BULGARIAN NATIONAL SCIENCE FUND

Ministry of Education and Science of Bulgaria

Institute of Experimental Morphology, Pathology and Anthropology with Museum

1962 BULGARIAN ANATOMICAL SOCIETY

IX NATIONAL CONFERENCE WITH INTERNATIONAL PARTICIPATION "MORPHOLOGICAL DAYS" 2026

ABSTRACT BOOK

JUNE 12-14, 2026, SOFIA, BULGARIA

ORGANIZING COMMITTEE

President

Prof. Nina Atanassova, PhD, DSc

Members

Prof. Svetlozara Petkova, PhD

Prof. Dimitar Kadiysky, PhD, DSc

Assoc. Prof. Ivelin Vladov, PhD

Assoc. Prof. Emilia Petrova, PhD

Assoc. Prof. Ekaterina Pavlova, PhD

Assoc. Prof. Yordanka Gluhcheva, PhD

Ass. Prof. Todor Kirov, PhD

Ass. Prof. Rosen Ivanov

The Conference is organized by

The Institute of Experimental Morphology, Pathology and Anthropology with Museum Bulgarian Academy of Sciences

The Bulgarian Anatomical Society

Financially supported by

Grant No. КП-06-МНФ/41 – 12.12.2025

Our sponsors

FOT LTD

MICROOPTICA

САВИМЕД ЕООД

GOLDEN SNAIL

PLENARY LECTURES

Combining development and evolution to understand the morphology of the ear

Abigail Tucker

King's College London, London, UK

E-mail: Abigail.tucker@kcl.ac.uk

Hearing as one of the five human senses is important not only for communication but also for our quality of life and integration into society, impacting on speech and language skills. We are able to hear due the unique and complex hearing organ that is the ear. The ear is a composite structure composed of different tissue types, derived from different tissue origins, that join together during development in order to form a functional unit. The mature ear can be divided into three regions, the outer, middle and inner ear, of which the eardrum, sandwiched between the middle and outer ear, is the focus of this talk. The eardrum is positioned where the different germ layers meet, and is composed of layers derived from the ectoderm, neural crest, and endoderm. The eardrum has evolved independently in several vertebrates: similarities in morphology, therefore, do not equate to evolutionary homology. Here we follow the development of the mammalian ear drum, investigating how signals integrate the different parts of the ear, using a suite of transgenic mice and comparisons across different mammals. The key role of the Fibroblast Growth Factor (FGF) family in uniting the external and middle ear is investigated. Once formed, we follow how stem cells are compartmentalised in the drum, the role of Wnt signalling, and how these cells are activated in response to repair. This work provides the mechanisms behind birth defects associated with the ear and provides new avenues for therapeutics for eardrum damage.

Key words: hearing, fibroblast growth factor, Wnt signalling, repair, developmental biology

Acknowledgments: Thanks to the funders (Medical research council, Royal National Institute for the Deaf, Wellcome Trust) and the Human Developmental Biology Resource

Transcriptomic analysis of primate stem cell niches

Anton B. Tonchev

[1]*Department of Stem Cell Biology, Research Institute, Medical University - Varna*
[2]*Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University - Varna*

E-mail: anton.tonchev@mu-varna.bg

The adult brain hosts neural stem cells (NSCs) in restricted microenvironments called NSC niches. The main NSC niche is the subventricular zone (SVZ) that extends along the cerebral lateral ventricles. The SVZ domains along the anterior horn of the lateral ventricle (SVZa) and the inferior horn of the lateral ventricle (SVZi) have differential neurogenic capacities. Cerebral ischemia can trigger uncommitted progenitor proliferation in both SVZa and SVZi, but neurogenesis occurs only in SVZa. The differences in the neurogenic capacity of SVZa and SVZi could be underlined by a differential gene expression in response to ischemia. We studied the transcriptional profiles of SVZa and SVZi following global cerebral ischemia to adult non-human primates and we were able to identify both common and differential gene signatures, illustrated in an open database: www.monkey-niche.org.

Key words: brain stem cell, neurogenesis, primate

Acknowledgments: Funded by Medical University – Varna, project 24026, and European Commission, Project TRANSTEM

Whole-body vibration after compressive spinal cord-injury in rats restores synaptic relations in the dorsal horn and alleviates pain-associated behaviour

Svenja Rink-Notzon[1], Martin Krueger[2], Milen Zamfirov[3], Diana Schaufler[4], Levent Sarikcioglu[5], Stefan Wagner[6], Süreyya Bilmen[7], Johannes Vogt[6], Lyudmila Belenska-Todorova[8], Stoyan Pavlov[9], Maria Eleni Manthou[10], Doychin N. Angelov[6*]

[1]Department of Prosthetic Dentistry, University of Cologne, Joseph-Stelzmann-Strasse 20, Cologne 50931, Germany; [2]Institute of Anatomy, Leipzig University, Liebigstraße 13, 04103 Leipzig, Germany; [3]Faculty of Educational Studies and the Arts, Sofia University, 69A Shipchenski Prohod Str., 1574 Sofia, Bulgaria; [4]University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Lung Cancer Group Cologne, Joseph-Stelzmann-Strasse 20, Cologne 50931, Germany; [5]Department of Anatomy, Faculty of Medicine, Akdeniz University, Dumlupınar Bulvarı 07058 Kampüs, Antalya, Turkey; [6]Department of Molecular and Translational Neurosciences, Institute of Anatomy II, Cluster of Excellence-Cellular Stress Response in Aging-Associated Diseases (CECAD), Center of Molecular Medicine Cologne (CMMK), University of Cologne, Joseph-Stelzmann-Strasse 20, Cologne 50931,Germany; [7]Vocational School of Health Services, Akdeniz University, Dumlupınar Bulvarı 07058 Kampüs, Antalya, Turkey; [8]Department of Biology, Medical Genetics, and Microbiology, Faculty of Medicine, Sofia University "St. Kliment Ohridski", Kozyak Str. 1, 1407 Sofia, Bulgaria; [9]Department of Anatomy and Cell Biology, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Prof. Marin Drinov Str.55, 9002 Varna, Bulgaria; [10]Laboratory of Histology and Embryology, Medical School, Aristotle University Thessaloniki, University Campus, 54124, Thessaloniki, Greece

*Corresponding author e-mail: angelov.anatomie@uni-koeln.de

Spinal cord injury (SCI)-associated neuronal loss and demyelination induce maladaptive neuronal circuits, the activation of which promotes severe sensory deficits including spasticity and persistent central neuropathic pain (PCNP). PCNP and spasticity in SCI-patients have been subject of numerous pharmacological, psychological and physiotherapeutic treatments, including whole-body vibration (WBV). However, a systematic assessment of WBV-associated alterations up to the synaptic level in the dorsal horn is missing and underlying mechanisms have not been explored. We assessed post-SCI pain-associated behavior index (PAB as assessed by established behavioral criteria) and compared the amounts of synapses (SYN^+, $VGLUT1^+$, $ChAT^+$, $VGAT^+$), $CGRP^+$- and SER^+-structures, as well as astro- and microglia in the dorsal horn of the lumbar spinal cord following thoracic SCI between WBV-treated and non-treated rats. Starting from the 3rd and continuing till the 12th postoperative week following SCI, WBV-treatment consistently reduced the PAB-index. Quantifications after STED-microscopy revealed that WBV increased the linear density of $VGAT^+$-and $VGLUT1^+$ perisomatic terminals and the quantity of SER^+ fibers. WBV-treatment diminished the amount of $CGRP^+$ structures in the dorsal horn, reduced the density of $CGRP^+$ perisomatic- and axon-axonic modulating synapses, and the amount of astro- and microglia. Our data show that the WBV-induced frequent (15-30 Hz) muscle contractions and proprioceptive impulses modulate spasticity (VGAT-related) and reduce the post-SCI hyperalgesia (CGRP-associated). In combination with the reduced astro- and microglia amounts, the described synaptic alterations are considered essential prerequisites for better motor recovery.These findings provide evidence for functional benefits of WBV in an animal SCI model and warrant further investigations to determine mechanisms underpinning this non-invasive, inexpensive and easily delivered rehabilitation therapy.

Key words: spinal cord injury, central neuropathic pain, whole body vibration, pain score, dorsal horn

Implementation of modern competences in teaching anatomy and development of the human sexual tract

Elisabeth Eppler*, Alessandro Bilella[1,2], Christian M. Hammer[2]

[1]*Research Group Didactic Morphology, Institute of Anatomy, University of Bern, Bern, Switzerland*
[2]*Anatomy Unit, Section of Medicine, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland*

*Corresponding author e-mail: elisabeth.eppler@unibe.ch

Medical health care professionals see themselves increasingly confronted with socio-cultural, environmental, and ethical issues regarding anatomy and development of the sexual organs. Also, anatomists observe an increased interest in sex- and gender-related topics by medical students who demand more in-depth information regarding genital organs and their variability, also across different life phases such as puberty and senium, to be able to better differentiate between physiologic variability and potentially pathologic conditions. The development of the sexual tract is particularly complex, being closely connected with the formation of the urinary tract and the organs of continence. For that reason, anatomy and embryology of sexual organs are relevant for many medical disciplines. In this lecture, in addition to various learning objectives, competences are presented grounding on general objectives within the PROFILES framework, which has been established for Swiss medical curricula. Medical students at the end of their pre-graduate course are expected to understand the developmental steps from the homologous anlagen to the sexually mature individual whereby modern competence-orientated teaching embraces the understanding that sexual development may display a remarkable degree of diversity. Nevertheless, particularly the current clinical classification of variants/differences of sexual development (DSD) remains to be fully integrated into modern anatomy and embryology education to foster a more accurate and inclusive understanding of human sexual development. This keynote lecture also provides some practical recommendations on how to incorporate these aspects in embryology and anatomy education, and includes recent findings regarding equal, diverse and inclusive anatomy teaching in the light of clinical relevance.

Key words: diversity, DSD-classification, embryology, medical education, life stages

Tears and dry eye – functional and clinical anatomy of the ocular surface

Friedrich Paulsen

Institute of Functional and Clinical Anatomy, Friedrich Alexander University Erlangen-Nürnberg, Erlangen, Germany

E-mail: friedrich.paulsen@fau.de

The ocular surface is a tightly integrated functional unit comprising the cornea, conjunctiva, lacrimal glands, meibomian glands, eyelids, and their neural connections. Its integrity depends on a stable tear film, which ensures optical quality, lubrication, and protection. This tear film consists of a lipid layer derived from meibomian glands, an aqueous component produced mainly by the lacrimal gland, and mucins secreted by conjunctival goblet cells and corneal and conjuctival epithelial cells. Dry eye disease (DED) is broadly classified into aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE), although overlap is common. ADDE results from insufficient lacrimal secretion and is often associated with aging, systemic autoimmune diseases, or drug-induced hyposecretion (e.g., anticholinergics, antihistamines). In contrast, EDE is primarily caused by excessive tear evaporation, most commonly due to meibomian gland dysfunction (MGD). MGD plays a central role in ocular surface pathophysiology. Anatomically, it involves obstruction of terminal ducts, acinar atrophy, and qualitative alterations of meibum. The resulting deficiency of the lipid layer leads to increased evaporation, tear film instability, and hyperosmolar stress. This triggers inflammatory signaling pathways, epithelial damage, and a vicious cycle of ocular surface deterioration. In addition to intrinsic gland dysfunction, multiple external and systemic factors contribute to DED. Aging leads to structural and functional decline of both lacrimal and meibomian glands. Contact lens wear alters tear film distribution and increases evaporation. Environmental conditions, particularly prolonged screen use in the context of “office eye syndrome,” reduce blink rate and promote tear film breakup. Furthermore, systemic medications may impair both aqueous and lipid secretion. Importantly, these factors rarely act in isolation. Instead, DED represents a multifactorial disease in which ADDE and EDE mechanisms interact. Dysfunction in one component of the ocular surface system propagates instability throughout the entire unit. A comprehensive anatomical and pathophysiological understanding of these interrelated mechanisms is essential for accurate diagnosis and for developing targeted therapies aimed at restoring tear film homeostasis and maintaining ocular surface health.

Key words: ocular surface, tears, dry eye disease, Meibomian gland, tear film

Nicotinamide Nucleotide Transhydrogenase (NNT) ablation, oxidative stress and testis function

Yaodi Liang, Marcel Jaklofsky, Maartje Verhoeven, Aleksandra Tsonkova, Misrat Masuma Parvez, Jaap Keijer, Katja Teerds*

Human and Animal Physiology, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

*Corresponding author e-mail: katja.teerds@wur.nl

Nicotinamide nucleotide transhydrogenase (NNT) is an inner mitochondrial enzyme that plays a critical role in maintaining cellular redox homeostasis through NADPH generation. Although NNT is essential for adrenal steroidogenesis, its role in testicular physiology and male reproductive function remains poorly understood. This study aimed to investigate the effects of NNT deficiency on redox balance and its consequences for Leydig cell function in mice. We evaluated oxidative stress markers, antioxidant enzyme expression, steroidogenic capacity, and seminiferous tubule morphology in 6-month-old NNT knockout (Nnt^{Δ}) and wild-type (Nnt^{wt}) 57CBL/6J mice by immunohistochemistry. NNT deficiency significantly increased the accumulation of oxidative stress markers, including 4-hydroxynonenal (4-HNE) and nitrotyrosine, by 7% ($p < 0.01$) and 10% ($p < 0.05$), respectively. Immunostaining of mitochondrial antioxidant enzymes SOD2 and PRDX3 was upregulated by approximately 20% ($p < 0.05$), along with an increase in IDH2 staining, indicative of activation of compensatory NADPH-production and antioxidant pathways. Despite these redox alterations, serum testosterone levels and the immunostaining of the key steroidogenic proteins, CYP11A1 and FDX1, remained unchanged. Histological analysis revealed a slightly reduced seminiferous tubule diameter and germinal epithelial thickness in Nnt^{Δ} mice compared to Nnt^{w} mice, accompanied by possible increased germ cell DNA damage as determined by yH2AX immunostaining. Furthermore, seminiferous tubule integrity was negatively correlated with SOD2 ($r=-0.626$, $p = 0.009$), PRDX3 ($r = -0.633$, $p = 0.009$), IDH2 ($r = -0.581$, $p = 0.018$), and oxidative stress markers 4HNE ($r = -0.557$, $p = 0.038$), Nitrotyrosine ($r = -0.576$, $p = 0.024$). In conclusion, NNT is essential for maintaining mitochondrial redox homeostasis in testicular Leydig cells. Although steroidogenic function appears resistant to NNT deficiency, increased oxidative stress is associated with small structural changes in the seminiferous epithelium. These findings highlight the importance of mitochondrial redox regulation in preserving the testicular microenvironment and supporting male reproductive health.

Key words: nicotinamide nucleotide transhydrogenase (NNT), Leydig cells, oxidative stress markers, seminiferous tubules

Plant-derived bioactive compounds and epigenetic cell reprogramming: dual strategies for diabetes treatment

Marija Đorđević*, Jovana Rajić, Ana Sarić, Jelena Arambašić Jovanović, Svetlana Dinić, Nevena Grdović, Aleksandra Uskoković, Mirjana Mihailović, Melita Vidaković

Department of Molecular Biology, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

*Corresponding author e-mail: marija.sinadinovic@ibiss.bg.ac.rs

Diabetes mellitus is a complex metabolic disorder characterized by impaired glucose homeostasis, β-cell dysfunction, and systemic oxidative stress. Natural plant-derived compounds have emerged as promising agents for the prevention and management of diabetes and its complications. Extracts from Gentianaceae species (*Gentiana dinarica* and *Gentiana utriculosa*) and *Centaurium erythraea* exhibit significant antidiabetic effects through multiple mechanisms, including the reduction of oxidative and nitrosative stress, improvement of glycemic control, preservation of pancreatic β-cell function, and protection against diabetes-associated cellular and organ damage in the liver, kidney, and erythrocytes. In addition, advanced drug delivery systems such as liposomal encapsulation enhance the bioavailability and therapeutic efficacy of compounds, emphasizing the importance of delivery optimization. Alongside these approaches, epigenome editing represents a distinct strategy aimed at restoring endogenous insulin production through targeted cell trans-differentiation. Using the EpiCRISPR system for targeted methylation of the *Arx* gene in pancreatic α-cells induces a phenotypic switch toward insulin-producing cells, demonstrating endocrine cell plasticity and its potential for therapeutic applications. Given the growing global prevalence of diabetes, advancing research into both plant-derived bioactive compounds and epigenetic reprogramming remains of critical importance. Plant-derived bioactive compounds offer an accessible, multifactorial approach for enhancing cellular protection and modulating metabolic imbalance and oxidative stress, whereas epigenetic reprogramming provides a precise, mechanism-driven strategy with the potential to directly restore endogenous insulin-producing cell populations.

Key words: antidiabetic, plants bioactive compounds, diabetic complication, epigenetic reprograming

Siponimod protects oligodendrocytes independently of adaptive immunity via S1PR5 signaling

Markus Kipp*, Newshan Behrangi, Emil Pril, Hannes Kaddatz

Institute of Anatomy, University Medical Center Rostock, Germany

*Corresponding author e-mail: markus.kipp@med.uni-rostock.de

Siponimod, a selective modulator of sphingosine-1-phosphate receptors (S1PR1 and S1PR5), is an approved therapy for multiple sclerosis and is primarily considered an immunomodulatory agent. However, accumulating evidence suggests additional direct effects within the central nervous system. Here, we investigated whether Siponimod exerts protective effects on oligodendrocytes independent of peripheral immune modulation. Using the cuprizone model of metabolic oligodendrocyte degeneration, we observed that Siponimod treatment significantly attenuates demyelination and oligodendrocyte loss. Notably, these protective effects were preserved in recombination-activating gene (RAG)–deficient mice, indicating that the observed neuroprotection occurs independently of adaptive immune cells such as B and T lymphocytes. To determine the receptor subtype mediating these effects, we examined S1PR5 knockout mice. In contrast to wild-type animals, Siponimod failed to confer protection in S1PR5-deficient mice. Moreover, S1PR5 knockout animals exhibited significantly more severe demyelination than wild-type controls, regardless of treatment, suggesting an intrinsic protective role of S1PR5 signaling in oligodendrocytes. Electrophysiological analyses revealed that action potential conduction following cuprizone-induced demyelination was markedly more impaired in S1PR5 knockout mice than in wild type mice. In vitro receptor assays demonstrated that Siponimod acts as an agonist at S1PR5 while exerting functional antagonism at S1PR1. S1P signaling pathways may, thus, represent a promising strategy to promote myelin preservation and functional recovery in demyelinating disorders.

Key words: Siponimod, S1PR5 signaling, oligodendrocyte protection, cuprizone model, demyelination

Acknowledgments: We thank all technical assistant of our Institute for their valuable support.

Genetic and epigenetic traits in cutaneous melanoma

Monica Neagu[1,2,3*], Elena-Georgiana Dobre[1], Carolina Constantin[1,2]

[1]*Immunology Laboratory, Victor Babes National Institute of Pathology, Bucharest, Romania*
[2]*Pathology Department, Colentina Clinical Hospital, Bucharest, Romania*
[3]*Doctoral School, University of Bucharest, Bucharest, Romania*

*Corresponding author e-mail: neagu.monica@gmail.com

The purpose of our study was to evaluate the genetic alterations that could affect the EGFR-RAS-RAF pathway in search of new druggable genetic targets. Droplet digital PCR (ddPCR) was used to evaluate cutaneous melanomas, benign nevi and normal skin samples. The samples subjected to ddPCR were analyzed also on RT-PCR and we found a perfect correlation between the data obtained on both technologies (Spearman correlation coefficient = 0.9913). The mutational status of healthy skin, nevi and melanoma specimens was assessed by ddPCR using BRAF V600, NRAS G12/G13, NRAS Q61, KRAS Q61 and EGFR exon 19 deletions Screening Assays according to manufacturer's instructions. We have found over 86% of the benign nevi harboring BRAF V600 mutations, while only 56% of skin melanomas harbored the same mutation. Benign nevi had a high expression of p16, p21, p53, bcl2 and cyclin D1 proving that cell cycle regulators are keeping the transformed melanocyte in an arrested cell cycle. In melanomas BRAF mutations significantly associated with Breslow index and tumor infiltrating lymphocytes, whereas NRAS mutations (NRAS Q61 and NRAS G12/G13) correlated with Breslow index and the mitotic index. Particularly, malignant transformation to a BRAF wild-type melanocytic tumor occurred in one of our cases involving a benign nevus with BRAF V600 positivity. ddPCR technique is sensitive so that we have detected in a normal skin sample NRAS G12/Q61 mutation, while the benign nevus excised from the skin harbored BRAF mutation. The high mutation profile of benign nevi was associated with a high expression of cell cycle regulatory proteins that could explain the melanocyte's arrested cell cycle within a benign nevus. Conclusion. The molecular profiling of cutaneous melanomas is of high importance in order to follow the dynamics of melanocyte transformation and orient the patient therapy.

Key words: melanoma, ddPCR, nevi, mutations

Acknowledgement. The study was financed by grants NASR, [PN 23.16.01.03] and ELI-RO 0_17/2024. Authors acknowledge the cooperation through COST Action CA21108 - European Network for Skin Engineering and Modelling, NETSKINMODELS.

