progenitor cells.

Highly dynamic changes in neonatal liver tissue reflect the current state of the disease and result from cholestasis, inflammation, and parenchyma remodelling. Our findings published in *J Hepatol* 2023 Oct;79(4):945-54 contribute to a current knowledge on histopathology of this rare disease.

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EVOLUTION OF THE TERM NAMING THE 8TH CRANIAL NERVE

Musil V.¹, Modrý M.¹, Stingl J.², Kachlík D.³

- ¹ Centre of Scientific Information, Third Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Department of Anatomy, Third Faculty of Medicine, Charles University, Prague, the Czech Republic
- ³ Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, the Czech Republic

vladimir.musil@lf3.cuni.cz

The cranial nerves were first mentioned in ancient anatomical works by Galen of Pergamum. This work aims to provide a historical overview of the term used to describe the vestibulocochlear nerve.

Anatomical works from the work of Vesalius to the official terminology were followed.

It also traces the number of cranial nerve pairs described and the positioning of the vestibulocochlear nerve among them, from Galen's time to the present. Additionally, it discusses the development of vernacular terms for the nerve in other languages.

Although this pair of cranial nerves is already described by Galen and systematized by Vesalius in 1543, the current valid term has been part of official terminologies only since 1960. Even though the term was codified in 1960 there are still authors who use the incorrect term to refer to this cranial nerve.

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USE OF FREE RADIAL FOREARM AND PRONATOR QUADRATUS MUSCLE FLAP: ANATOMICAL STUDY AND CLINICAL APPLICATION

Musilová Z., Kempný T., Knoz M., Joukal M., Lipový B., Holoubek J., Pöschl W. P., Cheng H. T.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

358583@mail.muni.cz

The radial forearm flap, often including the pronator quadratus muscle (PQM) flap, is widely used for reconstructing various soft-tissue surgical defects. The pronator quadratus muscle is primarily supplied by the anterior interosseous artery, with contributions from the radial and ulnar arteries. Consequently, the PQM flap can be harvested on the vascular pedicles of the radial artery. This study aimed to provide a detailed description of the arteries supplying the PQM, particularly the anatomical location of the radial artery branches perfusing the PQM.

The anatomical study involved the analysis of 13 fresh cadaver upper extremities, amputated at the forearm level. The radial, anterior interosseous, and ulnar arteries were cannulated and injected with colored silicone rubber, followed by macroscopic and microscopic dissection.

Muscular branches supplying the PQM originate directly from the arteries or via the palmar carpal arch, forming a rich anastomotic network. The anterior interosseous artery was identified as the primary supplying artery, with radial and ulnar muscular branches also contributing to PQM vascularization. We identified approximately four vascular pedicles from the radial artery. Most branches, averaging three, were found in the distal radial quarter. The strongest of these vessels, excluding the palmar radial branch, had an average diameter of 0.42 ± 0.1 mm and was present in 61.5% of cases within the distal radial quarter. Additionally, 12 patients underwent reconstructions of soft and bony tissue defects using a RFF + PQM (pedicled or free flap). The harvest site healed well in all cases, and except for one case with partial necrosis.

The distal radial quarter of the PQM muscle is supplied directly by radial vascular pedicles. The strongest branch was located approximately 32.6 ± 10.4 mm from the radial styloid process, while the midpoint of the PQM was 35.61 mm from the radial styloid process. Based on these results, the PQM flap should be harvested on the radial vascular pedicles within the range from the middle to the distal radial border of the muscle.

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VASCULAR SUPPLY OF THE DORSAL PORTION OF THE WRIST JOINT CAPSULE AND DORSAL CAPSULOTOMY

Musilová Z., Joukal M.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

358583@mail.muni.cz

Berger's dorsal radial capsulotomy is a universal surgical approach to the radiocarpal and mediocarpal joints aimed at treating advanced wrist osteoarthritis. Incisions made along the dorsal radiocarpal and intercarpal ligaments leave parts of the ligaments intact to preserve carpal stability but do not respect the coursing supplying vessels. This study aimed to detail the vascularization of the dorsal wrist joint capsule in relation to the ligaments and propose an optimal approach.

The anatomical studies were based on the dissection of upper limbs amputated at the forearm (31 limbs). The anterior interosseous artery (AIA), ulnar artery (UA), and radial artery (RA) were separately cannulated, flushed with water and alcohol, and injected with colored silicone (blue, yellow, red). The limbs were then fixed in an alcohol solution, followed by macroscopic and microscopic dissection with sodium hypochloride.

The dorsal wrist joint capsule is vascularized by three transverse arches formed by anastomosis of the terminal branches of the RA, AIA, and UA. The dorsal intercarpal arch (DIA) was present in 100% of cases, the dorsal radiocarpal arch (DRA) in 87.1%, and the basal metacarpal arch (BMA) in 77.4%. Supplying branches (average of 5) to the joint flap created by capsulotomy originated in 96.8% from the DIA, primarily supplied by the RA, and in 3.2% from the DRA, predominantly supplied by the AIA. The DIA ran distally from the dorsal intercarpal ligament, averaging 14.26 ± 3.93 mm from the triquetral bone.

The positions of the main supplying arches are near the dorsal extrinsic ligaments. Incisions along the ligaments during dorsal radial capsulotomy interrupt the vascular supply of the wrist joint capsule. Specifically, an incision along the dorsal intercarpal ligament interrupts the proximally running branches from the radial part of the DIA. Dorsal distal capsulotomy appears to be an optimal surgical approach.

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TERMINOLOGY OF THE GROWING LONG BONE: A HISTORICAL STUDY

Naňka O.², Bartoniček J.¹

¹ Department of Orthopedics, First Faculty of Medicine, Charles University and the Central Military Hospital, Prague, the Czech Republic

² Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, the Czech Republic

Ondrej.Nanka@lf1.cuni.cz

Division of the growing long bone into individual basic parts, i.e., diaphysis, metaphysis, physes and epiphyses, has been generally accepted and used. However, the origin of these terms is almost unknown. Therefore, we have made analysis of the literature in order to reveal their origin.

The terms epiphysis and apophysis have been used since the times of Hippocrates, although with a different meaning. In the times of Galen, the term apophysis was used to describe all types of bone processes, and epiphyses denoted articular ends. The term diaphysis denoting the middle cylindrical part of the long bone, was used for the first time by Heister in 1717.

The first to use the term metaphysis, was the Theodor Kocher in his books on gunshot wounds and on bone inflammations of 1895. Based on the Kocher's study, Lexer published a radiological study of vascular supply of bone where he defined metaphyseal blood vessels as a separate group supplying a particular part of the long bone.

Epiphyseal growth plate did not have a particular name from the time of its first description in 1836. In the second half of 19th century, this structure began to be called by different names. For this reason, the term "physis" was introduced in 1964 by the American radiologist Rubin in order to term distinctly the growth structure between metaphysis and epiphysis. One year later, the term physis appeared also in the radiological literature and during the following decades it spread also in the orthopedic literature.

Charles University Cooperatio Project, Morphological Disciplines of Medicine.

TAU PHOSPHORYLATION AND NEUROINFLAMMATORY RESPONSE TO A β IN CHOROID PLEXUS CELLS

Orviská P., EmamiAref P., Jeřábková K., Brázda V., Zamani A.

Alemeh Zamani Research Group, Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

petra.orviska@gmail.com

The pathogenesis of Alzheimer's disease is associated with the accumulation of amyloid-beta peptide and hyperphosphorylated Tau protein, leading to neuronal death. A β has been found to interfere with pathways that regulate the phosphorylation of Tau protein, especially in neuroinflammatory response. The blood-cerebrospinal fluid barrier and the choroid plexus play a key role in removing A β from the brain and may propagate its toxicity and Tau phosphorylation through neuroinflammatory reactions. The aim of the study was to confirm the relationship between neuroinflammation and increased Tau protein phosphorylation in a rat model cell line of the choroid plexus epithelium.

The rat choroid plexus epithelium cell line Z310 was cultivated with A β peptide for one and three day timepoints alongside non-treated control. Reverse transcription PCR was used to detect pro-inflammatory cytokines IL1 β , IL6, TNF α , and IFN γ , and immunocytochemistry was used to detect p-Tau at the phosphorylation sites T231, S199 and T181.

By calculating fold change values of RT-PCR, gene expression changes were observed. By the three day timepoint, a significant rise in expression was evident for most genes, particularly IL6 in the treated group. Notably, IL1 β exhibited prominent expression even after just one day of treatment, indicating its rapid response to A β exposure. Among the three Tau phosphorylation sites studied, the T231 site exhibited statistically significant changes in response to A β treatment upon calculating the corrected total cell fluorescence.

It was found that the mRNA expression of pro-inflammatory cytokines IL1 β , IL6, TNF α , and IFN γ , as well as p-Tau protein levels increased after A β treatment of the Z310 cells, supporting the main hypothesis that neuroinflammation in the choroid plexus epithelium is connected to Tau phosphorylation.

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POTENTIAL PROTECTIVE INTERACTION OF HYPERTHERMIA AND HYDROCORTISONE ADDITION ON FIBROBLASTS PROLIFERATION

Pavlíková Z.^{1,2}, Zahradníček O.³, Jelínek Michaelidesová A.³, Šrámek J.¹,
Davidková M.³, Hovoráková M.¹

- ¹ Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, the Czech Republic
- ³ Department of Radiation Dosimetry, Nuclear Physics Institute, Czech Academy of Sciences, Prague, the Czech Republic

zuzana.pavlikova@lf1.cuni.cz

Hyperthermia and hydrocortisone (HC) are known teratogens that can negatively affect embryonic development early in pregnancy. Cell proliferation is one of the crucial processes involved during early embryogenesis. Existing studies mostly evaluate one factor, but in pregnancy the factors do not act independently. In this study, we decided to test the teratological interaction of two different factors influencing the cell proliferation. We aimed to investigate the impact of elevated temperature and HC addition on fibroblasts proliferation in an in vitro culture.

Hamster lung fibroblasts (V79-4 cell line) were exposed to HC added in culture medium and cultured in vitro at 37 °C or 39 °C, respectively. To evaluate the effects of both factors on cell proliferation, various methods were used, including colony formation assay, generation of growth curves, computation of doubling times and mitotic index estimation.

Our results showed that hyperthermia slightly stimulated cell proliferation in the short term but suppressed it in the long term exposure. However, HC (0.1 mg/ml) acted as a stimulus of cell proliferation, when added in the in vitro culture medium. Strikingly, the interaction of HC and hyperthermia (39 °C) during long term exposition led to at least partial compensation of the negative effect of hyperthermia and to an increased proliferation when compared to cells cultured at 39 °C without the addition of HC.

Surprisingly, our results show that the interaction of two proven human teratogens is not necessarily additive or synergistic but it could lead to a partial rescue effect if the teratogens act in an opposite manner. This showed a need to consider the external factors interacting simultaneously during pregnancy as co-factors with different effects in comparison to their individual impact.

The work was supported by the Cooperatio Program of the Charles University, Prague, Czech Republic (207036-10 Morphological Disciplines of Medicine/LF1), and by the project OP RDE, MEYS (Reg. No.CZ.02.1.01/0.0/0.0/16_019/0000728) and by the long-term conceptual development of the research organization Nuclear Physics Institute of the Academy of Sciences of the Czech Republic (RVO: 61389005).

ANATOMICAL NOMENCLATURE FROM A LINGUISTIC PERSPECTIVE: A DATA-DRIVEN APPROACH

Požřková K.¹, Račanská M.²

¹ Language Centre, Faculty of Medicine Division, Masaryk University, Brno, the Czech Republic

² Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

porizkova@med.muni.cz

Teaching anatomical terminology at medical faculties in Central Europe has traditionally faced problems related to the different levels of students' initial knowledge. Furthermore, students appear to have little or no knowledge of the official language of standardised terminology, i. e., Latin. The aim of this study is to describe an innovative approach to teaching that combines and systematically addresses both problematic areas.

The applied research towards the stated goal is based on two independent methodological approaches. Firstly, a student's needs analysis aims to shed light on common problems in understanding the meaning and context of the use of anatomical terms. Subsequently, the methodology of corpus linguistics is assumed to bridge the gap between theoretical and practical use of terms, since such an approach, with the help of specialised information technology tools, enables to quantitatively and qualitatively explore how terminology words behave and accompany others in a large database of various medical texts.

The results of the survey analysis clearly reveal the students' need to grasp the meaning of anatomical terms via observing and exploring their real and typical context. Developing an electronic database of Czech clinical texts (that mainly includes anonymised patients' diagnostic summaries and narrative parts of discharge reports) meets the same needs and allows researchers and students to systematically search and analyse relevant terminological data.

This work will provide a brief excursion into the newly created medical terminology textbook, in which the main emphasis is put on understanding the construction of anatomical terms and their accurate application in professional communication. The textbook is based on interdisciplinary research of authentic clinical data using specialised tools of corpus linguistics. The aim is to systematically guide the students to recognize the basic patterns, acquire and apply them in alignment with professional communication objectives.

There was no specific funding for this work.

FACE APPROXIMATIONS OF THE CZECH COUNTESS SIDONIA VON SCHLICK

Shbat A.¹, Moraes C.², Malá-Zedníková P.¹, Beránek M.³, Kvasilová A.¹, Klepáček I.¹

- ¹ Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Ortoonline Treinamento em Desenvolvimento Profissional e Consultoria Ltda, Sinop, Brazil
- ³ The Cheb Museum, allowance organization of the Karlovy Vary Region, Cheb, the Czech Republic

andrej.shbat@lf1.cuni.cz

Facial reconstruction (face approximation) is important for fields such as anthropology, archaeology, medicine, art and modern 3D computer technology. It is used for the forensic identification of individuals, in oral and maxillofacial surgery, in the reconstruction of people from prehistoric and historic times, and for the transfer of faces and whole bodies of real people into the virtual space.

In 2015, the remains of Countess Sidonie Schlick (1560–1625) were recovered from the crypt of the Church of St. Nicholas and Elizabeth in Cheb (Czech Republic). We used standard anthropological methods to determine paleodemographic data (sex, stature, age). The skull was preserved, except for the absence of most teeth. We used three different approaches in reconstruction: drawing and physical face reconstruction based on classical anthropological and anatomical principles, and 3D computer approximation done by Blender software.

The metric differences between the reconstructions are not statistically significant, except for the nose and mouth regions. Here the differences are most noticeable.

Both plastic and 3D computer facial reconstruction have their pros and cons. However, the most complex reconstruction is always the nose, which has the highest variability. According to studies on human face recognition, it is the midface area of the nose and eyes, that is the most important. Therefore, its reconstruction is the most crucial in both plastic and 3D reconstruction.

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TARGETING THE IL-6 RECEPTOR ALPHA CHAIN TO MANIPULATE THE CANCER MICROENVIRONMENT

Smetana K., Jr.¹, Lacina L.^{1,2}, Raška M.³, Malý P.⁴

- ¹ Charles University, 1st Faculty of Medicine, Institute of Anatomy and BIOCEV, Prague, the Czech Republic
- ² Charles University, 1st Faculty of Medicine, Department of Dermatovenerology, Prague, the Czech Republic
- ³ Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, the Czech Republic
- ⁴ Institute of Biotechnology of the Czech Academy of Sciences, Vestec, the Czech Republic

karel.smetana@lf1.cuni.cz

Human tumors are complex ecosystems consisting of cancerous and non-cancerous cells, including cancer-associated fibroblasts (CAFs) and infiltrating immune cells. The use of immune checkpoint inhibitors in tumor treatment has led to an interest in possibly manipulating CAFs for therapy. Across different types of tumors, the secretion of IL-6 is a common characteristic of iCAFs. In this context, IL-6 is highly biologically active and affects the differentiation, proliferation, and migration of cancer cells. Therefore, targeting the IL-6 receptor appears to be a potentially effective approach for treating tumors and also severe inflammatory disorders.

We utilized biotechnology and protein engineering to develop a series of small proteins called NEF blockers with a strong affinity for the α subunit of the IL-6 receptor (IL6-R α). We demonstrated their predicted effect by inhibition of phosphorylation of the downstream protein STAT3. To examine the biological properties of the NEF blockers, we conducted tests using in vitro platforms such as iCelligence and IncuCyte. Through these methods, we studied the effects of the NEF blockers on the proliferation and migration of cancer cells. To verify their blocking function on the mouse version of the IL-6R α , we assessed their anti-inflammatory potential using a dextran-sulfate-induced acute model of mouse colitis.

NEF blockers are proven to be non-toxic. NEF blockers exhibited a negative effect on the proliferation and migration of cancer cells including melanoma and cells from ductal adenocarcinoma of the pancreas. Their application substantially suppressed intestinal inflammation such as the number of infiltrating cells, histological changes in mucosal architecture and secretion of inflammatory cytokines in mice.

NEF blockers are powerful inhibitors of IL6-R α . This outcome motivates us to continue the research with the potential for the practical application of NEF blockers.

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THE BLOOD-CEREBROSPINAL FLUID BARRIER AS A POTENTIAL ENTRY SITE FOR THE SARS-CoV-2 VIRUS

Solár P.^{1†}, Šerý O.^{2,3,4}, Vojtříšek T.^{2,5†}, Krajsa J.^{2,5}, Srník M.^{2,5}, Dziedzinská R.^{3,4}, Králík P.^{3,4}, Kessler M.^{3,4}, Dubový P.¹, Joukal A.¹, Balcar V. J.^{3,6}, Joukal M.¹

- ¹ Department of Anatomy, Faculty of Medicine, Masaryk University, the Czech Republic
- ² Department of Forensic Medicine, St. Anne's Faculty Hospital, Brno, the Czech Republic
- ³ Laboratory of Neurobiology and Pathological Physiology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, the Czech Republic
- ⁴ Laboratory of Neurobiology and Molecular Psychiatry, Department of Biochemistry, Faculty of Science, Masaryk University, Brno, the Czech Republic
- ⁵ Department of Forensic Medicine, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁶ Neuroscience Theme, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia

peter.solar@fnusa.cz

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is an RNA virus responsible for the coronavirus disease 19 (COVID-19). SARS-CoV-2 is thought to primarily attack the lungs and airways, but it can infect other organs including the central nervous system (CNS). The aim of the present study is to determine whether the choroid plexus could play a role in the entry of SARS-CoV-2 into the brain.

Tissue samples were obtained from 24 deceased COVID-19 positive individuals. We used reverse transcription real time PCR (RT-qPCR) test to look for SARS-CoV-2 viral RNA in selected brain regions including the choroid plexus. Following the RT-qPCR testing, two characteristic proteins of SARS-CoV-2 (spike protein S1 and nucleocapsid protein) were detected by immunofluorescence staining to visualize SARS-CoV-2 viral particles by confocal microscopy and to determine their cellular localization.

RT-qPCR of the choroid plexus followed by immunohistochemical staining showed the presence of SARS-CoV-2 viral particles in the epithelial cells. Double immunostaining demonstrated spike protein S1 of SARS-CoV-2 in the late endosomes of these epithelial cells.

These findings suggest that the blood-cerebrospinal fluid barrier serves as a site of entry of SARS-CoV-2 into the CNS.

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FINIS CORONAT OPUS

Stingl J.¹, Riedlová J.¹, Mrzálková J.¹, Musil V.²

- ¹ Department of Anatomy, Third Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Centre of Scientific Information, Third Faculty of Medicine, Charles University, Prague, the Czech Republic

josef.stingl@lf3.cuni.cz

The aim of the authors team was to present in the last presentation of this year's Morphology a group of examples of terminologically incorrect expressions occurring in morphology teaching, in medical communication and in the vocabulary of many patients. The authors anticipate that such a gathering of distinguished experts as the CAS membership would be in the best position to judge whether it is better to discard the collected material altogether or to include it in "the Golden Fund" of the Czech Clinical Anatomy.

Cooperatio 33 a 36.

LIGAMENTS OF THE TEMPOROMANDIBULAR JOINT: NEW CLASSIFICATION

Šedý J.^{1,2}, Kachlík D.^{1,2}, Žižka R.³

- ¹ Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Centre for Endoscopic, Surgical and Clinical Anatomy, Second Faculty of Medicine, Charles University, Prague, the Czech Republic
- ³ Department of Histology and Embryology, Medical Faculty of Palacky University, Olomouc, the Czech Republic

jirisedy@jirisedy.cz

Despite more than half of century of anatomical research, most anatomy textbooks still recognize only five ligaments of the temporomandibular joint – lateral, medial, sphenomandibular, stylomandibular and pterygomandibular. However, at least 11 ligaments have been already described, some of them clinically much more relevant than the repeatedly mentioned five. We have classified all ligaments into three groups: (1) intracapsular (intraarticular) group include medial discal, lateral discal anterior discal, discotemporal and discomalleolar ligaments, (2) capsular (juxtraarticular) group include medial and lateral (temporomandibular) ligaments and (3) extracapsular (extraarticular) group include sphenomandibular, stylomandibular, pterygomandibular ligaments together with tractus angularis, focusing on both anatomical principles and clinical relevance. We propose this classification to be routinely used for education of our students, mainly in dentistry and physiotherapy programs.

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SLOVAK AND CZECH ANATOMICAL TERMS WITH PREFIXES PRED- / PŘED-, I. E. IN FRONT OF, AND ZA-, I. E. BEHIND

Šimon František

Pavol Jozef Šafárik University in Košice, the Slovak Republic

frantisek.simon@upjs.sk

Some anatomical terms express a relative position, i. e. the name of an anatomical structure refers to another nearby anatomical structure. Just as in anatomical position, the right and left sides are seen differently from the observer's point of view and differently from the point of view of the object itself, so with some terms of extremity structures, the position in front of and in behind something can also be different depending on the direction of looking. In the Graeco-Latin nomenclature, the object's point of view is indicative, i. e. the metatarsus is behind the tarsus (Gr. meta- behind), in the Slovak and Czech we look at it the other way around, predpriehlavok (the Slovak term for metatarsus with the prefix pred-, in front of) is in front of priehlavok (Slovak term for tarsus). In the Czech term the starting term is nárt (Czech term for metatarsus), so the tarsus, Czech zánártí, is behind nárt. Similarly for the pair carpus and metacarpus, only in Slovak and Czech the two different anatomical structures are the starting point. The Slovak term for carpus, zápästie, and Czech zápěstí, is the bone behind the fist, Slovak term for metacarpus, záprstie, Czech záprstí, is the bone behind the fingers. The exception is the Graeco-Latin term antebrachium, which is formed from the point of view of the observer, i. e. the bone is located in front of the brachium. The more recent Slovak term for crus, predkolenie (Slovak prefix pred-, in front of), is formed from the point of view of the observer, that means it is the bone in front of the knee.