Forensic medical analysis of closed head acceleration–deceleration injuries

Natasha Davceva[1,2,3*], Joze Balazic[2]

[1]*Institute of Forensic Medicine, Faculty of Medicine, University "Ss. Cyril and Methodius", Skopje, North Macedonia*

[2]*Faculty of Medical Sciences, Goce Del˘cev University, ˘Stip, Macedonia*

[3]*Faculty of Medicine, University of Maribor, Slovenia*

*Corresponding author e-mail: drdavcevamk@yahoo.com

A key classification of closed head injuries, particularly relevant for reconstructing trauma biomechanics and of greatest medicolegal importance, is the distinction between contact injuries and acceleration–deceleration injuries. The two most characteristic acceleration–deceleration injuries are acute subdural hematoma (ASDH) and diffuse axonal injury (DAI), which both arise from acceleration forces applied to the head but differ in the duration of these forces. ASDH typically results from rapid deceleration against a firm surface, as occurs in falls, falls accompanied by blows, or traffic accidents in which the head strikes the ground directly, with cyclists and motorcyclists being particularly vulnerable. In contrast, DAI is associated with prolonged acceleration–deceleration forces, usually of lower magnitude, and occurs most frequently in vehicular traffic accidents, where deformable or padded surfaces extend the deceleration phase. DAI may also result from falls from considerable heights. This review discusses the development of knowledge regarding DAI, its definition, diagnostic criteria, and key considerations, based on a literature review. Post-mortem detection requires a comprehensive forensic-neuropathological examination of fixed brain tissue, with immunohistochemistry using antibodies against β-amyloid precursor protein (β-APP) remaining the method of choice to visualize damaged axons and distinguish traumatic from ischemic patterns of injury. In conclusion, although ASDH and DAI are both acceleration injuries and may occasionally co-occur, current evidence suggests that their coexistence more likely reflects complex injury mechanisms rather than a single, shared pathway.

Key words: acute subdural hematoma, diffuse axonal injury, acceleration–deceleration injuries, closed head injury

The carotid body: from a simple oxygen sensor to a polymodal integration hub

Nikolai Lazarov[1,2*], Dimitrinka Atanasova[2,3]

[1]*Department of Anatomy, Histology, and Embryology, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria*
[2]*Department of Synaptic Signaling and Communication, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Anatomy, Faculty of Medicine, Trakia University – Stara Zagora, Stara Zagora, Bulgaria*

*Corresponding author e-mail: nlazarov@medfac.mu-sofia.bg

The carotid body (CB) is the main peripheral arterial chemoreceptor, enabling rapid and sensitive detection of blood chemical levels and triggering appropriate respiratory and cardiovascular responses to their deviations, thereby leading to the restoration of blood gas homeostasis. Current advances in CB research have demonstrated its intricate internal structure and remarkable ability to respond to changes in the physical parameters of blood, as well as to other blood-borne stimuli such as circulating modulatory molecules and various endogenous substances including inflammatory mediators. Accordingly, the CB is increasingly recognized as a polymodal integrated metabolic sensor rather than solely an oxygen sensor. Moreover, neurobiological studies have confirmed that chemoreception is not simply a linear signaling pathway, but rather a complex, integrated process involving intercellular communication within a functional unit, the CB chemosensory system. Nonetheless, the role of glomus cells as multimodal sensors and the molecular mechanisms underlying their responsiveness to changes in plasma levels of metabolites such as glucose and lactate, hormones like insulin and leptin, or alterations in blood flow, temperature, and osmolality remain to be fully elucidated. Recent progress in cell fate experiments has further revealed that the CB is a neurogenic center with a functionally active germinal niche. This discovery may lead to the development of promising new candidate therapies to combat certain human sympathetic-related and cardiometabolic diseases, ultimately improving the quality of life. In conclusion, the CB is a significantly more complex structure than originally believed, with diverse roles in health and disease. Expanding knowledge of the pathophysiological mechanisms that alter its cell function will undoubtedly help to facilitate the translational CB research.

Key words: carotid body, chemoreception, hormonal and immune regulation, metabolic sensor, polymodal sensing
Acknowledgements: This work was supported by the European Union-NextGenerationEU through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0004-C01.

Apolipoproteins have a major role in cellular tumor dormancy in triple negative breast cancer: in-silico study

Zaynab El-Gammal[1], Usama Bakry[1], Ahmed F. El-Sayed[1,2], Toka A. Ahmed[1], Gehad Atef Oura[1], Shimaa E. Elshenawy[1], Nagwa El-Badri[3], Amin F. Romany[1], Khaled Amer[1], Tarek Elnagdy[1,4], Osama Azmy[1,5*], Tarek Taha[1]

[1]*Egypt Center for Research and Regenerative Medicine (ECRRM), Cairo, Egypt*
[2]*Microbial Genetics Department, Biotechnology Research Institute, National Research Centre, Giza, Egypt*
[3]*Center of Excellence for Stem Cells and Regenerative Medicine, Zewail City of Science and Technology, Egypt*
[4]*Military Medical Academy, Cairo, Egypt*
[5]*Reproductive Health Department, Medical Research and Clinical Studies Institute, National Research Centre, Giza, Egypt*

*Corresponding author e-mail: osamaazmy@nrc.sci.eg/ osamaazmy@ecrrm.ac.eg

Triple-negative breast cancer (TNBC) lacks estrogen, progesterone, and human epidermal growth receptors, with poor prognosis. The oocyte's embryonic milieu presents a unique tumor reversion microenvironment by inducing growth arrest. Oocyte extraction (OE) proteins induced the reversion of cancer cells from an aggressive to a dormant state. We filtered OE proteins based on their function, similarity, resolution, and the X-ray method. We acquired the OE protein structure from the Protein Data Bank. Subsequently, we used PyMOL to eliminate water and small co-crystallized molecules, then utilized DoGSiteScorer server to identify potential binding pockets. We performed docking analysis using the ClusPro server and HDock server and LigPlot+ and UCSF Chimera for complex interactions and visualizations. To assess the stability and flexibility of the proteins, we performed MD simulations using the GROMACS software. The proteomic profiling of OE revealed 478 proteins, with 29 proteins selected for molecular docking. Results showed low energy scores for complexes between OE proteins and four surface markers. APOA1 and APOC3 showed the highest stability and affinity with four surface markers namely, K1C14 (-3751.6, -4011.6, respectively), CLD3 (-3461.6, -3087.7, respectively) and CLD4 (-3301.1, -3031.7, respectively). The root mean square deviation (RMSD) for APOA1-K1C14 complex is the highest stable complex between (0.20 - 0.3 nm), followed by APOA1-CLD4 complex (between 0.30–0.35 nm), then APOA1-ITA6 complex (between 0.4–0.60 nm) and APOA1-CLD3 complex (between 0.4–0.70 nm). The radius of gyration ranges between 1.66 to 1.70 nm for the APOA1-CLD3 complex. The APOA1- K1C14 complex formed the highest number of hydrogen bonds (15-35 bonds). In conclusion, APOs most likely are the proteins involved in tumor dormancy induction. These proteins are involved in key different tumor-related pathways such as angiogenesis, proliferation, apoptosis, and migration. This explored a novel therapeutic options to induce dormancy in TNBC cells.

Tritrichomonas lectins with specificity for sialic acid. Biological functions and practical application.

Pavel Babál

Department of Pathology, Faculty of Medicine Comenius University, Bratislava, Slovakia

E-mail: pavel.babal1@gmail.com

Both *Tritrichomonas mobilensis* and *Tritrichomonas foetus* produce hemaglutinins with a property of lectins showing affinity exclusively for sialic acid. Both trichomonads induce diseases of their host animals: enterocolitis in squirrel monkeys and vaginitis and endometritis with infertility in cattle. Both lectins bind to NeuAc and NeuGc, in both α2-3, α2-6 and less in α2-8 linkage. The *T. mobilensis* lectin (TML) and the *T. foetus* lectin (TFL) are expressed on the parasites surface and function in the process of trichomonads adhesion to the mucosal surfaces. TML is highly stabile and thanks to its exclusive specificity for sialic acid it finds the use in sialylated compounds detection and isolation by affinity chromatography. Pathological processes like inflammation and neoplastic transformation are accompanied by changes in protein glycosylation with sialic acid. Neoplastic transformation of the thyroid gland is accompanied by over 2-fold increase of sialylated glycoconjugates expression, prevalently with sialic acid in the α2-3 linkage. The sialylation changes in the process of neoplastic transformation of the thyroid gland tissue is linked to changed sialyltransferases and neuraminidases expression, especially the overexpression of SIAT4c and SIAT4b. Increased sialylation is found in the skeletal muscle cells after invasion by the parasite *Trichinella spiralis*. On the contrary, prostate cancer shows decreasing levels of sialylation with increasing aggressivity of the neoplasm. In conclusion, it can be stated that: a) Neoplastic and also non-neoplastic pathological processes in tissues and organs are linked with changed levels of sialylated glycoproteins. b) Sialylation changes are accompanied with changed expression of sialyltransferases and neuraminidases. c) Evaluation of sialylation changes and different expression of sialyltransferases and neuraminidases may serve as useful diagnostic and prognostic tools in pathological conditions.

Key words: sialic acid, lectin, neoplasia, parasites

The male genital tract in motion - smooth muscle cells in male reproductive organs

Ralf Middendorff

Institute of Anatomy, Justus-Liebig University Giessen, Germany

E-mail: Ralf.Middendorff@anatomie.med.uni-giessen.de

The relevance of smooth muscle cells (SMCs) in male reproductive organs is underestimated. SMCs are rarely mentioned when talking about organ function and dysfunction. Aim of this study was the morphological and functional characterization of contractile structures in the testis, epididymis, seminal vesicle and prostate. For this, we used time lapse imaging, 3-dimensional immunostainings ("Clarity"), Micro-CT and a novel method of imaging analysis for the evaluation of multidirectional contractions. In the testis, an association of contraction patterns with spermatogenic stages was visualized. The second messenger cGMP shifted frequency spectra towards lower frequencies. In the epididymal duct, spontaneous contractions in the context of sperm transport were revealed. Surprisingly, such contractions were already present before puberty. Only the distal part of the adult cauda, relevant for storage and emission of sperm, did not contract spontaneously. Here we showed that not only noradrenaline but also oxytocin rapidly triggered the same complex emission-related contraction pattern. In the prostate, spontaneous contractility of gland-associated interstitial SMCs was described. The structure of the prostatic excretory ducts was defined and their SMCs were found to be without spontaneous contractions. Conventional treatment of benign prostate hyperplasia with alpha1-adrenergic blockers (e.g. tamsulosin) frequently elicits side effects on emission/ejaculation. We showed that tamsulosin disturbed noradrenaline-induced contractions of prostate ducts, seminal vesicle and cauda epididymidis. In contrast, the alternative drug tadalafil was without such negative effects. In summary, we could visualize and describe contraction patterns of SMCs in male reproductive organs revealing important functional aspects, underlying mechanisms and (side)effects of relevant drugs.

Key words: male reproductive organs, smooth muscle cells

Modes of inflammasome activity and their implications for the severity in human pathology

Soren Hayrabedyan*, Yuliia Mariienko, Ilka Tsvetkova, Krassimira Todorova

Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: shayrabedyan@ibir.bas.bg

Inflammasomes are cytosolic supramolecular platforms that translate cellular stress and pathogen-associated signals into inflammatory cytokine processing and regulated cell death. Our work identifies inflammasome activation as a shared pathogenic mechanism in reproductive and vascular pathology, with context-dependent effects on immune tolerance, tissue barrier function, and cell-death modality. In Sertoli cells, we previously demonstrated a functional NLRP3 inflammasome capable of modulating autophagy and cytokine production, supporting the concept that Sertoli cells are active innate immune regulators in male infertility rather than passive structural components of the seminiferous epithelium. NOD and TLR4-dependent danger signaling were associated with caspase-1 activation, IL-1β-related inflammatory output, autophagy remodeling, and potential disruption of testicular immune privilege. Moreover, inflammasome activation was shown to contribute also to apoptosis through functional caspase-1/caspase-3 interplay. This supports a hybrid inflammatory–apoptotic axis in which inflammasome signaling directs the fate of stressed cells beyond the classical pyroptotic paradigm. SARS-CoV-2 ORF3a induced ASC-associated inflammasome reorganization and caspase-1 engagement in microvascular lung endothelial cells, supporting endothelial inflammasome activation as a contributor to COVID-19 vascular injury. The observed canonical speck-like and distributed multifocal ASC/caspase-1 activation patterns suggest that ORF3a may promote heterogeneous inflammasome responses, ranging from controlled inflammatory signaling to extensive pyroptotic cell death. This duality may have important clinical implications for pathologies in which inflammasome activity supports tissue adaptation and immune surveillance, but may also drive infertility, endothelial injury, vascular inflammation, and severe disease progression.

Key words: inflammasome, NLRP3, caspase-1, Sertoli cells, SARS-CoV-2

Acknowledgments: Research data was generated using RI build within Consortium INFRAACT funded under Ministry of Education and Science Roadmap for RI program – grants #361/2023, #105/2025

Depicting layered anatomy of the temporal region in regards to surgical and non-surgical aesthetic interventions

Selcuk Tunali[1,2,3]

[1]*TunaliS Anatomy Institute, Ankara, Turkey*
[2]*John A. Burns School of Medicine, University of Hawaii, Honolulu, USA*
[3]*Faculty of Medicine, TOBB University of Economics and Technology, Ankara, Turkey*

E-mail: selcuk@hawaii.edu

The anatomy of the temporal region is one of the most complex and critical areas for aesthetic interventions, requiring a precise understanding of its layered architecture to ensure both safety and efficacy. This region is characterized by a "sandwich-like" arrangement of tissues, including the skin, subcutaneous fat, multiple fascial layers, and the temporalis muscle. A thorough understanding of these planes is essential for practitioners performing soft tissue filler injections, thread lifts, or surgical procedures. The layers are organized from superficial to deep: the skin, subcutaneous tissue containing superficial fat and reticular cutis, the superficial temporal fascia (temporoparietal fascia) including the rudimentary mimetic muscles around the auricle, the sub-fascial loose areolar tissue, and the deep temporal fascia, which splits into superficial and deep layers to enclose the temporal fat pad. The superficial temporal fascia is a direct cephalad extension of the galea aponeurotica, in other words the SMAS (Superficial Musculoaponeurotic System) and contains the superficial temporal artery and vein. The most significant risk in this region involves the frontal (temporal) branch of the facial nerve, which runs within or just deep to the superficial temporal fascia. Clinically, "temporal hollowing" is a hallmark of facial aging, caused by the depletion of both superficial and deep fat pads and bony remodeling. Aesthetic procedures aim to restore this volume to create a smooth transition between the forehead and the midface. To avoid vascular compromise and nerve injury, practitioners should be aware of the anatomy of the area. In conclusion, navigating the temporal region demands a rigorous adherence to anatomical boundaries. Mastery of these fascial layers and the precise localization of neurovascular structures allow clinicians to achieve harmonious facial rejuvenation while minimizing the risk of severe complications.

Key words: temple, anatomy, SMAS, superficial temporal fascia, deep temporal fascia

Tiny beams – new hope for cancer treatment

Valentin Djonov

Institute of Anatomy, University of Bern, Bern, Switzerland

E-mail: valentin.djonov@unibe.ch

Submillimeter Spatially Fractionated Radiotherapy (smSFRT) modulates the radiation dose on a micrometre scale by delivering the dose via tiny beams. This unique dose heterogeneity makes smSFRT highly efficient even for the treatment of radio/chemo/surgically-resistant tumours, while also displaying high normal, non-cancerous, tissue tolerance at the same time. In murine B16-F10 melanoma model, temporally fractionated smSFRT completely ablated 50% of tumors and prevented organ metastases and local recurrences for 18-months after treatment. In rat/mouse glioblastoma model, smSFRT, in combination with cisplatin, reduced the tumor volume 6-fold compared with cisplatin alone, and 60-fold compared with untreated mice. The radiation biology underlying the "SFRT effect" includes novel radiobiological mechanisms:
(1) vascular effects including (a) induction of selective vascular disruption of immature tumor vasculature (f.E. single smSFRT treatment reduced the blood vessel volume in orthotopic GBM by around 70%), or (b) induction of transient vascular "permeability window" in a dose-dependent manner.
(2) direct cellular damage in the microbeam path that elicits tissue-specific responses.
(3) induction of a unique, tumor-targeted immune response leading to local and systemic anti-tumor immune answer, process we call "*in situ* vaccination".
First studies indicate that smSFRT is clinically highly efficient even in treatment-resistant malignancies without other therapy options. SmSFRT demonstrates one of the best treatment outcomes ever achieved in preclinical models. At higher doses, smSFRT could serve as a very potent angio-disruptive and anti-angiogenic treatment. At lower doses, smSFRT increases transiently, the vascular permeability, while preserving vessel integrity, which is serving as a very efficient mechanism for drug delivery. In addition, smSFRT breaks the host anti-tumor immune response, by increased antigen presentation, activation and augmentation of Cytotoxic T Lymphocytes. These unique features support smSFRT as a novel therapeutic approach for the treatment of inoperable, and otherwise untreatable, radioresistant lesions.

Key words: radiotherapy, microbeams, combined radiotherapy treatment, SFRT

ORAL PRESENTATIONS

Comparative study of body composition in adolescent athletes practicing combat sports

Albena Borislavova Dimitrova[1,2]

[1]*National Sports Academy "Vassil Levski", Sofia, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Corresponding author e-mail: albena_84@abv.bg

Combat sports comprise a variety of disciplines focused on direct physical competition. While they differ in dynamics and workload, they consistently require rigorous technical training and high-intensity exercise. In combat sports, body composition is a critical factor in achieving success, as competitors are divided into weight classes. The optimal profile always includes low levels of body fat and high muscle mass, allowing the athlete to be as strong and fast as possible for the category. Most elite athletes maintain a low to normal body fat percentage while striving to develop skeletal muscle. The current study aimed to present a comparative analysis of segmental and whole-body composition between male adolescent athletes (22 judokas and 12 boxers). Whole- and segmental body analysis were performed by using bioelectrical impedance measurements (InBody 170 analyzer, Korea). Descriptive statistics were generated using SPSS 16 (IBM, USA), and the distribution of normality was assessed. The independent samples t-test was used to evaluate the differences in body composition. Significant differences in whole-body composition were observed for the mean values of muscle mass, fat-free mass and total body water with a particular emphasis on judokas. Segmental analysis of body composition showed a significantly higher muscle mass in all body segments of the judokas athletes and of the percent body fat in upper and lower limbs of boxers.

Key words: combat sports, adolescent athletes, body composition, segmental analysis

Anatomical connections of the tVTA/RMTg

Alessandro Bilella[1,2*], Elisabeth Eppler[1], Michel Barrot[3]

[1]*Institute of Anatomy, University of Bern, Bern, Switzerland*
[2]*Anatomy University of Fribourg, Fribourg, Switzerland*
[3]*Centre National de la Recherche Scientifique, Université de Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, Strasbourg, France*

*Corresponding author e-mail: alessandro.bilella@unibe.ch

The tail of the ventral tegmental area/rostromedial tegmental nucleus (tVTA/RMTg), is a bilateral cluster of GABAergic neurons located in the most caudal part of the ventral tegmental area (VTA). The tVTA/RMTg receives dense projections from the lateral habenula (LHb) and sends projections to the midbrain dopamine system (VTA, SNC) to the dorsal raphe (DR), to the extrapyramidal neurons, and the reticular formation in the midbrain. We performed a stereotactic injection in the tVTA/RMTg, in the globus pallidus (GP), nucleus accumbens (Acb) and ventral pallidum (VP) of adult male rats using dextrane biotin amine (BDA) as an anterograde tracer and Fluorogold as a retrograde tracer.We identified several brain regions, in the telencephalon, diencephalon and mesencephalon, that may play a role as relay centres between the tVTA/RMTG, the GP, AcbSh, AcbC and the VP.The LHb activity is modulated by the excitatory and inhibitory inputs arising from the GP, the VP and indirectly from the Acb. The VP, together with the LHb, the VTA and the SNC, modulates the limbic and cognitive stimuli integrating motivation and action. The relationship between the tVTA/RMTg and the basal ganglia leads us to better define the role of this pathway in modulating the midbrain dopamine system activity in relation to the aversive responses, the reward system and the locomotor activity.

Changes in the components of human somatotype due to the impact of type 1 and type 2 diabetes mellitus in Bulgarian patients

Atanas Baltadjiev*, Tsvetanka Petleshkova, Zdravka Harizanova, Kristian Argirov, Katerina Topalova

Department of Anatomy, Histology, and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

*Corresponding author e-mail: atanas.baltadjiev@mu-plovdiv.bg

The aim of the present study is to research the changes in the human somatotype components in adult Bulgarian patients with type 1 and type 2 Diabetes mellitus. More than 500 patients were involved in the study. They were divided into six groups by age and sex: male and female patients aged 20-40 years, male and female patients aged 41-60 years, male and female patients aged 61-80 years. Two hundred and forty healthy participants were enrolled in the study, divided into the same groups. Directly measured anthropological parameters were height, body weight, biepicondylar width of humerus and femur, contracted and relaxed arm, forearm, thigh and calf circumferences, and 9 skin folds. The regression equations introduced by Heath and Carter were used to define the somatotype components: endomorphy, mesomorphy and ectomorphy. The mean somatotype of female Bulgarian patients aged 20-40 years was defined as mesomorphic endomorph. However, the mean somatotype of the same aged healthy Bulgarian women was considered to be balanced endomorph. The mean somatotype of diabetic females aged 40-60 years was defined as mesomorph-endomorph while of the healthy women was considered to be mesomorphic endomorph. The mean somatotype of female patients aged 61-80 years was defined as endomorphic mesomorph while of healthy female controls at the same age mesomorph – endomorph. The somatotype of the male patients of the reported three groups was defined as endomorphic mesomorph. The impact of the Diabetes mellitus has been more expressed in the female Bulgarian patients than in the male Bulgarian patients.