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THE INTERPLAY OF INFLAMMATION AND PLACENTA IN MATERNAL DIABETES: INSIGHTS INTO HOFBAUER CELL EXPRESSION PATTERNS

Tauber Z.¹, Burianová A.¹, Koubová K.¹, Mrštík M.¹, Jirkovská M.², Čížková K.¹

¹ Department of Histology and Embryology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, the Czech Republic

² Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague, Prague, the Czech Republic

zdenek.tauber@upol.cz

Inflammation of the placenta is harmful to both the fetus and the mother. Inflammation is strongly associated with diabetes, a common complication of pregnancy. Hofbauer cells (HBCs), unique immune system cells of fetal origin in the placenta, play complex roles, including growth of placental villi and their branching, stromal remodelling, and angiogenesis.

Our study investigated the expression of IL-1 β , IL-10, CYP2C8, CYP2C9, CYP2J2 and sEH in HBCs from patients with type 1 diabetes mellitus (T1DM) and gestational diabetes mellitus (GDM) compared to healthy controls using immunohistochemistry. We also assessed the structure of the villus stroma.

In T1DM, HBCs showed inflammatory activation characterised by increased IL-1 β and decreased CYP epoxygenase expression compared to normal placentas. Conversely, significant inflammation in HBCs appeared less likely in GDM, as levels of IL-1 β and CYP epoxygenases remained stable compared to normal placentas. However, GDM showed a significant increase in sEH expression. Both types of diabetes showed delayed placental villous maturation and hypovascularisation, with GDM showing a more pronounced effect. These facts suggest an association of the CYP epoxygenase-EETs-sEH axis with IL-1 β expression as well as villous stromal hypovascularisation. Given the stable high expression of IL-10 in both controls and both types of diabetes, it appears that immune tolerance is maintained in HBCs. However, the expression of IL-10 in HBCs does not reflect the overall immunological tolerance at the fetal-maternal interface, as other structures such as the trophoblast and maternal decidua contribute significantly.

Based on our results, T1DM and GDM differ significantly.

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SINGLE BEST ANSWER (SBA) QUESTIONS FOR FORMATIVE AND SUMMATIVE ASSESSMENT IN HISTOLOGY AND EMBRYOLOGY CLASSES

Tonar Z., Vaňková L., Malečková A., Křížková V.

Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, the Czech Republic

zbynek.tonar@lfp.cuni.cz

Current evidence suggests that single best answer type (SBA) questions are an effective teaching and assessment tool for developing problem-solving and higher-order thinking skills in medical students. We would like to share our experience in creating and piloting SBA questions in our Histology and Embryology courses.

First, we formulated and published detailed learning outcomes, listing and organizing the core content into a list of explicit requirements that course graduates should meet. For three years, we have used a set of approximately 30 SBA questions for formative assessment of our courses. After collecting student feedback, we created a main set of 150 SBA questions that cover a substantial portion of the learning outcomes.

The analysis of the quiz questions provided useful hints for improving the formulations suitable for 1st and 2nd year students. Most of our students were new to the SBA concept, but they appreciated the motivational potential, the positive relationship to the subject, and the medical context of the cognitive and practical skills learned in the Histology and Embryology course.

We have found SBA questions to be quite effective in providing students with early formative feedback on whether they are on the right track in developing the necessary study habits, whether they have understood the material, and whether they are working towards successfully completing the course and meeting its learning outcomes. SBA questions seem to capture students' attention, help focus attention on important parts of the curriculum, and support decision-making and the ability to apply knowledge in context. SBA questions also encourage teacher-student interaction during our classes. The potential of the SBA for summative assessment remains to be tested.

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CASE OF THE PERSISTENCE OF THE FEMALE PROCESSUS VAGINALIS (CANAL OF NUCK) AND REVIEW OF LITERATURE

Vecanová J., Hvizdošová N., Hodorová I.

Department of Anatomy, P. J. Šafárik University in Košice, the Slovak Republic

janka.vecanova@upjs.sk

The canal of Nuck, which is the female equivalent of the male processus vaginalis, is a protrusion of parietal peritoneum that extends through the inguinal canal and terminates in the labia majora. The canal typically obliterates within the first year of life, but in some cases, the canal can partially or completely fail to close, potentially resulting in congenital inguinal hernias. We aim to increase awareness of this condition by reviewing the embryology, anatomy, and diagnosis of canal of Nuck disorders.

We report a case of a 70-year-old woman with cysts of the canal of Nuck bilaterally found in our department during routine anatomical dissection with first-year medical students. A comprehensive review of existing literature was conducted, focusing on the embryological development, anatomical features, and clinical significance of the canal of Nuck.

A small, deep seated, oval, soft tumor approximately 30 x 40 mm in size was found in the right and left inguinal regions of an older female cadaver, with suspected inguinal hernias. Deeper dissection showed cystic formations filled with fatty tissue, with no visible communication to the peritoneal cavity, compatible with a cyst (hydrocele) of the Nuck canal. Paparella et al. (2021) described a prevalence of 0,74% in 353 1-14-year old female patients with inguinal swellings. No data about the prevalence in adults are available. A recent classification subdivides the cyst of the canal of Nuck into four groups according to its anatomical position. Type A is located subcutaneously over the superficial inguinal ring or is extending to the labia majora, type B resides inside the inguinal canal, and type C is confined to the deep inguinal ring. Ultrasonography is the preferred diagnostic method. Surgical intervention is the treatment of choice, typically involving excision of the sac and closure of the internal inguinal ring.

Due to the rare clinical occurrence and the lack of literature, most health professionals are not aware of the existence of cysts of the canal of Nuck, and are often misdiagnosed. This entity should always be considered as a possible cause of suspected inguinal and genital swellings in females.

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NEUROPATHIC PAIN AND IMMUNOREACTIVE DYNAMICS: INVESTIGATING THE INFLUENCE OF PACLITAXEL IN BRAIN VENTRICLES

Voleská E., EmamiAref P., Kubíčková L., Joukal M., Zamani A.

Alemeh Zamani Research Group, Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

evelina.voleska@gmail.com

Neuropathic pain affects over half of chemotherapy patients, yet effective treatments are lacking. Inflammatory reactions significantly contribute to neuropathic pain, but the spread of inflammation or pain between the peripheral and central nervous systems remains unclear. The choroid plexus plays a significant role in linking the peripheral to central nervous system inflammation. This study hypothesized that immune cells in the choroid plexus release cytokines that influence macrophages in the cerebral ventricles following chemotherapy. The aim was to investigate changes in those macrophage numbers following chemotherapy.

A suitable *in vivo* rat model of paclitaxel-induced neuropathic pain was utilized, with control groups including untreated animals and those given only the drug vehicle. Activated (ED1+, proinflammatory) and resident (ED2+, antiinflammatory) macrophages of the ependymal layer of the cerebral ventricles were labeled using an immunohistochemical method with a fluorescent dye. Fluorescence microscopy was used for visualization and capturing microscopic images. Macrophages were quantified, and the number of macrophages per 1 mm² of tissue was determined, with graphs compiled from the data.

After an initial increase, the number of activated macrophages decreased over time but remained elevated at the end of the observation period. The number of resident macrophages tended to increase, continuing beyond the observed period. Towards the end of the monitored period, resident macrophages predominated over activated ones, with this shift occurring around the seventh day, marking a significant point in the study.

This work demonstrated that paclitaxel treatment alters the number of macrophages on the surface of the brain ventricles and mapped these changes. Additionally, the situation potentially affects the composition of the cerebrospinal fluid and choroid plexus functions, contributing to the further spread of neuroinflammation and neuropathy. The research also revealed a compensatory mechanism, providing new insights into developing therapeutics for chemotherapy-induced neuropathic pain.

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ULTRASOUND-GUIDED TREATMENT OF UPPER ARM MUSCLE SPASTICITY: AN ANATOMICAL AND RADIOLOGICAL STUDY

Vrzgula M.¹, Josayová L.², Lovásová K.¹, Hodorová I.¹

¹ Ústav anatómie, Lekárska fakulta UPJŠ, Košice, the Slovak Republic

² Neurologická klinika, Lekárska fakulta UPJŠ a UNLP, Košice, the Slovak Republic

marko.vrzgula@upjs.sk

The objective of our study was to determine a safe approach and identify optimal sites for botulinum toxin administration in selected upper arm muscles under ultrasonographic control. Administering botulinum toxin directly into the muscle is the preferred method for treating focal, multifocal, and segmental spasticity, most often following a stroke. This method provides pain relief, reduces involuntary movements, and helps restore both passive and active muscle functions.

An anatomical muscle dissection (52-year-old male cadaver) was performed at the Department of Anatomy to define suitable sites for the application of botulinum toxin into the pronator teres, flexor carpi radialis, palmaris longus, flexor carpi ulnaris, flexor pollicis longus, flexor digitorum superficialis, and flexor digitorum profundus. Ultrasound visualization of the selected upper arm muscles on a 24-year-old male volunteer, along with the evaluation of the proposed approaches, was conducted out at the Department of Neurology.

All muscles in our study were optimally visualized using ultrasound, and the proposed locations for needle placement under ultrasonographic guidance were evaluated as safe and satisfactory. The needle should be inserted into all muscles from the front, approximately in their proximal half, perpendicular to the long axis of the upper limb. Attention should be paid to the surrounding blood vessels and nerves to avoid damage.

When botulinum toxin is applied intramuscularly, it is crucial to deliver it precisely into the intended muscle, without injuring surrounding structures. To achieve this, several localization techniques are employed, such as palpation, electromyography, electrical stimulation, and radiological modalities. Ultrasonography allows detailed localization of the muscle and determination of its size. Its primary advantage is the ability to directly visualize the needle and surrounding structures, which is particularly beneficial in revealing muscular and neurovascular anatomical variations during the implementation of interventional procedures.

There was no specific funding for this work.

IN VITRO STUDY OF MATERIALS FOR FUNCTIONAL REPLACEMENTS IN NERVE TISSUE REGENERATION

Zamani A.¹, Kocourková K.², Kadlečková M.², Mikulka F.², Minaříková R.², Smolka P.², Rabova A.¹, EmamiAref P.¹, Kubickova L.¹, Hašanová K.¹, Joukal M.^{1*}, Minařík A.^{2*}

¹ Department of Anatomy, Masaryk University, Brno, the Czech Republic

² Department of Physics and Material Engineering, Tomas Bata University in Zlín, the Czech Republic

zamani@med.muni.cz

Sciatic nerve injuries pose a significant clinical challenge, often resulting in severe functional impairment and reduced quality of life. Precise 3D printing fabrication of scaffolds that replicate the complex architecture of native nerve tissue emerges as a promising solution for further nerve tissue regeneration. Schwann cells play a crucial role in supporting neurons by promoting axonal growth and facilitating myelination, which is essential for effective nerve regeneration. This study focuses on printable polymer-based biocompatible materials, enabling Schwann cell adhesion, proliferation, and differentiation in vitro.

The growth of cells and the extension of cell processes were evaluated using immunocytochemistry for beta-actin.

Among the tested materials, the initial synthetic biocompatible material demonstrated low cell adhesion, resulting in poor cell viability on the surface. In contrast, the enhanced composite scaffolds seem to be more suitable, supporting better cell growth and cell process extension. Our results provide a platform of suitable materials for the growth of Schwann cells.

These findings offer a promising foundation for further research and development of complex conduits for nerve tissue regeneration.

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INVESTIGATE THE ROLE OF CHOROID PLEXUS IN ALZHEIMER'S DISEASE

Jeřábková K., Orviská P., Zareravasan A., Dianat M., Joukal A., EmamiAref P., Brázda V., [Zamani A.](#)

Alemeh Zamani Research Group, Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

zamani@med.muni.cz

Alzheimer's disease is a progressive neurodegenerative disorder characterized by the gradual worsening of dementia over several years, eventually becoming completely debilitating in its later stages. The three hallmark features of AD are amyloid-beta (A β) aggregation, tau hyperphosphorylation, and neuroinflammation. However, the precise sequential relationship among these phenomena remains unclear. The Choroid Plexus, which forms the blood-cerebrospinal fluid barrier, plays a crucial role in the clearance of A β from the brain. In Alzheimer's disease, insufficient A β clearance and increased inflammatory changes in the choroid plexus and cerebrospinal fluid are observed. This study investigates the underlying mechanism by which the choroid plexus is altered during Alzheimer's disease.

We used an in vitro model of Alzheimer's disease in Z310 cells developed in our lab. The choroidal epithelial cells, Z310 cells, were incubated with A β peptide for various time points, and molecular techniques were used to investigate the Z310 cell alterations.

We showed the molecular structure alteration of Z310 cells as early as 1 hour after the A β treatment. We observed a significant increase in molecular alteration with time. Alzheimer's disease-associated molecules, such as amyloid precursor protein (APP) and inflammatory mediators, were found to be among the molecules that exhibited alterations. Furthermore, proteins responsible for the barrier properties were demonstrated to be influenced by treatment with A β .

Our results provide valuable insights into the importance of understanding the role of the choroid plexus in the early stages of the disease. This includes its contribution to disease progression as well as its protective functions.

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MORPHOLOGICAL CHARACTERISTICS OF DEVELOPMENTAL ANOMALIES OF THE THYROID GLAND: A PROSPECTIVE AUTOPSY-BASED STUDY

Zavhorodnia I.¹, Samec M.², Hešková G.¹, Straka L.³, Janík M.³, Výbohová D.¹

- ¹ Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic
- ² Department of Medical Biology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic
- ³ Department of Forensic Medicine and Medicolegal Expertises, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic

zavhorodnia1@uniba.sk

Thyroid diverticulum – precursor of the thyroid gland (TG) develops between the first and second pharyngeal pouches, and descends in front of laryngeal cartilages to the level of CV to T1 vertebra via the thyroglossal duct. By the 6th week of gestation the thyroglossal duct starts to obliterate. Its incomplete obliteration causes occurrence of anatomical variations such as pyramidal lobe (PL) and levator glandulae thyroideae (LGT).

The current study aims to highlight occurrence and morphological characteristics of PL and LGT in 86 non-embalmed, non-frozen cadavers (66 male and 20 female). PL and LGT were evaluated according to the developed evaluation criteria.

Developmental anomalies of the TG were observed in 63.95 % of cases. PL was detected in 59.3 % of cases. LGT was observed in 47.67 % of cases. PL and LGT occurred more frequently in males (60.61 % and 48.48 %, respectively) compared to females (55 % and 45 %, respectively). Both variable anatomical structures prevailed on the left side of the TG. Several types of PL were recognized: junctional in 58.82 %, isthmal in 27.45 %, lobar in 13.73 % of cadavers. The average height, width, and area of PL were estimated 23.81 mm (SD ± 9.95 mm), 13.13 mm (SD ± 5.16 mm), 320.16 mm² (SD ± 203.64 mm²), respectively. Most frequent ratio of height to width of PL 1.3 : 1 was observed in 13.73 % of detected PLs. Macroscopic evaluation of LGT showed prevalence of fibrous fibers in 65.85 % of LGTs. Muscular fibers were observed in 26.83 %. Three types of LGT were observed: hypopyramidal in 70.73 %, thyreoglandular in 19.51 %, and hyoglandular in 9.76 % of samples.

Knowledge gained from this study is applicable in the surgical field to reduce risk of intraoperative and postoperative complications in head and neck surgery.

There was no specific funding for this work.

A HISTOLOGICAL OBSERVATION OF HUMAN IMMATURE PERMANENT TOOTH AFTER FAILED REGENERATIVE ENDODONTIC TREATMENT AND SUBSEQUENT ROOT CANAL TREATMENT

Žižka R.^{1,2}, Šedý J.³, Tauber Z.²

- ¹ Department of Histology and Embryology, Medical Faculty of Palacky University, Olomouc, the Czech Republic
- ² Institute of Dentistry and Oral Sciences, Medical Faculty of Palacky University, Olomouc, the Czech Republic
- ³ Department of Anatomy, Second Faculty of Medicine, Charles University, Prague, the Czech Republic

loupaczech@gmail.com

Regenerative endodontics is currently one of the possible alternatives of permanent teeth treatment with unfinished development. In the case of the success of therapy and the formation of newly produced mineralized tissue, we have limited information on the histological character of such tissue. This case report brings histological evaluation of unsuccessful regenerative endodontic treatment.

The patient who was treated with unsuccessful regenerative endodontic treatment and subsequent root canal treatment of the upper left permanent middle incisor appeared after 21 months with a horizontal root fracture. The tooth was extracted, demineralized in mixture of 45% formic acid with 17% sodium citrate for 4 weeks, embedded in paraffin and the 5 µm thick serial sections of the apical part were made. After staining with hematoxylin-eosin and Brown-Brenn (Taylor modification) the histological evaluation was performed.

In apical third of the root there was abundant deposition of newly produced mineralized tissue which had character of cementum with lacunae. There was transition basophilic line between dentin and newly produced tissue. There was only isolated part with tubular structure which was not connected to secondary dentin.

Despite the fact that treatment modality is called regenerative endodontic treatment, the histological outcome is rather reparative.

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A CASE OF FAHR'S DISEASE MISDIAGNOSED WITH MULTIPLE SCLEROSIS

Abna Z., Moghadasi A.N.

Multiple Sclerosis Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran

abna.zohreh90@yahoo.com

Idiopathic basal ganglia calcification or Fahr's disease is a rare neurodegenerative disorder that mainly affects basal ganglia and cerebellum. The age of onset is around the fourth to fifth decade of life and patients present with various neurological symptoms including movement disorders.

Here, we report a 35 year old woman presented by limb paresthesia that she was misdiagnosed as multiple sclerosis (MS) and was treating with interferon β 1a. There was no history of using tobacco, alcohol or illicit substance. Family history was remarkable in that the patient's sister suffered from a disabling neurological disease and was bedridden but unfortunately, her medical documents were not available. General and neurological examination of our patient was normal. Routine blood tests and renal and liver function tests were normal. Other laboratory investigations consisting of serum calcium, phosphate, magnesium, parathyroid hormone (PTH) and 25hydroxy vitamin D3 were all within the normal limits. Brain MRI revealed bilateral involvement of basal ganglia, thalamus, periventricular and subcortical white matter in favor of calcium deposits. Total findings suggested the diagnosis of Fahr's disease. Treatment with interferon was stopped and symptomatic therapy with gabapentin was started for the patient.

One of the most common causes of central nervous system involvement in young patient presenting with sensory symptoms is MS; however, imaging findings is an appropriate guide in limiting the differential diagnosis and achieving the correct diagnosis. Differential diagnosis for basal ganglia calcification is very wide including certain infections, neoplasms, metabolic diseases such as diabetes mellitus, hyperparathyroidism, and pseudohyperparathyroidism. Therefore, in the absence of any secondary etiology for brain calcification in young adult with positive family history of the same neurologic condition Fahr's disease should be considered.

There was no specific funding for this work.

EXPANSION AND MORPHOLOGICAL CHARACTERIZATION OF PATIENT-DERIVED PANCREATIC DUCTAL ADENOCARCINOMA CELLS CULTURED IN PLANAR CONDITIONS AND THREE-DIMENSIONAL SPHEROIDS

Acimovic I.¹, Vašíčková K.¹, Vaškovicová N.¹, Vodinská M.^{2,3}, Eid M.⁴, Moravčík P.³, Vlačný J.⁵, Kala Z.³, Vaňhara P.^{1,6}

- ¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² Molecular Oncology - Solid Cancer, CEITEC, Brno, the Czech Republic
- ³ Surgery Clinic, University Hospital Brno, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁴ Internal Hematology and Oncology Clinic, University Hospital Brno, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁵ Department of Pathology, University Hospital Brno, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁶ International Clinical Research Center, St. Anne's University Hospital, Brno, the Czech Republic

acimovic.ivana@gmail.com

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent type of pancreatic cancer. PDAC cells derived from the primary tumors of PDAC patients are a valuable source for development of three-dimensional (3D) in vitro models such as spheroids. These models can be used for disease modeling, personalized medicine and drug testing. One of the factors linked to the progression of PDAC is endoplasmic reticulum (ER) stress. We aimed in characterization of morphological properties of patient-derived PDAC cells in planar (two-dimensional, 2D) conditions and in 3D spheroids and their response to modulation of ER stress in vitro.

Patient-derived PDAC cells were obtained upon surgical resections and propagated in planar conditions. Multi-well agarose molds were used for spheroid formation. Effect of induction and suppression of ER stress on spheroid formation was analyzed after the treatments with tunicamycin and tauroursodeoxycholic acid (TUDCA), respectively. To quantify the observed morphological differences, we analyzed the spheroid parameters, area and circularity. Scanning electron microscope (SEM) was used to access the spheroids' morphology in detail.

Cells were successfully expanded from the resected tumors of PDAC patients and propagated in vitro. We identified morphological and molecular differences of tumor-expanded cells cultured in standardized 2D conditions. In addition, tumor-expanded cells exhibited differential propensity to form multicellular spheroids, as documented by total area and circularity of spheroids. This propensity was driven by induction/alleviation of cell stress response integrated at the ER and can be attributed to individual variability in PDAC patients.

ER stress-dependent morphological differences of tumor-expanded PDAC cells and assembly to 3D multicellular spheroids were identified. Our results indicate the feasibility of 3D PDAC modeling to capture the differential response to ER stress in individual PDAC patients.

This study was supported by AZV ČR (NU23-08-00241) and Masaryk University (MUNI/A/1598/2023).

EFFECT OF Wi-Fi ON MESONEPHROS DEVELOPMENT IN THE CHICKEN EMBRYO

Almášiová V., Andrašková S., Holovská K.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

viera.almasiova@uvlf.sk

Wi-Fi, mobile phones and other wireless devices are currently very popular, because they offer many benefits to their users. On the other hand, their excessive use creates an environmental burden, electrosmog. The developmental period of an individual, where a prominent cellular division and differentiation occur, is extremely sensitive to various environmental impacts. The aim of the current study was observation of the effect of Wi-Fi radiation on the structure of the mesonephros in the chicken embryo on day 9 of its development.

Fertilized eggs were divided into the control (n=6) and Wi-Fi (n=6) groups. On day 9 of incubation, the embryological material was routinely processed for preparation of hematoxylin and eosin histological sections.

Continuous exposure of Wi-Fi radiation at a frequency of 2.4 GHz and power density of 300 $\mu\text{W}/\text{m}^2$ during a 9-day incubation period did not affect general histological structure of the mesonephric kidneys. In both, the control and Wi-Fi groups, we observed mesonephric renal corpuscles and tubules, as well as mesonephric ducts. There were no significant structural changes in the mesonephric parenchyma except for the congestion of the blood vessels in the interstitium and slight enlargement of the urinary space in the renal corpuscles of the Wi-Fi group. Vascular congestion is mostly registered in the organs, which are particularly susceptible to the thermal effect of non-ionizing radiation due to their structural specificities, such as high water content, prominent capsule and superficial position in the body.

Morphological changes detected in the current work point to the fact that Wi-Fi, which also belongs to the spectrum of non-ionizing radiation, can interfere with the delicate developmental mechanisms that take place during organogenesis.

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HISTOGENESIS OF GASTRIC GLANDS IN THE CHICKEN EMBRYO

Andrašková S., Almášiová V., Holovská K.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

sandra.andraskova@uvlf.sk

Although the histogenesis of birds has its own specificities, the basic developmental processes are in principle the same as in mammals. The avian stomach is composed of two histologically and physiologically distinct regions, a glandular portion or proventriculus which is responsible for peptic digestion, and a muscular portion, the gizzard. The present study monitors histogenesis of the proventricular glands and secretory activity of glycosaminoglycans in 9 and 14-day-old chicken embryos.

The collected material was processed by histological routine, stained with hematoxylin-eosin for analysis of the basic structure of the glandular stomach. We used the PAS histochemical technique to analyse neutral glycosaminoglycans.

On day 9 of incubation, the proventricular glands were formed by simple tubules lined by pseudostratified columnar epithelium and showed no branches. In 14-day-old chicken embryos an increase in the thickness of the proventriculus wall and enlargement of the stomach glands were clearly visible. The branching of glands was evident and secretory epithelium differentiated into a simple columnar epithelium. In both developmental stages, we identified the glandular epithelium as mucus-secreting. Histochemical characteristics of proventricular gland epithelium showed low production of glycosaminoglycans on embryonic day 9, but their production increased progressively on day 14 of incubation.

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DIFFERENCES IN NESTIN EXPRESSION IN THE HEART OF YOUNG AND AGING SPONTANEOUSLY HYPERTENSIVE RATS

Artykov T.¹, Čížková D.¹, Žurmanová J. M.², Galatík F.², Šilhavý J.³, Pravenec M.³, Mokry J.¹

¹ Department of Histology and Embryology, Faculty of Medicine in Hradec Kralove, Charles University, Hradec Kralove, the Czech Republic

² Department of Physiology, Faculty of Science, Charles University, Prague, the Czech Republic

³ Institute of Physiology, Czech Academy of Sciences, Prague, the Czech Republic

artykovt@lfhk.cuni.cz

Cytoskeletal intermediate filament nestin is a protein detected in cells of some developing and regenerating tissues. It is expressed in the myocardium briefly during fetal development. However, current studies demonstrate the presence of nestin+ cells in the adult heart under pathological conditions such as myocardial hypertrophy and infarction.

In this study, we immunohistochemically detected nestin in the left ventricle and interventricular septum of the heart of 5 and 15-month-old spontaneously hypertensive rats (SHR) and rats of a derived conplastic SHR-mtBN strain with the mitochondrial genome of normotensive individuals. The aim was to compare nestin expression and distribution in the heart of young and aging SHR and SHR-mtBN rats.

Infrequent nestin+ cardiomyocytes and interstitial cells located near larger blood vessels were detected in the myocardium of 5-month-old rats of both strains. Although nestin expression was slightly more abundant in the SHR group, the differences were only small. Greater differences were observable in the age category of 15-month-old rats. In the SHR group, nestin positivity was detected in a larger number of cardiac cells evenly situated in the free wall of the left ventricle and a bit more in the interventricular septum, while in the conplastic strain this protein was expressed more in groups of cells in certain areas and less in other parts of the myocardium.

This study confirms differences in the occurrence of nestin in the heart of spontaneously hypertensive rats depending on age and mitochondrial genome. Less frequent nestin re-expression reflects a lower intensity of cytoskeletal remodelling of myocardial cells more resistant to hypoxia in SHR-mtBN rats. It can be assumed that nestin plays a role in the process of complex pathological changes of the heart in hypertension, and its re-expression may indicate the ability of myocardial regeneration.

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ANATOMY OF THE HUMAN BODY IN REAL IMAGES - PHOTOGRAPHIC ATLAS

Boleková A., Vecanová J., Hvizdošová N., Hodorová I.

Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, the Slovak Republic

adriana.bolekova@upjs.sk

In the recent period, the visualization of anatomy teaching by using of educational photos and videos has grown enormously. Despite the existence of various anatomical atlases, visual documentation directly from the dissecting room greatly help the students in preparing for practical learning. The intention of the presented project is the preparation and production of a new teaching aid called "Anatomical photographic atlas for practical lessons, part 1" (hereinafter Atlas), in which real images of organs, their parts and topographical areas of the human body, obtained from specimens and cadavers used in practical anatomy lessons. Atlas is usable within a separate study at practical lessons as well as at home.

The preparation of cadavers and selected organs according to the schedule of the winter semester of the subject Anatomy 1 will be proceed. The next step will be photographic documentation and the completion of video recordings. Creation the definitive form of Atlas will made in the last phase of the project.

Understanding the complexity of the human body using real images from dissections will help in the successful study of the subject, students will more easily and clearly acquire the knowledge they will need not only to pass the anatomy exam, but also to study clinical subjects and finally for their future clinical application. This material will serve not only students, but also young assistants in preparation for practical lessons, and also other teachers as a source of knowledge that they will pass during teaching.

Learning with photos and videos is comfortable, easier and more effective, reducing the time spent on learning while allowing understanding the subject in depth. With the help of the Atlas, anatomy will be easier, simpler and, above all, more effective to learn, thus the audio-visual work of the Atlas will contribute to the considerable facilitation of the study of this difficult subject. his multimedia atlas will serve not only students, but also young assistants in preparation for practical lessons and other teachers and health workers as a source of knowledge during their professional life.

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DIAGNOSIS OF SELECTED HEREDITARY EYE DISEASES IN DOGS BY PCR

Bučan J., Holečková B.

University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic

jaroslav.bucan@student.uvlf.sk

Collie eye anomaly (CEA) is a serious inherited eye disease in dogs, affecting predominantly collie breeds. Primary signs include choroidal hypoplasia and the occurrence of colobomas. The disease is caused by a deletion of a 7.8 kb stretch in the 4th intron of the NHEJ1 gene. Progressive retinal atrophy (PRA) is a set of diseases of hereditary origin that can be divided into several groups. The RCD3 form, which is caused by a mutation in the PDE6A gene, manifests as progressive early degeneration of rods and cones. It is sometimes difficult to make a diagnosis in these breeds based on ophthalmological examination alone, and therefore genetic testing should be performed to supplement the diagnosis in these breeds.

In our research, we focused on the diagnosis of these diseases in a group of 40 dogs belonging to predisposed breeds using the conventional PCR method. 29 Australian Shepherds and 11 Longhaired Collies were diagnosed.

Of the diseases, only mutations causing CEA were confirmed. There were 11 positive individuals (a/a) and 2 heterozygous carriers of the disease (A/a). The other 27 individuals were negative (A/A). Progressive retinal atrophy was not confirmed in any dog.

Our research confirmed a higher frequency of CEA in long-haired collies, as all positive individuals belonged to this breed. In spite of this predisposition, the course of the disease is mild in these individuals, without serious visual impairment, but their use in breeding is not recommended, because of the transmission of the disease to the offspring. Only carriers have been diagnosed in Australian Shepherds, which are unlikely to develop the disease if bred, provided they are mated to a genetically healthy individual.

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INFLUENCE OF Wi-Fi RADIATION ON THE ORGANOGENESIS OF CHICKEN EMBRYO

Bučko M., Andrašková S., Holovská K., Almášiová V.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

matus.bucko@student.uvlf.sk

Electrosmog originating from the electromagnetic radiation of Wi-Fi routers, mobile phones and other wireless electronic devices has created a big burden on living organisms and the environment over the last decades. The rise of Wi-Fi usage has been attributed to its high popularity and efficiency, introducing it to shopping centres, schools and other public spaces.

The main goal of the present work was to observe the effect of Wi-Fi radiation on the histological structure of the organs in the developing chicken embryo on the 9th day of incubation. Wi-Fi group was exposed to a non-ionizing radiation at a frequency of 2.45 GHz and average power density of 300 $\mu\text{W}/\text{m}^2$ during the entire incubation period. Embryological material from the control (n=6) and Wi-Fi (n=6) group was commonly processed for preparation of histological sections.

Wi-Fi radiation (2.45 GHz, 300 $\mu\text{W}/\text{m}^2$) did not cause any significant morphological changes within individual organs and tissues of the chicken embryos. However, we noted some variations between the control and exposed groups. In the parenchymatous organs such as the lungs, liver, spleen, kidneys, gonads and also in the developing connective tissues, we observed blood vessels congestion, which is very likely a consequence of the thermal effect of Wi-Fi on the tissues. In the preparations stained by histochemical methods we found reduced production of PAS positive substance in the cavity of the muscular stomach, as well as increased production of the collagen fibres in the perichondrium of the cartilage precursor of the developing bone in the Wi-Fi group.

Structural changes recorded in the Wi-Fi group point to the the thermal as well as non-thermal effect of radiation on the tissues of a living organism. Recorded morphological changes may pose a potential health risk to the developing organism exposed to the influence of Wi-Fi radiation.

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INVESTIGATING THE ROLE OF ENDOPLASMIC RETICULUM STRESS AND UNFOLDED PROTEIN RESPONSE IN PATIENT DERIVED PANCREATIC ADENOCARCINOMA CELLS

Cakmakci R. C., Acimovic I., Jan L., Portakal T., Eid M., Vlažný J., Moravčík P., Kala Z., Vaňhara P.

- 1 Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- 2 Internal Hematology and Oncology Clinic, University Hospital Brno, Brno, the Czech Republic
- 3 Department of Pathology, University Hospital Brno, Brno, the Czech Republic
- 4 Surgery Clinic, University Hospital Brno, Brno, the Czech Republic
- 5 International Clinical Research Center, St. Anne's University Hospital, Brno, the Czech Republic

riza.can.cakmalci@med.muni.cz

Pancreatic adenocarcinoma (PDAC) is a prevalent and deadly cancer, often diagnosed at advanced stages. Research of ER stress and the unfolded protein response (UPR) in PDAC cells offers new treatment perspectives. Rapid PDAC cell proliferation and interactions with tumor microenvironment induce protein misfolding in the ER and initiation of integrated stress response including UPR. This study investigates patient-specific expression changes of proteins associated with ER stress (BiP, CHOP) and PDAC carcinogenesis (TUSC3, E-cadherin, N-cadherin, EGFR, TGF β R) to provide novel insights to molecular circuitry relevant to PDAC diagnostics, follow-up, and molecular targeting. A 3D culture model was developed to replicate PDAC cell heterogeneity in vitro for better study and treatment.

Primary tumor cells from patient resections were cultured in DMEM with 10% FBS at 37°C and humidified atmosphere. Cell viability was determined by MTT assay. Intracellular levels of ER stress regulators and membrane receptors in PDAC samples were analyzed by Western Blot. Tunicamycin (TN, 0.5 and 1 μ M) induced ER stress by inhibiting N-glycosylation. Tauroursodeoxycholic acid (TUDCA, 0.5 μ M) and Salubrinal (SAL, 500nM) were combined with TN to inhibit ER stress. Agarose and hydrogel were used for investigations in 3D culture conditions.

We demonstrated differential UPR activation with increased expression of BiP and CHOP modulators in TN, TUDCA+TN, and SAL+TN groups. TUSC3 decreased in TN groups. E-cadherin expression decreased in all groups except TUDCA. N-cadherin expressions were contradictory. EGFR expression increased in TN, TUDCA+TN and SAL+TN groups. TGF- β R expression showed no changes. Spheroid size and circularity varied between agarose and hydrogel scaffolds.

This study highlights patient-specific differences in expression of tumor-driving genes associated with ER stress response, transmembrane receptors and cell junctions.

Differential ER stress response altering dynamics of crucial signaling molecules can contribute to phenotypic differences of patient-specific PDAC cells.

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SHH DESCENDANT CELLS IN THE TOOTH-BONE INTERFACE

Dalecká L.^{1,2}, Kindlová M.^{1,3}, Pavlíková Z.^{1,3}, Hovořáková M.¹

- ¹ Institute of Histology and Embryology, 1st Faculty of Medicine, Charles University, Prague, the Czech Republic
- ² Department of Cell Biology, Faculty of Science, Charles University, Prague, the Czech Republic
- ³ Department of Anthropology and Genetics, Faculty of Science, Charles University, Prague, the Czech Republic

linda.dalecka@lf1.cuni.cz

Sonic Hedgehog (Shh) is a signalling molecule crucial for the development of all organ systems, particularly during early embryogenesis, and it also plays a significant role in regenerative processes in adulthood. Shh plays a role in inducing osteoclasts, which are essential for defining the size of the tooth germ formation region in the alveolar bone. However, its expression has not been directly observed in the cells of bone tissue. During prenatal development, the growth of the tooth germ is restricted by the surrounding tissues and the processes in tooth-bone interface play an important role for tooth eruption.

We aimed to analyse bone formation during the late prenatal and postnatal development of the mouse maxilla and mandible, focusing on cells expressing Shh and their descendants at the tooth-bone interface of both mouse jaws. We took advantage from transgenic mouse strains (TdTomato crossed with ShhEGFP) allowing cell lineage tracing of Shh descendants and their visualization in the offspring. Surprisingly, we detected a population of Shh descendant cells in the area of the forming alveolar bone in TdTomato/ShhEGFP mouse heads at E18.5. Using immunohistochemistry combined with RNAscope we evaluated the association of these Shh descendant cells with populations of osteoblasts, osteoclasts and osteocytes respectively.

Interestingly a distinct population of osteoblasts and osteocytes seems to be TdTomato positive. Thus it appears that Shh descendant cells participate in the bone formation at the tooth-bone interface. In contrast, the osteoclasts were TdTomato negative proving their origin in a cell population not related to the Shh expressing cells. The origin of Shh descendants in the tooth-bone interface needs to be further elucidated.

Understanding the origin and a role of Shh descendant cells in the alveolar bone formation may enhance our understanding of the pathogenesis and regeneration processes in the tooth-bone interface, and also help to understand the defects such as ankylosis.

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EVALUATION OF ANGIOGENIC POTENTIAL AND BIOCOMPATIBILITY OF A POROUS BIOMATERIAL USING AN EX-OVO QUAIL CHORIOALLANTOIC MEMBRANE (CAM) MODEL

Demčišáková Z.¹, Tirpáková Z.², Luptáková L.², Medvecký Ľ.³, Hurníková J.¹, Šimaiová V.¹, Teleky J.¹, Petrovová E.¹

¹ Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

² Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

³ Institute of Materials Research, The Slovak Academy of Sciences, the Slovak Republic

zuzana.demcisakova@uvlf.sk

The chorioallantoic membrane (CAM) of chicken embryos has been extensively used to assess the biocompatibility and angiogenic potential of biomaterials. However, the quail embryo model offers enhanced experimental efficiency and the potential for larger sample sizes due to a shorter developmental period and smaller size. The quail-specific marker of endothelial cells (QH1) suggests that quail could represent an equally valuable model for angiogenic studies as chicken embryos. The objective of the presented study was to assess the angiogenic potential of an acellular porous polyhydroxybutyrate/chitosan (PHB/CHIT) based scaffold in combination with pro- and anti-angiogenic substances on an ex ovo quail CAM model.

On embryonic day (ED) 6, the experimental biomaterials were applied on CAM and tested itself or submerged previous to application in vascular endothelial growth factor (VEGF-A; application dose 25 ng), saline solution (PHY); or angiogenesis inhibitor (Angiostatin; 4.7 mg/ml; 1:100 in PBS). After 72 hours (ED9), we conducted a deep morphological, histological, immunohistochemical and molecular study of angiogenic potential and biocompatibility of acellular PHB/CHIT scaffold. The formation of vessels as a sign of the ongoing angiogenesis was analysed in the surrounding area of the scaffold and inside of the pores of the implants, using the markers of embryonic endothelium (WGA, SNA), myofibroblasts (alpha-SMA), endothelial cells (VE-CADH) and hemangioblasts (QH1).

The morphological and histochemical analysis showed strong angiogenic potential of untreated scaffold without additional effect of the angiogenic factor VEGF-A. The lowest angiogenic effect was observed in the scaffold soaked in angiostatin. Gene expression of pro-angiogenic growth factors, i.e., VEGF-A, ANG-2, and VE-CAD, was upregulated in untreated scaffolds after 72 hours, indicating a pro-angiogenic environment. The results proved strong endogenous angiogenic potential of PHB/CHIT composite.

We provide the methodology to investigate angiogenic potential of biomaterials using quail CAM ex ovo assay as a suitable alternative animal model compared to the

commonly used chicken CAM platform for rapid screening of biomaterials in the field of tissue engineering.

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COURSE OF PREPARATION, PRESERVATION AND PLASTINATION TECHNIQUES - A NEW OPTION FOR FURTHER EDUCATION OF AUTOPSY TECHNICIANS AND PREPARATORS

Frišhons J., Šandor O., Hána M., Joukal M., Dzetkuličová V.

Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

203563@mail.muni.cz

The education of autopsy technicians and preparators is important for their work at morphological institutes when maintaining collections or creating new teaching specimens. In recent years, there have been several educational programmes focused on special dissection techniques. Currently, the only course that involves teaching a wide range of preparation, preservation and plastination techniques is offered at the Department of Anatomy in Brno. The pilot cycle of this course took place this year under the auspices of the National Restoration Plan and Masaryk University.

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STUDY OF THE STRUCTURE OF THE GILLS, EYE AND MUSCLES OF NEOCARIDINA DAVIDI AFTER EXPOSURE TO MOSPILAN® 20 SP

Galajda M.¹, Almášiová V.², Andrašková S.², Galajda R.³

- ¹ University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic
- ² Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic
- ³ Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

Matus.Galajda@student.uvlf.sk

Within the framework of the European Convention on the Protection of Vertebrates Used for Experimental and Other Scientific Purposes, one of the main requirements is to limit the number of experiments on vertebrates, which requires the search for suitable species among invertebrates. Freshwater shrimp of the species *Neocaridina davidi* represents a suitable and promising organism for testing the toxicity of various pollutants, because it reacts very sensitively to various environmental changes. In the work, we focused on histological structure of gills, eye and muscles of *Neocaridina davidi* after exposure to the pesticide acetamiprid in the water environment. Acetamiprid contained in the preparation Mospilan® 20 SP belongs to the group of neonicotinoids, which are used in agriculture to protect plants from harmful insects.

During acute toxicity testing over a period of 5 days aimed at determining LC 50 (EPA), the structure of gills, eye and muscles of shrimps exposed to a concentration of 1mg/l Mospilan® 20 SP in the water environment and shrimps from the control group were evaluated. 7 µm thick longitudinal sections of shrimps were stained with the hematoxylin-eosin method and evaluated with a conventional light microscope.

In individuals exposed to acetamiprid, we noted significant deformation of the gills, eye and muscles. The gills had discontinuous epithelial lining, and significantly dilated blood vessels were present in the connective tissue cores. In the eyes we found a violation of the layers of the retina and muscle fibers in the muscle tissue were broken. These obvious morphological changes point to a very damaging effect of Mospilan® 20 SP on the structure of freshwater shrimp.

In our work, it was shown that the freshwater shrimp *Neocaridina davidi* is suitable as a model organism for evaluating the effect of pesticides on organisms in the aquatic environment.

This work was supported by the VEGA project 1/0166/21.

STUDY OF THE STRUCTURE OF THE HEPATOPANCREAS OF NEOCARIDINA DAVIDI AFTER EXPOSURE TO ACETAMPRID IN THE AQUEOUS ENVIROMENT

Galajda R.¹, Almášiová V.²

¹ Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

² Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

richard.galajda@uvlf.sk

The use of pesticides in agriculture brings with it the risk of harming non-target organisms, damaging the environment and contributing to the reduction of biodiversity. Freshwater shrimp of the species *Neocaridina davidi* represent a suitable and promising organism for testing the toxicity of various xenobiotics, as they react very sensitively to various environmental changes. In the work, we focused on the study of the hepatopancreas after exposure to the pesticide acetamiprid from the group of neonicotoids in the aquatic environment in the Mospilan® 20 SP.

As part of a 5-day acute toxicity test aimed at determining the LC 50 (EPA), the structure of the organs of individuals exposed to a concentration of 1mg/l Mospilan® 20 SP in the aquatic environment and individuals from the control group were compared at the level of light microscopy. Histological sections were made and stained with the hematoxylin-eosin method.

Endodermal region of the digestive tract the system in *N. davidi* consists of intestine and hepatopancreas. The hepatopancreas is a tubular organ involved in digestion, absorption of nutrients, accumulation of reserve material and also in the excretion and detoxification of xenobiotics. Therefore, it is highly sensitive to physiological and environmental changes that occur in natural habitats. This was also shown after 5 days of exposure to acetamiprid, where changes were observed in epithelial damage and shrinkage of hepatopancreatic tubules in shrimp from the experimental group compared to the control group.

Our observation showed that the hepatopancreas in the model organism *N. davidi* is suitable for assessing changes after exposure to pesticides in the aquatic environment. For further research, however, it will be important to study various organs also at the ultrastructural level.

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MANIFESTATIONS OF FACIAL DIRECTIONAL ASYMMETRY IN YOUNG ADULTHOOD

Harnádková K.^{1,2}, Hřebcová E.¹, Měšťák J.³, Dupej J.¹, Morávek A.⁴, Velemínská J.¹

- ¹ Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, the Czech Republic
- ² Department of Histology and Embryology, Third Faculty of Medicine, Charles University, Prague, the Czech Republic
- ³ Department of Plastic Surgery, 1st Faculty of Medicine and Bulovka Hospital, Charles University, Prague, the Czech Republic
- ⁴ Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, the Czech Republic

harnadkk@natur.cuni.cz

The goal of our research was to describe facial directional asymmetry in pre-adult individuals. Knowing how facial features develop during this period is crucial for many scientific and medical areas, but pre-adult individuals are generally under-researched.

In this study, we describe semi-longitudinal trends in the development of facial directional asymmetry in healthy 56 pre-adult individuals aged 15 to 19 years. The 3D facial models were analysed by geometric morphometrics. Both, neutral facial expressions and smiles which represents facial dynamics were included.