Key words: Diabetes mellitus, Bulgarians, somatotype, components

Exposure to novel environment enhances memory stability in the retrosplenial cortex of Wistar rats

Bogomil Peshev[1*], Desislava Krushovlieva[1], Pavel Rashev[1,2], Dimitrinka Atanasova[1,3], Jana Tchekalarova[1]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences (BAS), Sofia, Bulgaria*
[2]*Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Anatomy, Faculty of Medicine, Trakiya University, Stara Zagora, Bulgaria*

*Corresponding author e-mail: bogpeshev@gmail.com

It has been demonstrated that environmental novelty enhances the stabilization (initial consolidation) of memories for specific events (episodic memory) in the dorsal hippocampus of the brain in mammals. This modulatory effect of novelty has been related to substantial increase in the levels of the catecholaminergic neurotransmitter dopamine and the initiation of *de novo* synthesis of plasticity-related proteins. The retrosplenial cortex (RSC) is a cortical region that is particularly active during the early stages of episodic memory formation. However, it remains unclear if novelty-induced increase in dopamine levels could also enhance initial consolidation in the RSC. To study this particular matter, we conducted a series of experiments that combined a low-intensity version of the contextual fear conditioning (CFC) paradigm with subsequent short-lasting exposure to a novel enriched environment (EE) cage. In brief, experimental animals (Wistar rats) first underwent the CFC training to establish a weak short-term memory, which was followed by a subsequent exposure to the EE cage 30 minutes later. We hypothesised that the EE session will increase dopamine release in RSC, therefore enabling the stabilization of the weak CFC memory. To examine the role of dopamine and protein synthesis for this potential enhancement of memory stability, a subset of experimental animals received intracranial infusion of the dopamine D1 receptors antagonist, SCH 23390, or the protein synthesis inhibitor, Anisomycin, in the anterior part of the RSC immediately after the behavioral procedures. After 24 hours the experimental animals were subjected to a planned test session to evaluate their behavioral responses. We observed markedly better performance in rats that were exposed to novelty, but were not subjected to SCH 23390 or Anisomycin infusion. Overall, our experiments provide key evidence for novelty-induced enhancement of initial consolidation in aRSC. This effect is similar to what was previously described in the dorsal hippocampus.

Key words: retrosplenial cortex, novelty, initial consolidation, dopamine, environmental enrichment
Acknowledgments: This work is funded by the National Science Fund of Bulgaria (research grants: КП-Н06-81/7; КП-06-Н61/12)

Hydra peptide pedin stimulates neurorepair and remyelination in mice

David Lutz[1,2]

[1]*Department of Anatomy and Cell Biology, Medical University Varna, Varna, Bulgaria*
[2]*Vascular Biology Research Group (RenEVA), Research Institute, Medical University – Varna, Varna, Bulgaria*

Corresponding author e-mail: david.lutz@mu-varna.bg

Cnidarians exhibit remarkable regenerative capacity, supported by a diverse repertoire of small signaling molecules that coordinate whole-body and neural repair. Whether such molecules can be leveraged to promote neuroregeneration in higher organisms remains unknown. Here we identify pedin, a Hydra-derived foot morphogen peptide, as a potent inducer of myelination and neurorepair in mice. Pedin enhances neurite outgrowth in dorsal root ganglion and cortical neurons, promotes process extension in Schwann cells, and drives Golgi apparatus remodeling. At the functional level, pedin modulates neuronal electrical impedance and calcium dynamics, increases glial ensheathment, and promotes neurite–glial alignment, thereby facilitating myelination in vitro. In a mouse model of femoral nerve transection, pedin guides axonal regrowth across the lesion gap and restores gait function. These findings establish that cnidarian-derived peptides can enhance neurorepair and counteract demyelination in mammals, revealing a previously unrecognized class of pro-regenerative signals. More broadly, they point to the conservation of ancient peptide-mediated repair mechanisms that engage fundamental neuroglial programs shared across metazoan evolution.

Key words: demyelination, neurorepair, small-molecule therapeutics, neurodegeneration

Acknowledgments: This research was funded by the NextGenerationEU via the Bulgarian National Recovery and Resilience Plan, Project #BG-RRP-2.004-0009-C03 (to D.L.), and by the National Scientific Program "Excellent Research and People for the Development of European Science" 2021 (VIHREN) of the Bulgarian National Science Fund, contract #KP-06-DV/7 from 17.12.2024 (to D.L.).

The role of cholesterol and oxidative stress on the morphology of flagellated cells

Desislav Dinev*, Anton Kolarov, Vladislav Lazarov, Irina Chakarova, Valentina Hadzinesheva, Katerina Kavaldzhieva, Kalina Belemezova, Nikola Mladenov

Department of Biology, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria

*Corresponding author e-mail: d.dinev@medfac.mu-sofia.bg

Trypanosomes and animal spermatozoa have little in common, except that both are eukaryotic cells existing in suspension and moving freely via a single flagellum. Cell morphology, including that of the flagellum as a defining structural element, is sensitive to changes in membrane composition. Cholesterol levels can alter membrane characteristics such as fluidity, rigidity, stability, and the boundaries between cell domains. Oxidative stress is another factor affecting membranes, both directly through the oxidation of unsaturated fatty acids and indirectly through its overall influence on the cell. These membrane-related factors can lead to pronounced alterations in cellular shape, motility, and structural integrity. We subjected mammalian spermatozoa and *Trypanosoma equiperdum* to two types of treatments: cholesterol enrichment and oxidative stress induction. We then observed the morphology of Giemsa-stained treated cells to detect and analyze the resulting changes, as well as their similarities and differences between the two cell types.

Key words: membranes, cholesterol, oxidative stress, Trypanosomes, morphology

Acknowledgements: This work was supported by Grant No. D-163/04.06.2025 of the Medical University of Sofia.

Antiganglioside antibodies titer in serum of patients with Alzheimer's disease

Vera Kolyovska[1*], Elica Mihaylova[1,2], Iskra Sainova[1], Sonya Ivanova[2], Todor Belev[3], Desislava Drenska[4], Dimitar Maslarov[4,5]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2]*University Hospital for Neurology and Psychiatry "St. Naum", Sofia, Bulgaria*

[3]*Medical University of Sofia, Sofia, Bulgaria*

[4]*Neurology Clinic, First MHAT "St. John Krastitel", Sofia, Bulgaria*

[5]*Medical College "Y. Filaretova", Medical University of Sofia, Neurology Clinic University First MHAT, "St. John Krastitel", Sofia, Bulgaria*

*Corresponding author e-mail: verakol@abv.bg

Two decades ago, the diagnosis of Alzheimer's disease (AD) was made only at autopsy. Today, the pathology is much better understood and the diagnosis is often made by a combination of clinical signs and biomarkers. Especially in the early stages, when clinical manifestations often overlap with other forms of dementia. Gangliosides are a class of acidic glycosphingolipids, which are 10% of the lipids in the nervous system. The titer of the different antiganglioside antibodies is a marker for different types of pathological disorder: GM1 – a marker for damage to the myelin sheath, GM3 – a marker for the presence of an inflammatory process, GD1a – a marker for neuronal damage. Using the ELISA technique, we have studied the titer of antiganglioside antibodies in the blood serum of patients with PET-proven AD. These values have been compared with those of patients with other types of dementia and people with diabetes, as well as with parameters in healthy people.Our results show that anti-GM1 IgG antibodies are elevated in both patients with AD and diabetes. Anti-GM3 IgG antibodies are less elevated in both patients with AD and patients diagnosed with diabetes. Anti-GD1a IgG antibodies are elevated in patients with other types of dementia and diabetes and react with a slightly elevated titer in AD. The result for antibodies against common gangliosides is interesting – they react with a high titer, but only at a dilution of 1:40 in samples from AD patients. These antibodies react with a high titer in patients with high sugar. AD can be accompanied not only by protein accumulation, but also by vascular changes, for example in patients with long-term elevated glucose. In our next analyses, our goal is to check whether this indicator can distinguish AD from other types of dementia.

Key words: antiganglioside IgG antibodies, Alzheimer's disease, serum, ELISA, patients with diabetes

Molecular characterization of endometrial cell lines

Evgenija Sotirovska[1*], Jong-Hun Park[1], Pavel Videv[1], Kalina Ganevska[1], Stefani Kovachka[1], Rosen Ivanov[5], Liliya Koleva[2], Anatoly Kolev[2], Kristian Chechev[3], Bozhidar Karamishev[3], Daria Koleva[3], Stefani Petrova[1], Yordanka Gluhcheva[5], Vesela Karamisheva[3,4], Svetla Petrova[1], Kirilka Mladenova[1], Jordan Doumanov[1]

[1]*Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd., Sofia, Bulgaria*
[2]*UMBHALSM "N.I.Pirogov", 21 Totleben Blvd., Sofia, Bulgaria*
[3]*Medical University – Sofia, 15 Acad. Ivan Geshov Blvd., Sofia, Bulgaria*
[4]*SBALG "Maichin Dom", 2 Zdrave St., Sofia, Bulgaria*
[5]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Acad. Georgi Bonchev Str., Bl. 25, 1113 Sofia, Bulgaria*

**Corresponding author e-mail: evgenija.sotirovska188@outlook.com*

Endometrial cancer (EC) is the most common gynecological malignancy worldwide and the sixth most frequent cancer in women. It arises from uncontrolled proliferation and invasion of cells in the uterine lining. EC is classified into two main types. Type I (endometrioid adenocarcinoma) is low-grade, estrogen-dependent, and often progesterone-sensitive. It typically develops in the context of endometrial hyperplasia, is diagnosed at an early stage, and has a favorable prognosis with effective treatment. In contrast, Type II (serous carcinoma) is high-grade, estrogen-independent, and associated with deep myometrial invasion and metastasis. It arises from atrophic endometrium or precursor lesions and has a poor response to hormone therapy, resulting in a worse prognosis. Estrogen mediates its effects through two nuclear receptors - estrogen receptor α (ERα) and estrogen receptor β (ERβ), which exert opposing roles in endometrial biology. The balance between biological activity of these receptors is essential for maintaining endometrial homeostasis, and disruption of this equilibrium may contribute to malignant transformation. Cell lines derived from the endometrial cancer are essential for studying tumorigenesis mechanisms *in vitro*. In this study, we examined the expression of ERα and ERβ in normal and malignant endometrial tissues using immunohistochemistry, as well as in our newly established endometrial cell lines. We further analyzed the expression and subcellular localization of caveolin-1 (CAV1) and zonula occludens-1 (ZO-1) using immunofluorescence to assess changes in cellular morphology, polarity, and junctional integrity in the new established cell lines. CAV1 was additionally evaluated for its potential role in estrogen receptor signaling. By integrating histopathological, molecular, and structural analyses, this study enhances understanding of endometrial cancer biology and tumor heterogeneity.

Key words: endometrial cancer, estrogen receptor, caveolin1, ZO-1, immunohistochemistry, immunofluorescence
Acknowledgements: This work was supported by grant from Bulgarian Science Fund № KP-06-N83/7, 05.12.2024 and partially by grants from the Ministry of Education and Science of Bulgaria under contract DO1-361/2023 and from Sofia University Science Fund № 80-10-170, 22.04.2026 and 80-10-166, 22.04.2026.

Testicular and sperm alterations induced by exposure to polystyrene microplastics during juvenile development in Wistar rats

Hristiyana Kanzova[1*], Stefan Manchev[2], Rosen Ivanov[3], Despina Pupaki[2], Desislava Ankova[2], Hristo Gagov[4], Iliyana Sazdova[4], Milena Mishonova[4], Neli Raikova[4], Lea Koceva[4], Andrey Ivanov[1,5], Todor Kirov[3,6], Dimitrinka Atanasova[1,7], Pavel Rashev[2], Madlena Andreeva[1]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[4]*Department of Animal and Human Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*
[5]*University Hospital "Saint Anna"- Sofia, Nephrology Clinic, Sofia, Bulgaria*
[6]*Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University – Sofia, Sofia, Bulgaria*
[7]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*

*Corresponding author e-mail: hristiyanakanzova@gmail.com

Emerging environmental contamination with microplastics (MPs) has raised significant concerns regarding their potential impact on reproductive health, particularly during critical developmental windows. This study aimed to evaluate the effects of subchronic oral exposure to polystyrene microplastics (PS-MPs) of different sizes on the male reproductive system in Wistar rats, starting in the juvenile period. Male rats at 30 days of age were exposed to PS-MPs (1 µm and 5 µm) for 30 days, covering the late stages of sexual maturation. Sperm motility and morphology, as well as histological alterations in the testes, were assessed. In addition, the distribution and accumulation of microplastics within testicular tissue were examined using fluorescence-based imaging techniques. Exposure to PS-MPs resulted in mild, size-dependent impairments in sperm quality, including reduced motility and an increased frequency of morphological abnormalities, more pronounced in animals exposed to smaller particles. Histological analysis revealed early degenerative changes, such as slight disorganization of the seminiferous epithelium and reduced germ cell density more prominent in the 1 µm group rather than the 5 µm group, without severe structural damage. Fluorescence imaging confirmed the presence and accumulation of microplastics in the testes, indicating their ability to penetrate and persist in reproductive tissues even after short-term exposure. In conclusion, 30-day oral exposure to PS-MPs starting in the juvenile period induces early, subclinical, and size-dependent alterations in the testes, affecting sperm parameters and tissue structure.

Key words: polystyrene microplastics (PS-MPs), male reproductive toxicity, sperm quality, testicular histopathology, microplastic accumulation

Acknowledgments: This work was supported by Grant № KP 06-M83/2, National Science Fund, Bulgaria

A rare case of spinal dysraphism in Early Iron Age burial

KaloyanVassilev

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

Corresponding author e-mail: vasilev.kaloian@abv.bg

During rescue archaeological excavations of a cemetery from the late 19th and early 20th centuries, in the "Kaleto" area near the center of the modern town of Svishtov, conducted in September 2025, a pit was investigated, at the bottom of which skeletal remains of two individuals were discovered. Ceramic fragments dating from the late Iron Age were registered in the pit filler. The skeletal remains of both individuals are completely preserved and in good condition, allowing for a complete anthropological analysis. The first individual is lying in an arcuate position along the periphery of the pit. The second is lying on his back, with his limbs extended, slightly turned outward at the elbow joints, his lower limbs bent at the knee joints. Reddish pigmentation was found in the area of the feet, on the bodies of the long bones of the limbs and on the skull, probably due to the use of red ochre during the funeral ritual. The posture and use of red ochre in burial are well known in the Early Bronze Age, which necessitated the use of C^{14} to refine the dating. Anthropological analysis showed that the remains belonged to a female (individual 1) and a male (individual 2). The two individuals were in their mature age of 40-50 years. The height of both individuals was calculated using the Trotter-Gleser formulas, respectively 160.38 cm for individual 1 and 170.39 cm for individual 2. Some variations and defects were observed in the bone remains, and in individual 2, the rare defects of fusion of the vertebral arches in the cervical and lumbar spine, which can also be considered as spina bifida, were detected.

Key words: anthropology, archaeology, pathology, variation, spinal dysraphism

Prolonged “ex situ” liver preservation using an original perfusion device

Keti Tsomaia[1,2], Nino Inauri[1], Otar Philishvili[1], Nodar Khodeli[1,2]

[1]*Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*

[2]*Alexandre Natishvili Institute of Morphology, TSU, Tbilisi, Georgia*

*Corresponding author e-mail: ketie.tsomaia@tsu.ge

Prolonged *ex situ* liver preservation remains a critical challenge in transplantation, particularly for marginal donor organs. Recent advances emphasize the importance of maintaining physiological perfusion conditions and minimizing hemolysis during long-term perfusion. The aim is to develop an original perfusion device, scheme, and method for prolonged *ex situ* liver preservation and to evaluate its advantages over standard approaches. Experimental studies were conducted on porcine models (20–22 kg) using a self-designed perfusion machine based on a universal two-chamber atraumatic blood pump capable of generating both continuous and pulsatile flow. Several experimental groups were established, including standard perfusion, pulsatile perfusion, combined liver–kidney perfusion, and prolonged (24-hour) preservation using a specialized organ container. Hemodynamic, biochemical, and morphological parameters were monitored. Pulsatile perfusion demonstrated clear advantages over standard methods, including absence of hemolysis, stable hematological parameters, improved oxygenation, and significantly increased bile production. The integration of a donor kidney into the perfusion circuit enabled effective metabolic clearance, replacing the need for a dialyzer. The developed organ container maintained near-physiological temperature and humidity, preventing tissue damage and enabling stable organ metabolism. Successful preservation was achieved up to 24 hours, with maintained liver function (lactate clearance, glucose metabolism, coagulation activity) and preserved histological architecture without necrosis. The developed perfusion system, incorporating a pulsatile flow pump, simplified circuit design, and physiological organ container, enables effective prolonged *ex situ* liver preservation. The approach is technically feasible, cost-effective, and provides a platform for improving donor organ utilization and future regenerative strategies.

Key words: liver preservation, machine perfusion, pulsatile flow, transplantation, ex situ perfusion

Acknowledgments: This study was supported by the Shota Rustaveli National Science Foundation of Georgia (Project №FR-24-18604).

Apelinergic signaling in the hippocampus tracks memory dysfunction in the cuprizone model

Kristina Petrova[1*], Lyubomir Gaydarski[1], Nikola Stamenov[1], Tsvetko Obetsanov[1], Alexandar Iliev[1], Stancho Stanchev[1], Pavel Rashev[2], Despina Pupaki[2], Milena Mourdjeva[2], Ivanka Kostadinova[3], Milen Hristov[4], Boycho Landzhov[1]

[1]*Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria*
[2]*Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Pharmacology, Pharmacotherapy and Toxicology, Medical University of Sofia, Sofia, Bulgaria*
[4]*Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria*

*Corresponding author e-mail: kpetrova@medfac.mu-sofia.bg

Multiple sclerosis is a chronic demyelinating disease of the central nervous system frequently associated with cognitive impairment, including deficits in learning and memory. The hippocampus plays a critical role in memory processing. The Apelinergic system, comprising Apelin and its receptor APLNR, has been implicated in neuroprotection. However, its role in hippocampal alterations during demyelination and remyelination remains insufficiently understood. Thirty 8-week-old male C57BL/6 mice were randomly assigned to three groups (n = 10): CO (normal water), DE (0.2% cuprizone in drinking water for 5 weeks), and RE (0.2% cuprizone for 5 weeks followed by 3 weeks of normal water). Immunohistochemical analysis was performed using anti-APLNR and anti-Apelin antibodies. Quantification of marker expression was conducted using ImageJ. Cognitive function was assessed using the passive avoidance test. Statistical analysis was performed using GraphPad Prism. Data distribution was evaluated using the Shapiro–Wilk normality test. Group comparisons were conducted using the Kruskal–Wallis test followed by Dunn's post hoc test with significance level of $\alpha < 0.05$. Significant differences in APLNR and Apelin expression were observed between the experimental groups, indicating dynamic modulation of the Apelinergic system in response to demyelination and remyelination. Behavioral testing revealed a pronounced impairment in memory during demyelination, with the lowest latency times recorded at the fifth week of cuprizone exposure. During the remyelination phase, a significant improvement in memory performance was observed, with latency times increasing progressively and reaching their highest values after 3 weeks of recovery. Despite this improvement, memory performance in the RE group remained significantly reduced compared to the CO group. Demyelination is associated with both altered Apelinergic signaling in the hippocampus and significant memory deficits, while remyelination leads to partial recovery. These findings support a potential involvement of the Apelinergic system in hippocampal plasticity and suggest its relevance in mechanisms underlying cognitive impairment in demyelinating conditions.

Key words: Apelin, APLNR, passive avoidance test, Multiple sclerosis, demyelination, remyelination
Acknowledgment: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0004-C01 Strategic research and innovation program for development of Medical university—Sofia.

Experimental rodent models of atherosclerosis. Aortic endothelium changes in Lipofundin model in rats

Lazar Jelev

Department of Anatomy, Histology and Embryology, Medical University – Sofia, Sofia, Bulgaria

Corresponding author e-mail: ljelev@medfac.mu-sofia.bg

Atherosclerosis, a chronic, progressive inflammatory vascular disease and the leading cause of cardiovascular disease, mortality, and morbidity worldwide, is the subject of intensive experimental research. Understanding its pathophysiology, location specificity, and effective treatment requires the development and testing of numerous experimental animal models. Small laboratory rodents (mice, rats, hamsters, guinea pigs) are commonly used in experimental atherosclerosis research, mainly because of practical and economic advantages. The vascular pathology observed in the various experimental models can be used to study the different stages of atherosclerosis in humans. An interesting fast experimental model in rats of early endothelial changes in atherosclerosis can be achieved by intravenous administration of Lipofundin-S, a lipid emulsion used clinically for parenteral nutrition. For the experimental protocol, male Wistar rats (3-month-old, weighing 250–260 g) received Lipofundin MCT/LCT 20% (B. Braun Melsungen AG, Germany) via a surgically implanted femoral vein catheter in a daily dose of 1 ml/100 g bodyweight/5 min, for 10 days. On the en face preparations of the rat aortas can be observed morphological changes in the endothelial lining (presence of binucleated cells, small denuded areas), and also high number of adherent mononuclear cells, foci of lipid accumulations and rarely foam cells.

Key words: atherosclerosis research, experimental models, rats, Lipofundin, aortic endothelium

Human glioblastoma multiforme – searching for new approaches in anticancer treatment

Lora Dyakova[1*], Tanya Zhivkova[2], Abedulkadir Abudalleh[2], Hrisrto Hristov[2], Daniela-Cristina Culita[3], Gabriela Marinescu[3], Teodora Mocanu[3], Emanuil Naydenov[4], George Miloshev[5], Milena Georgieva[5], Radostina Alexandrova[2]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Physical Chemistry "Ilie Murgulescu", Romanian Academy, Bucharest, Romania*
[4]*"St.Ivan Rilski"Hospital, Bulgaria*
[5]*Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: sigma13@abv.bg

The high degree of malignancy, high heterogeneity, high invasiveness and limited access of antitumor agents to the tumor are still some of the challenges in the treatment of human glioblastoma multiforme. The side and toxic effects of conventional anticancer drugs are no less a problematic. The search for new approaches in brain cancer treatment continues. The aim of our study was to evaluate the potential cytotoxic/antitumor effect of newly-synthesized Zn(II)/Au(I) complexes with Schiff base ligands in cultured primary and permanent human glioblastoma multiforme cells. Methods with different targets in cells and mechanisms of action were used in our experiments: i) short-term (24-72 h) - MTT test, Neutral red uptake cytotoxicity assay, crystal violet staining technique, double staining with acridine orange and propidium iodide, Annexin V/FITC, Comet assay; ii) long-term (37 days)- 3D colony forming method. Two type of experimental models were used: permanent cell lines- 8MGBA, U251MG (human glioblastoma multiforme cells), Lep-3 (human non-tumor lung fibroblastoid embryo cells) and primary human glioblastoma cells. The results obtained show the promising antitumor activity of the compounds investigated. Metal complexes are more effective compared to the ligands alone. The higher cytotoxic/antitumor activity exhibit ZndmenAu even compare to the effect of cisplatin, oxaliplatin and vincristine. The compounds induce cytopathological changes, morphological changes, characeristic for early and late stages of apoptosis and inhibit the 3D growth of tumor cells in a semi-solid medium. A protocol for obtaining primary cell cultures of human glioblastoma multiforme was optimized and the model was successfully used to demonstrate the promising cytotoxic potential of newly synthesized Zn(II)/Au(I) complexes with Schiff base ligands.