Our results showed that facial directional asymmetry changes with age, with the most significant changes occurring in the lower third of the face. On the other hand, certain areas of the face exhibited asymmetry consistently: protrusion of the left forehead and retrusion of the right upper lip were observed. In smiles, a consistent protrusion of the left cheek was observed, suggesting more pronounced involvement of the left side of the face. Additionally, we found that asymmetry is more prominent in boys.

In conclusion, facial directional asymmetry evolves and changes with age, especially in the lower third of the face, and boys exhibit greater asymmetry. The manifestation of asymmetry during facial expressions are particularly evident in cheek areas.

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SHANK3 DEFICIENCY AFFECTS NEURITE LENGTH AND BRANCHING IN DOPAMINERGIC NEURONS IN VITRO

Havránek T.^{1,3}, Bogyová E.³, El Falougy H.¹, Polák S.¹, Báčová Z.³, Bakoš J.^{2,3}

- ¹ Department of Anatomy, Faculty of Medicine, Comenius University, Bratislava, the Slovak Republic
- ² Department of Physiology, Faculty of Medicine, Comenius University, Bratislava, the Slovak Republic
- ³ Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, the Slovak Republic

tomas.havranek@fmed.uniba.sk

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by social impairments and restricted, repetitive behaviors. Mutations in SH3 and multiple ankyrin repeat domains 3 (SHANK3) gene have been associated with ASD conditions. SHANK3 proteins are scaffolding proteins involved in synapse formation and function.

In this study, we explored the impact of SHANK3 deficiency on the neurite morphology of primary dopaminergic neurons isolated from the tegmentum of neonatal (postnatal day 1) mice lacking the SHANK3 gene. Additionally, we examined the effects of transiently silencing SHANK3 (siSHANK3) in rat primary dopaminergic neuronal cultures. This transient silencing was achieved using the lipid transfection reagent DharmaFECT combined with siRNA targeting Shank3. We used tyrosine hydroxylase staining to identify dopaminergic neurons and applied Sholl's analysis to evaluate neurite arborization.

Neurites of dopaminergic neurons isolated from SHANK3-deficient mice were shorter and had fewer branches compared to wild-type controls. Transient silencing of SHANK3 gene in rat dopaminergic neurons also led to a significant reduction in neurite branching.

These findings suggest that SHANK3 proteins play a critical role in regulating neurite growth and morphology, possibly through modulation of cytoskeletal proteins. Genetic variants of SHANK3 proteins may therefore contribute to synaptic plasticity deficits in dopaminergic neurons, which could underlie some of the behavioral features of ASD.

This study was supported by APVV-21-0189 and VEGA 2/0057/23 funding.

DIRECTED DIFFERENTIATION OF PLURIPOTENT STEM CELL-DERIVED, EXPANDABLE LUNG EPITHELIAL PROGENITORS (ELEP) FOR INVESTIGATION OF REGENERATION AND CELL STRESS RESPONSE

Herůdková J.^{1,3}, Havlíček V.¹, Pelková V.^{1,3}, Vaškovicová N.¹, Portakal T.¹, Pečinka L.^{2,3,4}, Moráň L.¹, Sedláková V.¹, Porokh V.¹, Kotasová H.^{1,2}, Hampl A.^{1,2,3}, Vaňhara P.^{1,2,3}

- ¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² International Clinical Research Center, St. Anne's University Hospital Brno, Brno, the Czech Republic
- ³ University Hospital Brno, Brno, the Czech Republic
- ⁴ Department of Chemistry, Faculty of Science, Masaryk University, Brno, the Czech Republic

jarmila.herudkova@med.muni.cz

Human lungs have only a moderate capacity to regenerate, and this is even more pronounced during aging or lung injury. Stimulation of alveolar regeneration through reactivation of tissue-residing stem cells can induce lung regeneration. Experimental approaches to address lung stem cell biology are, non-trivial, small-scale, or originate in non-physiological tissues, e.g., lung adenocarcinoma. Alveolar type I (AT1) cells responsible for gas exchange, surfactant-producing alveolar type II (AT2) cells, and recently identified transitional cell states of these two cell types are all involved in recovery of lungs. Here we report a novel model of lung progenitors derived from pluripotent stem cells that maintain their epithelial characteristics over time and can produce terminally differentiated alveolar epithelia when properly stimulated.

In our experiments, we used various biology methods, including immunofluorescence and electron microscopy, to detect structures and proteins of pneumocytes, proliferation, viability, and cell stress.

We investigated human embryonic stem cell (hESC)-derived expandable lung-like epithelia progenitor (ELEP) cells that express early lung transcription factor NK2 Homeobox 1 (NKX2.1). ELEPs also possess abundant lamellar bodies and are positive for surfactant proteins. We cultivated ELEP cells in air-liquid interface (ALI) conditions in different types of media to induce differentiation to more mature phenotypes. Specifically tuned conditions, such as airlift interval of culture medium, significantly increased cell polarity proteins and nascent extracellular matrix production in ALI culture. We confirmed morphological changes induced by cultivation at ALI, specific medium-directed differentiation, and substrate stiffness by transmission and scanning electron microscopy.

Interestingly, differentiated ELEPs expressed a transitional phenotype between AT2 and AT1 cells, which could reflect prolonged integrated stress response induced by

culture conditions. We found that endoplasmic reticulum (ER) stress pathways were activated during differentiation, mediated by XBP1s, ATF4 and ATF6 transcription factors.

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EFFECT OF WI-FI ON FIBROUS COMPONENTS IN THE CHORIOALLANTOIC MEMBRANE OF CHICKEN EMBRYO ON EMBRYONIC DAYS 9 AND 14

Holovská K.¹, Andrašková S.¹, Molnár J.², Almášiová V.¹

¹ Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

² Department of Theoretical and Industrial Electrical Engineering, Faculty of Electrical Engineering and Informatics, Technical University in Kosice, the Slovak Republic

katarina.holovska@uvlf.sk

The aim of our study was to observe the effect of Wi-Fi radiation on the fibres that form the wall of the main blood vessels in the chorioallantoic membrane (CAM).

Fertilized chicken eggs were divided into the control group (CO, n=20) and two groups continuously exposed to Wi-Fi radiation with a frequency of 2.4 GHz with an average power density of 300 $\mu\text{W}/\text{m}^2$ during 9 (Wi-Fi 9, n=10) and 14 (Wi-Fi 14, n=10) days. The samples of CAM from all groups were routinely processed for light microscopy. Specific stainings were used to identify collagen (Picrosirius red), elastic (Weigert long method), and reticular (Gordon-Sweet) fibres in the blood vessels of CAM.

On embryonic day 9, all types of fibres were developed in the major blood vessel in the control group, however, the reticular fibers were less stained in comparison to the elastic and collagen fibres. On embryonic day 14, the amount of all three types of fibres significantly increased compared to ED 9. The Wi-Fi radiation had no significant effect on the overall structure of the CAM, but the change in quantity of fibres was evident in both Wi-Fi groups. In the Wi-Fi 9, collagen fibres stained more intensively, while elastic fibres stained less intensively compared to the control group. There was no change in the reticular fibres quantity. In the Wi-Fi 14, the amount of collagen fibres did not change in comparison to the controls, but there was a decrease in the amount of elastic as well as reticular fibres.

Our results confirmed the effect of non-ionizing electromagnetic radiation, the source of which is Wi-Fi, on the amount of collagen, elastic as well as reticular fibres in the wall of the blood vessels in dependence of its different duration and different stage of development of the embryo.

The study was supported by the VEGA 1/0036/22.

USE OF THE CAM METHOD IN CHICKEN EMBRYOS FOR THE STUDY OF HUMAN TUMORS

Houdek M.¹, Szotkowská T.¹, Buchtová M.^{1,2}, Kolísková P.^{1,2}

- ¹ Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, the Czech Republic
- ² Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, the Czech Republic

mojmir.houdek@seznam.cz

Chorioallantoic membrane (CAM) is formed by fusion of allantois and chorion layers during the development of bird embryo. This structure creates a rich vascular network that provides interface for gas exchange, calcium uptake and waste excretion. CAM allows to study tissue transplants, angiogenesis, growth and invasiveness of tumors, distribution of drugs or toxicological studies. Here, we use CAM assay to evaluate the effect of potential treatment on tumorigenesis of pancreatic cancer. Malignant tumors of the pancreas are among the most aggressive tumors and they are the fourth most common cause of death from cancer worldwide. Over 2,300 new cases are diagnosed annually in the Czech Republic, unfortunately the prognosis is very poor, with a 95% mortality rate. This is mainly due to late diagnosis, because this disease is difficult to detect at an early stages. Surgery is possible only in a small number of patients and oncological treatment has not yet resulted in a significantly longer survival rate. The main aim is therefore to develop a targeted effective anti-tumor treatment.

Casein kinase is important in biological processes necessary for proper functioning of the organism (e.g. cell proliferation, differentiation, DNA damage and apoptosis) and it represents a promising target in cancer therapy. Gemcitabine is an antitumor antimetabolite that exhibits significant activity against a broad spectrum of solid tumors.

The aim of our study is to evaluate the *in vivo* efficacy of the casein kinase inhibitor (CI-001) alone or in combination with gemcitabine on human pancreatic tumor initiated from the MIA PaCa-2 cell line using the CAM method. The samples were histologically processed and immunofluorescence was performed. We tested proliferative activity by detection of Ki67, apoptosis by analyses of Caspase 3 and TUNEL method, and tumor growth and ability to metastasize by E-cadherin and vimentin.

We observed several differences between treated tumors and controls which confirmed anti-tumor effect of this drugs.

CK1 inhibitors were provided by company CasInvent Pharma.

EFFECT OF WI-FI ON DEVELOPING HYPOPHYSIS IN THE CHICKEN EMBRYO

Hudáková P., Andrašková S., Holovská K., Almášiová V.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

pahudakova@gmail.com

The rapid proliferation and differentiation of embryonic tissues makes them extremely sensitive to various risk factors of the environment. The massive expansion of wireless technologies in recent times has caused concerns about possible health impairment, especially related to the prenatal development. The aim of this study was to investigate the effect of non-ionizing electromagnetic radiation on pituitary gland in Wi-Fi exposed chicken embryos.

On day 9 of incubation, embryos from the control (n=6) and exposed (n=6) groups were removed from the eggs and processed for light microscopy purposes.

9-days continuous exposure of Wi-Fi radiation at a frequency of 2.4 GHz and power density of 300 $\mu\text{W}/\text{m}^2$ did not cause any obvious structural abnormalities in the developing hypophysis. In both, the control and Wi-Fi groups, we registered normal structural organization of the infundibulum and median eminence with its typical ependymal, fibrous and palisade layers. Developing adenohypophysis was composed of epithelial cells arranged in cords with an intervening mesenchyme and capillary network. While in the control group the pituitary vessels were normal, in the exposed group we noted their obvious congestion. Similar vascular congestion was found in the organs that are very sensitive to the thermal effect of non-ionizing radiation such as the brain, eye or testicles. This is due to their high water content, prominent capsule and superficial position in the body.

The structural changes we found indicate a combination of non-thermal and thermal effects of Wi-Fi radiation on developing pituitary tissue. We hypothesize that Wi-Fi radiation can interact with various developmental processes involved in organogenesis. Therefore, it is necessary to continue in study of these specific mechanisms and to apply prevention in the given area.

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MALIGNANT MELANOMA IN 15 YEARS OLD HUNGARIAN WARMBLOOD

Hudáková P., Korim F., Almášiová V.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

pahudakova@gmail.com

Melanomas are very common skin tumours that usually occur in older grey horses (over 7-9 years of age). Some can be benign for a long time before they transform into a malignant form but more than 80% of melanoma lesions will become malignant at some point. In some cases they can spread into internal organs and had fatal consequences as happened in our case. Horse was presented with several lesions on ventral tail and perineum in 2019 and undergo partial amputation in 2021 to remove only the largest lesion. After two years he was presented with multiple organ failure.

During autopsy we have noticed multiple lesions on the liver, spleen, lungs, pancreas, kidneys and hearth accompanied with pleural and peritoneal effusion. Histopathological examination of samples were made.

Macroscopical findings revealed multiple ovoid lesions in size from 1x1cm to 9x10cm. Some of them were well pigmented and some has lost its pigment. Microscopic examination presented multinodular, badly demarcated epithelioid melanocytes arranged in nests and sheets with obvious pleomorphism. They showed mild mitotic activity and high stage of necrosis.

The index of malignancy is not determined by the size of the melanoma. Even small tumours can be highly malignant and the larger ones can be benign for a long time. That is the legitimate reason to remove all of them before they get bigger, difficult and more dangerous.

There was no specific funding for this work.

THE COMBINATION OF CHITOSAN-BASED BIOMATERIAL AND CELLULAR THERAPY FOR SUCCESSFUL TREATMENT OF DIABETIC FOOT – PILOT STUDY

Humeník F.^{1,*}, Vdoviaková K.¹, Krešáková L.¹, Danko J.¹, Giretová M.², Medvecký L.², Lengyel P.³, Babík J.³

- ¹ Department of Morphological Sciences, University of Veterinary Medicine and Pharmacy in Košice, Košice, the Slovak Republic
- ² Division of Functional and Hybrid Systems, Institute of Materials Research of SAS, Košice, the Slovak Republic
- ³ Clinic of Burns and Reconstructive Medicine, AGEL Hospital, Košice, the Slovak Republic

filip.humenik@gmail.com

Diabetic foot ulceration are one of the most common complications in patients treated for diabetes mellitus. The presented pilot study describes the successful treatment of diabetic ulceration of the heel with ongoing osteomyelitis in a 39-year-old patient after using a combination of modified chitosan-based biomaterial in combination with autologous mesenchymal stem cells isolated from bone marrow and dermal fibroblasts. The isolated population of bone marrow mesenchymal stem cells fulfilled all the attributes given by the International Society for Stem Cell Research, such as fibroblast-like morphology, high expression of positive surface markers (CD29 - $99.1 \pm 0.4\%$; CD44 - $99.8 \pm 0.2\%$ and CD90 - $98.0 \pm 0.6\%$) as well as the ability of multilineage differentiation. Likewise, the population of dermal fibroblasts showed high positivity for the widely accepted markers collagen I, collagen III and vimentin confirmed by immunocytochemical staining. Moreover, we were able to described newly formed blood vessels shown by angio CT and almost complete closure of the skin defect after 8 month of the treatment.

Autologous mesenchymal stem cells for transplantation was characterized according to essential criteria of ISSCR (morphology, multilineage potential and presence of surface markers). Same autologous dermal fibroblast was isolated and characterized by using of immunocytochemistry staining for confirmation of presence typical fibroblast markers (COL I, COLIII and Vimentin). The patient was admitted to the Department of Burns and Reconstructive Surgery of the Agel Hospital Košice – Šaca with an extensive foot ulcer of the right pelvic limb due to the indication for amputation of part of the limb. Clinical examination showed necrotic changes of the foot, lytic changes of Lisfrank, dislocation in II.-V., subluxation of I. Stp. Fr. II. and III. MTT. After tangential necroctomy in the area of the planta and calcaneus of the right foot we applied of chitosan-based biomaterial with adhered autologous BM-MSK and skin fibroblasts (8×10^4 BM-MSK/cm² and 15×10^3 fibroblasts/cm²) as well as the local intramuscular application of autologous BM-MSK to the foreleg area (2×10^6 cells/10ml of 0.9%NaCl). Cells of both lines for application came from the second

passage. The secondary layer of the outer covering was a sterile gauze bandage soaked in suspensio visnievski cum balsamo peruviano and the tertiary layer of the outer covering was synthetic cotton wool. Re-bandages of second and tertiary were performed at an interval of every 72 h, taking into account the condition and progress of wound secretion. Due to the extent of the defect, on the seventh day from the beginning of the therapy, we proceeded to take the autograft (dermal graft) from the thigh area. The procedure was performed under general anesthesia, the thickness of the graft was 0.254 mm and the size was 80 cm² and it was fixed by suture. The primary layer of the outer covering was a sterile gauze bandage soaked in suspensio visnievski cum balsamo peruviano and the secondary layer of the outer covering was synthetic cotton wool. Re-bandages of second and tertiary were performed at an interval of every 72 h, taking into account the condition and progress of wound secretion

- In the present study, we successfully isolated mesenchymal stem cells from the bone marrow. The yield of isolated cells was 17.9×10^6 cells/mL. hBM-MSC show high expression of CD29 ($99.1 \pm 0.4\%$), CD44 ($99.8 \pm 0.2\%$), CD90 ($98.0 \pm 0.6\%$) and low expression of CD34 ($0.8 \pm 0.2\%$) and CD45 ($0.3 \pm 0.1\%$).
- We were also able to isolate and cultivate a cell culture of dermal fibroblasts. The yield of isolated cells was 1.5×10^6 cells/mL. Cells from cultivated population showed a spindle shape and 85 – 95 μm in size and according to results of immunocytochemistry staining, they showed positivity for Vimentin, Collagen I and Collagen III.
- We observed pain relief after intramuscular application of autologous hBM-MSC, enhancement of healing process activation, formation of blood vessels and almost complete healing of the defect after skin graft transplantation after 8 months from the start of therapy.
- The success of the treatment in this case can be explained by the synergistic positive healing effect of both the used material and the cell components, since fibroblasts and MSC show significant immunomodulating and paracrine potential, which plays an important role in the wound healing process. In the same way, chitosan-based biomaterials are also used in the healing process of refractory wounds, which directly affects the hemostatic, proliferative and immunomodulating phase.

The presented study describes the positive impact of autologous bone marrow-derived mesenchymal stem and dermal fibroblast in combination of chitosan-based biomaterial in the diabetic foot treatment. We observed pain relief after intramuscular application of autologous hBM-MSC, enhancement of healing process activation and almost complete healing of the defect after skin graft transplantation after 8 months from the start of therapy.

This research was funded by Slovak Research and Development Agency (APVV-23-0360) and Scientific Grant Agency of the Ministry of Education Slovak Republic (VEGA 1/0237/23).

VISUALIZATION OF rAAV VECTOR ACTIVITY ON ALTERNATIVE CHICK CAM MODEL

Hurníková J., Demčíšáková Z., Šimaiová V., Teleky J., Petrovová E.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, the Slovak Republic

julia.hurnikova@student.uvlf.sk

The chorioallantoic membrane (CAM) is an extraembryonic tissue layer generated by the fusion of the chorion with the vascularized allantoic membrane. The CAM of the bird embryo is a suitable alternative model for the study of biocompatibility and angiogenesis. Due to these aspects, the CAM model is recognized in biomaterial testing, tissue engineering, and many other fields. Viral vectors are the most effective means of gene transfer to modify specific cell types or tissues, with recombinant adeno-associated viral vectors (rAAV) being the leading platform for in vivo gene therapy.

In the present research, we applied and subsequently visualised activity of rAAV vectors carrying three different reporter genes (lacZ, RFP, GFP) on chick (*Gallus gallus*) CAM using the ex ovo approach. The application on the CAM was made on ED7, where 20 µl of vector was applied within a silicone ring placed on a suitable CAM zone. Visualization was made after 24 hours (ED8), using fluorescent microscopy for the RFP (TRITC filter) and GFP (FITC filter) vectors and β-galactosidase staining kit for the lacZ vector.

Visualization of the lacZ rAAV vector was possible after some modifications, as the kit was designed for cell cultures and fixed samples. The staining solution caused hemorrhages and destruction of blood vessels at the place of application. We overcame this hurdle by diluting the solution with distilled water in a ratio of 1,8 (H₂O):1 (kit solution). Fluorescence visualization of the rAAV RFP was not possible due to interference with the blood cells' fluorescence signal present under the same filter. In comparison, GFP rAAV visualization was successful and clear, without any signal interference.

The chick CAM is a suitable alternative model for rAAV vector testing. The best solution for studying rAAV vector activity would be a vector carrying the reporter gene GFP, which offers simple and reliable visualization.

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ANATOMICAL DISSECTION OF THE HUMAN ORBIT: DETAILED STRUCTURAL ANALYSIS AND EDUCATIONAL IMPLICATIONS

Hvizdošová N., Vecanová J., Hodorová I.

Department of Anatomy, Pavol Jozef Šafárik University in Košice, Košice, the Slovak Republic

natalia.hvizdosova@upjs.sk

The objective of this study was to perform a detailed anatomical dissection of the human orbit and to provide a comprehensive analysis of its contents, aiming to improve anatomical education.

The study involved the detailed dissection of human cadaveric orbits obtained from anatomical donation programs. Dissections were conducted using standard anatomical techniques, with careful documentation of each step. High-resolution photographs were taken throughout the dissection process. Key anatomical structures, including the eyeball, extraocular muscles, optic nerve, ophthalmic vessels, and associated connective tissues, were identified and labeled.

The dissection showed a detailed and accurate view of the orbital contents. Each structure was clearly identified and described, spatial relationships documented. The extraocular muscles, including the superior, inferior, medial, and lateral rectus muscles, as well as the superior and inferior oblique muscles, were carefully defined. The optic nerve and its sheath, the ophthalmic artery and its branches, and ophthalmic veins were examined in detail.

This study presents a comprehensive and detailed anatomical dissection of the human orbit, providing valuable knowledge for education. The findings improve the understanding of the anatomy of the orbit and are helpful for individual education of students.

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REGENERATION OF BONE DEFECTS IN SHEEP ANIMAL MODEL WITH CALCIUM PHOSPHATE ENRICHED WITH CALCIUM SULFATE HEMIHYDRATE

Ivašková S., Krešáková L., Vdovíaková K., Korim F., Humenník F., Medvecký L.

Katedra morfológických disciplín UVLF v Košiciach, Ústav materiálového výskumu SAV, the Slovak Republic

sonja.ivaskova@student.uvlf.sk

Orthopedic bone defects, stemming from trauma, infections, neoplasms, congenital conditions, or aging, require grafts to ensure a tight connection between the implant and host bone. The materials used must provide an ideal environment for bone healing.

We used a biomaterial based on calcium phosphate enriched with calcium sulfate hemihydrate. In vivo testing involved 6 sheep weighing 50-80 kg aged 2-2.5 years. Under general anesthesia, a 10 mm wide and 10 mm deep defect was drilled in the proximal epiphysis of the left tibia using a 9 mm diameter drill bit. The biomaterial, mixed aseptically with a curing liquid, was applied to the defect. After six months, we evaluated the results macroscopically, histologically using hematoxylin-eosin staining, and via X-ray and CT examination.

Macroscopically, we observed a smooth bone surface at the biocomposite cement application site, with compact bone tissue seamlessly integrating with the surrounding tissue. We did not observe any inflammatory reaction of the surrounding tissue or residual biomaterial. CT scans confirmed the biomaterial's integration and new bone tissue growth over the surface. Histologically, we noted fibrous and lamellar bone tissue at the biomaterial edges without any inflammatory cells.

Our biomaterial demonstrated good bone biocompatibility, with new fibrous bone forming around it. There were no inflammatory reactions or pathological changes in the surrounding tissue. Biodegradation and bioresorption of the material were partial, and the paste remained firmly fused in the bone tissue, maintaining its integrity, indicating good bioactivity. In conclusion, this biomaterial is highly compatible with bone tissue, promoting effective integration and healing.

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PRESENTATION OF 3D VIRTUAL SPECIMENS FOR TEACHING OF ANATOMY

Janoušek T., Kuchař M., Hylmarová M., Machek T.

Department of Anatomy, Charles University, Faculty of Medicine in Hradec Králové, the Czech Republic

janousetom@lfhk.cuni.cz

The integration of 3D virtual models in anatomy education marks a significant advancement in medical and biological learning. Traditional methods of teaching anatomy, which rely heavily on physical dissection and static images, are increasingly being augmented and, in some cases, replaced by interactive 3D models. These models offer dynamic and detailed views of anatomical structures, providing students with enhanced understanding and engagement. Interactive educational applications such as Complete Anatomy, Labster, AnatomyLearning, or specialized virtual table platforms like Anatomage enable students to explore the human body in detail, perform virtual dissections, and visualize dynamic processes.

To create and share custom 3D models, we utilized photogrammetry to digitize real anatomical specimens. Photogrammetry involves capturing multiple high-resolution photographs from different angles and reconstructing these images into detailed 3D models using Agisoft Metashape. These models were then refined and optimized in Blender or MeshLab to clean up the models, making them suitable for interactive applications. The refined 3D models were integrated into interactive platforms using Three.js and the Unity engine.

The trial application allows for the integration of anatomical chapters with virtual models, text, supplementary illustrations, videos, and other elements. It can be customized to meet specific study plan requirements. To reinforce learning, the application may include various interactivity as quiz questions on anatomical structures, enabling students to repeatedly practice and review the material on specific models. In the future, the application could serve as a comprehensive interactive textbook.

The use of interactive educational applications and online platforms has shown to significantly improve the comprehension of anatomical structures among students. Custom applications developed with Three.js or Unity may provide tailored educational experiences that align with specific instructional goals, further increasing student engagement and motivation.

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INVESTIGATING MICROGLIAL ROLES IN CEREBELLAR DEGENERATION OF LURCHER MICE

Yakushko O.¹, Cendelin J.², Jelínková D.², [Kolinko Y.](#)¹

¹ Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, the Czech Republic

² Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University, the Czech Republic

yaroslav.kolinko@lfp.cuni.cz

Lurcher mice are known to undergo extensive degeneration of almost all cerebellar Purkinje cells and a majority of cerebellar granule neurons. Although this model has been widely studied, we found a lack of research specifically examining the state of microglia during such extensive degeneration. Therefore, our project's aim was to perform a stereological analysis of microglial distribution across different cerebellar layers in both Lurcher and wild-type mice. Additionally, we were motivated by the potential role of microglia in the progression of degeneration and its implications for treatment.

The frozen cerebellum of 5 pairs of 3-month-old mice was stained into 40- μ m-thick serial sections and stained using anti-allograft inflammatory factor 1 (Iba1) antibodies (Jackson Immuno Research, UK). The stereology approaches were used to collect data for the total volumes of cortical layers, white matter, and cerebellar nuclei, along with the quantification of the total number of microglial cells and the lengths of their process in these layers.

Our preliminary data determined that Lurcher mice showed fewer microglia cells and shorter processes but higher cell density across various cerebellar compartments compared with wild-type mice. In wild-type mice, microglial density was lowest in the molecular layer, intermediate in the granular layer and white matter, and highest the cerebellar nuclei. However, microglial distribution in Lurcher mice's cerebellum was disrupted.

Our findings suggest an active microglial response to Purkinje cell degeneration, as well as differences in microglia density between the two mouse strains.

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THE ROLE OF PRIMARY CILIA IN THE PROGRESSION OF ORAL SQUAMOUS CELL CARCINOMA (OSCC)

Kolářská P.^{1,2}, Moldovan Putnová B.^{2,3}, Hurník P.^{2,4}, Štembárek J.^{2,5}, Buchtová M.^{1,2}

- ¹ Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, the Czech Republic
- ² Laboratory of Molecular Morphogenesis, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Brno, the Czech Republic
- ³ Department of Pathological Morphology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno, the Czech Republic
- ⁴ Institute of Clinical and Molecular Pathology, Faculty of Medicine, University of Ostrava, Ostrava, the Czech Republic
- ⁵ Department of Maxillofacial Surgery, University Hospital Ostrava, Ostrava, the Czech Republic

petra.cela@gmail.com

Squamous cell carcinoma develops in the squamous epithelium of the lip or oral cavity lining. Oral squamous cell carcinoma (OSCC) constitutes 90% of all the subtypes of oral malignancies. More than 380,000 new cases are diagnosed, and over 170,000 deaths are reported each year worldwide. Despite improvements in the diagnosis, surgical techniques, and various strategies for treatment, the 5-year survival rates globally have remained unchanged. OSCC is characterized by dysregulated cell cycle processes closely associated with primary cilia (PC) signaling. PC sense and transmit signals to the inside of the cells, and their presence on the cell surface is typical only for certain stages of the cell cycle. Therefore dysfunction in primary cilia can lead to cell cycle dysregulation, contributing to various diseases, including cancer. PC frequency and size are reduced in many tumor types, but information about PCs in OSCC is limited.

Here, we evaluated PC frequency and morphology in primary human OSCC samples (collected at University Hospital Ostrava). PC in OSCC samples were detected by immunofluorescence (IF) labeling. Next, we selected two genes with possible roles in OSCC, Polo-like kinase 1 (PLK1) and Never in mitosis A (NimA)-related kinase (NEK2), which possess function in cell division and in ciliogenesis.

IF analysis revealed a small proportion of ciliated cells in OSCC with very short PC compared to normal gingival tissue. Detection of PLK1 and NEK2 in patient samples and in oral tumor cell line SCC-25 by IF showed changes in expression in comparison to control samples.

The dysregulation of PC in OSCC might represent a novel approach for understanding OSCC pathogenesis. NEK2 and PLK1 genes, which are assumed to play a key role in the regulation of primary cilia, and in the development of OSCC, are potential therapeutic targets and have to be tested in detail in the future.

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RADIOLOGIC EVALUATION OF THE CALCIUM PHOSPHATE BASED MATERIAL DEGRADATION IN PIG ANIMAL MODEL

Korim F.¹, Vdoviaková K.¹, Ivašková S.¹, Krešáková L.¹, Vrzgula M.², Giretová M.³, Medvecký Ľ.³

¹ University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic

² Faculty of Medicine, Pavol Jozef Šafárik University in Košice, the Slovak Republic

³ Slovak Academy of Science, Institute of Materials Research, the Slovak Republic

filip.korim@student.uvlf.sk

Material biodegradation is one key factor in the regenerative medicine of bone tissue defects. Currently, there are many materials that differ in their biodegradation properties, osteoinductive and osteoconductive properties.

In our pilot study, we monitored the dynamics of the degradation of tetracalcium phosphate/monetite material with the formation of new bone tissue in artificial defects of the tibia tuberosity in the 2nd, 4th and 10th week after the implantation of the material in three pigs. After the observation period, pigs were humanely euthanized under deep anaesthesia at 2nd, 4th, and 10th week after implantation of the material. Immediately after euthanasia, the limbs were disarticulated and subjected to computed tomography (CT – Philips Brilliance).

Based on the CT examination, we can state the gradual biodegradation of the calcium phosphate material. Material was present at all weeks of healing and had a density of approximately 1500 HU (Hounsfield Unit). On the 4th week, there are present newly formed bone with density of 500 – 600 HU on the margins of the material. After 10 weeks after implantation, we observe a half-degradation of the material with full growth of new bone into the original bone.

It is necessary to point out that each material has its characteristic degradation time, which depends on the physico-chemical reactions and cellular mechanisms of the organism. In order to further understand the process of biodegradation and the formation of new bone tissue at the implantation site, it is necessary to carry out many more similar experimental studies with a larger number of animals and materials.

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INTERMEDIATE EARLY GENES' PRODUCTS AND THEIR AGE- DEPENDENT CHANGES IN PREFRONTAL CORTEX IN ACUTE AND CHRONIC STRESS RAT MODELS

Kovács L. Á.^{1,2}, Golgol A.¹

¹ University of Pécs, Medical School, Department of Anatomy, Hungary

² Centre for Neuroscience, Pécs, Hungary

ifjkovacs14@gmail.com

FOS proteins (FOS, FOSB/ Δ FOSB) are early responding gene products contribute to form the activator protein-1 (AP-1). The occurrence of FOS proteins can be induced by acute restraint stress, while the presence of FOSB/ Δ FOSB proteins can be induced/changed by chronic variable mild stress (CVMS). The prefrontal cortex and hippocampus play pivotal role in stress and development of mood disorders, moreover both cortical fields show robust age dependent morphological changes. Since there is limited information about the age-dependency of ARS or CVMS-induced IEGs' products in the prefrontal cortex, we aimed to semi-quantify the ARS induced FOS and FOSB and CVMS induced FOSB/ Δ FOSB content in three areas of prefrontal cortex (Anterior cingulate (AC), prelimbic (PL) and infralimbic (IL) in rat model.

In the ARS model we used eight main age groups (1 months old [M], 1.5M, 3M, 6M, 12M, 18M, 24M) versus controls. In the CVMS model we use the last six age groups versus controls. Rats were clustered subgroups per age: controls, ARS and CVMS. To test the stress efficacy, we measured the total body, thymus, and adrenal glands' weight. Wistar rats We performed a diaminobenzidine FOS or FOSB/ Δ FOSB immunolabelling on the coronal sections of the hippocampus.

The PL region showed ARS-induced FOS rise (compare to their respective controls) in the pubertal young adult, and adulthood periods. The CVMS induced FOSB/ Δ FOSB content did not show significant changes. The amount of IEGs decreased by ageing but the stress induce occurrence did not show age-dependent changes in the examined cortical areas.

Corresponding to our previous results, the occurrence/presence of IEGs' products is also function of the age, but prefrontal cortex function (response) do not altered by ageing that show a strong contrast to hippocampus and subcortical stress orchestrating areas such as diencephalon. Further investigation needed to understand the age-characterized changes in the stress machinery.

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EFFECTS OF A HIGH-METHIONINE DIET ON THE CEREBELLUM OF FEMALE RATS

Kovalská M.¹, Fábryová M.¹, Hnilicová P.², Kalenská D.³, Lehotský J.⁴

- ¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ² Biomedical Centre in Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ³ Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ⁴ Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic

maria.kovalska@uniba.sk

Methionine (Met) is an essential amino acid, and its metabolism produces homocysteine (Hcy) as an intermediate. Elevated levels of Hcy, known as hyperhomocysteinemia (hHcy), are associated with neurotoxicity. The cerebellum is vital for normal brain function and is implicated in various neurological disorders that affect cognition, mood, and behaviour. Given the scarcity of research on cerebellar neurodegeneration and building on previous studies, we aimed to investigate the effects of induced hHcy via a high-methionine diet on the cerebellum of laboratory animals.

To induce mild hHcy, adult female Wistar rats (n = 6) were fed a high-methionine diet at a dose of 2 g/kg of body weight per day for four weeks. Other 6 female rats served as a control group. Following the induction of mild hHcy, the animals on the 28th day of diet underwent MRI analysis, and on the 29th day were sacrificed, and their cerebellums were processed for paraffin embedding and subsequent histological and immunohistochemical analyses. To evaluate potential neurodegenerative patterns, we used Cresyl violet (CV) staining, the Bielschowsky (BS) method, and immunohistochemistry with Neurofilament light chain (NFL) and Glial fibrillary acidic protein (GFAP) antibodies.

MRI and MRS analyses detected cerebellar changes in the volume and metabolic ratio in hHcy animals. Histo-morphological analyses revealed decreased vitality of Purkinje cells (PCs), with swollen or shrunken cell bodies of PCs. The BS method indicated no plaques in female hHcy rats. Reduced NFL immunolabeling and the presence of reactive astrocytes were noted in hHcy animals.

Our findings demonstrate that mild hHcy can create a toxic environment detrimental to PCs. These results contribute to a better understanding of how modifiable risk factors like hHcy can affect clinically relevant neurological disorders.

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THE EXPANDING REACH OF BORRELIA MIYAMOTOI IN SLOVAKIA AND CZECH REPUBLIC

Kozlov D.^{1,2}, Majláthová V.², Majláth I.², Myslivcová Fučíková A.³, Pipová N.², Vargová B.⁴, Bjelková K.³

¹ Faculty of Medicine in Hradec Králové Charles University, the Czech Republic

² Faculty of Science, Pavol Jozef Šafárik University in Košice, the Slovak Republic

³ University of Hradec Králové, the Czech Republic

⁴ University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic

bubanovd@lfhk.cuni.cz

Borrelia miyamotoi, a bacterium belonging to the relapsing fever group of *Borrelia*, has garnered attention as an emerging pathogen with a global distribution. Predominantly found in the northern hemisphere, it often co-occurs with the causative agent of Lyme borreliosis. The recent recognition of *B. miyamotoi* as a human pathogen has spurred intensified research into its ecology and pathogenic potential. However, knowledge regarding its circulation in nature remains limited.

To address this gap in knowledge, a comprehensive study was conducted, encompassing 2 370 *Ixodes ricinus* ticks (encompassing nymphs, females, and males) collected from various localities in eastern Slovakia and the northern Czech Republic. Additionally, 160 deer keds of the genus *Lipoptena*, 40 rodent samples from the genus *Apodemus*, 63 samples from *Lacerta viridis*, and 32 samples from *Lacerta agilis* were examined. All samples were screened for the presence of *B. miyamotoi*, targeting the *glpQ* gene, a hallmark of the relapsing fever group of *Borrelia*.

B. miyamotoi was detected in *I. ricinus* ticks, with a total of 29 positive identifications across the nymph, female, and male ticks. The overall infection prevalence in ticks was 1.1% in Slovakia and 1.6% in the Czech Republic. While no human cases have been documented in either country to date, the widespread presence of *B. miyamotoi* in the examined locations highlights the potential risk of human infection.

This study underscores the importance of continued research into the occurrence of *B. miyamotoi* to gain a comprehensive understanding of its prevalence and distribution within tick populations. Such knowledge is paramount for developing effective prevention strategies and ensuring timely diagnosis of *B. miyamotoi*-induced human illness.

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GLUTATHIONE PEROXIDASE 6 IN FEMALE GENITAL ORGANS OF RAT

Krehelová A.¹, Čurgali K.², Mihalik J.¹, Hodorová I.¹

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, the Slovak Republic

² Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, the Slovak Republic

andrea.krehelova@upjs.sk

Glutathione peroxidases are antioxidant enzymes, that belong to the defense system against reactive oxygen species (ROS). ROS are able to harm cells and cause many types of pathologies, including the infertility.

Glutathione peroxidase 6 (GPx6) was detected in female rat genital organs. Sprague Dawley female rats were mated with males of same strain. After successful mating (presence of vaginal plug), females were overdosed by anesthetic on the first, third and fifth day of pregnancy (D1, D3, D5), and ovaries, oviducts and uterine horns were removed. For GPx6 detection, standard immunohistochemical procedure was performed by using anti-GPx6 antibody (MyBioSource, USA).

GPx6 was detected in all examined organs in many cell types. In the ovary, GPx6 was found in the granulosa-lutein cells cytoplasm of corpus luteum, but follicular cells and oocyte cytoplasm were negative. In the oviduct, we detected GPx6 in the epithelial cells cytoplasm. In the ovary and oviduct, we did not observe any differences between days of pregnancy. On D1, the uterus shown positive cytoplasm of luminal epithelium. Epithelial cells of uterine glands shown positive cytoplasm and cytoplasmic granules. On D3, the luminal epithelium was negative. The uterine glands epithelium had rare occurrence of positive cytoplasmic granules. On D5, the luminal epithelium was negative and epithelial cells of uterine glands almost completely lost positive cytoplasmic granules. The cytoplasm of fibroblasts was mostly negative on D1 and D3, but mostly positive on D5.

GPx6 may defend cells of corpus luteum, and epithelial cells of uterine lumen and uterine glands against the oxidative stress. Because positive granules were detected in the epithelium of uterine glands, one can assume, that this enzyme may be secreted into the uterine lumen. This may indicate, that GPx6 may play a certain role during the preimplantation period of pregnancy in rat.

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MORPHOLOGICAL EVALUATION OF THE EFFICACY OF BIOCEMENT WITH THE ADDITION OF PHYTIC ACID/PHYTASE IN THE THERAPY OF OSTEOCHONDRAL DEFECTS

Krešáková L.¹, Vdoviaková K.¹, Humeník F.¹, Ivašková S.¹, Korim F.¹, Medvecký L.²

¹ Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, Kosice, the Slovak Republic

² Division of Functional and Hybrid Systems, Institute of Materials Research of SAS, Kosice, the Slovak Republic

lenka.kresakova@uvlf.sk

Hyaline articular cartilage has unique physiology, biological and biomechanical properties with very limited self-healing ability, which makes the process of cartilage regeneration extremely difficult. Therefore, research is currently focused on finding new and potentially better treatment options. Our study aimed to clarify the therapeutic effect and regenerative potential of the tetracalcium phosphate/monetite biocement hardened with phytic acid/phytase mixture on osteochondral defects in animal model. The results were compared with untreated defects.

A sheep animal model was used to perform in vivo experiments with osteochondral defects created on the trochlea ossis femoris, where macroscopical, histological analysis as well as X-ray, computed tomography and magnetic resonance imaging were applied.

After 6 months, biocement showed excellent biocompatibility with the surrounding cartilaginous and bone tissue with no observed signs of inflammatory reaction. In contrast to the fibrous or fibrocartilaginous tissue on the untreated side, treatment with biocement resulted in the formation of tissue with a dominant hyaline cartilage structure, although fine fibers were observed ($p < 0.001$, modified O'Driscoll score). Continuous formation of subchondral bone and hyaline cartilage layers was present even though residual biocement was observed in the trabecular bone. Integration of the biocement with adjacent bone and cartilage was confirmed. The treated osteochondral defects were filled with tissue that had a well-defined surface with fine surface irregularities. The thickness of newly formed tissue was comparable to the adjacent cartilage. The structure of the regenerated cartilage was mainly homogeneous with isointense signal and showed no cracks and fissures. Areas with hypointense or hyperintense signal were rarely observed. There weren't any pathological changes, or anomalous tissue formation.

We believe that novel biocement with addition phytic acid/phytase may provide a promising material for the functional osteochondral defect reconstruction and further research.

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DIABETES MELLITUS AND CELLULAR CHANGES IN THE CHOROID PLEXUS

Kročka E.^{1,2}, Kubíčková L.¹, Joukal M.¹

¹ Department of Anatomy, Faculty of Medicine, Masaryk University, the Czech Republic

² Department of Internal Medicine, Hospital Nové Město na Moravě, the Czech Republic

erik.krocka@med.muni.cz

Diabetes mellitus (DM) is one of the most common chronic metabolic disorders worldwide. Besides the best-known organ-specific complications of DM, there is another important condition called diabetic encephalopathy (DE), which is expressed by cognitive impairment, psychiatric and motor disorders. Pathogenesis of DE is complex and one of the possible mechanisms is lesion of the brain barriers. We aimed to investigate whether DM induces cellular changes in the choroid plexus representing the blood-cerebrospinal fluid barrier (BCSFB).

In response to our previous results, we continued to research other types of brain border-associated macrophages in the choroid plexus: M1 (CCR7+), M2 (CD206+), and microglia OX-42 (CD11b/c) respectively. This study used Wistar rats (8–10-week-old males) divided into two groups: the diabetic group (n=3) and the control group (n=2). DM was induced by intraperitoneal injection of streptozotocin (80 mg/kg), the control group received only vehicle. Animals were euthanized by CO₂ inhalation three weeks after induction of DM or application of a vehicle. The animals were perfused by Zamboni's fixative solution and the brain was harvested. The coronal brain sections were immunostained with anti-CCR7, anti-CD206 and anti-CD11b/c antibodies and the number of positive cells per mm² was examined and statistically evaluated.

There is significant difference in the population of the choroid plexus macrophages between diabetic and control group. The number of OX-42 and M1 cells was increased in DM group compared to control group. There is also increased number of M2 cells in DM group. Our results indicate inflammatory reaction of BCSFB in diabetic environment.

DM causes alteration in immune profile of the choroid plexus. This condition could lead to damage of the BCSFB with consequences to disrupt brain microenvironment and form DE.

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IN VITRO MODELS FOR STUDYING MITOCHONDRIAL RESPIRATION IN NON-ALCOHOLIC FATTY LIVER DISEASE

Kučera O., Maseko T. E., ElkalSaf M., Peterová E., Staňková P., Melek J., Čížková D., Bezrouk A., Červinková Z.

Charles University, Faculty of Medicine in Hradec Králové, the Czech Republic

kucerao@lfhk.cuni.cz

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder (affecting up to 70% of obese people), and its prevalence is still increasing. Its pathogenesis is complex, but insulin resistance plays a central role. Nonetheless, mechanistic studies that assess additional fundamental questions are necessary. The lack of relevant in vitro models of NAFLD limits a deeper understanding of the pathophysiological mechanisms developing this disease. The main aim of this work was to create suitable in vitro models of NAFLD for assessing changes in mitochondrial respiration using two human hepatoma cell lines (HepaRG and HepG2) exposed to a combination of free fatty acids (FFAs) oleate (OA) and palmitate (PA).

HepaRG and HepG2 cell lines were cultured according to the recommended protocols, and then the cells were exposed to 1mM OA/PA (2/1) and 2mM OA/PA (1/1). Subsequently, changes in energy metabolism were evaluated by measuring oxygen consumption (Agilent Seahorse XFe96 analyser) and the activity of respiratory complexes. Ultrastructural changes in the morphology of mitochondria (transmission electron microscopy) and changes in the expression of selected genes related to lipid metabolism (qRT-PCR) were also evaluated.