Key words: glioblastoma multiforme, newly-synthesised metal complexes, Schiff bases, cytotoxic/ antitumor effect

Acknowledgments: Grant№ КП-06-М61/3 from 13.12.2022, National Science Fund, Bulgarian Ministry of Education and Science; Project Number IC-RO/04/2026-2028, bilateral project between Bulgarian Academy of Sciences and Romanian Academy.

APLNR signaling at the crossroads of astrocyte activation and remyelination of the septum in the cuprizone model

Lyubomir Gaydarski[1*], Kristina Petrova[1], Nikola Stamenov[1], Tsvetko Obetsanov[1], Alexandar Iliev[1], Stancho Stanchev[1], Pavel Rashev[2], Despina Pupaki[2], Milena Mourdjeva[2], Ivanka Kostadinova[3], Milen Hristov[4], Boycho Landzhov[1]

[1]*Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria*
[2]*Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Pharmacology, Pharmacotherapy and Toxicology, Medical University of Sofia, Sofia, Bulgaria*
[4]*Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University of Sofia, Sofia, Bulgaria*

*Corresponding author e-mail: lgaydarski@medfac.mu-sofia.bg

Cuprizone-induced demyelination is a widely used experimental model of multiple sclerosis that allows detailed investigation of glial responses and repair-associated mechanisms in the central nervous system. The APLNR has been implicated in neuroglial regulation. Regardless, its relationship with astrocytes during demyelination and remyelination remains incompletely understood. This study aimed to assess changes in APLNR expression and its association with GFAP-positive astrocytes in the septum of cuprizone-treated mice. Thirty male 8-week-old C57BL/6 mice were divided into control, demyelination, and remyelination groups (n = 10 per group). Demyelination was induced by administering 0.2% cuprizone in drinking water for 5 weeks, followed by a 5-week recovery period for the remyelination group. Motor performance was evaluated using the rotarod test. APLNR and GFAP expression were analyzed using immunohistochemistry and confocal immunofluorescence, with quantitative image analysis performed in ImageJ. Statistical analysis was conducted using GraphPad Prism, applying the Shapiro–Wilk test for normality and Kruskal–Wallis analysis with Dunn's post hoc test ($\alpha < 0.05$). Significant alterations in APLNR expression were observed across experimental conditions. GFAP-positive astrocytes were markedly increased during demyelination, decreased during remyelination, and were lowest in controls. A significant association between APLNR and GFAP expression was identified specifically during demyelination. Colocalization between the two markers was most prominent during demyelination and remained detectable, though reduced, during remyelination, while minimal overlap was observed in controls. Functionally, demyelination resulted in impaired motor coordination, with the lowest rotarod latency recorded at week 3. During remyelination, performance progressively improved, approaching control levels by week 5. These findings indicate that demyelination in the septum is associated with pronounced astroglial activation and dynamic modulation of APLNR expression. The observed relationship between APLNR and GFAP suggests a role for apelinergic signaling in astrocyte-mediated responses during injury and subsequent recovery.

Key words: APLNR, GFAP, Multiple sclerosis, motor coordination, rotarod test

Acknowledgment: This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0004-C01 Strategic research and innovation program for development of Medical university—Sofia.

Frontal Fibrosing Alopecia – clinical and diagnostic criteria

Mary Gantcheva

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Science, Sofia, Bulgaria*
[2]*Acibadem City Clinic Mladost, Sofia, Bulgaria*

*Corresponding author e-mail: mary_gant@yahoo.com

Frontal fibrosing alopecia (FFA) is a relatively recently recognized condition. It is a form of primary lymphocytic scarring alopecia characterized by fronto-temporal hair recession and in half of the patients with eyebrow hair loss. FFA occurs most often in postmenopausal woman but in the last decades it has worldwide widespread affecting also men and younger women of all skin types. The pathogenesis of the disease is multifactorial - genetic predisposition, hormonal and environmental factors and inflammatory pathways, with notable involvement of PPAR-γ and mammalian target of rapamycin (mTOR). Clinical presentation has tree specific forms –linear, diffuse and pseudo-fringe sign – pattern, all of them with different prognosis. Diagnosis is usually made clinically with the help of a noninvasive procedure – trichoscopy, that help us to prevent scalp biopsy. Typical trichoscopic findings include: loss of follicular openings, perifollicular erythema and scaling and white cicatricial zones. We emphasize the importance to recognize this disease presenting a case of FFA, which helps for better understanding and proper disease management.

Key words: Frontal fibrosing alopecia, trichoscopy

Chemical composition and *in vivo* antitumor activity of the aqueous ethanol extract from aerial parts of *Epilobium parviflorum* Schreb. in a mouse model of Ehrlich's ascites carcinoma

Mashenka Dimitrova[1*], Inna Sulikovska[1], Katerina Dimitrova[1], Ivaylo Ivanov[2]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2]*Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria*

[*]Corresponding author e-mail: mashadim@abv,bg

The aim of the present study was to determine the chemical composition of the 80 % aqueous ethanol extract from the leaves and twigs of *Epilobium parviflorum* Schreb. (smallflower hairy willowherb) of Bulgarian origin and to test its antitumor activity in a mouse model of Ehrlich's ascites carcinoma (EAC). Secondary metabolites in the extract were identified by LC-HRMS. The main component was found to be the macrocyclic ellagitannin oenotein B. The extract also contained six compounds yet undescribed in this herb of other sources. The effect of the extract on the EAC cell line was tested first *in vitro*. The *in vivo* experiments were performed on mice, injected i.p. with EAC cells and developing ascites form of Ehrlich's carcinoma. They were divided into 3 groups: untreated with the extract (negative controls), treated daily with 30 mg/kg b.w. extract and treated once with 15 mg/kg b.w. 5-fluorouracil (5-FU) (positive controls). After the end of experiment, the animals were sacrificed by decapitation. Smears of the ascites cells were made and colored with May Grunwald Gimsa to estimate cellular morphology. Blood was collected for hematological and biochemical analyses. The results showed a visible pro-apoptotic activity of the extract in both EAC cells and the ascites smears. The blood analyses demonstrated improvement of the measured parameters after application of the extract in comparison to the extract-untreated animals. Moreover, a decrease of the enzymes' levels, demonstrative for the functioning of liver and kidney was observed in biochemical analyses. From those results, it can be concluded that the aqueous ethanol extract from aerial parts of *E. parviflorum* has a noticeable pro-apoptotic and organ protective activity and is a candidate as an auxiliary natural therapeutic.

Key words: *E. parviflorum,* LC-HRMS analyses, Ehrlich's ascites carcinoma, mouse model

Acknowledgement: This research was funded by the Bulgarian National Science Fund; Grant number KP-06 N31/1.

Double karyosphere, a rare finding in mouse oocytes at germinal vesicle stage

Maya Markova*, Anton Kolarov, Irina Chakarova, Valentina Hadzhinesheva, Ralitsa Zhivkova, Stefka Delimitreva, Venera Nikolova

Department of Biology, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria

*Corresponding author e-mail: m.markova@medfac.mu-sofia.bg

Resumption of meiosis by prophase-arrested (germinal vesicle stage) mammalian oocytes is characterized by the formation of the so-called surrounded (rimmed) nucleolus, or karyosphere. It is a sphere of heterochromatin at the nucleolar periphery that gradually includes all chromosomes, and is thought to be important for the correct spindle assembly and chromosome segregation during the first meiotic division. Oocytes in published reports have only one karyosphere, and its presumed function to hold the chromosomes together implies that it should be single. However, our observations reveal that almost 13% of prophase mouse oocytes have two karyospheres, usually of equal size, but sometimes one much smaller than the other. Cells with double karyosphere do not differ visibly in other respects from cells with a single karyosphere, but since they were fixed for observation, their meiotic and developmental potential is unknown. The double karyosphere is an interesting phenomenon which requires further study.

Key words: oogenesis, oocyte meiotic maturation, karyosphere, surrounded nucleolus, germinal vesicle

Acknowledgments: This work was supported by Grants No. D-97/24.06.2020 and D-137/14.06.2022 of the Medical University of Sofia.

Anthropological and archaeological investigation of a medieval necropolis near the town of Chirpan (Southern Bulgaria)

Nadezhda Atanassova[1*], Vladislav Todorov[1], Petar Leshtakov[2], Lilia Ovnarska-Kolibarska[1]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2]*National Archaeological Institute with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: naditimeva@gmail.com

The archaeological site is covered an area of approximately 5 acres and is located 1.5 km northeast from the town of Chirpan, Stara Zagora district. The rescue excavations are carried out in the period 2020 - 2022. Over 600 archaeological structures from five historical eras are registered. The AMS analysis of human bone remains from the earliest grave (No 114) dated it between 5620 and 5482 cal BC (95.4%). The most active habitation was during the Early Chalcolithic era, when a settlement arose in the first half of the 5th millennium BC. During the period of the 11th - 13th centuries AD, a Medieval settlement arose on the site, from which 28 dwellings, outbuildings, a large number of food storage pits and 116 graves (?) have been preserved. All deceased are buried in the Christian custom. According to the orientation of the burial pits, higher mortality was recorded (46%) in the winter period, average in spring and autumn (37%) and lowest in summer (17%). The aim of the present study is to establish main anthropological characteristics and paleopathological changes of the buried in the Medieval necropolis. Established anthropological methods are used to achieve this aim. The anthropological investigation includes 121 identified individuals from 118 grave structures with an inhumation burial practice. The ratio of subadults to adults is almost equal (1:0.86), however 54% the infant mortality rate was 47%. The male/female distribution (1.06:1) among the adults buried in the necropolis, is also almost equal, with a very slight predominance of male skeletons. During the paleopathological examination, multiple morphological bone changes of the cranial and postcranial skeletons are identified, morphological bone changes indicating congenital and acquired diseases are identified, which are indicative for poor quality of life in this Medieval population.

Key words: anthropology, archaeology, Medieval times, necropolis

PAS-diastase histochemistry for glycogen detection in the myenteric plexus of the rat colon

Nikolay Genov[1*], Nikolay Dimitrov[1], Dimitrinka Atanasova[1,2]

[1]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: nikolay.genov@trakia-uni.bg

Periodic acid-Schiff (PAS) staining is a well-known histochemical method for demonstrating polysaccharides in tissues. When differentiation between glycogen and other PAS-positive substances is required, diastase digestion may be incorporated into the procedure (PAS-D). Although rarely used in enteric nervous system studies, this method may help assess glycogen-associated PAS reactivity in the myenteric plexus and adjacent tissues. This study aimed to determine whether PAS-D staining can be applied to the myenteric plexus of the distal colon in adult rats. Tissue samples from the distal colon of three-month-old male Wistar rats were fixed in 4% paraformaldehyde for 48 h. Sections 6 µm thick were prepared and mounted on chrome-gelatin-coated microscope slides. Glycogen digestion was performed using alpha-amylase derived from *Bacillus licheniformis* (Browin, Łódź, Poland). The enzyme was diluted 1:10 and 1:20 in 0.9% NaCl and applied for 2 h at 37°C in a humidified chamber. Enzymatic digestion produced concentration-dependent changes in PAS staining. At the 1:20 dilution, PAS reactivity was not significantly reduced. By contrast, the 1:10 dilution reduced both staining intensity and PAS-positive area around the myenteric plexus by 45%. In the smooth muscle layer adjacent to the ganglia, PAS positivity decreased by 62%, based on staining intensity and area fraction. These results indicate that PAS-D histochemistry with alpha-amylase is a reliable approach for demonstrating glycogen-dependent PAS reactivity in the intestinal wall and in structures associated with the myenteric plexus.

Key words: myenteric plexus, alfa-amylase, PAS, diastase, PAS-D

Acknowledgement: This research was supported by project No. BG-RRP-2.004-0006-C02 under the National Recovery and Resilience Plan of Bulgaria, Component “Innovative Bulgaria”, and by the Faculty of Medicine, Trakia University, Stara Zagora (Grant No. 8/2023).

A container for “ex situ” machine-assisted liver preservation

Nino Inauri[1*], Keti Tsomaia[1,2], Manana Kakabadze[2], Otar Philishvili[1], Avto Dgebuadze[1], Nodar Khodeli[1,2]

[1]*Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*

[2]*Alexandre Natishvili Institute of Morphology, TSU, Tbilisi, Georgia*

*Corresponding author e-mail: ninainauri1@gmail.com

One of the key directions in modern transplantation science is the improvement of donor organ quality through optimization of pre- and post-transplant conditions. Recent advances indicate that prolonged *ex situ* liver preservation under near-physiological conditions with controlled perfusion parameters may not only improve marginal organs but also stimulate regenerative processes, enabling the generation of functional grafts from partial liver segments. The aim is to provide maximal physiological conditions for the liver graft during prolonged *ex situ* preservation. Five experiments of prolonged machine liver preservation were conducted using a porcine model (20–22 kg). A modified thermally insulated container (up to 20 L) equipped with a Peltier thermoregulation system was used. The organ was placed on a soft support over an air-cushion mattress simulating diaphragmatic motion. Normothermic protein solution was continuously recirculated through a heat exchanger. Arterial inflow (hepatic artery) was provided via pulsatile oxygenated blood; portal inflow simulated splanchnic circulation; venous outflow was achieved via the inferior vena cava. Internal conditions (temperature, humidity) were continuously monitored. In three experiments, stable preservation for 24 hours was achieved, demonstrated by stable hemodynamics (hepatic artery: 70 mmHg, 125 ml/min; portal vein: 12 mmHg, 450 ml/min), adequate oxygenation, and metabolic parameters. Two experiments were terminated early due to technical issues. A 10–12% increase in liver weight was observed, likely due to relative hyperperfusion. Continuous bile production was recorded in successful cases. The system maintained intra-abdominal-like humidity and temperature conditions. Artificial replication of physiological conditions and homeostatic parameters is critical for successful prolonged *ex situ* liver preservation and maintenance of organ viability.

Key words: liver preservation, machine perfusion, ex situ, transplantation, regeneration

Acknowledgments: This study was supported by the Shota Rustaveli National Science Foundation of Georgia (Project №FR-24-18604).

Digital bone templates for generation of personalized 3D printed implants for clinical medicine

Petar Valchanov

Medical University of Varna, Department of Anatomy and cell biology, Varna, Bulgaria

Corresponding author e-mail: pvulchanov@gmail.com

A major part of modern medicine is the production of high fidelity personalized medical devices, which combines the amazing spatial accuracy of 3D technologies with the advanced properties of composite materials. They provide high level of customization and more options in surgical treatment of a broad range of bone related conditions like chronic fractures or malignant tumors.

The personalized medical implants are designed on templates, generated through the 3D segmentation of CT and MRI series of images and represent with high degree of detail the surface of the bones. On those templates the schematic of the implant is drawn directly, including the margins and the positions of the fixation screws. In this way a broad range of personalized medical devices are generated and manufactured with 3D printing, including polymer cutting and drilling guides, thermoplastic 3D printed models for preoperative planning and titanium personalized surgical plates. The final medical devices match perfectly the surface of the bones of interest and provide better results in multiple operative parameters, including surgical time, average intraoperative blood loss, average intraoperative X-rays and the whole effectiveness of the surgery. In this work the whole workflow for the generation of the personalized devices is presented, including the scanning, designing, 3D printing, postprocessing, sterilization, quality control and the application in actual surgical cases.

Key words: 3D printing, Digital twins, templating, 3D modelling, personalized medicine

Cell cultures as model systems in cytocompatibility assessment of new wound healing materials and skin care products

Orlin Alexandrov[1], Eliya Stoilova[2], Silvia Mancheva[2,3], Tanya Zhivkova[2], Lora Dyakova[4], Abedulkadir Abudalleh[2], Elena Vassileva[5], Hristo Hristov[2], Erika Adomavičiūtė[6], Virginija Jankauskaitė[6], Ioana Baldea[7], Monica Neagu[8], Merve Erginer Haskoylu[9], Radostina Alexandrova[2*]

[1]*Faculty of Medicine, Medical University – Sofia, Sofia, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*New Bulgarian University, Sofia, Bulgaria*
[4]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[5]*Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*
[6]*Kaunas University of Technology, Kaunas, Lithuania*
[7]*University of Medicine and Pharmacy, Cluj-Napoca, Romania*
[8]*"Victor Babes" National Institute of Pathology, Bucharest, Romania*
[9]*Istanbul University-Cerrahpaşa, Istanbul, Türkiye*

*Corresponding author e-mail: rialexandrova@hotmail.com

The skin is the largest organ in the human body, performing vital protective, regulatory and sensory functions. Skin care (for both healthy and damaged skin) is extremely important for our overall health and well-being. The aim of our study was to summarize the role of different cell cultures (human keratinocytes, human and mouse fibroblasts) as well as the challenges in testing the cytocompatibility of new wound healing and skin care materials in laboratory conditions. The impact of wound healing materials (polyzwitterionic hydrogels, cellulose-based materials) and skin care products (collagen-based formulations) with different structures and chemical/physicochemical properties was evaluated in our study. Direct experiments (cells were cultured directly in/on the materials) and indirect experiments (cells were grown in a culture medium in which the tested materials were pre-inocubated) were performed with various cytotoxicity, cytological and biochemical methods, including MTT test, double staining with acridine orange and propidium iodide and scratch test. The advantages and limitations of cell cultures used as model systems in these type of investigations were summarized as well as strategies for addressing the challenges of these in vitro cell models and studies.

Key words: skin models, cell cultures, wound healing materials, skin care products, cytocompatibility

Acknowledgements: The study was supported by Bulgarian Natioanal Science Fund (Grant N КП-06-КОСТ/18 from 12.08.2024), Bulgarian Academy of Sciences (Bilateral project between the Bulgarian Academy of Sciences and the Lithuanian Academy of Sciences, project IC-LT/04/2025-2027) and COST Action CA21108 „NETSKINMODELS“ and COST Action CA21108 „NETSKINMODELS“.

Diabetes mellitus induced in early life affects spermatogenesis in developing rat and fertility

Ekaterina Pavlova[1], Rosen Ivanov[1*], Desislava Abadjieva[2], Yordanka Gluhcheva[1], Emilia Petrova[1], Ivelin Vladov[1], Emilia Lakova[3], Nina Atanassova[1]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

[2]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

[3]*Department of Physiology and Pathophysiology, Medical University of Pleven, 5800 Pleven, Bulgaria*

*Corresponding author e-mail: rosen.lyubomirov.ivanov@gmail.com

Diabetes mellitus (DM) impairs spermatogenesis and testosterone biosynthesis resulting in male infertility. The effect of diabetes on the testis was investigated mainly in adulthood, hence we aimed to study the impact of DM, induced in early postnatal life on the testis and fertility. Neonatal (NDM) and pre-pubertal diabetes (PDM) were induced in rats by streptozotocin administration on day 1 or day 10, respectively. Germ and somatic cells (Leydig and Sertoli cells) of developing testis were quantified on day 25 (puberty) and day 45 (end of puberty) and sperm characteristics on day 65 (adulthood). Serum glucose, insulin and testosterone (T) levels were measured. Androgen production by Leydig cells (serum testosterone levels) and action (protein expression of androgen receptor - AR) were evaluated. Glucose levels were elevated in tandem with decreased insulin secretion more pronounced in PDM than in NDM. Insulin resistance index (HOMA) in post-pubertal animals was increased in NDM implying development of DM type 2 whereas PDM rats are not insulin resistant, suggestive for development of DM type 1. Total germ cell number was significantly reduced in 45-day-old PDM rats as a result of incomplete spermatogenesis manifested by lack of elongating spermatids. T levels were reduced on days 45 and 65 more pronounced in PDM. On day 45, Leydig cell number was reduced more pronounced in PDM corresponding to the changes in T levels. Sertoli cell number was not significantly changed by DM but AR protein expression on day 45 was altered in PDM but not in NDM. Sperm concentration and motility were reduced in both groups. Elevation of round (undifferentiated) cells in semen samples was more expressed in NDM than PDM. Our comparative evaluation of NDM and PDM provided new knowledge on differential effects of two types of diabetes on proceeding and completion of spermatogenesis via altered androgen production and action.

Key words: Diabetes mellitus, testis, spermatogenesis, testosterone, androgen receptor

Acknowledgements: The study was supported by Grant No KP-06-N71/7 from the Bulgarian National Science Fund.

Downregulation of Neuraminidase 1 expression in mouse skeletal muscles as a response to infection with *Trichinella spiralis* (Owen, 1835) – more questions than answers

Rositsa Milcheva[1*], Svetlozara Petkova[1], Zuzana Hurníková[2], Ani Georgieva[1], Katerina Todorova[1], Ivelin Vladov[1], Inna Sulikovska[1], Elitsa Mihailova[1], P. Babál[3]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum – BAS, Sofia, Bulgaria*
[2]*Institute of Parasitology – SAS, Košice, Slovakia*
[3]*Department of Pathology, Faculty of Medicine Comenius University, Bratislava, Slovakia*

*Corresponding author e-mail: rosicamilcheva@abv.bg

For the first time, the expression of neuraminidase 1, a multifunctional enzyme, was examined in an experimental model of trichinellosis in skeletal muscle tissue. Downregulation of protein and mRNA transcripts was observed after *in vivo* infection, as well as in C2C12 myotubes treated with *Trichinella spiralis* protein extract, suggesting that this event was not a reaction of the host but was induced by the parasite. The biological significance of this finding, even if analyzed in different aspects, is still unclear. Nevertheless, this work provides further evidence of *Trichinella´*s capacity to manipulate the transcriptional program of its host. Given that NEU1 upregulation has been reported in several cancer diseases, further studies are warranted to investigate whether *Trichinella* can similarly induce targeted changes in other biological models, which may refine our understanding of the parasites modulatory potential.

Key words: neuraminidase 1, skeletal muscles, *Trichinella spiralis*

Acknowledgments: This work was financially supported by the Bulgarian Academy of Sciences and the Slovak Academy of Sciences under grant IC-SK/03/2023-2024.

Scoliosis in 12 y.o. adolescent male with CMT disease

Sofia Fursova*, Atanas Katsarov

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: Sophia.fursova11@gmail.com

Charcot-Marie_Tooth disease is an hereditary degenerative disorder of the central and peripheral nervous system that causes muscle atrophy and loss of proprioception. CMT disease can be autosomal dominant (most common) or X-linked recessive or autosomal recessive. Common orthopaedic deformities include cavovarus foot deformity, hip dysplasia, spinal deformities. Spinal deformities are present in approximately 25% of the patients with CMT disease. Scoliosis is uncommon, occurring in up to 30% of the patients. Scoliosis is defined as a three-dimensional and rotational deformity of the spine (in sagittal, frontal and coronal planes). Approximately 75% of the patients with CMT disease have hereditary motor and sensory neuropathy. Generally, spinal deformities can be managed by the same techniques used for idiopathic scoliosis that includes observation, physiotherapy, bracing and operative treatment. This case report represents an adolescent with diagnosis CMT disease inherited demyelinating polyneuropathy with chronic axonal loss with thoracolumbar scoliosis. The results of the treatment show improved posture and enhanced quality of life.

Key words: Charcot-Marie-Tooth disease, scoliosis, deformity

Internal consistency of a foot and eye dominance scale in male subjects

Vasil Kotetarov[1], Stefan Sivkov[2*], Katerina Akabalieva[3], Asen Beshkov[4], Valentin Akabaliev[3]

[1]*Research Institute at Medical University Plovdiv, Bulgaria*

[2]*Department of Anatomy, Histology and Cytology, Faculty of Medicine, Medical University Pleven, Bulgaria*

[3]*Department of Psychiatry and Medical Psychology, Faculty of Medicine, Medical University, Sofia, Bulgaria*

[4]*State Hospital of Psychiatry, Pazardzhik, Bulgaria*

*Corresponding author e-mail: st.sivkov@yahoo.com

The aim was to investigate the reliability (internal consistency) of a Combined Foot and Eye Dominance Scale in men - schizophrenia patients and healthy subjects. We used a Combined Foot and Eye Dominance Scale to assess a sample of 86 men - schizophrenia patients (mean age 44.51 years (SD = 10.73, range 23-67) and healthy subjects (Mean age 34.70 years (SD = 16.82, range 18-79). The scale consisted of two subscales: Foot Dominance Subscale and Eye Dominance Subscale. Scale reliability statistics, non-parametric Mann-Whitney test and Spearman's rank correlation coefficient were used. Considerable differences were found in the contribution of the single items to the Combined Scale. Some items show greater means (0, 14 - 0, 80), suggesting greater phenogenetic component and greater contribution to the total scale mean. The mean correlation between the items is positive (0, 30), indicating good internal consistency of the scale. There is strong consistence and coherence between the two component subscales as well as between the individual dominance tests of the scale in the male population. The Combined Scale strongly and objectively reflects leftedness and provides an easier and more accessible way for researching laterality in men.

Metal complexes of salinomycin with Zn (II), Co (II), Cu (II), Mn (II) and Gd (III) express promising cytotoxic activity in cultured human cervical cancer and melanoma cells

Tanya Zhivkova[1*], Hristo Hristov[1], Lora Dyakova[2], Abdulkadir Abudalleh[1], Peter Dorkov[3], Juliana Ivanova[4], Radostina Alexandrova[1]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Chemistry Department, R&D, BIOVET JSC, Peshtera, Bulgaria*
[4]*Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*

*Corresponding author e-mail: tani413@abv.bg

Salinomycin is a polyether ionophore antibiotic isolated from Streptomyces albus that is widely used in veterinary medicine as coccidiostatic and antibacterial agent. The anticancer activity of salinomycin has been demonstrated in various cell culture model systems as well as its selectivity against cancer stem cells. The aim of our study was to evaluate the influence of Salinomycin (SalH) and five newly synthesized complexes of SalH with Zn (II), Co (II), Cu (II), Mn (II) and Gd (III) on viability and proliferation of cultured human cervical cancer and melanoma cells. The following human permanent cell lines were used as model systems in our study: HeLa (cervical carcinoma), A-375 (malignant melanoma), SH-4 (derived from pleural effusion of patient with metastatic melanoma), HaCaT (keratinocytes) and Lep-3 (non-tumor embryonic fibroblasts). The investigations were performed by MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, double staining with propidium iodide and acridine orange, scratch assay (in short-term experiments, 24-72 h) and 3D colony forming method (in long-term experiments, 30-35 days). The results obtained reveal that the compounds examined possess significant dose- and time- dependent cytotoxic activity when applied at a concentration range from 0.5 to 50 μg/ml in both short-term (with monolayer cell cultures) and long-term (with 3D cancer cell colonies) experiments. The cytotoxic effect of the metal complexes of SalH is comparable to the effect of SalH tested alone (in HeLa cells) or more pronounced (in A375, SH−4 cells) and surpasses that of the commercial antitumor agents cisplatin and oxaliplatin.

Key words: salinomycin, metal complexes of salinomycin, cervical cancer and melanoma cells

Acknowledgments: support by European Union Next Generation EU through the National Recovery and Resilience Plan of the Republic of Bulgaria, project no. BG-RRP-2.004-0008 as well as by the National Science Fund in Bulgaria, grant no КП-06-КОСТ/18 from 12.08.2024

The anatomical basis of transpedicular screw placement in spinal fusion surgery, presented through photorealistic 3D models in virtual reality

Toma Spiriev[1*], Martin Trandzhiev[1], Francesco Carbone[2], Michael Vollenbröker[3], Vladimir Nakov[1], Ivan Maslarski[4], Jan Frederick Cornelius[5]

[1]*Department of Neurosurgery, Acibadem City Clinic University Hospital Tokuda, Sofia, Bulgaria*
[2]*Department of Neurosurgery, Karlsruher Neurozentrum, Städtisches Klinikum Karlsruhe, Karlsruhe, Germany*
[3]*Institute for Anatomy I, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University, Düsseldorf, Germany.*
[4]*Department of Anatomy and Histology, Pathology, and Forensic Medicine, University Hospital Lozenetz, Medical Faculty, Sofia University, Sofia, Bulgaria*
[5]*Department of Neurosurgery, Medical Faculty & University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

*Corresponding author e-mail: spiriev@gmail.com

The advancement of technologies for photorealistic 3D scanning of anatomical specimens has allowed for the generation of 3D models that preserve the spatial characteristics and texture of the object. This has led to the creation of specially designed anatomical and surgical educational materal, that helps training and education of these disciplines. The aim of this study is to present the neuroanatomical basis for transpedicular spine screws placement through the use of photorealistic 3D models. Dedicated anatomical dissections of paraspinal musculature were 3D scanned. Additionally, bone 3D scanning of individual spinal vertebrae and whole spine models were done. The models were additionally uploaded to an immersive VR environment, where a set of virtually instruments was created. Teaching interactive sessions with neurosurgery orthopedic residents presenting the anatomy of the back region, vertebrae morphometry and surgical simulation of approaches were done. The photorealistic 3D models enabled high-fidelity visualization of vertebral anatomy, accurately representing pedicle morphology, cortical boundaries, and spatial relationships to adjacent neural structures. Segment-specific variations in pedicle angulation, diameter, and trajectory were clearly identifiable and reproducible within the virtual environment. Integration into an immersive VR platform allowed dynamic manipulation, including real-time transparency adjustment, facilitating simultaneous visualization of surface landmarks and internal pedicle trajectories. This enabled direct correlation between anatomical entry points and optimal screw trajectories, enhancing spatial understanding using the virtual instrument allowing repeated, standardized simulation of transpedicular screw placement. The use of virtual reality technology not as a replacement, but as an adjunct to traditional teaching methods. It is associated with high user engagement and interactivity. The combination of photogrammetry models of spine segments with a dedicated 3D-modelled instrument set allowed for the conduction of an innovative training of spine anatomy, as well as spinal screws placement.

Key words: transpedicular screw placement, pedicle anatomy, photorealistic 3D models, virtual reality, surgical simulation, anatomy education

Integrin receptors mediated tissue remodeling

Volodia Georgiev

Manhattanville University, School of Arts and Sciences, New York, USA

Corresponding author e-mail: georgievv@mville.edu

Integrin receptors for the extracellular matrix activate intracellular signaling pathways that are critical for tissue development, homeostasis, and regeneration/repair, and their loss or dysregulation contributes to many developmental defects and tissue pathologies. Cell adhesion to the extracellular matrix is fundamental to tissue integrity and human health. Integrins are the main cellular adhesion receptors that through multifaceted roles as signaling molecules, mechanotransducers and key components of the cell migration machinery are implicated in nearly every step of cancer progression from primary tumor development to metastasis. Altered integrin expression is frequently detected in tumors, where integrin receptors have roles in supporting oncogenic growth factor receptor (GFR) signaling and GFR-dependent cancer cell migration and invasion. In addition, integrins determine colonization of metastatic sites and facilitate anchorage-independent survival of circulating tumor cells. The diversity of integrin receptors repertoire allows for adhesion to nearly all components of extracellular matrix. Since the pattern of integrins expression is a key determinant of cell behavior in response to microenvironmental cues, deregulation of integrins caused by various mechanisms has been causally linked to cancer development and progression. Understanding the integrin signalosome and the key pro-oncogenic pathways elicited by integrins, as well as uncovering the mutational and transcriptomic landscape of integrin-encoding genes across human cancers will elucidate the critical points of tumor development and progression. In addition, the integrin-mediated control of cancer stem cell and tumor stemness in general, such as tumor initiation, epithelial plasticity, organotropic metastasis and drug resistance. With insights into how integrins contribute to the stem-like functions, there is better understanding of the integrin receptors' role in cancerogenesis, which will greatly assist novel therapeutic development and more precise clinical decisions.

Key words: integrin receptor, GFR

POSTER PRESENTATIONS

From LSIL cytology to p16-positive cervical lesion: a case report and management approach

Alexandra Gerdjikova[2], Gergana Trenova[2], Desislava Bojkova[2], Neli Doneva[1], Ekaterina Uchikova[1], Nikoleta Parahuleva[1], Mariana Parahuleva[3], Mariya Koleva[2], Yana Merdjanova-Gargova[1], Ani Paunova[1], Angelina Mollova-Kysebekirova[2]

[1]*Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

[2]*Department of General and Clinical Pathology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

[3]*Universitätsklinikum Gießen und Marburg Standort Marburg, Marburg, Germany*

Cervical cancer screening has evolved with the integration of cytology, high-risk human papillomavirus (HR-HPV) testing, and biomarker analysis to improve detection of clinically significant cervical lesions. Biomarkers such as p16 and Ki-67 are increasingly used to identify transforming HPV infections and guide patient management. A 30-year-old nulliparous woman presented for routine cervical cancer screening using liquid-based cytology (LBC). Cytological evaluation revealed a low-grade squamous intraepithelial lesion (LSIL). Subsequent high-risk human papillomavirus (HR-HPV) testing performed by polymerase chain reaction (PCR) was positive. As part of further risk stratification, p16/Ki-67 dual-stain cytology was performed and showed positive results, indicating a transforming HPV infection. The patient was referred for colposcopic examination, which revealed suspicious cervical changes, and a directed cervical biopsy was obtained. Histopathological evaluation demonstrated epithelial abnormalities with strong and diffuse p16 immunohistochemical expression, consistent with an HPV-associated transforming cervical lesion. Based on the integration of cytological findings, HR-HPV PCR results, biomarker positivity, and histopathological confirmation, cervical conization was recommended as the most appropriate therapeutic management. Diagnostic evaluation included cervical cytology, high-risk HPV testing by PCR, and histopathological examination of endometrial curettage with hematoxylin–eosin staining. Immunohistochemical analysis for PTEN, PAX2, and AMHRII was performed. The case was analyzed within an institutional research protocol approved by the ethics committee (approval No. P-KHE-4/28.10.2024). This case highlights the value of combining cytology, HR-HPV PCR testing, and biomarker analysis such as p16/Ki-67 dual staining in the evaluation of cervical lesions. The use of multiple diagnostic modalities improves risk stratification and supports timely clinical decision-making.

Key words: LSIL, HPV, P16, Ki67

The effect of chronic exposure to cobalt chloride on mouse liver

Anastasija Ilovska, Rosen Ivanov, Ekaterina Pavlova, Yordanka Gluhcheva*

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

*Corresponding author e-mail: ygluhcheva@hotmail.com

Cobalt (Co) is a widely distributed essential microelement but it is also an environmental pollutant. Prolonged exposure to cobalt may cause damage to the liver, kidneys, circulatory and nervous systems, primarily through mechanisms involving oxidative stress, inflammation and apoptosis. Cobalt's extensive use in cosmetics, medical implants, chemical industries and as a food supplement has raised concern about its biological effects on cells, tissues and organs. Cobalt chloride ($CoCl_2$) is a widely used, water-soluble compound known as an effective hypoxia-mimicking agent. Our aim was to investigate the effect of chronic $CoCl_2$ exposure on the development of liver inflammation in immature (18-day-old) and mature (45-day-old) mice treated with low (75 mg/kg) and high (125 mg/kg) doses of cobalt chloride. Pregnant ICR mice were subjected to 75 mg or 125 mg/kg body weight $CoCl_2x6H_2O$ daily 2–3 days prior delivery and treatment continued 45 days after birth. Pups were sacrificed on postnatal days 18 and 45. In our studies, the chronic treatment with $CoCl_2$ exerted pronounced dose- and age-dependent toxic effects on the liver. The administration of low and high doses of cobalt chloride led to significant accumulation of cobalt ions in the hepatic tissue in a dose-dependent manner, more pronounced in mature animals. $CoCl_2$ treatment disrupted hepatic homeostasis and induced hepatocellular injury via hypoxia, apoptosis and local immune activation resulting in Kupffer cell activation and leukocyte infiltration in the damaged areas. Immature animals exhibited more pronounced apoptosis and structural damage, whereas mature mice showed a stronger inflammatory reaction. The age-dependent differences observed suggest that immature animals are more sensitive to the toxic effects of cobalt ions due to their poorly developed immune system.

Key words: cobalt chloride, mouse, chronic exposure, liver, inflammation

Ultrastructural characterization of the three subnuclei of the rat spinal trigeminal nucleus using electron microscopy

Andrey Ivanov[1,3*], Dimitrinka Atanasova[1,2], Nikolai Lazarov[1]

[1]*Department of Synaptic Signaling and Communication, Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Department of Anatomy, Faculty of Medicine, Trakia University – Stara Zagora, Stara Zagora, Bulgaria*
[3]*University Hospital "St. Anna" Sofia, Clinic of Nephrology, Hemodialysis Ward, Sofia, Bulgaria*

*Corresponding author e-mail: andreyivanovmd@gmail.com

The spinal trigeminal nucleus (SpV) is a key structure in the processing of orofacial sensory information, including nociception, temperature, and touch. It is anatomically and functionally subdivided into three distinct subnuclei: the oral (SpVo), interpolar (SpVi), and caudal (SpVc) regions. This study employs scanning electron microscopy to investigate the ultrastructural organization of these three subnuclei, aiming to elucidate differences in synaptic architecture, neuronal morphology, and glial interactions that may underlie their functional specialization. Ultrathin sections of rat brainstem tissue containing the SpV were prepared and analyzed using transmission electron microscopy using the Walton contrasting procedure. Distinct ultrastructural features were observed across the subnuclei. The SpVo exhibited a higher density of myelinated axons and organized synaptic arrangements, consistent with its role in processing discriminative touch. In contrast, the SpVi displayed intermediate characteristics, with a mixture of myelinated and unmyelinated fibers and diverse synaptic profiles, suggesting integrative sensory functions. The SpVc, known for its involvement in nociceptive processing, demonstrated a predominance of unmyelinated fibers, numerous asymmetric synapses, and increased glial cell presence, indicative of active modulation and plasticity. Additionally, variations in vesicle types, synaptic cleft morphology, and mitochondrial density were noted, reflecting differences in metabolic and neurotransmission demands. These findings provide detailed insight into the microanatomical heterogeneity of the SpV subnuclei and support their distinct functional roles in sensory processing. This ultrastructural characterization contributes to a deeper understanding of trigeminal sensory pathways and may inform future studies on pain mechanisms and therapeutic targets.

Key words: spinal trigeminal nucleus, electron microscopy, ultrastructure, nociception, synaptic organization

Endothelial activity in pig intrarenal arteries after using of compound 48/80

Angel Vodenicharov[1*], Branimir Nikolov[2], Nikolay Tsandev[1,3]

[1]*Department of Preclinical Science, Faculty of Veterinary Medicine, Medical University, Pleven, Bulgaria*
[2]*Department of Preclinical Science, Faculty of Veterinary Medicine, Medical University, Pleven, Bulgaria*
[3]*Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria*

*Corresponding author e-mail: angel.vodenicharov@mu-pleven.bg

Compound 48/80 is a histamine releaser, used to provoke the release of histamine from mast cells. The aim of the study was to study its effect on the endothelium of the arterial intrarenal vessels in the domestic pig. Kidneys from 1 male and 1 female 8-month-old pigs, 90 – 100 kg/b.w., were treated with Compound 48/80 (Sigma, USA), diluted with isotonic solution - 100µg/ml, 36^0 C. It was introduced through one of the two main branches of *A. renalis*, and then through the other branch 36^0 C isotonic solution was introduced (for control). After 10 min, the kidneys were perfused with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After 30 min, pieces ($1mm^3$) with blood vessels were prepared, placed in the fixative for 2 h, washed in buffer, post-fixed in 1% OsO_4 and embedded in Durcupan. Semi-thin (1 µm) sections were prepared and stained by a modified method of Humphrey and Pittman (1974). Ultrathin sections were made from appropriate areas, which were contrasted with uranium acetate and lead citrate. The observations showed that along with mast cell reactivity after treatment with Compound 48/80 - release of granules and evaginates of the plasmalemma, in separate areas the intimal endothelial cells of the arteries and arterioles were visibly activated, which was best manifested in the interlobar arteries. The activity of the endothelium was demonstrated by: 1) prominence of the nucleated part of the cells in the lumen, with the remaining part being greatly thinned; 2) „optically empty" round or elliptical areas in the nucleated or remaining part of adjacent cells, without disruption of the connection with the endothelial layer. Similar findings were also observed in transmission electron microscopic studies. In conclusion, compound 48/80 activates the endothelium of intrarenal arteries.

Key words: compound 48/80, intrarenal arteries, pig, endothelial activity

Geometrics in anatomy – "forgotten" neck triangles and their clinical significance

Bistra Blagova

Department of Anatomy, Histology, and Embryology, Medical University of Sofia, Bulgaria

Corresponding author e-mail: dr_blagova@abv.bg

The neck is a specific region of the body that can be examined and treated using anatomical triangles. Several of these triangles have been identified, some of which are well-known. In contrast, others have been largely "forgotten", such as *Beclard's* triangle, *Farabeuf's* triangle, *Lesser's* triangle, *Pinaud's* triangle, and *Pirogov's* (*Pirogoff's*) triangle. These anatomical references could be helpful for surgeons performing procedures in the neck. A search was conducted using both electronic databases and hand-searched resources in the area of neck anatomy and surgery. The collected information was summarized and illustrated through photos and schemes. By analyzing the anatomical characteristics and contents of the studied regions, along with surgical experience, conclusions were drawn regarding the importance and usefulness of *Beclard's*, *Farabeuf's*, *Lesser's*, *Pinaud's*, and *Pirogov's* triangles in clinical practice. The neck not only connects the head to the rest of the body but also contains structures that carry blood and nerves to and from the brain. While many triangles in the neck have been described, some have become less commonly known. The discussed triangles were found to be reliable and consistent landmarks that are valuable to surgeons specializing in head and neck surgery. A thorough understanding of the described neck triangles could improve the efficiency of surgical procedures and help prevent intraoperative complications. This detailed review of their anatomical features and clinical significance provides practical benefits for surgeons.

Key words: *Beclard's* triangle, *Farabeuf's* triangle, *Lesser's* triangle, *Pinaud's* triangle, *Pirogov's* (*Pirogoff's*) triangle

Resistin and visfatin are two adipokines that are potential markers in patients with endometrial and ovarian cancer - immunohistochemical study

Blagovesta Mitkova[1*], V. Kenovska[2], P. Koleva[3]

[1]*Dept. of Anatomy and cellular biology, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria*
[2]*Dept. of Anatomy and cellular biology, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria*
[3]*Head of the Department of Clinical Pathology, SBGAL, "Prof. Dr. D. Stamatov", Varna, Bulgaria*

*Corresponding author e-mail: blagovesta.nanova@abv.bg

Inflammation and altered immune response are important components of obesity and contribute greatly to the presentation of metabolic complications associated with adipose tissue, overweight, obesity, and carcinogenesis. Adipokines are now being considered and studied as potential biomarkers for the risk of developing complications. One of these complications is the development of cancers directly related to metabolic disorders. Elevated levels of resistin are associated with insulin resistance and have been shown to increase with obesity, supporting its role in metabolic dysfunction. It has been associated with increased risk of progression, angiogenesis, and metastasis in various cancer models. Visfatin is a proinflammatory cytokine involved in cellular metabolism and chronic inflammation, provoking neoplasia. The aim of the study is the influence of perivisceral adipose tissue on the processes of tumorigenesis due to the altered neoplastic microenvironment by establishing the localization of the adipokines resistin and visfatin and their cytotoxic effect. Material: biopsy specimens from patients with endometrial (70) and ovarian carcinoma (38) were provided by the Department of Clinical Pathology, SBGAL, Prof. Dr. D. Stamatov, Varna. A classical immunohistochemical technique was applied, using monoclonal antibodies resistin-C 10 and anti-visfatin [EPR21980] (ab236874). Results: In patients with endometrial carcinoma, a moderate to weak immune reaction was observed in the cytoplasm of the affected glandular cells, as well as in macrophages. Adipocytes show weak to negative expression of anti-resistin antibody, while the intensity of the immune reaction to anti-visfatin is strongly positive. Estrogen-dependent endometrial tumors show strong expression of estrogens. In patients with ovarian carcinoma, the intensity of the immune reaction to resistin is weak to negative, while for visfatin it is strong in intensity. Conclusion: the antitumor effect of resistin and visfatin activity involved in tumorigenesis and cancer progression (both adipokines are potential biomarkers for obesity risk).