Oxygen consumption was more stimulated in FFAs-treated HepaRG cells compared to FFAs-treated HepG2 cells (basal ($p < 0.01$) and maximal respiration ($p < 0.01$), spare respiratory activity ($p < 0.01$), and proton leak ($p < 0.001$)). Glycolysis was more stimulated in both the basal ($p < 0.001$) and compensatory ($p < 0.001$) states in FFAs-treated HepaRG cells than in FFAs-treated HepG2 cells. In FFAs-treated HepaRG cells, succinate-activated respiration in permeabilized cells exposed to FFAs (OXPHOS, ETS, and leak states) was stimulated. In contrast, succinate-activated respiration was inhibited in FFAs-treated HepG2 cells. S-oxidation and complex I and II substrate-stimulated respiration were associated with increased complex I and II activities in HepaRG cells (but not in HepG2 cells) exposed to FFAs ($p < 0.05$). Incubation of cells of both lines with FFAs dose-dependently disrupted mitochondrial morphology. HepaRG cells were more sensitive to the lipotoxic effect of FFAs than HepG2 cells (cytotoxicity tests, activities of caspase-3 and caspase-9, reactive oxygen species production).

These findings suggest that HepaRG cells are more suitable for developing in vitro models assessing mitochondrial respiratory adaptations in the early stages of NAFLD.

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ARTERIOLAR DENSITY IN VENTRICLES OF PATIENTS WITH HEART FAILURE

Kučera T.¹, Kuzárová L.¹, Melenovský V.²

¹ Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague, Prague, the Czech Republic

² Institute for Clinical and Experimental Medicine-IKEM, Department of Cardiology, Prague, the Czech Republic

tkucer@lf1.cuni.cz

Pathophysiological processes during the progress of heart failure affect the morphology of the heart on the macroscopic and microscopic level. An important factor for proper functioning of myocardium is efficient blood circulation in the microvascular bed, which also includes arterioles. Right ventricular dysfunction (RVD) is a situation that can affect the progress of heart failure. We evaluated density of arterioles (AD) in the hearts of patients with terminal heart failure with respect to concomitant RVD.

For analysis, we used the myocardial tissue from explanted hearts of heart transplant recipients with heart failure. Therefore, we obtained the samples of left (n=17) and right ventricles (n=18). The samples were immediately fixed with 4% formaldehyde, embedded into paraffin and then cut for the purpose of immunohistochemical labeling. An antibody against smooth muscle actin was used to visualize vascular smooth muscle cells in order to better identify arterioles in the myocardium. Histomorphometric measurements were performed using the program ImageJ.

In the left ventricular myocardium of patients with heart failure, the AD was $1,59 \pm 0,46/\text{mm}^2$, while in the right ventricular myocardium the AD was $1,21 \pm 0,43/\text{mm}^2$. When we divided the samples of right ventricles according to presence of RVD, the AD values were $1,28 \pm 0,63/\text{mm}^2$ for the RVD group and $1,15 \pm 0,63/\text{mm}^2$ for the non-RVD group. The differences between the groups were not statistically significant.

Samples of left and right myocardia from patients with terminal heart failure had similar AD. Furthermore, in our set of patients we did not observe a significant difference of AD between RVD and non-RVD groups. This corresponds with our findings of capillary density. To provide more conclusive data, additional samples should be evaluated and comparison with available control myocardia from transplant donors should be also included.

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NATIVE ACETABULAR ANTEVERSION PREDICTED FROM THE ORIENTATION OF TRANSVERSE ACETABULAR LIGAMENT

Kuchař M.¹, Hylmarová M.¹, Morávek A.¹, Kučera T.², Henyš P.³

¹ Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, the Czech Republic

² Department of Orthopedics, University Hospital Hradec Králové, the Czech Republic

³ Institute of New Technologies and Applied Informatics, Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, the Czech Republic

kucharm@lfhk.cuni.cz

Precise positioning of the acetabular component during total hip replacement is the key to achieving optimal implant function and ensuring long-term patient comfort. However, different anatomical variations, degenerative changes, dysplasia, and other diseases make it difficult. In this study, we discuss a method based on the three-dimensional direction of the transverse acetabular ligament (TAL), predicting native acetabular anteversion with higher accuracy.

Angular positions of the acetabulum and direction of the transverse ligament were automatically calculated from routine computed tomography data of 270 patients using a registration algorithm. The relationship between acetabular angles and ligament direction and their relationship with sex, age, and pelvic tilt were sought. These relationships were then modelled using multilinear regression.

Including the direction of the transverse ligament in the sagittal and transverse planes as a regressor in the multilinear model explained the variation in acetabular anteversion ($R^2=0.76$ for men, $R^2=0.63$ for women; standard deviation in prediction: men, 3.92° and women, 4.00°). The results indicate that the ligament was suitable as a guidance structure almost insensitive to age.

This study aimed to increase the accuracy of the relationship between TAL and acetabular planes. We found no correlation between the ligament angle and OI. However, we confirmed the correlation between the TAL and operative and anatomical anteversion. Further, for the first time, we presented an estimation of native acetabular anteversion based on the three-dimensional orientation of TAL. The study outcomes were minimally affected by patient age and pelvic tilt.

There was no specific funding for this work.

ATRIAL MYOCARDIAL ARCHITECTURE: IS DEVELOPMENT OF THE PECTINATE MUSCLES HOMOLOGOUS TO DEVELOPMENT OF THE VENTRICULAR TRABECULAE?

Kvasilová A., Neradilová C., Sanková B., Olejníčková V., Neffeová K., Sedmera D.

Institute of Anatomy, First Faculty of Medicine, Charles University, the Czech Republic

alena.kvasilova@lf1.cuni.cz

Ventricular trabeculae and pectinate muscles are morphological hallmarks of chamber myocardium differentiation. However, it is unclear to which extent they are homologous concerning the mechanisms of their formation. Pectinate muscles start to form between stages 23-24 in the chick, and around ED11 in the mouse. The trabeculae appear before the pectinate muscles during cardiogenesis.

The internal structure of the cardiac chambers was studied by histology and whole mount confocal microscopy. The activation sequence was visualised by optical mapping. In vitro mechanical unloading and rescue attempts by silicone oil droplet injection were performed. Proliferation was evaluated using BrdU or EdU pulse, and proliferative history was assessed by labelling dilution of tritiated thymidine.

The ratio of pectinate muscles to free wall in the chick embryo increased steadily between stages 24 and 38 from 6 and 12 up to 62 and 69% of the left and right atrial myocardium, respectively. Complete mechanical unloading inhibited their formation, which was not rescued by injection of the silicone oil droplet. Left atrial ligation at stage 21 prevented their formation in the ligated part of the atrium, caused a slowing of the impulse propagation, and induced ectopic pacemaker activity. There was a concomitant decrease in the expression of the haemodynamically-dependent gene KLF2. Unlike ventricular trabeculae, which show a considerably slower proliferative activity than the compact myocardium, the proliferative structure of the atria was more homogeneous, with an even higher cell division rate in the pectinate muscles. Atrial wall growth involved thickening of the pectinate muscles with the free wall remaining relatively thin.

There is some structural and functional homology between the ventricular trabeculae and the atrial pectinate muscles, but there are also significant differences between the atria and the ventricles in terms of myocardial morphogenesis.

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ULTRASOUND SCAN OF COMMON CAROTID ARTERY AND INTERNAL JUGULAR VEIN

Lovasová K.¹, Vrzgula M.¹, Mihalik J.¹, Hudák V.², Firment J.², Boleková A.¹, Hodorová I.¹

¹ Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, the Slovak Republic

² 1st Department of Anaesthesiology and Intensive Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, the Slovak Republic

kvetuse.lovasova@upjs.sk

Ultrasound anatomy of vessels is important for diagnostic process of vascular pathology as vessels for vascular access. Diagnostic ultrasound informs about the normal and abnormal structure of the vessel, its patency and blood flow.

Patient lies horizontally with the head either in a neutral position or slightly turned to the opposite side. In cases of good intravascular volume status, the head may be slightly elevated; in hypovolemia, the patient may need to be placed in Trendelenburg position with elevated lower extremities. During sonographic visualization of the common carotid artery and internal jugular vein a linear probe with a high frequency setting is applied transversely on the neck approximately at the level of the cricoid cartilage or just below the thyroid cartilage, ensuring the centre of the probe is placed on the pulsating common carotid artery and the position of the internal jugular vein. Another probe position is in a longitudinal-cross section view.

In the ultrasound image, it is important to locate the pulsating common carotid artery. The easily compressible internal jugular vein lies above the artery or just laterally. The sternocleidomastoid muscle lies above the vessels.

Detailed knowledge about vascular anatomy; the blood vessels location and topography supplemented by a high quality of anatomical images is a prerequisite for a successfully performed ultrasound diagnostic examination and for central venous cannulation, as a part of major surgical procedures or in patients requiring intensive care treatment.

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THE EFFECT OF IONIZING RADIATION ON THE POPULATION OF GLIAL CELLS IN THE NEUROGENIC REGIONS OF THE BRAIN

Macková L.¹, Bálentová S.¹, Kalenská D.², Muriň P.³, Kecskés I.³

- ¹ Department of Histology and Embryology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ² Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, the Slovak Republic
- ³ Department of Radiotherapy and Oncology, Martin University Hospital, Martin, the Slovak Republic

mackova154@uniba.sk

Backgrounds: The most widely used radiation treatment for patients with multiple brain metastases is fractionated whole-brain irradiation (fWBI). The appropriate use of fWBI can provide rapid attenuation of many neurologic symptoms and improve quality of life. However, with increasing median survival rates, post-therapeutic changes in neurocognitive functions and quality of life have gained more importance. As the age of patients rises, the risk of late radiation-induced changes manifesting in histological and functional changes increases. In this study, we investigated the delayed effect of fWBI on the distribution of glial cells in selected brain regions.

Adult male Wistar rats (4 months old) received fWBI with a total dose of 32 Gy administered in 4 fractions (dose 8 Gy per fraction) once a week on the same day for 4 consecutive weeks. After being sacrificed five months following fWBI, histopathological changes in two brain neurogenic regions were monitored: the hippocampal dentate gyrus (DG) and the rostral migratory stream (RMS).

Although histological image analysis of brain slides is still in progress, the results suggest subtle changes in the distribution of astrocytes and microglia within the RMS and DG five months after fWBI.

We can tentatively conclude that fWBI led to subtle histological changes in astrocytes and microglia in neurogenic areas. For definitive conclusions, it is necessary to correlate the data with other results of histological analysis and behavioral testing.

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INSIGHTS INTO CHANGES IN ENDOSOMAL PROTEOM IN ALZHEIMER'S DISEASE USING SORL1-DEFICIENT CORTICAL NEURONS AS A MODEL

Mečava M., Fojtík P., Vajrychová M., Raška J., Plešingrová K., Sedmík J., Kadaková S., Pospíšilová V., Váňová T., Soukup O., Bohačiaková D.

¹ Department of Histology and Embryology, MED MUNI, Brno, the Czech Republic

² Biomedical Research Centre, Faculty Hospital Hradec Kralove, Hradec Kralove, the Czech Republic

markomecava.wrk@gmail.com

Alzheimer's disease (AD) causes the most common form of dementia in the elderly, characterized by the deposition of neurotoxic amyloid-beta (A β) plaques, hyperphosphorylation of Tau protein, and the formation of neurofibrillary tangles. Dysfunction of endosomes and the endo-lysosomal pathway are increasingly recognized as critical contributors to AD and are even considered the initiators of this pathology by some. On a cellular level, this dysfunction manifests as swelling of early endosomes. However, the mechanisms behind the enlargement of endosomes and the resulting changes in the composition of the endosomal proteome remain unclear. Sortilin-related receptor 1 (SORLA, encoded by the SORL1 gene) was shown to play a pivotal role in endosomal trafficking, particularly in regulating the processing route of the amyloid precursor protein (APP), and is increasingly recognized as one of the causal genetic risk factors for AD. Importantly, dysfunctional SORLA results in enlarged endosomes in neurons, creating a phenocopy of this early cellular anomaly observed in AD.

To investigate the protein composition within the early endosomes, we isolated early endosomes from healthy and SORL1-deficient cortical neurons derived from induced pluripotent stem cells using a commercially available Trident Endosome Isolation Kit (Genetex). Differential and semiquantitative proteomics were conducted on isolated endosomal fractions using Tandem Mass Spectrometry (MS).

Endosomal isolation protocol and sample preparation from iPSCs derived cortical neurons and their endosomal fractions for MS optimized. Overall, 829 proteins were identified that differed among compared groups (knock-in, knock-out and wild type) and showed statistically significant differences. A trend of accumulation of proteins inside KI and KO endosomes was observed. Many of these proteins are involved in synaptic, neuronal functions, cell transport and sorting mechanisms, signalling, and the pathological processes associated with AD.

Our findings indicate a profound disruption in the proteomic landscape of endosomes due to SORL1 dysfunction. This suggests that functional SORLA is not only critical for the sorting of APP protein but also affects a plethora of other biological processes required for neuronal health, thus strengthening the hypothesis that the compromised endo-lysosomal pathway is an underlying cause of Alzheimer's disease.

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LYSOSOMAL-MEDIATED DRUG RESISTANCE - FACT OR ILLUSION? (THE ROLE OF pH GRADIENTS)

Mlejnek Petr

Faculty of Medicine and Dentistry, Palacky University Olomouc, the Czech Republic

mlejnek_petr@volny.cz

pH gradients across cell membranes affect the intracellular distribution of hydrophobic weak-base anticancer agents. This significantly reduces their availability at target sites, reducing cytotoxic effect and leading to drug resistance. This hypothetical in vitro study evaluates the impact of two important pH gradients on the intracellular distribution of such drugs in tumor cells.

Calculations.

Simplified models clearly show that even a relatively small pH gradient between cytosol and extracellular environment can dramatically reduce the intracellular concentration of weak-base drugs. This is only slightly affected by the pKa or number of cells. However, if the latter is enormous and the volume ratio between extracellular environment and target site (cytosol) decreases significantly, the pH gradient has little effect on the drug concentration at target site. In contrast, even a large pH gradient between lysosomes and cytosol has only marginal impact on the intracellular drug distribution. The effects of the drug pKa or number of cells are also small here. Only extremely acidic lysosomal pH or enormous lysosome size have substantial impact on the target site concentration of weak-base drugs, although the significance of these effects is questionable.

The volume ratio between the drug “trapping” and target sites is a crucial factor in the ability of pH gradients to change target site drug concentrations. Accordingly, the pH gradient between cytosol and extracellular environment has a much greater impact on the intracellular distribution of hydrophobic weak-base drugs than that between lysosomes and cytosol under standard conditions in laboratory experiments.

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ALLEVIATION OF FOLLICULOGENESIS DISORDERS BY QUERCETIN IN NICOTINE-TREATED RATS

Mohammadghasemi F.¹, Sayad E.¹, Faghani M.¹, Keshavarz P.²

¹ Cellular & Molecular Research Center, Department of Anatomy, School of Medicine, Guilan University of Medical sciences, Rasht, Iran

² Department of Genetics, School of Medicine, Guilan University of Medical sciences, Rasht, Iran

parsahistolab@gmail.com

Smoking can have detrimental effects on folliculogenesis, ovulation, and the formation of ovary hormones. The purpose of this study was to examine the impact of quercetin, a natural flavonoid, on ovary histophysiology alterations in rats treated with nicotine.

Adult rats were subdivided into four groups. Control, nicotine, quercetin, and co-treated nicotine and quercetin group. Ovarian histological changes, estradiol and progesterone serum levels were evaluated using histopathology and ELISA respectively. Homogenized ovary tissue underwent colorimetric analysis to assess the activity of lipid peroxidation and antioxidant enzymes. Immunohistochemistry was utilized to assess the estrogen receptor alpha (ESR1) index in the ovary tissue.

Nicotine alone decreased the numbers of primary, pre-antral and antral follicles and serum level of estradiol ($p < 0.05$) in compare with the control group. Co-treatment with nicotine-quercetin resulted in an increase in antral follicles and corpus luteum and a decrease in atretic follicles when compared with nicotine treated group ($p < 0.05$). It also increased estradiol level ($p < 0.05$). Different groups had no statistical differences in progesterone levels. Nicotine-quercetin co-treatment resulted in a decrease ($p = 0.01$) in MDA and an increase ($p = 0.02$) in total antioxidant capacity (TAC) for the ovary when compared to the nicotine group. Nicotine alone was shown to significantly diminish ESR1 levels in ovarian stroma ($P = 0.02$) and corpus luteum ($P = 0.04$) compared to the control group. In comparison with the nicotine group ($p < 0.001$), co-treatment with nicotine-quercetin resulted in a higher ESR1 index in the ovarian stroma and corpus luteum.

Quercetin-nicotine co-treatment protects folliculargenesis and ovulation through an increase in serum estradiol, decrease in MDA, elevation of ovarian TAC, and upregulation of ESR1 in ovary as demonstrated in this study. It is advisable for women who smoke to take quercetin as a natural antioxidant.

Guilan University of Medical Sciences.

BONE MINERAL DENSITY AS A SEX DETERMINATION FEATURE

Morávek A.¹, Henyš P.², Kuchař M.¹, Harnádková K.³, Velemínská J.³, Prstková K.¹

- ¹ Department of Anatomy, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, the Czech Republic
- ² Institute of New Technologies and Applied Informatics, Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Liberec, the Czech Republic
- ³ Department of Anthropology and Human Genetics, Faculty of Science, Charles University in Prague, Prague, the Czech Republic

moravekal@lfhk.cuni.cz

In forensic sciences, estimating sex and age through bone radio-density, expressed in Hounsfield units, has considerable potential. This method can be used independently or to complement traditional anthropological analyses. This research enhances this methodology, using the hyoid bone as a case study.

We collected seventy-three CT scans of healthy adults (44 females, 29 males) from clinical sources. For data processing, we utilized statistical machine learning and data reduction techniques. By creating a hyoid bone template and applying bijective mapping, we analyzed the spatial bone density of each bone in the dataset. To examine spatial differences in bone density, we developed a tetrahedron-wise and global partial least squares regression sex classification model.

The study found that sex can be classified from various regions of the bone with a maximum accuracy of 79% and an average of 69%. Due to extreme spatial collinearity, considering multiple points does not enhance sex classification. However, by reducing data dimensionality using partial least squares analysis and discriminant analysis while considering whole bones, sex can be predicted from density with high accuracy, exceeding 98.5%.

This study introduces an innovative approach to analyzing bone radio-density, demonstrating its applicability for osteobiography. The promising results from the hyoid bone characterization indicate the method's potential for application to other bones in the human body, either to study their variability or for forensic purposes.

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MORPHOLOGY OF THE LEFT AND RIGHT ATRIAL AURICLE IN THE HUMAN

Lexová M., Bílková L., Naňka O., Sedmera D.

Institute of Anatomy, First Faculty of Medicine, Prague, the Czech Republic

Ondrej.Nanka@lf1.cuni.cz

Atria of the human heart generally arise from different embryonic anlagen, the original embryonic atria gradually transforming into the so-called auricles. The auricles, unlike the rest of the atrium, are trabeculated (pectinate muscles). The left auricle (LAu) is classified according to its external shape, while the right auricle (RAu) is classified according to the arrangement of the pectinate muscles.

The group consisted of 107 hearts from donor bodies for the study of anatomy. The mean age was 73 years, 67 females and 40 males. We studied the arrangement and size of the right and left auricles, the arrangement and number of pectinate muscles.

In the group of left auricles we identified the cactus type in 22%, the chicken wing type in 40%, the wind sock type in 15% and the cauliflower type in 24%. The average number of pectinate muscles in LAu was 10. In the group of right auricles we identified type I in 22%, type II in 22%, type III in 7.5%, type IV in 38%, type V in 3% and type VI in 8.5%. The average number of pectinate muscles in RAu was 15.

The exact morphology may be clinically important during atrial catheterization procedures and in association with embolic stroke.

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TETRALOGY OF FALLOT: PHYSIOLOGICAL AND MORPHOLOGICAL CHANGES IN CONDITIONAL JAGGED1 MUTANT MICE

Neffeová K., Zábrodská E., Olejníčková V., Sedmera D., Kolesová H.

Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, the Czech Republic

Kristyna.Neffeova@lf1.cuni.cz

The Notch signaling pathway is essential for embryonic development. Mutations in the human Jagged1 (Jag1) gene, which encodes a ligand for the Notch receptor, cause the Alagille syndrome. Symptoms of this inherited disease may include various forms of Tetralogy of Fallot.

Here, we generated Jag1flox/flox Islet1Cre/+ mice with conditional Jag1 deletion in the cardiac outflow tract to investigate the impact of Jag1 mutations on cardiac morphology.

Mice with conditional deletion exhibited severe cardiac malformations typical for Tetralogy of Fallot. Islet1 is also expressed in sinoatrial and atrioventricular nodes, therefore we used the optical mapping to visualize changes in patterning of the cardiac conduction system. The analysis of E14.5, E16.5 embryos and adult mice showed changes in the activation pattern. In controls, we showed matured activation from apex to base with two separate activation centres. Mutant embryonic hearts revealed activation only from the left ventricle, indicating a perturbed function of the right bundle branch. In mutant adult mice, activation occurred at additional activation centres, distinguishing them from controls where excitation is conducted from a single site at the apex. Vevo ultrasound imaging physiological analysis was performed only on adult heterozygotes, because of the postnatal mortality of the homozygotes. Most of the monitored hemodynamical parameters did not show significant differences. However, spackle-based strain analysis revealed vulnerable areas of contractile defect that generate mechanical dyssynchrony pronounced mostly at anterior wall.

In our study, we demonstrated morphological and electrophysiological alterations resulting from conditional deletion of Jag1. Embryonic mice exhibited malformations and irregular activation patterns. Severe malformations were less prevalent in adult mice, primarily due to the survival of heterozygotes and an increased mortality rate among mice displaying severe congenital defects. Nevertheless, surviving animals exhibited abnormal electrophysiological changes along with physiological alterations resulting in dyssynchronous myocardial contractions observed during strain analysis.

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ANATOMICAL VIDEO ATLAS „LOWER LIMB” AS A SUITABLE TOOL FOR SELF-STUDING

Pavliuk-Karachevtseva A., Hodorová I.

Department of anatomy, Medical Faculty of Pavol Jozef Šafarik University, Košice, the Slovak Republic

andriana.pavliuk-karachevtseva@upjs.sk

"Anatomia fundamentum medicinae est" remains relevant, emphasizing the importance of anatomy in medical education. The current two-semester system at the Faculty of Medicine necessitates extensive educational resources such as books, atlases, scripts, video atlases, and mobile applications. These materials support both practical lessons in dissecting rooms and further independent study. Educational recordings offer self-study opportunities, targeted learning, and accessibility for students with special needs.

The videos feature real human bones, ligaments, muscles, and cadavers from the Department of Anatomy at Pavol Jozef Šafarik University. Filming was conducted in collaboration with the Department of Medical Informatics. English dubbing was added using VideoPad Video Editor.

Twelve educational videos, each up to 10 minutes long, were created. These are organized into five units: 1. Bones of the lower limb (4 videos), 2. Joints of the lower limb (4 videos), 3. Muscles of the lower limb (2 videos), 4. Vascular supply of the lower limb (1 video), and 5. Innervation of the lower limb (1 video). The content aligns with the entire anatomy course and is continually updated to meet student needs. The videos are accessible via MS Teams and SharePoint, with additional Latin terminology versions on the Educational Portal. They are available on all mobile devices and benefit both general and dental medicine students.

A solid understanding of human anatomy is crucial for medical education and the advancement of medicine. The lower limb is a clinically significant area, and video atlases provide an effective tool for topic review, practical test preparation, and exam readiness. English-dubbed videos enhance comprehension and offer quality self-study opportunities outside the dissecting room.

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ROLE OF GPX5 IN HUMAN COLORECTAL ADENOCARCINOMA

Pavliuk-Karachevtseva A.¹, Benetinová Z.², Bohuš P.², Mihalik J.¹, Hodorová I.¹

¹ Department of anatomy, Medical Faculty of Pavol Jozef Šafarik University, Košice, the Slovak Republic

² Department of Pathology, Medical Faculty, Pavol Jozef Šafarik University, Košice, the Slovak Republic

andriana.pavliuk-karachevtseva@upjs.sk

Glutathione peroxidase 5 (GPx5) is well-known as Epididymal Secretory Glutathione Peroxidase protecting cells from oxidative damage by reducing hydrogen peroxide and organic hydroperoxides. Oxidative stress is a critical factor in the development and progression of colorectal cancer. Reactive oxygen species (ROS) can induce DNA damage, promote mutations, and contribute to the carcinogenic process. GPx5 helps mitigate oxidative stress and maintain cellular homeostasis, but its role and functions is still less studied.

In our study we used the three-step indirect immunohistochemical method of GPx5 detection in 25 specimens of the human colorectal adenocarcinoma and 6 specimens of healthy colon tissue. The samples were obtained from the Institute of Pathology, Louis Pasteur University Hospital Košice, Slovak Republic.

In healthy tissue all specimens (100%) showed high expression of GPx5 in basal cells and cylindrical epithelium of the colon mucosa. High diffuse expression was found in 18 specimens of adenocarcinoma (72%). In these cases, the enzyme was detected in cylindrical epithelium of pathologically changed crypt. In 7 adenocarcinoma specimens (28%) the negative GPx5 expression was detected.

GPx5 probably plays a significant role in colorectal cancer by managing oxidative stress within cells. Its expression levels and activity could influence tumor behavior, progression, and response to treatment. GPx5 represents an important focus for ongoing research in colorectal cancer as a potential prognostic marker and therapeutic target. Understanding its precise role and developing targeted interventions could improve outcomes for patients with this type of cancer.

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IMPORTANCE OF VARIATIONS IN ANTERIOR DIVISIONS OF THE BRACHIAL PLEXUS

Petanjek T.L.^{1,2,3}, Blažević A.^{1,4}, Prkačin M.V.^{1,4}, Grgurević L.^{4,5,6}, Jalšovec D.^{4,7}, Hladnik A.^{1,4}

- ¹ Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia
- ² Department of Physiology, University of Zagreb School of Medicine, Zagreb, Croatia
- ³ Department of Neuroscience, University of Zagreb School of Medicine, Zagreb, Croatia
- ⁴ Department of Anatomy and Clinical Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia
- ⁵ Centre for Translational and Clinical Research, Department of Proteomics, University of Zagreb School of Medicine, Zagreb, Croatia
- ⁶ BIMIS – Biomedical Research Centre Šalata, University of Zagreb School of Medicine, Zagreb, Croatia
- ⁷ Department of Thoracic Surgery Jordanovac, Clinical Hospital Centre Zagreb, University of Zagreb School of Medicine, Zagreb, Croatia

tin.petanjek@mef.hr

The anterior divisions of the brachial plexus are integral to the innervation of the upper limb, encompassing both motor and sensory functions. Variations in these divisions could have a profound effect on clinical practices, especially in upper limb surgery and diagnostics. As such a thorough understanding of these variations is essential.

For this study, a total of 58 upper extremities previously dissected from 29 formaldehyde-phenol preserved cadaveric specimens from the Department of Anatomy and Clinical Anatomy at School of Medicine, University of Zagreb, were analyzed in April 2022. Each cadaver was evaluated separately for the left and right sides, recognizing that the anatomy of the brachial plexus can differ between the two sides.

Variations from the pattern of the brachial plexus were observed in 67% of upper extremities. The most observed variation is an additional communicating branch from one of the cords towards the opposite cord, root, or branch (50%). Another group of commonly observed variation were communications between the musculocutaneous and median nerve (14%). Unique formations of the brachial plexus were also observed in 2 upper extremities. In one, the lateral cord was formed exclusively from the upper trunk and in the second, the anterior division of the middle trunk was completely included in the formation of the medial root of the median nerve.

Cases where fibers from the middle trunk do not contribute to the formation of the lateral cord, consequently to the musculocutaneous nerve, raise questions in which amount does the C7 spinal segment truly participate in the musculocutaneous nerve

formation as traditionally described in anatomical textbooks. This is supported by unaffected function of anterior arm muscles in C7 spinal injury. The potential clinical significance of these findings pertains to surgical procedures in the axillary region and the anterolateral approach to the humerus, as well as to the differential diagnosis of causes of muscular weakness and sensory changes. The observed number of variations also raises the question of whether the anatomy typically described in textbooks, truly represents the standard anatomy.

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MASSIVE MIGRATION OF PROSPECTIVE TELENCEPHALIC GABAERGIC NEURONS THROUGH THE MARGINAL ZONE IN MONKEY AND HUMAN FETUSES

Čavka M.^{1*}, Zanze Beader M.^{1*}, Esclapez M.², [Petanjek Z.](#)¹, Hladnik A.¹

¹ School of Medicine University of Zagreb, Department of Anatomy and Clinical Anatomy & Croatian Institute for Brain Research, Zagreb, Croatia

² INSERM, INMED, Institut de Neurobiologie de la Méditerranée, Marseille, France

zdravko.petanjek@mef.hr

The marginal zone (MZ) is the most superficial layer of the developing telencephalon which will become cell sparse adult cortical layer I. By the end of the first year, it already resembles adult cytoarchitectonic features. However, during gestation, the MZ represents a dynamic compartment of multiple transient phenomena, which are reflected in the profound structural changes of this layer.

In this study, the MZ was examined on Nissl, Golgi, and immunohistochemically processed human and cynomolgus monkey fetal brain tissue. The focus was on structural changes related to the migration of prospective GABAergic neurons that are heterogeneous populations with a crucial role in the coordination and integration of cortical processing.

At postconceptional week (pcw) 15 in human and embryonic day (E) 64 in monkey, small cells expressing calretinin, calbindin, somatostatin, or glutamate-decarboxylase (GAD) were more frequently found in the MZ. By the end of the second trimester (pcw22 and E90), the MZ showed a further increase in thickness and became populated by densely arranged small migratory-like cells forming several sublayers. These cells invaded the MZ from the proliferative zones located at the junction of the pallium (cortex) and subpallium (ganglionic eminence): subcallosal and retrosplenial regions of the medial telencephalic wall, septal eminence, and zone around the temporal horn of the lateral ventricle.

Obtained data strongly suggests that by the end of the middle trimester of gestation, the MZ represents the main pathway for GABAergic neuron migration in monkeys and humans. We expect that even subtle lesions within the MZ, in cases of fetal distress or prematurely born infants, might significantly influence the formation of the cortical GABAergic network. Therefore, systematic morphological analysis of the MZ in the last trimester of gestation might have implications in understanding the pathogenesis of epilepsy, autism, or schizophrenia.

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EVALUATION OF VASCULAR DAMAGE OBSERVED ON THE CHICKEN CHORIOALLANTOIC MEMBRANE INDUCED BY THE VENOM OF A BRAZILIAN ENDEMIC SNAKE SPECIES BOTHROPS LEUCURUS

Petrilla V.^{1,2}, Polláková M.¹, Bekešová B.¹, Vlčková R.¹, Sopková D.¹, Petrillová M.³, Petrovová E.⁴, Legáth J.^{5,6}

- ¹ Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy, Košice, the Slovak Republic
- ² Zoological Department, Zoological Garden Košice, Košice, the Slovak Republic
- ³ Department of General Competencies, University of Veterinary Medicine and Pharmacy, Košice, the Slovak Republic
- ⁴ Department of Morphological Disciplines, The University of Veterinary Medicine and Pharmacy, Košice, the Slovak Republic
- ⁵ Department of Pharmacology and Toxicology, University of Veterinary Medicine and Pharmacy, Kosice, the Slovak Republic
- ⁶ Department of Biotechnology and Bioinformatics, Faculty of Chemistry, Rzeszow University of Technology, Rzeszów, Poland

vladimir.petrilla@uvlf.sk

The modern scientific approach has transformed the potentially deadly nature of snake venoms into medically significant components. In our pilot study, we monitored and evaluated the haemotoxic effects of the native venom from the Brazilian endemic snake species *Bothrops leucurus*, employing the HET-CAM bioassay developed by Lüpke.

The unfractionated venom was applied at two concentrations (20 000 and 2000 µg/ml). Each testing group consisted of 4 fertilised chicken eggs. 50 µl of the prepared venom solution was applied to the exposed blood vessels of the chorioallantoic membrane of each egg. In a control group, 50 µl of sterile saline solution was applied instead of venom. Changes in the blood vessels, specifically hyperaemia, haemorrhage, and clotting, were subsequently monitored and evaluated at 5-minute intervals after application.

The vascular damage was dose-dependent, as the higher concentration of 20 000 µg/ml induced the onset of haemorrhage and blood clots formation along the chorioallantoic vessels. In contrast, the lower concentration of 2000 µg/ml resulted only in haemorrhage. These differences between concentrations are reflected in the calculated cumulative score and the resulting classification of irritant potential. For the concentration of 20 000 µg/ml, the irritant potential value was determined to be 10.5, which according to Lüpke's grading system is classified as having strong irritant potential. For the lower concentration of 2000 µg/ml, a lower irritant potential of 4.5 was calculated, which corresponds to Lüpke's classification as slight. No occurrence of hyperaemia was recorded in either group. On the contrary, after the application of

venom solutions of both concentrations, it was possible to observe gradual vasoconstriction of both large and small vessels, which was most pronounced in the fifth minute post-application.

Given the vascular damage and haemotoxic effects of the tested venom, it can be assumed that the venom of *Bothrops leucurus* contains components with the ability to affect the cardiovascular system. Based on these findings, we propose to proceed with a proteomic analysis, isolation and identification of haemotoxic components, followed by repeated testing of these isolated fractions.

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STUDYING THE PATHOLOGICAL SORLA VARIANT AND ITS INVOLVEMENT IN ALZHEIMER'S DISEASE DEVELOPMENT

Plešingrová K.¹, Raška J.^{1,2}, Satková M.¹, Fojtík P.^{1,2}, Pospíšilová V.¹, Sedmík J.¹, Cesnáriková S.¹, Amruz Černá K.¹, Bártová S.¹, Hříbková H.¹, Bohačiaková D.^{1,2}

- ¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² International Clinical Research Center (ICRC), St. Anne's University Hospital, Brno, the Czech Republic

klara.plesingrova@mail.muni.cz

Alzheimer's disease (AD) is a progressive neurodegenerative disease. Mutations in specific genes such as APP and PSEN1/2 can directly lead to AD development, also via amyloid beta accumulation. Recent studies show that the SORL1 gene, coding the SORLA protein, could have a similar effect. It was discovered that SORLA regulates the recycling of amyloid precursor protein and is involved in the degradation of amyloid beta. However, how specific AD-causing mutations in the SORL1 gene alter the biology of neurons remains unknown.

This project is focused on investigating the impact of pathogenic mutations in the fibronectin type III domain of SORLA using in vitro modeling. SORL1 G1732A mutation was introduced by CRISPR/Cas9 technology into induced pluripotent stem cells (iPSCs) and these cell lines were further differentiated into 2D neurons and 3D cerebral organoids. AD pathology and disease development were studied by a variety of molecular approaches – gene expression, protein levels, immunocytochemistry, and electron microscopy.

Our results showed impaired SORLA maturation and trafficking – decreased SORLA levels and alternations in SORLA shedding. Induced neurons exhibit endosomal swelling suggesting impairments in the endolysosomal pathways and altered APP processing, which directly leads to amyloid beta accumulation.

This study provides evidence supporting the critical role of SORLA mutations in AD development.

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LPS TRIGGERS UPR IN EXPANDABLE LUNG EPITHELIA (ELEPS) AND ENHANCES CELL MIGRATION BY ALTERING N-CADHERIN AND E-CADHERIN

Portakal T., Havlíček V., Herůdková J., Pelková V., Cakmakci R.C., Hampl A., Vaňhara P.

- ¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² International Clinical Research Center, St. Anne's University Hospital, Brno, the Czech Republic

turkan.portakal@med.muni.cz

Lipopolysaccharide (LPS), of gram-negative bacteria, causes a lung inflammation, however, specific effects of LPS on lung alveolar cells are unclear. Phenotypic changes of alveolar epithelium, such as epithelial-to-mesenchymal transition (EMT) contribute to lung fibrosis and respiratory failure. Endoplasmic reticulum signaling and unfolded protein response (UPR) were reported to be involved in lung fibrosis through adaptation to cell stress, phenotypic changes or cell death. LPS role associated with endoplasmic reticulum (ER) in lung cells through is virtually unknown. This study investigates effects of LPS on EMT and ER in expanable lung-like epithelial cells (ELEPs).

ELEP cells, were derived from hESC CCTL14 line as described elsewhere (Kotasova et al., 2022). Briefly, ELEPs were directed to the foregut endoderm and to lung trajectory To investigate effects of LPS, ELEPs were treated with different concentrations of LPS and Tunicamycin (TN) in 2D and 3D culture conditions. CRISPR/Cas9-mediated TUSC3 knockout (KO) ELEP cells were also used to study UPR. ER stress response was analyzed by qRT-PCR, Western blot, and immunofluorescence microscopy.

LPS and TN increased ELEP cell migration. qRT-PCR revealed upregulation of SLUG and SNAIL after LPS treatment, suggesting propensity to EMT. qRT-PCR, WB, and IF results showed UPR activation with increased BiP and CHOP in TN-treated cells. TN also increased E-cadherin and N-cadherin expression with abnormal cytoplasmic accumulation. LPS-induced ER stress was confirmed with increased BiP and CHOP. IF and WB results showed increased N-cadherin due to LPS, while qRT-PCR results showed increased E-cadherin. In 3D studies, TN and TUSC3 KO accelerated spheroid formation, while LPS resulted in larger spheroids. qRT-PCR results of 3-day-old spheroids showed upregulation of BiP, CHOP, E-cadherin, and N-cadherin.

LPS induced EMT in ELEP cells, through the activation of UPR, and alteration of TUSC3 and adhesion molecules.

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MUSCLES OF THE FOREARM AND HAND IN THE ANUBIS BABOON (PAPIO ANUBIS) – ANATOMICAL CASE REPORT

Pračková I.^{1,2}, Vargová L.¹, Páral V.², Vymazalová K.¹

¹ Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

² Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, the Czech Republic

245553@muni.cz

The anatomy of the muscles of the forearm and hand in humans has been studied in great detail, including existing anatomical variations. For animals, the description of the structures and anatomical variations is still not complete. The aim of this case study was to describe the anatomy of the muscles of the forearm and hand with emphasis on the origin, insertion and innervation of the muscles in one individual of the Anubis baboon. Obtained information were compared with the available data on this animal and with the anatomical standard and possible variations in humans.

The biggest differences were found in flexor digitorum profundus muscle, flexor pollicis longus muscle, extensor digiti minimi muscle, abductor pollicis longus muscle and in the group of the muscles of the middle space.

Any work describing anatomy in detail is an important contribution to the stabilisation of the anatomical standard and variability in these exotic species. Detailed description of the anatomical structures is crucial for improving techniques in the field of veterinary surgery and orthopaedics.

There was no specific funding for this work.

CELLULAR DISTRIBUTION AND SEMI-QUANTITATIVE CHANGES OF DELTA OPIOID RECEPTORS IN THE SPINAL DORSAL HORN OF A MOUSE SPARED NERVE INJURY MODEL

Rábová A., Dubový P., Kubíčková L., Joukal M.

Department of Anatomy, Cellular and Molecular Research Group, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

rabova@med.muni.cz

The primary sensory neurons (PSN) of the dorsal root ganglia (DRG) play a pivotal role in the afferentation of peripheral nociception into the spinal cord. The majority of the central branches of PSNs terminate on the neurons of the spinal dorsal horn (SDH). The aim of present experiments was to study semi-quantitative changes of DOR protein levels in lumbar (L-SDH) and cervical (C-SDH) of both sides (ipsilateral and contralateral) in a mouse model of neuropathic pain.

We used unilateral mouse spared nerve injury model (n=8) with spared tibial nerve (SNIt). In sham-operated animals (n=8), the left sciatic nerve was only exposed. Both SNIt- and sham-operated animals were left to survive for 7 and 21 post-operation days (POD). Four mice without any operation were used as naive control. The spinal cord segments in the range of L3-L5, C4-C6 were dissected. A set of cryostat sections (12µm) was immunostained with polyclonal DOR primary antibody under the same conditions. Double immunostaining with IB4-FITC and DOR antibody was to distinguish DOR distribution in layers I and II.

Levels of DOR-IF were similar in both C-SDH and L-SDH of naive mice. Sham or SNIt operations induced bilateral increases in DOR-IF in L-SDH at POD7 and POD21. The increased DOR-IF was found in C-SDH on ipsilateral side after sham, but on contralateral side after SNIt operation at POD7. However, no significant changes were found in C-SDH after sham or SNIt at POD21.

The results indicate that both sham or SNIt operations resulted in bilateral changes in DOR protein levels in both C-SDH and L-SDH.

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VENTRAL PREPROSTATIC SPACE – UROLOGIST’S NIGHTMARE

Rejtarová O.¹, Štefková K.¹, Endlicher R.¹, Brodák M.²

¹ Department of Anatomy, Charles University in Prague, Faculty of Medicine in Hradec Králové, the Czech Republic

² Department of Urology, University Hospital in Hradec Králové, the Czech Republic

rejto@lfhk.cuni.cz

Introduction and aim: The ventral preprostatic space (VPS) is an anatomical area located anterior to the prostate, ventral to the isthmus prostatae. The isthmus is clinically known as the anterior fibromuscular stroma (AFMS). Traditionally, the AFMS is described as non-glandular regions composed primarily of fibrous tissue and smooth muscle fibers. Moreover, the prostate lacks a capsule in the AFMS part; thus, VPS is an immediate continuation of AFMS ventrally. However, recent studies have indicated the presence of glandular tissue in the AFMS region. In an effort to enhance the understanding of this critical area, urologists and anatomists are continuously working to grasp better and describe the structure and boundaries between the various parts of the prostate. Our research specifically targeted the AFMS and the VPS, aiming to accurately delineate the boundaries and describe the individual parts and structures. This detailed anatomical mapping is intended to help surgeons identify the most precise resection line during prostatectomy, thereby improving surgical outcomes and minimizing complications, as well as the recurrence of disease.

Methods: The first set of samples, connective tissue from patients' periprostatic space, was collected at the Urology Clinic of FNHK during robotic prostatectomy, and the second set of samples, VPS and AFMS, was obtained from male cadavers at the Anatomy Department LFHK. All samples were histologically processed using HE staining, Green Trichrome staining, immunohistochemistry, and microscopically observed.

Results: Our pilot data indicate that glandular tissue is present not only in the AFMS but also in the connective tissue of VPS.

Conclusion: If our observations regarding the presence of both malignant and benign glands in this space are confirmed, the resection line and the overall approach to prostatectomy and subsequent treatment will need to be reconsidered. This underscores the critical need for further research to optimize clinical outcomes.

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CDK13 AS A COORDINATOR OF PRIMARY CILIA FUNCTION IN LIMB DEVELOPMENT

Romanová A.¹, Jandová N.¹, Pushkarov V.², Dubajic M.^{1,2}, Kavková M.³, Nováková M.⁴, Kohoutek J.², Buchtová M.^{1,2}, Hampel M.^{1,2}

- ¹ Institute of Animal Physiology and Genetics, CAS, Brno, the Czech Republic
- ² Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, the Czech Republic
- ³ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁴ Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, the Czech Republic

533884@mail.muni.cz

The development of vertebrate limbs is a highly coordinated process that relies on the precise regulation of signaling pathways. Primary cilia are essential organelles that serve as signaling centers for pathways such as Sonic Hedgehog (Shh), which is crucial for proper limb patterning. Cyclin-dependent kinase 13 (CDK13) has been identified as a crucial regulator of these ciliary functions. In this study, we explored the function of CDK13 in the context of limb morphogenesis, focusing on its role in Shh signaling.

To study defective embryonic development of limbs as a result of Cdk13-deficiency, we use embryonically lethal Cdk13 hypomorphic and knockout mutant embryos. To assess morphological changes, histological methods and microCT were used. Gene and protein expression alterations were detected using qPCR, RNAScope, RNASequencing, WB, IHC and ICC. Primary cilia defects and Shh pathway protein localization were detected using fluorescence confocal microscopy followed by image analysis using Imaris software.

In this study, we investigated the role of CDK13 in coordinating primary cilia function during limb development. Using genetic and biochemical approaches, we demonstrated that CDK13 modulates the Shh pathway by influencing the stability and localization of its components within primary cilia. CDK13 deficiency resulted in aberrant ciliary architecture and disrupted Shh signaling, leading to limb malformations.

Our findings highlight CDK13 as a critical player in the integration of ciliary signals necessary for limb morphogenesis, providing new insights into the molecular mechanisms underlying developmental disorders associated with ciliary dysfunction.