Key words: immunohistochemistry, adipokines, endometrial, ovarian cancer

Inflammatory pathway in aging Leydig cells

D. Barbutska[1*], Y. Koeva[1], M. Shishmanova-Doseva[2], P-P Petrov[1]

[1]*Department of Anatomy, Histology and Embryology, Medical University of Plovdiv, Plovdiv, Bulgaria*
[2]*Department of Pharmacology, Toxicology and Pharmacotherapy, Medical University of Plovdiv, Plovdiv, Bulgaria*

*Corresponding author e-mail: Darina.Barbutska@mu-plovdiv.bg

Aging of the male reproductive system is associated with progressive structural and functional changes in the testis that lead to reduced production of the male sex hormone testosterone. Steroid-producing Leydig cells (LCs) play a key role in maintaining the endocrine function of the testis as the main source of testosterone. In recent years, several studies suggest that inflammatory mediators, including tumor-necrosis factor alpha, TNF-α, are significantly involved in testicular aging. TNF-α is a pro-inflammatory cytokine that is expressed by various cells in the testis and can modulate the function of Leydig cells by influencing signaling pathways regulating steroidogenesis, cell proliferation and apoptosis. The accumulation of TNF-α in the aging testis is associated with an increased level of chronic inflammation, which in turn suppresses the expression of key steroidogenic enzymes, such as StAR, 3β-HSD, P450scc; induces oxidative stress and apoptotic cascades. This leads to impaired cellular viability and functional activity of Leydig cells to synthesize testosterone. This study aims to describe the immunoexpression of TNF-α in the LCs of rats during aging. We used an experimental model of male Wistar rats at different ages (5, 15 and 21 months), whose testis were handled and subjected to the immunohistochemical study with anti- TNF-α antibody. The immunohistochemistry indicates an increase in the intensity of the expression for TNF-α in LCs in the testis of aged rats. These results correlate with the morphological changes in the aging testis with hematoxylin-eosin staining. Our results reveal the role of TNF-α in the processes of testicular aging, with special emphasis on its influence on morphology, signaling mechanisms and steroidogenic function of Leydig cells. Understanding these mechanisms could contribute to the development of new therapeutic strategies to limit age-related hypogonadal dysfunction.

Key words: TNF-α, aging, Leydig cells, inflammation, steroidogenesis

Anatomical variation of the thyrocervical trunk: Absence of inferior thyroid artery with dominant supply from the right superior thyroid artery

Iren Bogeva[1*], Desislava Marinova[1], Lyubomira Angelova[2], Stefan Trifonov[1]

[1]*Department of Anatomy, Histology, Cytology and Biology, Faculty of Medicine, Medical University – Pleven, Bulgaria*
[2]*Medical student, Medical University – Pleven, Bulgaria*

*Corresponding author e-mail: irenbogeva@outlook.com

Variations in the branching pattern of the thyrocervical trunk are of considerable anatomical and clinical importance, particularly in surgical procedures involving the thyroid gland and larynx. The inferior thyroid artery typically represents a major source of blood supply to the thyroid gland, and its absence is uncommon, especially when observed bilaterally. During routine anatomical dissection of the cervical region in a cadaver at the Department of Anatomy, Medical University – Pleven, a bilateral absence of the inferior thyroid arteries (ITAs) was identified. The superior thyroid arteries were present bilaterally and followed their typical anatomical course, arising from the external carotid arteries and supplying the thyroid gland. The right superior thyroid artery was the dominant arterial source for the gland, giving glandular branches to both the upper and lower parts of the entire thyroid gland. In contrast, the left superior thyroid artery was less developed and supplied predominantly the lower portion of the gland, mainly on the left side. The thyrocervical trunk did not give rise to ITAs. Instead, an aberrant branch originated directly from the thyrocervical trunk on each side. These arteries ascended medially toward the larynx without providing branches to the thyroid gland. No accessory arterial supply, such as a thyroidea ima artery, was identified. The arterial supply to the thyroid gland was therefore maintained primarily by the superior thyroid arteries. This case represents a rare bilateral anatomical variation characterized by the absence of the inferior thyroid arteries and the presence of aberrant arterial branches arising directly from the thyrocervical trunk. In this context, the right superior thyroid artery plays a dominant role in vascularizing the gland. Recognition of such vascular patterns is essential for surgeons performing thyroidectomy and other cervical procedures, as unanticipated variations may increase the risk of intraoperative complications.

Key words: inferior thyroid artery, inferior laryngeal artery, subclavian artery, thyrocervical trunk, human anatomy variation

De novo primer design and PCR optimization for detection of alternatively spliced mouse ChAT mRNA isoforms

Desislava Marinova*, Stefan Trifonov

Department of Anatomy, Histology, Cytology and Biology, Faculty of Medicine, Medical University – Pleven, Bulgaria

*Corresponding author e-mail: desislava.mmarinova@mu-pleven.bg

Acetylcholine is a major neurotransmitter that regulates diverse functions in both the somatic and autonomic nervous systems. The cholinergic system plays a central role in motor control, emotional behavior, and higher cognitive processes, including learning and memory. Acetylcholine biosynthesis is catalyzed by choline acetyltransferase (ChAT), which serves as a reliable molecular marker for cholinergic structures. The complexity of ChAT gene expression is further increased by alternative splicing, generating multiple mRNA isoforms with distinct regulatory and functional properties. The aim of the present study was to perform *de novo* primer design and PCR optimization for the selective detection of alternatively spliced mouse ChAT mRNA isoforms. Primer pairs were designed to target R1, R3, R4, N1, N2, M, peripheral ChAT, and common ChAT variants. Primer specificity and optimal PCR conditions were evaluated using specially designed synthetic DNA fragments (gBlock-1 and gBlock-2) and a plasmid containing isoform-specific nucleotide sequences. PCR reactions were performed under optimized conditions, including primer pair-specific annealing temperatures and the addition of dimethyl sulfoxide (DMSO) when primers encompassed exon-exon junctions. Amplification specificity was assessed using melting curve analysis and agarose gel electrophoresis. The applied optimization strategy enabled reliable identification between closely related ChAT splice variants and effectively eliminated nonspecific amplification products. In conclusion, the *de novo* designed primers and optimized PCR conditions provide a robust and reproducible molecular approach for detecting alternatively spliced mouse ChAT mRNA isoforms. This methodological framework facilitates precise analysis of cholinergic gene regulation and may support future studies investigating cholinergic dysfunction in physiological and pathological conditions, as well as the development of targeted pharmacological and gene therapy strategies.

Key words: choline acetyltransferase, alternative splicing, primer design, PCR optimization, cholinergic system

Hepatocellular remodeling after microplastic exposure

Despina Pupaki[1*], Dessislava Ankova[1], Nikolay Genov[2], Dimitrinka Atanasova[2,3], Pavel Rashev[1]

[1]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*

[3]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: dpupaki@ibir.bas.bg

Microplastics are emerging environmental contaminants with potential adverse effects on liver structure and function. The present study aimed to evaluate time-dependent morphological and morphometric changes in hepatocytes, including recovery dynamics, following exposure to polystyrene microplastics (PS-MPs). A total of 54 six-week-old male Swiss albino mice were randomly assigned to control and experimental groups (3 controls and 6 experimental animals per week). The experimental animals received 1 μm PS-MPs via drinking water at a dose of 0.1 mg/day for four weeks, followed by a two-week recovery period without exposure. Histological (H&E), histochemical (PAS), and morphometric analyses of hepatocyte cross-sectional area were performed at weeks 1, 4, and 6. Statistical evaluation included Kruskal–Wallis test with Dunn's post hoc analysis. At week 1, a significant increase in hepatocyte area was observed in the treated groups compared to controls ($p < 0.0001$), indicating an early cellular response. By week 4, pronounced hepatocellular ballooning was detected, with an approximate 35% increase in cell area ($p = 0.0064$), consistent with hypertrophic alterations. Following the two-week recovery period (week 6), partial regression of these changes was observed, with no statistically significant differences between groups. Histologically, hepatocyte ballooning occurred without evidence of steatosis and was accompanied by mild inflammatory infiltration. PAS staining demonstrated a progressive increase in glycogen accumulation, reaching approximately a sevenfold elevation by week 6 ($p < 0.0001$). These findings indicate that microplastic exposure induces dynamic and partially reversible hepatocellular alterations, suggesting adaptive responses of the hepatic parenchyma following cessation of exposure.

Key words: polystyrene microplastics, hepatocellular hypertrophy, liver histology, glycogen accumulation

Acknowledgments: This research was funded by the Bulgarian National Science Fund (Grant No. КП-06-Н81/2).

Sex differences in size and shape of the coxal bone in Bulgarians

Diana Toneva[1*], Silviya Nikolova[1], Dora Zlatareva[2], Nevena Fileva[2], Georgi Milenov[2]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[2]*Medical University – Sofia, Sofia, Bulgaria*

*Corresponding author e-mail: ditoneva@abv.bg

The human pelvis exhibits the most pronounced sexual dimorphism in the skeleton, largely due to its functional role in childbirth. The coxal bones constitute the major part of the pelvis and demonstrate size and shape characteristics that are highly valuable for sex estimation in forensic contexts. This study employs both metric and geometric morphometric approaches to investigate sexual dimorphism of the coxal bone and to evaluate the effectiveness of size and shape variables in sex estimation in the Bulgarian population. A total of 276 abdominal computed tomography (CT) scans of Bulgarian adults (136 males and 140 females) were analyzed. Three-dimensional surface models of the pelvis were generated from the CT images using InVesalius, and 34 anatomical landmarks located on the coxal bones were digitized in MeshLab. An extended set of linear measurements describing the coxal bones was derived from the landmark coordinates. The landmark configurations were further analyzed using generalized Procrustes superimposition, followed by Procrustes ANOVA to assess sex differences in centroid size and shape. Principal component analysis (PCA) was then applied to explore shape variation within the sample. Finally, the potential for sex classification was evaluated using machine learning algorithms (support vector machines and logistic regression). The statistical analyses revealed significant sex differences in both size and shape of the coxal bone. PCA revealed a clear separation between male and female individuals along the primary principal component axis. Shape-based classification achieved an accuracy of up to 98%, substantially outperforming that based on centroid size. The machine learning models based on coxal bone measurements achieved classification accuracies ranging from 95% to 100%. These findings highlight the pronounced sexual dimorphism of the coxal bones and confirm the high reliability of measurements and shape variables for accurate sex estimation.

Key words: hip bone, sexual dimorphism, computed tomography, geometric morphometrics, machine learning

Acknowledgments: The study was funded by the Bulgarian National Science Fund, Grant number КП-06-Н51/4–11.11.2021.

Primo Vascular System under artificial circulation: a pilot hypothesis-based study

Dimitri Kordzaia*, Nodar Khodeli, Keti Tsomaia, Lika Svanadze

Alexandre Natishvili Institute of Morphology, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia

*Corresponding author e-mail: dimitri.kordzaia@tsu.ge

The Primo Vascular System (PVS) is a recently described putative circulatory network composed of primo vessels and nodes forming interconnected pathways. It has been proposed as an additional microcirculatory system with superficial (subcutaneous) and deep components, including intravascular and intralymphatic structures. The superficial pathways have been suggested to correspond to acupuncture meridians. Despite growing evidence, a key paradox remains: PVS structures are typically identifiable only in living, anesthetized organisms and not in postmortem conditions. To evaluate whether PVS structures can be identified in organisms lacking native cardiac or cerebral activity but maintained under artificial circulation. Pilot experiments were conducted on rabbit models using an original extracorporeal perfusion system. Two conditions were studied: (1) heart-dead and (2) brain-dead organisms under controlled artificial circulation. Visualization of intralymphatic primo vessels followed established staining and microsurgical techniques with defined morphological criteria. Preliminary observations demonstrated that intralymphatic primo vessel-like structures could be identified under artificial circulation despite absent native cardiac or cerebral activity. These findings suggest that PVS detectability depends not strictly on organismal viability, but on functional circulation and microenvironmental conditions. The results align with previous studies demonstrating continuity of intralymphatic primo vessels and extend these observations to artificially maintained systems. Artificial machine perfusion models may provide a novel platform for investigating the Primo Vascular System. The findings support the hypothesis that functional circulation, rather than viability per se, is critical for PVS visualization, opening new directions for controlled experimental studies.

Key words: primo vascular system, artificial circulation, machine perfusion, lymphatic system, hypothesis

Acknowledgments: This study was supported by the Shota Rustaveli National Science Foundation of Georgia (Project №FR-24-18604).

Arc immunoreactivity and Arc/pCREB distribution in the dorsal hippocampus after β2-adrenergic activation of the basolateral amygdala

Dimitrinka Atanasova[1,2*], Bogomil Peshev[1], Pavel Rashev[3], Lidia Kortenska[1], Milena Mourdjeva[3], Despina Pupaki[3], Nikolay Dimitrov[2], Nikolaos Efstratiou[2], Jana Tchekalarova[1]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[3]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*

[*]Corresponding author e-mail: d.atanasova@inb.bas.bg

Activity-regulated cytoskeleton-associated protein (Arc) is a widely recognised marker of experience-dependent neuronal plasticity and early cellular activation during memory formation. Although the basolateral amygdala (BLA) is known to modulate hippocampal memory consolidation, its influence on the distribution of Arc expression across dorsal hippocampal subfields remains unclear. This study examined Arc immunoreactivity and the cellular relationship between Arc and phosphorylated CREB (pCREB) following post-training β2-adrenergic activation of the BLA. Adult male Wistar rats were assigned to three experimental groups: control, clenbuterol-treated (Clen), and SCH 23390 + clenbuterol-treated (SCH/Clen). After object-location training, Clen was infused bilaterally into the BLA, while SCH 23390 was administered before Clen infusion in the combined-treatment group. Animals were perfused 60 minutes after training and treatment. Coronal paraffin sections (6 μm) were processed for Arc immunohistochemistry and double immunofluorescence for Arc and pCREB. Arc immunoreactivity was observed mainly in the cytoplasm and proximal dendrites, showing staining patterns ranging from granular to diffuse, whereas nuclear expression was rare. In control animals, Arc-positive cells were scarce. Clen treatment increased Arc expression in CA3c, CA3b, CA3a, CA2, and CA1, with the strongest effect in CA3a, CA3b, and CA1. Pretreatment with SCH 23390 attenuated the Clen-induced increase in Arc expression in CA3b, CA3a, CA2, and CA1. Double immunofluorescence in CA1 revealed cellular co-expression of Arc and pCREB, but only limited subcellular co-localisation. Arc was localised predominantly in the perisomatic and perineuritic compartments, whereas pCREB remained mainly nuclear. These findings demonstrate a region-specific and compartment-specific pattern of Arc expression in the dorsal hippocampus following BLA stimulation and support a role for dopaminergic signalling in regulating plasticity-related protein expression in selected hippocampal subfields.

Key words: Arc, pCREB, dorsal hippocampus, basolateral amygdala, immunofluorescence
Acknowledgement: This research was funded by the Bulgarian National Science Fund (Grant No. КП-06-Н61/12).

From adult morphology to developmental hypothesis: Variation of the adult lateral wrist extensors – a developmental viewpoint

Dimo Stoyanov[1, *], Tsvetomir Kachovski[2], Lora Veleva[1], Martin Ivanov[1], Kamelia Bratoeva[3,4], Anton Tonchev[1, 4], Emil Kovachev[2], Stoyan Pavlov[1]

[1]*Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University of Varna, Varna, Bulgaria*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University of Varna, Varna, Bulgaria*
[3]*Department of Physiology and Pathophysiology, Medical University of Varna, Varna, Bulgaria*
[4]*Vascular Biology Research Group (RenEVA), Research Institute, Medical University of Varna, Varna, Bulgaria*

*Corresponding author e-mails: dimostoyanov1990@gmail.com or dimo.stoyanov@mu-varna.bg

Observing anatomical variations is a common occurrence in the dissection course for medical students and is especially important during surgical interventions. The importance of studying anatomical variability and introducing this concept during early medical education is stressed by the fact that medical malpractice due to variations is reported to be approximately between 10% and 25%. Here we present a systematic study of the radial wrist extensors in 58 human upper limbs, including two foetal specimens at GW 12 and GW 17. Our findings can be separated into three groups. First, we noticed that if an interconnecting tendon arises from ECRL (m. extensor carpi radialis longus), it always inserts below the main tendon of ECRB (m. extensor carpi radialis brevis) ('L-to-B' connection). If it originates from the ECRB, it always inserts medial to the main tendon of ECRL ('B-to-L' connection). These findings are in line with the existing literature. Second, the origin of the interconnecting tendons is also highly repetitive. They start from the ventral portion of ECRL or the latero-dorsal aspect of ECRB, respectively. Third, we analysed the distribution of only a single interconnecting tendon being present ('B-to-L' or 'L-to-B') versus both occurring in the same specimen. Our observations suggest that both tendons are much more likely to be present. This prompted us to perform a chi squared test of the observed distribution versus the expected one. The analysis revealed that both tendons are present much more often that one would expect from separate events, therefore, interconnecting tensons are formed co-dependently. We conclude by suggesting that the oblique mode of tendon fission may lead to the observed distribution. The foetal limbs ECRL and ECRB showed anatomy similar to that of the adult specimens.

Key words: upper limb development, radial wrist extensors variability, interconnecting tendons, anatomy, variations

Doxorubicin-induced neurotoxicity: histopathological and immunohistochemical evidence from acute models

Liana Gogiashvili[1,2], Elene Nikobadze[1*], Tamuna Gvianishvili[1,3]

[1]*TSU, A. Natishvili Institute of Morphology, Tbilisi, Georgia*
[2]*D. Tvildiani Medical University, Tbilisi, Georgia*
[3]*Tbilisi State Medical University, Tbilisi, Georgia*

*Corresponding author e-mail: elene.nikobadze@tsu.ge

Chemotherapy-induced cognitive impairment (CICI), or "chemobrain," is characterized by deficits in memory, attention, learning, and executive function, significantly affecting patients' quality of life. Despite its clinical importance, the underlying mechanisms remain unclear. Doxorubicin (DOX), an anthracycline widely used in cancer therapy, exerts antitumor effects through DNA intercalation, inhibition of topoisomerase II, oxidative stress, and activation of apoptotic pathways. Although DOX has limited penetration across the blood–brain barrier (BBB), growing evidence suggests it may induce central nervous system toxicity. The aim of this study is to investigate the acute effects of DOX on the cerebral cortex and BBB, focusing on histopathological and immunohistochemical changes. Adult male Wistar rats (170–200 g) were used. Controls were maintained under standard vivarium conditions (GALAS, 2023). Experimental groups received DOX (5–15 mg/kg) under different regimens. Brain tissues were analyzed using histological and immunohistochemical methods. DOX induced dose-dependent alterations in neurons, glia, and BBB components, including cytoskeletal degeneration, cellular swelling, extracellular matrix depletion, and disruption of intercellular junctions. Marked reactive gliosis, astrocytic hypertrophy and hyperplasia, and increased GFAP immunoreactivity were observed. Anti-MBP (F6) expression decreased in oligodendrocytes, with associated myelin loss and fragmentation. Neuronophagia was prominent, with oligodendrocytes acting as satellite cells in grey matter. Numerous vacuolated neurons and necrotic foci were identified. In the choroid plexus, vascular abnormalities included luminal dilation, hyperemia, hemolysis, and hemo- and plasmorrhagia, along with ependymal swelling and partial fusion with pia mater structures. In conclusion, acute DOX exposure causes significant brain cortical cytoarchitecture changes, including neuronal damage, reactive gliosis, BBB disruption, and enhanced oligodendrocyte involvement in neuronophagia, suggesting morphological substrates of chemobrain and cognitive impairment.

Key words: doxorubicin, chemotherapy-induced cognitive impairment, CNS glia, immunohistochemistry

Structural characteristic of the *pars pelvina urethrae* in domestic pig

Genadi Kostadinov[1*], Angel Vodenicharov[2], Rosen Dimitrov[1], Nikolay Tsandev[1,2]

[1]*Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria,*

[2]*Department of Preclinical Science, Faculty of Veterinary Medicine, Medical University, Pleven, Bulgaria*

*Corresponding author e-mail: genadi.kostadinov@trakia-uni.bg

The aim of this study was to determine the structural features of the pelvic urethra in male pigs. Pelvic urethral sections of 6–8 month, 100–110 b.w. male pigs were collected immediately after slaughter in slaughterhouse and fixed in formalin, Carnoy's and Bouen's liquids. Sections 5–6 µm were stained with hematoxylin-eosin, orcein, Van-Gieson and Masson trichrome. Light microscopic studies revealed that the muscle bundles of the *m. urethralis* surrounded the parenchyma of the *pars disseminata prostatae*, which had a relatively compact structure under the muscle, while in depth it showed a clearly pronounced lobar structure. Between the individual lobes connective tissue septa and excretory ducts that opened into the lumen of the urethra, were located. The parenchyma was represented mainly by glandular tubules with a wall of a single layer of cubic epithelium. Some of the tubules showed an independent course, while these, for the most part, connected with neighboring tubules. At the place of their connection an expansion of a different shape was usually observed. The blood vessels in the lobes were represented by capillaries, most often with two, and less often with three endothelial cells along the circumference of the sections through them. Transverse and tangential sections through arteries and veins of different calibers were observed, and nerves with a relatively large thickness, reaching 100 µm. They were located in the middle of connective tissue with mostly of collagen fibers of different orientation, as well as between bundles of *m. urethralis*, in which the transverse striation in the cytoplasm of cells of the *textus muscularis striatus syncytialis* was visible. Selective staining demonstrated more clearly the collagen and elastic fibers of the connective tissue, blood vessels and muscles, nerves, and glandular parenchyma. On some of the sections through arteries, both *lamina elastica externa* and *interna*, were clearly visible.