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USING HUMAN PLURIPOTENT STEM CELL-BASED GLIAL MODELS TO STUDY ALZHEIMER'S DISEASE

Satková M.¹, Sedmík J.¹, Raška J.^{1,2}, Plešingrová K.¹, Fojtík P.^{1,2}, Boháčiková D.^{1,2}

¹ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

² International Clinical Research Center (ICRC), St. Anne's University Hospital, Brno, the Czech Republic

miriam.satkova@med.muni.cz

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by two pathological features: the buildup of amyloid- β plaques and Tau neurofibrillary tangles. Importantly, ~5% of AD cases have been linked to mutations in the APP, PSEN1, PSEN2, and SORL1 genes. Notably, the SORLA protein, encoded by the SORL1 gene, functions as an intracellular sorting receptor, playing a crucial role in transporting the amyloid precursor protein (APP). The impact of SORLA mutations has been primarily studied in neurons, where we and others showed it significantly affects the biology of endosomes and the recycling of APP protein within neurons (Jensen, Raska, et al., accepted in PNAS). However, how these AD-causing mutations affect glial cells, and their function remains unknown.

To study the role of SORLA protein in human glial cells, we started by optimizing the protocols for the differentiation of human induced pluripotent stem cells (iPSCs) into both 2D astrocytes and 3D-astrocytes-containing spheroids. These were then characterized by the immunofluorescence and qPCR. Furthermore, specific pathological mutations of the SORL1 gene were introduced into the iPSCs via the CRISPR/Cas9 technology.

We have established a protocol for differentiating 2D astrocytes from iPSCs using combined NEUROG2 overexpression and the Astrocyte Basal Medium approach and characterized them by immunofluorescence and qPCR to confirm the expression of astrocyte-specific markers. The iPSCs-derived neurons and astrocytes were further used to generate more functionally relevant 3D models – Brain microphysiological systems and “Asteroids”, neuronal spheroids enriched for mature astrocytes. These 3D models are currently being characterized.

We have successfully created a relevant 2D glial cell model consisting of astrocytes, along with 3D models such as brain microphysiological systems and asteroids. We will now combine these optimized protocols with CRISPR/Cas9-edited iPSCs with defective or absent SORLA protein to study SORLA-dependent pathologies further.

This work is supported by the EU Joint Programme - Neurodegenerative Disease Research (JPND2023-087), Faculty of Medicine, Masaryk University (MUNI/A/1598/2023), Czech Science Foundation (Grant No. GA24-12028S; Grant No. GA24-11364S) and by the Czech Health Research Council (AZV project No. NU22J-

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EVALUATION OF BONE TISSUE REGENERATION IN THE AREA OF THE MANDIBLE USING A HYDROXYAPATITE BONE PLATE

Šimaiová V., Teleky J., Demčišáková Z., Humníková J.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Košice, the Slovak Republic

veronika.simaiova@uvlf.sk

Substitute biomaterials for bone research that have been applied to the area of defects on the mandible are increasingly the subject of interest of scientists around the world. Hydroxyapatite ceramic scaffolds have fully acceptable phase composition, microstructure and compressive strength for their use in regenerative medicine.

Five adult females of the Large White breed (n=5) were used in the work. Unilateral cortical bone defects were created in animals using an extraoral approach to the mandible. A sterile hydroxyapatite (HA) ceramic plate 1.5 cm long, 0.7 cm wide, and 0.3 cm thick was inserted to fill the marginal defect on the right side. After the end of the monitoring period, the animals were killed at different time periods of 3, 4, 5 and 6 months. Tissue samples were taken from the site of the bone defect and evaluated histologically and immunohistochemically.

Microscopically, we observed the regenerating compact bone tissue with the presence of bone cells osteoblasts, osteocytes and mineralized matrix. The bone surface was relatively smooth without remnants of HA ceramic plates. In the place of the defect, the ceramic plate was completely degraded and the defect was filled with newly formed, regenerated bone tissue. Immunohistochemical detection in the bone defect on the body of the mandible 3, 4, 5 and 6 months after implantation of a HA ceramic plate in a pig showed the presence of type I collagen in the bone matrix of compact bone, in cancellous bone, around bone marrow cavities, as well as in the surrounding area and inside osteons and lacunae of osteocytes.

In experimentally created defects on the body of the lower jaw, we confirmed the regeneration of bone tissue after the application of the HA bone plate to the defect site.

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TEMPOROMANDIBULAR DISC IN PIG - THE BEST ANIMAL MODEL FOR THE HUMAN?

Teleky J., Šimaiová V., Demčišáková Z., Hurníková J.

Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic

jana.teleky@uvlf.sk

The temporomandibular joint (TMJ) is located in the facial area of the skull, and ensures the connection of the mandible with immobile skull bones. TMJ plays the essential role in chewing, breathing, swallowing, in humans, for speaking, among other common daily activities of living. Part of the joint is the articular plate (discus articularis) of a fibrous nature, the task of which is to minimize the discrepancy between the mandibular condyle and the temporal bone. The purpose of this work was to assess the disc in the domestic pig morphometrically and to evaluate its use as an animal model for humans.

We used 16 heads of freshly slaughtered pigs of Slovak White improved breed, which came from Kosice slaughterhouse. The age of the pigs was 6 months, the animals were female and all were clinically healthy. In order to obtain TMJ discs for their subsequent morphometric assesment, an anatomical dissection was performed.

By examining 32 articular discs, we found that the discus articularis appeared as a rigid, elastic, pale pink to white structure. The shape of the disc was oval from the dorsal view, biconcave from the side view. By measuring the discs, the average value of the right disc was 28.04 mm x 16.86 mm, the left disc was 29.03 mm x 18.36 mm.

Anatomically the disc of pig is very similar to that of man. According the literature there is also functional similarity in the oral apparatus in pig and man. It means that the pigs can represent a reasonable animal model for studies on the temporomandibular joint in human.

The study was supported by VEGA 1/0373/24.

3D-PRINTED MODELS OF ANEURYSMS BASED ON PATIENTS' DATA TO STUDY HEMODYNAMIC

Torkashvand M.¹, Nováková L.², Hejčl A.³, Brunátová J.⁴, Hron J.⁴, Víteček J.⁵, Vítečková Wünschová A.¹

- ¹ Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² Faculty of Mechanical Engineering, J.E. Purkinje University, Prague, the Czech Republic
- ³ Department of Neurosurgery, J. E. Purkinje University, Masaryk Hospital, Ústí nad Labem, the Czech Republic
- ⁴ Faculty of Mathematics and Physics, Mathematical Institute, Charles University, Prague, the Czech Republic
- ⁵ Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, the Czech Republic

553124@mail.muni.cz

Cerebrovascular diseases, including hemorrhagic strokes, are among the most fatal conditions globally, often triggered by ruptured brain aneurysms. Aneurysms, characterized by the weakening and ballooning of blood vessel walls, primarily require treatments like clipping and coiling, both involving brain surgery with significant risks. Understanding the mechanisms and factors influencing brain aneurysms is crucial for developing effective prevention and treatment strategies. Notably, changes in hemodynamic conditions can lead to endothelial dysfunction, an aneurysm development and its rupture.

CT scans of six cerebral aneurysms with known rupture sites were used to create virtual meshes. Computational Fluid Dynamics (CFD) simulations were conducted to analyze flow dynamics. Two aneurysms' meshes from this set were 3D printed to verify CFD simulations. These in vitro models were connected to a pump for Particle Image Velocimetry (PIV) measurements.

Four of six aneurysms showed ruptures near a vortex with low wall shear stress (WSS) and a high oscillatory shear index (OSI). One ruptured in a high WSS flow jet, while another in a significant bleb without distinct hemodynamic parameters. The PIV measurements confirmed the CFD results.

Our study confirmed that aneurysms rupture points are associated with at least three distinct hemodynamic conditions. Areas with low WSS exhibiting recirculation, while those with high WSS showed jet-like flow patterns. The rupture point in the bleb was without distinct hemodynamic parameters. These findings indicate a need for larger study of aneurysms hemodynamic to be able to predict their rupture.

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THE USE OF MICRO-CT AND HISTOLOGICAL ANALYSIS IN THE DIAGNOSIS OF PAGET'S DISEASE

Vaňátková K.¹, Vargová L.¹, Horáčková L.², Němečková A.³, Vymazalová K.¹, Macháček J.⁴, Vopálenský M.⁵, Adámková I.⁵, Jilmová D.⁶

- ¹ Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² Department of Kinesiology, Faculty of Sports Studies, Masaryk University, Brno, the Czech Republic
- ³ Department of Histology and Embryology, Faculty of Medicine, Charles University, Pilsen, the Czech Republic
- ⁴ Department of Archaeology and Museology, Faculty of Philosophy, Masaryk University, Brno, the Czech Republic
- ⁵ Institute of Theoretical and Applied Mechanics, Czech Academy of Sciences, Telč, the Czech Republic
- ⁶ City Museum and Gallery Břeclav, the Czech Republic

katerina.vanatkova@med.muni.cz

Paget's disease is a metabolic disease of unclear etiology, characterized by excessive bone resorption followed by the formation of structurally deficient bone. The most commonly affected bones are the vertebral column, pelvis, femur, skull, and sacrum. The affected bone is more prone to deformities and fractures. The disease manifests itself in the fifth or sixth decade of life. The current incidence of Paget's disease in the Czech Republic is approximately 3 % and it is one of the most common metabolic diseases. However, findings on historical skeletal remains are still rare. During archaeological research in Líbivá near Břeclav, possible manifestations of Paget's disease were observed on one of the skeletons at a burial site from an earlier phase of the Migration Period.

A skeleton with possible manifestations of Paget's disease was subjected to anthropological and palaeopathological analyses. Macroscopic, histological, and micro-CT examinations were used to evaluate pathological changes.

The skeletal remains of a male about 60 years old had significant pathological changes on the skull, vertebrae, long bones, and rib. The lesions were localized on the skull and vertebrae. Fractures healed by the pseudoarthrosis were observed on the 5th lumbar vertebrae and the 1st rib. On the postcranial skeleton, the spongiosis of the long bones was quite abundant, diploe of the skull was wide and showed a pumicelike structure. The histological examination of bone tissue sections showed a mosaic pattern, a hallmark of Paget's disease. Micro-CT of the cranial vault showed the typical bone structure in Paget's disease. The bone has lost its homogeneous structure, with a clearing at the site of osteolytic foci, thickening at the site of new bone formation, and

only sporadically there are fragments of normal bone tissue. The boundary between compact and spongiosis bone is not recognizable.

The used methods confirmed the diagnosis of Paget's disease.

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3D MODELS OF INTRACRANIAL CAROTID STENOSES

Vítečková Wünschová A.¹, Hejčl A.², Hron J.³, Víteček J.^{2,4}

- ¹ Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² International Clinical Research Center, St. Anne's University Hospital, Brno, the Czech Republic
- ³ Faculty of Mathematics and Physics, Mathematical Institute, Charles University, Prague, the Czech Republic
- ⁴ Institute of Biophysics of the Czech Academy of Sciences, Brno, the Czech Republic

andrea.wunschova@med.muni.cz

Intracranial carotid stenosis due to atherosclerotic plaque poses a high risk, particularly if the plaque is unstable, as it may lead to ischemic stroke. Current literature indicates a link between the hemodynamics in the stenosis and its further development. The goal of this study was to set up a computational fluid dynamics (CFD) model and a laboratory model to evaluate the hemodynamics of intracranial carotid stenosis.

CT scans of patients with 70% or higher stenosis of the internal carotid artery who underwent endarterectomy were used to create a virtual 3D mesh for the CFD models. Additive manufacturing, also known as 3D printing, was employed to create laboratory models. The hemodynamics in the laboratory model were evaluated using particle image velocimetry.

Both the CFD model and the laboratory model revealed the formation of flow jets accompanied by high wall shear stress within the stenosis. This was followed by a turbulent region with vortices immediately downstream of the stenosis. The good accordance between the CFD and laboratory models indicated a reasonable setting of boundary conditions for the flow computation.

The CFD model, together with the laboratory model, can precisely reconstitute the flow conditions in the intracranial stenosis. Furthermore, the laboratory model can serve to verify the boundary conditions for the flow computation.

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IMMUNOHISTOCHEMICAL DETECTION OF GPX4 IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS: A PILOT STUDY INVESTIGATING ITS PRESENCE IN WELL-DIFFERENTIATED TUMORS

Vrzgula M.¹, Mihalik J.¹, Briškárová V.², Hodorová I.¹

¹ Ústav anatómie, Lekárska fakulta UPJŠ, Košice, the Slovak Republic

² Ústav súdneho lekárstva, Lekárska fakulta UPJŠ, Košice, the Slovak Republic

marko.vrzgula@upjs.sk

Our study aims to detect the immunoreactivity of GPx4 in human pancreatic ductal adenocarcinoma (PDAC) cells. For many years, glutathione peroxidases have been recognized for their role in converting H₂O₂ and organic hydroperoxides into water or their corresponding alcohols. To date, eight glutathione peroxidases (GPx1 - GPx8) have been identified in mammals, each exerting antioxidant functions in diverse cellular locations. Among them, GPx4 stands out for its specific role in protecting membranes against oxidative stress. It exhibits a unique ability to reduce H₂O₂, small hydroperoxides, and even complex lipid hydroperoxides, such as those found in phospholipids and cholesterol within biomembranes.

The research involved four formalin-fixed, paraffin-embedded tissues of well-differentiated PDAC, and the immunoreactivity of GPx4 was analyzed using the three-step indirect immunohistochemical method under a light microscope.

In all samples of PDAC, a positive immunoreaction for GPx4 was observed with diffuse expression in the tumor cells.

GPx4 is an intracellular monomeric selenium-containing protein. It serves as an antioxidant enzyme, effectively reducing lipid hydroperoxide production during lipid peroxidation. This process generates various end molecules, such as malondialdehyde and 4-hydroxynonenal, which can disrupt the function and activity of DNA and proteins by forming cross-links. Additionally, excessive lipid peroxidation not only interferes with molecular signal transduction but also triggers cell death, notably ferroptosis, a type of regulated cell death dependent on iron. Consequently, genetic or pharmacologic inhibition of GPx4 expression or activity may induce ferroptosis across different tissues and cell types, with potential therapeutic implications for cancer treatment.

This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic and the Slovak Academy of Sciences VEGA 1/0173/19.

ANATOMICAL BACKGROUND FOR CONSTRUCTION OF PALATE PROTECTIVE TRAY FOR CLEFT PALATE OPERATIONS IN PEDIATRIC PATIENTS

Vymazalová K.¹, Košková O.², Marcián P.³, Richtrová M.⁴, Joukal A.¹, Matysková D.¹, Bryšová A.^{1,5}, Joukal M.¹, Štourač P.^{4,6}

- ¹ Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ² Department of Burns and Plastic Surgery, Joint workplaces with the University Hospital Brno - workplaces of the Bohunice and Mater. Hospital, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ³ Department of Biomechanics, Faculty of Mechanical Engineering, Brno University of Technology, the Czech Republic
- ⁴ Department of Paediatric Anaesthesiology and Intensive Care Medicine, Institutions shared with the Faculty Hospital Brno (paediatric medicine), Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁵ Department of Stomatology, Institutions shared with St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University, Brno, the Czech Republic
- ⁶ Department of Simulation Medicine, Theoretical Departments, Faculty of Medicine, Masaryk University, Brno, the Czech Republic

vymazalova@med.muni.cz

Cleft lip and palate belong to the most common congenital development defect with a significant effect on an infant's life. Anesthesiologic management, especially intubation, is often difficult due to the defect's nature with high incidence of complications. Therefore, the protective tray covering the defect in the oral cavity may significantly improve a visualization of the entrance to the larynx, and thus to ensure better intubation conditions. The major goal of proposed project is to increase patient's safety during anesthesia and to improve anesthesiologic management by intubation facilitation and tissue protection. To reach the aim of the project, we will develop a methodology for a design and construction of palate protective tray used in anesthesiologic management. Pilot project performed on the cadaver from collection of the Department of Anatomy, Faculty of Medicine, Masaryk University with cleft lip and palate was performed to assess feasibility of the proposed project.

The cadaver used on the pilot project was male newborn with atypical cleft lip and palate with non-developed premaxilla. 3D scan of the upper jaw including cleft palate, alveolar process and premaxilla was performed using intraoral 3D scanner and 3D virtual design of the protective tray was prepared. Based on the 3D virtual protective tray, the mold formed by negative unique impression of the upper jaw and palatoalveolar impression for casting of a protective tray was created on a 3D printer. In order to test the protective tray, cuts were made from the angles of the mouth to the

lower edge of the auricles. Next, exarticulation of the temporomandibular joint was performed.

The protective silicone tray fitted precisely on the upper jaw giving ideal background for tissue protection during intubation. The entire process from the scanning to the protective tray preparation took 24 hours.

A morphological analysis based on measuring the dimensions on palate 3D scans is currently underway, which aims to design a universal protective tray.

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MACROSCOPIC AND MICROSCOPIC EVALUATION OF DEVELOPMENTAL ANOMALIES OF THE THYROID GLAND: CASE REPORT

Zavhorodnia I.¹, Samec M.², Hešková G.¹, Straka L.³, Janík M.³, Výbohá D.¹

¹ Department of Anatomy, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic

² Department of Medical Biology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic

³ Department of Forensic Medicine and Medicolegal Expertises, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, the Slovak Republic

zavhorodnia1@uniba.sk

Thyroid gland (TG) develops from a pouched thyroid diverticulum between the first and second pharyngeal pouches during the 4th week of gestation. Eventually it descends via the thyroglossal duct from the level of foramen caecum linguae in front of the hyoid bone, thyroid and cricoid cartilages, until it reaches level between CV and T1 vertebra. Due to distant descend, and complete or partial obliteration of thyroglossal duct, TG is objected to the formation of developmental variations. This study summarizes a case report of rare occurrence of several pyramidal lobes (PLs) and levators glandulae thyroideae (LGTs) in one TG.

Dissection of a 45-year-old non-embalmed, non-frozen female cadaver was conducted as a part of our prospective cohort study. En bloc dissection method for evisceration of cervical and thoracic complex of organs and further infero posterior approach for precise dissection of PL and LGT were applied. Samples were stained with HE and analyzed under magnification of 40x, 100x, 200x, and 400x.

Macroscopic evaluation of TG showed three unusual morphological structures: one on the right side, and two on the left side. On the right side, short PL and thyreopyramidal LGT were observed. On the left side, hyopyramidal LGT was located medially, and PL of atypical shape of "hourglass" with fibrous band was detected laterally. Microscopic evaluation revealed that the LGT on the right side and medial LGT on the left side are muscular structures without thyroid follicles in it. Fibrous band of the PL on the left side confirmed presence of the thyroid follicles, muscular fibers as well as collagen fibers.

Knowledge gained in this study is applicable in the thyroid surgery, as incomplete resection of PL and LGT may increase risk of thyroid tissue residual in situ. The latter, therefore, may initiate the formation of the thyroglossal duct cysts and reoccurrence of the thyroid neoplasm.

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QUANTITATIVE IMMUNOHISTOCHEMICAL ANALYSIS OF ALPHA-SM ACTIN POSITIVE ELEMENTS IN HUMAN INFERIOR VENA CAVA, PORTAL VEIN AND COMMON ILIAC VEIN, A PILOT STUDY

Zavřelová M.¹, Kunčarová K.¹, Mik P.^{1,2}, Červenková L.², Hanáček L.³, Hošek P.², Eberlová L.

¹ Department of Anatomy, the Czech Republic

² Biomedical Centre, the Czech Republic

³ Department of Forensic Medicine Faculty of Medicine in Pilsen, the Czech Republic

mariezavrelova11@icloud.com

Based on our previous study, we aimed to compare the structure of the human with the pig veinous analogues. Contractile proteins in the wall of the human supra- and infrahepatic inferior vena cava (IVC), the portal vein (PV), and the common iliac vein (CIV) were evaluated.

Tissue samples were obtained in cooperation with the Institute of Forensic Medicine, Medical Faculty in Pilsen, from 15 donors (8 men and 7 women), aged 47 – 85 years. Samples were stitched to cork plates, fixed in 10% buffered formalin, and processed into 3 µm paraffin sections. Contractile elements were visualized using immunohistochemical reaction against alpha-smooth muscle actin. Based on stereological principles, the area fraction (AA) of the alpha-smooth muscle actin (SMA) was estimated within the intima and media. The point-counting method and the Ellipse software (ViDiTo, Košice, Slovakia) was used for the quantification, the paired t-test, Mann-Whitney U test and bootstrapping for the statistical analysis.

The mean AA of SMA was 34.07% ± 5.85% (mean ± SD) in the infrahepatic IVC, 32.89% ± 10.48% in the suprahepatic IVC, 41.41% ± 9.08% in the PV, and 41.80% ± 13.29% in the CIV. The intraindividual difference was statistically significant between the SMA positivity of the intima and media in the infrahepatic IVC and the portal vein ($p = 0.02$). In agreement with the porcine veins from our previous experiment, the proportion of actin-positive cells was greater in the PV to the IVC grafts. When compared to the porcine IVC (9.34% ± 8.13%), the human IVC had significantly larger AA (SMA) both supra- and infrahepatically ($p < 0.01$).

AA (SMA) was greater in the PV in pigs and humans. Human IVC had significantly larger AA (SMA) both in supra- and infrahepatic segments.

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Schedule at a Glance

| | | | Lecture Hall A | Lecture Hall B |
|---------------------------------|-------------|-------------------------------|--|---|
| Sunday September 8 | 15:00–17:00 | Registration | | |
| | 17:00–19:00 | | Opening Ceremony | |
| | 19:00–20:30 | | Welcome evening | |
| Monday September 9 | 8:00 | | Invited Lecture | |
| | 8:40–10:00 | | Molecular and Cell Biology | Experimental Morphology |
| | 10:00 | Coffee Break | | |
| | 10:30 | | Invited Lecture | |
| | 11:10–12:25 | | Clinical Anatomy | Neurosciences I |
| | 12:15 | Lunch | | |
| | 13:15 | Congress Photo | | |
| | 13:30–15:30 | | 13:30–15:00 Developmental Morphology | 13:30–15:30 Symposium on Quality of Teaching Anatomy |
| | 15:00–16:00 | Coffee Break - Poster Session | | |
| | 16:00–17:15 | | 16:00–17:00 Oral Morphology | 16:00–17:15 Clinical Morphology |
| | 17:15–17:45 | | Meetings of society committees of the CAS and CSHC | |
| | 17:45–18:15 | | Plenary meetings of the CAS and CSHC | |
| | 19:30 | Social evening | | |
| Tuesday September 10 | 9:00–10:30 | 10:30 Coffee Break | 9:00–10:30 Neurosciences II. | 9:00–10:20 Anatomical Terminology |
| | 11:00–12:00 | | Varia | |
| | 12:00–12:15 | | Closing ceremony | |
| | 12:15 | Lunch | | |