Key words: pig, pelvic urethra

Morphological dynamics of somitic development in *Gallus gallus*: a pilot study

Georgi Petrov*, Desislava Abadjieva

Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: georgi@ibir.bas.bg

Maternal resource allocation is a key determinant of embryonic development in oviparous species, with eggshell properties representing an important component of the embryonic microenvironment. While the role of eggshell-derived calcium in later developmental stages is well established, its potential association with early morphogenetic processes remains insufficiently explored. The present pilot study aimed to investigate whether variation in eggshell structural properties, assessed through a proxy-based classification approach, is associated with early embryonic morphogenesis in *Gallus gallus*. Fertilized hen eggs (n = 5) were incubated under standardized conditions and embryos were analyzed at early developmental stages corresponding to Hamburger–Hamilton stages HH12-HH14. Morphometric analysis was performed using digital image processing techniques, including somite count (SC), somitic row length (SRL), and bilateral symmetry index (BSI). A strong monotonic association was observed between proxy-based classification of eggshell properties and somite count ($r_s = 1.00$, $p \leq 0.017$), with consistent positive trends observed for SRL and BSI. Embryos derived from higher-ranked eggs exhibited increased axial elongation and improved bilateral symmetry. These results suggest that maternal resource allocation may influence the precision of early morphogenesis. The integration of proxy-based assessment, digital morphometry, and histological analysis provides a reproducible framework for investigating developmental variability in avian models.

Key words: *Gallus gallus*, somitogenesis, morphometrics

Effects of systemic lupus progression on oocyte meiosis in MRL/lpr mice

I. Chakarova[1*], V. Hadzhinesheva[1], R. Zhivkova[1], M. Markova[1], V. Nikolova[1], A. Kolarov[1], N. Mladenov[1], G. Boneva[2], S. Bradyanova[2], J. Prechl[3], N. Mihaylova[2], A. Tchorbanov[2], S. Delimitreva[1]

[1]*Department of Biology, Medical University of Sofia, Sofia, Bulgaria*

[2]*Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[3]*R&D Laboratory, Diagnosticum Zrt, Budapest, Hungary*

*Corresponding author e-mail: i.chakarova@medfac.mu-sofia.bg

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the activation of the immune response against self antigens. Numerous reproductive complications, including reduced birth rate and complications for the mother and the fetus during pregnancy, have been observed in women with SLE. In the present study, we aimed to investigate the effect of SLE development on oocyte meiosis in lupus-prone mice. Lupus-prone MRL/lpr mice were used for the experiments: disease-free (4 weeks of age) and sick (20 weeks of age, virgin and postpartum). The immune response was monitored by flow cytometry, ELISpot, ELISA, and histology. Oocytes were analyzed by fluorescence microscopy based on chromatin, tubulin, and actin structures. The lupus-prone MRL/lpr mice developed age-dependent symptoms of SLE with increased levels of various autoantibodies, proteinuria, and renal infiltrates and a tendency for the immune response to worsen with changes in cell populations and the cytokine profile. The number and quality of oocytes were also affected, and the successful pregnancy rate of MRL/lpr mice was limited to only 60%. Isolated oocytes showed severe structural changes in all studied groups. Systemic alterations in immune homeostasis in SLE affect the quality of developing oocytes, which is evident from a young age. The data obtained is in line with the trend of reduced fertility in lupus-prone MRL/lpr mice. The phenomenon can be explained by changes in the microenvironment of the relevant organs and close connection between ovulation and inflammatory processes.

Key words: oogenesis, systemic lupus, meiotic spindle, actin cap

Depletion of vascular adaptive mechanisms and mast cell-driven fibrosis in established and advanced hypertensive heart disease

Ivan Angushev[1*], Lyubomir Gaydarski[1], Kristina Petrova[1], Tsvetko Obetsanov[1], Nikola Stamenov[1], Stancho Stanchev[1], Tihomir Dikov[2], Vesela Ivanova[2], Boycho Landzhov[1], Alexandar Iliev[1]

[1]*Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria*
[2]*Department of General and Clinical Pathology, Medical University of Sofia, Sofia, Bulgaria*

*Corresponding author e-mail: iangushev@medfac.mu-sofia.bg

Hypertensive heart disease involves complex structural and vascular remodeling of the myocardium. While mast cells are implicated in pro-fibrotic and pro-inflammatory pathways, vascular endothelial growth factor (VEGF) and neuronal nitric oxide synthase (nNOS) are vital for compensatory angiogenesis and the regulation of vascular tone. This study investigates the interplay between these factors during the progression of hypertension-induced myocardial injury. The study utilized 6-month-old and 12-month-old Spontaneously Hypertensive Rats (SHR), representing established and advanced stages of hypertensive heart disease, respectively. The number of mast cells (MCN) in the right and left ventricles was quantified using toluidine blue, tryptase, and c-kit staining. Concurrently, the myocardial expression of VEGF and nNOS was evaluated using semi-quantitative immunohistochemistry. As hypertensive injury progressed from 6 to 12 months, MCN significantly increased in the myocardium of both the left and right ventricles. This accumulation of mast cells was positively correlated with enhanced interstitial fibrosis. Conversely, the expression of vascular adaptive molecules declined as the disease advanced. VEGF expression was markedly stronger in the 6-month-old SHR but decreased significantly in the 12-month-old group across the examined ventricular zones. Similarly, nNOS immunoreactivity exhibited a slight downregulation in both ventricles of the older SHR compared to the younger animals. The progression from established to advanced hypertensive heart disease is characterized by a significant accumulation of pro-fibrotic mast cells alongside a simultaneous depletion of compensatory vascular mechanisms, evidenced by the downregulated expression of VEGF and nNOS. This relationship suggests that the exhaustion of adaptive angiogenic and vasodilatory pathways, coupled with mast cell-driven fibrosis, plays a critical role in the maladaptive transition toward heart failure.

Key words: nNOS, VEGF, hypertensive heart disease, angiogenesis, myocardial remodeling

Birth order and its influence on the anthropometric sizes of the newborn

Ivaila Ivanova–Pandourska*, Yanitsa Zhecheva

Institute of Experimental Morphology, Pathology And Anthropology With Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: anthropologyvaila@gmail.com

Birth weight is one of the important parameters for survival, growth and development of the child. Genetic, socio-economic, environmental factors influence the prenatal period, but parental factors (morpho-functional status of mother and father, age, etc.), as well as birth order being a great importance for newborn sizes. The aim of the study is to evaluate the influence of birth order on the basic anthropometric sizes of newborns. Data on 1435 (747 /52.1%/ boys and 688 /47.9%/ girls) livebirth singleton neonates born in 2020, as well as data on their mothers, pregnancy and delivery, were collected from the birth registry of the Obstetrics and Gynecology Hospital "St. Sofia", Sofia. According birth order the newborns were divided into three groups: first-born, second-born and third-or-later-born. The collected data were analyzed by statistical software SPSS 16.0 and Z-score transformation was applied to birth weight and length of neonates. One-way ANOVA analyses with post hoc Tukey Honestly Significant Difference Test were performed and significance level was set at $P \leq 0.05$. The highest percentage of neonates are first-borns (56.0%), followed by second-born babies (36.0%) and third-or-later-born babies (8.0%). Birth order has a significant influence on the newborn body weight and length ($p=0.004$). The sizes of neonates increased with birth order, as Z-score varied from -0.07 to 0.20 SDS. The first-born babies have the smallest birth sizes. Boys and girls born from second and third-or-later birth have weight and length above and around the average. Their dimensions significantly differ ($p \leq 0.05$) from those of first-born babies. Second and third-or-later-born infants weigh an average of 68.6 grams and 125.7 grams more at birth than first-born children, respectively. The babies born second and third or later are significantly longer than these born first. The birth order significantly affects the anthropometric characteristics of neonates. The babies from third-or-later-births have larger body sizes.

Key words: newborn weight, newborn length, birth order, maternal age, Sofia

Acknowledgments: The work was supported by the Bulgarian National Science Fund: Grant КП-06-Н51/7 from 11.11.2021

Combined memantine and environmental enrichment enhance neurogenesis in the subgranular zone of the dentate gyrus in Wistar rats

Ivaylo Stefanov[1*], Bogomil Peshev[2], Pavel Rashev[3], Nikolay Dimitrov[1], Nikolaos Efstratiou[1], Nikolai Genov[1], Lidia Kortenska[2], Milena Mourdjeva[3], Jana Tchekalarova[2], Dimitrinka Atanasova[1,2]

[1]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: ivaylo.stefanov@trakia-uni.bg

Adult hippocampal neurogenesis represents an important form of structural plasticity in the mature brain and occurs primarily in the subgranular zone (SGZ) of the dentate gyrus. Understanding how different external factors influence this process remains a key area of research. In this context, combining pharmacological and environmental interventions may provide deeper insight into the mechanisms regulating adult neurogenesis. The present study aimed to evaluate the effects of memantine administration combined with environmental enrichment (EE) on cell proliferation and neuronal differentiation in the SGZ of the dentate gyrus in adult Wistar rats. The animals were divided into two groups: veh-veh-HC, maintained under standard housing conditions, and veh-MEM-EE, which received memantine and was exposed to an enriched environment. The EE conditions included increased cage space, as well as various objects promoting sensory, motor, and social stimulation. Immunohistochemical analysis was performed using Ki67 as a marker of proliferating cells and doublecortin (DCX) as a marker of immature neurons. Compared to controls, the veh-MEM-EE group demonstrated a clear increase in Ki67-positive cells within the SGZ, indicating enhanced proliferative activity. In parallel, DCX expression was elevated, suggesting an expansion of the population of newly generated immature neurons and stimulation of early neuronal differentiation. Taken together, these findings suggest that the combined application of memantine and environmental enrichment promotes key stages of adult neurogenesis in the dentate gyrus. From a morphological perspective, this multimodal approach induces measurable changes within the main hippocampal neurogenic niche. Such a model could be useful for further studies investigating the structural basis of learning and memory, as well as mechanisms involved in neurodegenerative disorders.

Key words: adult neurogenesis, subgranular zone, dentate gyrus, Ki67, doublecortin (DCX)
Acknowledgement: This research was funded by the Bulgarian National Science Fund (Grant No. КП-06-Н61/12)

Combination between CT-scan and photogrammetry in the creation of printable bone models

Ivelina Ivanova[1*], Nikolay Genov[1], Nikolay Dimitrov[1], Dimitrinka Atanasova[1,2], Ivaylo Stefanov[1], Kristina Miteva[3]

[1]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Medical student, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*

*Corresponding author e-mail: ivelina.ivanova@trakia-uni.bg

The increasing shortage of cadaveric specimen for dissections as well as the rigorous regulatory requirements and the need of special laboratory equipment essential for the bone extraction has created a big gap which the usage of new technologies such as AI-driven segmentation software, CT-scans and 3d printers could fill. We have compared the usage of raw DICOM data from patients' CT-scans to photogrammetry in the development of an anatomically accurate model of femur. The model was classified as correct if it features 23 elements important in the anatomical curriculum of medical students based on Atlas der Anatomie des Menschen (Sobotta/Becher) 23rd edition. With the implication of CT-scans of lower limbs and pelvis studied with the free, open-source software, 3D Slicer 5.10.0, we have managed to create a 3d printable femur showing an anatomical accuracy of 56.5% failing to provide fine bony prominences and fossae. The model that was derived by using photogrammetry identified 21 of 23 elements, showing 91.3% accuracy, but lacks inherent absolute scaling, requiring manual calibration to match anatomical proportions. To overcome these limitations, we developed a hybrid methodology, that utilizes the CT-derived 3d object as the spatial backbone of the model while the photogrammetry mesh is integrated to provide an increased resolution and high anatomical fidelity. The combination between these to relatively easy methods for creating 3d printable objects could serve as an important step in the creation of an accurate substitute of human bones and organs in the study of medical students.

Key words: CT-scan, femur, 3d models, 3d printing, photogrammetry

Dilated cardiomyopathy *in vivo* and *in vitro* modelling with a focus on enzymatic pheno- and genotyping

Katerina Todorova[1*], Any Georgieva[1], Rositsa Milcheva[1], Zdravka Petrova[1], Inna Sulikovska[1], Volodymyr Balatskyi[2], Viktor Navrulin[2], Wiktoria Laprus[2], Paweł Dobrzyń[2]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

[2]*Nencki Institute of Experimental Biology - Polish Academy of Sciences, 02-093 Warszawa, Poland*

*Corresponding author e-mail: katerinagencheva@yahoo.com

Cardiomyopathies are a group of inherited diseases or, rarely, acquired conditions. Derived cardiomyopathy cases are characterized by genetic heterogeneity, where more than 200 genes were evaluated to be responsible. On the other hand, acquired cardiomyopathies develop over time, due to coronary artery disease, high blood pressure, infections, toxins or other external factors that affect the structure and function of the heart muscle. Dilated cardiomyopathy (DCM) is one of the frequent forms of cardiomyopathies worldwide. It is well known that the contractions of the myocardium are a subsequence of multiple cell surface glycoproteins including calcium, potassium and sodium ion channels that propagate the electrical impulses and action potentials. In the process of sialylation – the modification of proteins, where glycoproteins are formed, several enzymes from the family of sialyltransferases take role. There are some data about altered glycoprotein sialylation, particularly in human DCM. The data explained above and the prevalence of DCM cardiomyopathy forms in animals and humans, provoked our interest to assess the role of several factors in glycoprotein sialylation in the pathogenesis of cardiomyopathy via simulated *in vivo* and *in vitro* models, that are not genetically predisposed and to compare with research data published on the topics of pathophysiological and clinical heterogeneity of DCM. The results showed that the adapted models have potential to resemble DCM disease, and significantly improve the possibilities for specific research in DCM field.

Key words: dilated cardiomyopathy, *in vivo* and *in vitro* models

Acknowledgments: This work was supported by the project № IC-PL/16/2026-2027, between BAS and PAS.

Morpho-kinematic and biochemical profiling of x- and y-bearing spermatozoa in rams (*Ovis aries*)

Krasimir Kotzev*, Desislava Abadjieva

Institute of biology and immunology of reproduction "Akad. K. Bratanov", Bulgarian academy of sciences, Sofia, Bulgaria

*Corresponding author e-mail: krasimir.kotzev9@gmail.com

The aim of the present study is to perform a comprehensive morpho-functional characterization of two subpopulations of ram spermatozoa (*Ovis aries*), separated in a Percoll density gradient according to the sex chromosome they carry (X or Y). Through an integrated analysis of morphometric parameters (ASMA), enzymatic activity (ALP, GGT, LDH, CK), and kinematic characteristics (SCA), the study seeks to identify differences between the two groups and to determine specific biomarkers for the quality of sexed gametes. The results identify the Y group as having an elongated morphotype and increased head roughness, directly associated with an altered enzymatic status, expressed through a strong positive correlation with ALP ($r = 0.74$) and GGT ($r = 0.927$) activity. In the X group, morphometric parameters do not exert a significant influence on kinematics and metabolism. The kinematic profile shows higher variability and increased VCL and BCF in gametes carrying the Y chromosome, as well as a pronounced negative correlation between Roughness and LIN ($rs = -0.82$). The correlation matrices confirm functional instability in the same fraction, where morphological deviations directly determine sperm motility. In summary, the integrated approach demonstrates that morphological pathology is a determining factor for the biochemical and kinematic profile of spermatozoa, which necessitates the use of combined diagnostic methods for the evaluation of sex-sorted semen.

Key words: spermatozoa, morphology, kinematics, enzyme activity, rams

Study of certain anthropometric indicators in Bulgarian men and women with hypertension

Kristian Argirov[1*], Atanas Baltadjiev[1], Bistra Dobreva-Yatseva[2], Tsvetanka Petleshkova[1]

[1]*Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Bulgaria*

[2]*First Department of Internal disease, Section of Cardiology, Faculty of Medicine, Medical University of Plovdiv, Bulgaria*

*Corresponding author e-mail: kristian.argirov@mu-plovdiv.bg

The role of obesity in the pathogenesis of hypertension has been the subject of extensive research. The aim of this study is to research the classic anthropometric indicators (height, weight, waist and hip circumference) and indices the ratio between waist circumference and hip circumference (WHR) and the ratio between waist circumference and height (WHtR) between patients with arterial hypertension and healthy controls. The study comprised of 40 male and 40 female subjects diagnosed with arterial hypertension, in addition to a control group of 35 males and 35 females. The anthropometric parameters measured in both groups were height, body weight, waist circumference and hip circumference. The results demonstrate that, with the exception of height, all analyzed indicators demonstrate statistically significantly higher values in males and females diagnosed with arterial hypertension ($p<0.001$). The values of both indices are significantly higher in male and female patients than in healthy control subjects ($p<0.001$). The results of the study demonstrate that central obesity plays a significant role in the development of arterial hypertension.

Key words: arterial hypertension, anthropometric indices, Bulgarians

Morphological changes in the mouse brain after chronic exposure to cobalt chloride

Lora Kamenska, Yordanka Gluhcheva, Emilia Petrova*, Rosen Ivanov, Ekaterina Pavlova, Ivelin Vladov, Nina Atanassova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: emiliapetrova@abv.bg

Cobalt overexposure is associated with neurotoxicity and adverse effects on the central nervous system. The present study aims to investigate morphological changes in the mouse brain following chronic exposure to cobalt chloride ($CoCl_2$). ICR mice were treated prenatally with $CoCl_2$ 2-3 days before birth and during the postnatal period at a daily dose of 75 mg/kg body weight until day 90. Studies were performed on postnatal days 18, 45 and 90. Histopathological changes were observed in both the cerebrum and the cerebellum in all age groups, most pronounced in immature mice. In the cerebral cortex, shrunken neurons with pyknotic nuclei, enlarged perineuronal, periglial and perivascular spaces as well as reactive vascular endothelium were observed. In the cerebellar cortex, structural disorganization was evident, including necrotic and autolytic Purkinje cells (PC), reduced PC number, and abnormal PC localization within the granular layer. Immunohistochemical analysis demonstrated increased glial fibrillary acid protein (GFAP) expression in all treated groups, indicating the development of reactive astrogliosis in response to cobalt-induced injury. The observed dynamics of GFAP upregulation highlight the key role of astroglia in both adaptive and pathological processes under cobalt-induced neurotoxicity. Transferrin receptor 1 (TfR1) immunoreactivity increased in the treated groups, and was localized in the endothelial cells of brain capillaries, choroid plexus epithelial cells, cortical neurons, and Purkinje cells. These findings suggest dysregulation of iron metabolism, likely associated with the hypoxia-mimicking effects of cobalt chloride.

Key words: cobalt chloride, mouse brain, histopathological changes, GFAP, TfR1

Automated evaluation of gene expression in chromogenic in situ hybridization images using spectrum-normalized pseudo-channel

Lora Veleva*, Stoyan Pavlov

Department of Anatomy and Cell Biology, Medical University of Varna, Varna, Bulgaria

*Corresponding author e-mail: lora.veleva@mu-varna.bg

The inferior subventricular zone (SVZi) alongside the temporal horn of the lateral ventricle demonstrates a distinct pattern of gene expression changes in a primate model of temporary global cerebral ischemia. A well-established method to visualize these transcriptomic changes in histological samples is chromogenic RNA in situ hybridization (CISH), based on preliminary RNA sequencing data. However, manual quantification of CISH images is time-consuming and highly prone to individual biases. Alternatively, fully automated approaches require consistent experimental and image acquisition conditions, which can limit their widespread application. In the present study, we attempt to solve this problem by developing an ImageJ-based algorithm for image normalization to address the variations in the staining and the imaging setup. As proof of concept, we used NBT/BCIP CISH images of genes that are upregulated after ischemia in the primate brain. The spectral response of the pseudo-filter (defined as sensitivity 1 in the boundaries of 540 nm –660 nm, and 0 otherwise) was fitted as a weighted linear combination of the relative sensitivity spectra of Zeiss Axiocam Mrc rev.3 (extracted from the camera documentation).

$$PseudoCh = 0 + \beta_R * R + \beta_G * G + \beta_B * B + \varepsilon$$

The coefficients of the model were used as weights for the calculation of the pseudo-filtered NBT-formazan image. As a normalization strategy, we next apply a modified optical density (OD) transformation that uses the topological estimate of the illumination field instead of the maximal or modal grey value as an approximation for incident light intensity. This approach corrects for aberrations in the light source between images as well as considers the OD of the local environment of each pixel. The regions of interest were semi-automatically defined by manually outlining the ventricular surface and using Euclidian distance mapping in ImageJ to select the ependymal layer and the subependymal region.

Key words: gene expression, inferior subventricular zone, in situ hybridization, image analysis, optical density

Prognostic and predictive IHC markers in urinary bladder carcinoma

Lucia Krivosikova[1], Pavel Babal[1*], Sebastian Kern[1], Mikus Kuracinova Kristina[1], Stanislav Ziaran[2], Branislav Trebaticky[2], Andrea Janegova[1], Miriam Bollova[1], Pavol Janega[1]

[1]*Department of Pathology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*
[2]*Department of Urology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

*Corresponding author e-mail: pavel.babal@fmed.uniba.sk

Bladder carcinoma is the ninth leading cause of death in men. Diagnosis involves histological classification of surgical specimens, the most frequent being urothelial carcinoma, categorized by grade (low/high) and invasiveness. Because specific subtypes correlate with distinct protein-expression profiles, this retrospective study evaluates the prognostic value of several such markers. According to WHO 2022 criteria, 258 urothelial bladder carcinoma cases were diagnosed. Immunohistochemistry was used to evaluate the expression of CK 5/6, CK7, CK20, p63, E-cadherin, AIF (apoptosis inducing factor), survivin, and Ki67. The intensity (0-3) and percentage of positive cells (1-10) were calculated for cytoplasmic/membrane expressions, nuclear expressions were evaluated as percentage only. Expression profiles were statistically correlated with tumor stage and grade. With the exception of AIF, staining intensity lacked significant correlation with tumor stage or grade. Cytokeratin-positive cell percentages differed between stage pTa and pT1 or pT2 but not between tumor grades. AIF, survivin, and Ki67-positive cells were significantly more prevalent in high-grade versus low-grade tumors, while p63 showed an inverse trend. Survivin and Ki67 expression increased significantly across stages pTa, pT1, and pT2. Evaluation of cytokeratins 5/6, 7, and 20 is of limited diagnostic or prognostic significance. Conversely, AIF-positive cell counts correlate with tumor grade. Survivin and Ki67 expression increased with both stage and grade, serving as significant prognostic and predictive markers for bladder carcinoma.

Key words: urothelial carcinoma, survivin, Ki67, AIF, prognostic markers, predictive markers

Acknowledgments: Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V03-00046

Morphological characteristics and increased expression of LAMP1 and LC3 in patients with autism spectrum disorder

Maria Gevezova[1,2], Tsvetomira Ivanova[1,2], Iliana Pacheva[3,4], Yordan Sbirkov[1,2], Ivan Ivanov[3,4], Victoria Sarafian[3,4]

[1]*Department of Medical Biology, Medical University of Plovdiv, Plovdiv, Bulgaria*
[2]*Research Institute, Medical University of Plovdiv*
[3]*Department of Pediatrics, Medical University of Plovdiv, Plovdiv, Bulgaria*
[4]*Pediatrics Clinic, St. George University Hospital, Plovdiv, Bulgaria*

*Corresponding author e-mail: mariya.gevezova@mu-plovdiv.bg

Autism spectrum disorder (ASD) is among the most severe childhood psychiatric disorders in terms of clinical status, outcome, impact on family and society. The etiology at the molecular, cellular and systemic levels remains incompletely understood, and autophagy and lysosomal function are poorly investigated. The present study aimed to evaluate the gene expression of Lysosomal Associated Membrane Protein 1 (LAMP1) and Microtubule-associated protein 1A/1B-light chain 3 (LC3), as key markers of autophagy, in patients with ASD compared to healthy controls (HC). The study included a cohort of children with idiopathic ASD and age-matched HC. Diagnosis and clinical severity were assessed using the Autism Diagnostic Observation Schedule (ADOS). Gene expression was analyzed by quantitative PCR (qPCR). Cell samples were processed by cytospin centrifugation, fixed on slides and subjected to immunofluorescence staining with antibodies against LAMP1 and LC3. Morphological analysis was performed using a BioTek Cytation C10 fluorescence microscope. The results showed a statistically significant increase in the gene expression of LAMP1 and LC3 in patients with ASD compared to HC. Immunofluorescence analysis demonstrated a more intense signal in cells from the patient group, suggesting increased autophagic activity and changes in the lysosomal system. The obtained data support the hypothesis of the involvement of autophagy and lysosomal function in the pathophysiology of ASD and emphasize the importance of morphological analysis as a complementary approach to molecular studies.

Key words: Autism Spectrum Disorder, LAMP1, LC3
Acknowledgments: Project No BG-RRP-2.004-0007-C03

The possible mast cell role in the etiopathogenesis of microscopic collagenous colitis

Maria Popivanova[1,2,*], Veselin Belovezhdov[1,2], Dorian Dikov[1], Alexander Ivanov[1,2]

[1]*Medical University of Plovdiv, Plovdiv, Bulgaria*
[2]*UMHAT "St. George", Plovdiv, Bulgaria*

*Corresponding author e-mail: Mariya.Popivanova@mu-plovdiv.bg

Microscopic colitis (MC) is a disease, combining symptoms of chronic watery diarrhoea, abdominal pain and nausea due to inflammation of the colon with no macroscopic endoscopic evidence but clearly seen in biopsies under the microscope. Due to the histology findings it can be classified mainly as microscopic collagenous colitis (MCC) or lymphocytic colitis (MLC). However, the etiology and pathogenesis of MC are largely unknown. In this study, we aimed to observe mast cell counts and their possible role for the pathological changes in the colonal mucosa in patients with MCC. We investigated 10 biopsy samples of patients with proven MCC based on 10 μ width of the basement membrane, expressed inflammatory reaction in lamina propria and focal destruction of the outer enterocytic layer. Mast cell count evaluation was made with immunohistochemistry marker CD117 and then compared with endoscopic biopsy samples of patients with no clinical, nor histological data of MCC. Mast cells found under the thickened basement membrane outnumber those in the biopsies with no such pathology in control group. Increased number of mast cells in colonic mucosa is seen in other studies of inflammatory bowel diseases not related to MCC as well. These facts suggest concluding that the presence of mast cells is contributing in the inflammatory process and morphology changes seen in histology samples and thus is defining by implication the clinical manifestation. Under that impression mast cells might play critical role in the etiopathogenesis of microscopic collagenous colitis.

Key words: mast cells, microscopic, collitis, collagen, etiopathogenesis

Impact and modern molecular identification of Nematodes in wildlife

Mariana Panayotova-Pencheva[1*], Delka Salkova[1], Rasa Binkienė[2], Gražina Stanevičiūtė[2], Virmantas Stunžėnas[2]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[2]*Nature Research Centre, Lithuanian Academy of Sciences, 08412 Vilnius, Lithuania*

*Corresponding author e-mail: marianasp@abv.bg

The prevalence of parasitic nematodes among wild fauna induces significant ecological and economic consequences. Extensive invasions often escalate into increased morbidity and lethality, acting as a determinant for population decline in wildlife. Parasitoses also function as predisposing factors for secondary pathological conditions, such as nutritional deficiencies, opportunistic infections, and reproductive dysfunction. These processes not only erode biodiversity but also inflict direct damage on the hunting and game management sectors. The synergistic use of grazing territories by both wild and domestic species facilitates interspecies transmission cycles, thereby threatening the agricultural sector. Furthermore, anthropogenic animal introduction and natural migratory processes serve as vectors for the dissemination of allochthonous parasitic agents. Effective control of these pathogens correlates directly with the precision of the diagnostic process. Traditional diagnosis of nematodoses relies primarily on the morphological determination of macroscopic forms (adult helminths), as identification based on eggs and larvae is often taxonomically uncertain. In modern parasitology, the so-called integrative taxonomic approach is becoming established, combining classical morphometric analyses with molecular genetic methods. This study provides a critical review of contemporary molecular methodologies for characterizing nematodes in wildlife. Data from recent research were analyzed, focusing on parasite life cycles, host range, and optimized DNA extraction protocols. Particular attention is given to the selection of primer pairs and the expected sizes of the resulting amplicons. The analysis confirms the dominant role of ribosomal DNA markers - partial fragments of the 18S and 28S rRNA genes, as well as the ITS1-5.8S-ITS2 intergenic spacers. Additionally, the utility of the MSP1 gene and mitochondrial loci, such as COI and 12S, for species-level differentiation is examined. PCR-based techniques adapted for both non-invasive fecal samples and post-mortem lung tissue analysis are summarized. These standardized molecular tools could serve as a practical guide for future studies in the field of wildlife parasitology.

Key words: DNA extraction protocols, ITS spacers, molecular diagnostics, PCR-based techniques, ribosomal DNA markers

Acknowledgments: The financial support from the Bulgarian Academy of Sciences (Bilateral agreement between BAS and LAS, № IC-LT/01/2025-2027) is gratefully acknowledged. Project: "An integrative taxonomic approach to studying etiology of helminthoses in wild animals in Bulgaria and Lithuania"

Preparation and making of an anatomical trophy from a European bison head (Bison bonasus)

Martin Haddadin*, Rosen Dimitrov

Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Thracian University, Stara Zagora, Bulgaria

*Corresponding author e-mail: martin.haddadin@trakia-uni.bg

The European bison (Bison bonasus), also called the European bison, is a large mammal of the order Artiodactyls. The anatomical object was obtained after an autopsy procedure. The preparation of the permanent preparation from bison head bones went through eight stages. The resulting trophy from a bison skull was presented in the collection of the anatomical museum at the Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Thracian University, Stara Zagora.

Key words: anatomy, craniology, taxidermy, trophy

Acknowledgments: The authors express their gratitude to all colleagues and institutions who assisted in the collection and processing of the materials.

Morphological changes in the ageing kidney after Aronia melanocarpa supplementation

Mina Pencheva[1], Elena Daskalova[2*], Fanka Gerginska[2], Slavi Delchev[2], Milena Georgieva[3], Petko Denev[4]

[1]*Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University - Plovdiv, Plovdiv, Bulgaria*

[2]*Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

[3]*Laboratory of Molecular Genetics, Epigenetics and Longevity, Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria*

[4]*Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Plovdiv, Bulgaria*

*Corresponding author e-mail: elena.daskalova@mu-plovdiv.bg

Klotho is a key anti-aging protein predominantly expressed in the kidney, with a well-established decline during ageing. The present study aimed to evaluate morphological changes in renal tissue and the expression pattern of Klotho following supplementation with *Aronia melanocarpa* in aged rats. Male Wistar rats were divided into three groups: young controls, aged controls (27 months), and aged animals supplemented with aronia juice (10 ml/kg) for three months. Immunohistochemical analysis demonstrated strong and uniform Klotho expression in the tubular epithelium of young animals, characterized by diffuse cytoplasmic localization. In contrast, aged control rats exhibited a marked reduction in Klotho expression, with heterogeneous, focal staining and disrupted tubular organization. Morphological alterations included irregular tubular profiles and uneven epithelial structure. In aronia-supplemented animals, partial restoration of Klotho expression was observed, with increased staining intensity and more homogeneous distribution within tubular epithelial cells. Additionally, improved preservation of tubular morphology was noted, suggesting attenuation of age-related structural alterations. These findings indicate that *Aronia melanocarpa* supplementation may exert a protective effect on the ageing kidney, potentially through modulation of Klotho expression. Further quantitative analysis is required to confirm these observations and to elucidate the underlying mechanisms.

Key words: Klotho, ageing, kidney, Aronia melanocarpa, immunohistochemistry

Detection of microplastics in human placental tissue using Nile Red staining and fluorescence microscopy

Miriam Bollová*, Pavol Janega, Lucia Krivošíková

Institute of Pathological Anatomy, Faculty of Medicine, Comenius University in Bratislava and University Hospital Bratislava, Bratislava, Slovakia

*Corresponding author e-mail: miriam.bollova@fmed.uniba.sk

The presence of microplastics in human tissues has raised increasing concern regarding their potential impact on health. Most previous studies have relied on Raman microscopy or flow cytometry, often using homogenized samples or animal models, which do not allow precise histological localization. This study aimed to detect and localize microplastics in human placental tissue using a fluorescence-based method while preserving tissue architecture. Formalin-fixed, paraffin-embedded placental tissue samples from 23 physiological pregnancies were deparaffinized and stained with Nile Red. The staining solution was prepared in dimethyl sulfoxide and ethanol, and samples were incubated at 50–60°C to enhance fluorescence signal. Strict contamination control measures were applied throughout the process. Fluorescence microscopy was used to identify microplastic particles based on their characteristic signal. A total of 624 particles were detected across 690 visual fields. The majority were found in chorionic villi (54%), followed by decidua (18%), intervillous space (14%), extravillous trophoblast (9%), and vascular walls (5%). Statistical analysis demonstrated significantly higher accumulation of particles in the intervillous space ($p < 0.001$ to $p < 0.0001$), suggesting preferential localization within specific placental compartments. These findings indicate the possible presence of microplastics in multiple structures of the human placenta. Although no direct association with placental pathology was established, the preservation of tissue architecture provides a valuable basis for future research. Further studies are needed to investigate potential biological effects, mechanisms of interaction, and possible implications for pregnancy outcomes.

Key words: microplastics, placenta, Nile Red, fluorescence microscopy, histology

Osteogenesis in the late fetal period in rats

Nadezhda Kadreva*, Nadya Penkova, Pepa Atanassova

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

*Corresponding author e-mail: nadezhda.kadreva@mu-plovdiv.bg

Detailed studies of the processes of osteogenesis are necessary due to the widespread use of bone and biopolymer implants in various fields of medicine—dental implantology, neurosurgery, and orthopedics. The aim of our study is to examine the skeleton of 18-day-old mouse embryos to demonstrate the different types of osteogenesis. Five 18-day-old mouse embryos, obtained through controlled insemination and humane euthanasia of the mothers according to the Bulgarian Food Safety Agency (BFSA) protocol, were examined histologically using routine hematoxylin-eosin staining. The microscopic preparations allow us to demonstrate desmal and chondral osteogenesis in the various bones of the skeleton—the future flat and long bones. Advanced processes of desmal osteogenesis are observed in the region of the calvaria. The vertebrae of the spinal column, the ribs, the pelvic bone, and the phalanges of the fingers are still at the cartilaginous stage of chondral osteogenesis. Studies of mouse embryos and fetuses provide an opportunity to elucidate the early stages of osteogenic processes and to conduct subsequent experiments with various biopolymer materials used in clinical practice and ones still under investigation.

Key words: rat fetuses, desmal osteogenesis, chondral osteogenesis

Characteristics of the Bulgarian population based on anthropogenetic tests

Nadezhda Paraskova[1*], Zorka Mitova[2]

[1]*Department of Zoology and Anthropology, Faculty Of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: nadjia_para@yahoo.com

Anthropophysiological tests are genetically encoded and are under the regulation of the central nervous system. The studied group is from a region with complex ethnogenesis in historical terms. Some tests have been studied among the population of Northeastern Bulgaria. The following anthropogenetic tests were analyzed: Hand clasping, Arm folding and Clapping. The results obtained were compared with data for the country as a whole. Generally accepted methodologies have been applied (Luby, 1908; Weyrer,1932; Blincoe, 1962). Hand clasping: In the contingent of the test, the left type of the test is most often detected, followed by the right type. Arm folding: In the studied population, the left-handed type dominates over the right-handed type. Clapping: In this test, the neutral type is the most common in the sample examined. The hand clasping test is characterized by almost identical values of the type of left hand and the type of right hand and by a relatively high percentage of individuals indifferent to the test. For the arm folding test the data shows more frequent left-wing characteristics. In the clapping test, the order of the typical values differs from the normal distribution in the Bulgarian population. The similar presentation of the left and right hands and the high percentage of the neutral type are regional specifics of the hand clasping test. The neutral type prevails in the clapping test with almost equal values of the left-hand type and significantly lower values of the right-hand type.

Key words: hand clasping, arm folding, clapping

High origin and superficial course of ulnar artery: a case report

Nikola Stamenov[1*], Iva Dimitrova[2], Georgi Georgiev[3], Lyubomir Gaydarski[1], Kristina Petrova[1], Boycho Landzhov[1]

[1]*Department of Anatomy, Histology, and Embryology, Medical University of Sofia, Bulgaria*
[2]*Department of Cardiology, University Hospital "St. Ekaterina", Medical University of Sofia, Bulgaria3*
[3]*Department of Orthopaedics and Traumatology, University Hospital Queen Giovanna-ISUL, Medical University Sofia, Bulgaria*

*Corresponding author e-mail: nstamenov@medfac.mu-sofia.bg

Anatomical variations in the arterial system of the upper limb are relatively frequent. One such variation, the superficial ulnar artery (SUA), can originate from the axillary, brachial, or superficial brachial arteries and typically follows a course superficial to the forearm muscles. Its reported incidence ranges from 0.7% to 9.4%. This report details a notable case of a superficial ulnar artery identified during a routine anatomical dissection of the left upper limb in a Caucasian female cadaver. In this instance, the variant artery exhibited a high origin, branching from the brachial artery in the mid-arm. It then traversed the forearm superficially before terminating normally in the hand. The presence of a superficial ulnar artery is clinically significant for several reasons - Vulnerability: Its superficial position increases the risk of accidental trauma and iatrogenic injury during orthopedic surgeries; Clinical Utility: Conversely, its accessibility makes it a potential candidate for cannulation; Procedural Awareness: Knowledge of this variation is essential for nursing staff, orthopedic surgeons, and interventional cardiologists to avoid complications during intravenous access or surgical interventions.

Key words: superficial ulnar artery, variation, clinical significance

Region-specific pCREB immunoreactivity in the dorsal hippocampus following β-adrenergic activation of the basolateral amygdala

Nikolay Dimitrov[1], Nikolaos Efstratiou[1], Bogomil Peshev[2], Pavel Rashev[3], Lidia Kortenska[2], Milena Mourdjeva[3], Despina Pupaki[3], Nikolay Genov[1], Jana Tchekalarova[2], Dimitrinka Atanasova[1,2*]

[1]*Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: d.atanasova@inb.bas.bg

The basolateral amygdala (BLA) plays a key role in modulating hippocampus-dependent memory consolidation. However, little is known about how noradrenergic stimulation of the BLA influences the regional pattern of transcriptional activation within the hippocampus. The present study aimed to determine the distribution of phosphorylated cAMP response element-binding protein (pCREB) in the dorsal hippocampus after training following noradrenergic activation of the BLA, and to assess whether dopaminergic D1/D5 receptors are involved in this effect. Adult male Wistar rats were divided into three experimental groups: control, clenbuterol (Clen) treated, and SCH 23390 + clenbuterol (SCH/Clen) combination treated groups. After training the rats in the object-location task, clenbuterol was directly infused into the basolateral amygdala (BLA) bilaterally, whereas in the combined-treatment group, BLA stimulation was preceded by SCH 23390 treatment. Animals were perfused 60 min after training. Brain tissues were paraffin-embedded, cut into 6 μm-thick sections, and immunohistochemically stained for phospho-CREB (Ser133). The number of positive neurons were counted in the granular layer of the dentate gyrus (GrDG), CA3 subfields, CA2, and CA1. Clenbuterol treatment markedly increased the number of pCREB-positive nuclei in the GrDG, CA2, and CA1 regions of the dorsal hippocampus, whereas no significant changes were observed in the CA3 subfields. Pre-treatment with SCH 23390 almost completely blocked the clenbuterol-induced increase in pCREB immunoreactivity, restoring the values in the responsive regions to levels close to those of the controls. These findings demonstrate that noradrenergic stimulation of the basolateral amygdala induces a region-specific pattern of pCREB activation in the dorsal hippocampus. They further suggest that dopaminergic D1/D5 receptors are involved in this response selectively in the GrDG, CA2, and CA1, pointing to a distinct anatomical organisation of the early molecular events underlying hippocampal plasticity associated with memory.

Key words: basolateral amygdala, dorsal hippocampus, pCREB, immunohistochemistry, memory consolidation
Acknowledgement: This research was funded by the Bulgarian National Science Fund (Grant No. КП-06-Н61/12).

3D colony-forming method in the search for new anticancer agents

Orlin Alexandrov[1*], Hristo Hristov[2], Eliya Stoilova[2], Silvia Mancheva[2,3], Tanya Zhivkova[2], Lora Dyakova[4], Abedulkadir Abudalleh[2], Ildiko Buta[5], Daniela-Cristina Culita[6], Gabriela Marinescu[6], Teodora Mocanu[6], Radostina Alexandrova[2]

[1]*Faculty of Medicine, Medical University – Sofia, Bulgaria*
[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*New Bulgarian University, Sofia, Bulgaria*
[4]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[5]*"Coriolan Drăgulescu" Institute of Chemistry, Timisoara, Romania*
[6]*"Ilie Murgulescu" Institute of Physical Chemistry, Romanian Academy, Bucharest, Romania*

[*]Corresponding author e-mail: orlinalexandrov02@gmail.com

Monolayer (2D) cell cultures have long been established as useful models in the field of biomedicine and biotechnology, including in the identification of new antitumor agents. Along with their many advantages (relatively well-studied, cheap and convenient to work with), 2D cell cultures also have several limitations, mainly due to the inability to adequately recreate the characteristics and behavior of the tumor in vivo. Our study aims to optimize the conditions for conducting the 3D colony-forming method and to compare the data obtained with its help with the results of cytotoxic tests conducted with 2D cell cultures. Three groups of substances were used: metal complexes with different Schiff bases; nanostructured materials obtained by green synthesis; commercial antitumor agents (cisplatin, oxaliplatin). Cell lines from human cervical carcinoma (HeLa) and melanoma (A375), as well as from retrovirus-induced transplantable rat sarcoma (LSR-SF-SR). Two types of experiments were carried out: i) short-term experiments (24-72 hours, with monolayer cultures) using the MTT test, neutral red uptake assay, crystal violet staining, acridine orange and propidium iodide staining; ii) long-term experiments (duration over two weeks) by the 3D colony forming assay in a semi-liquid medium. The conditions for conducting the 3D colony-forming method with various chemical compounds and cell lines were optimized (culture medium, agarose concentration, cell number, feeding frequency, etc.). In most cases, a good correlation was found between the results obtained in the course of the short-term and long-term experiments. Determining an effective inhibitory concentration that completely suppresses 3D tumor cell growth is an important indicator that reflects the ability of the investigated compounds to irreversibly suppress the survival and/or proliferation of tumor cells in the long term in laboratory conditions.

Key words: 2D and 3D cell cultures, cell lines, cancer, cytotoxicity, antitumor activity
Acknowledgements: The study was supported by the Bulgarian National Science Fund (Grant N КП-06-КОСТ/18 from 12.08.2024 and КП-06-Н91/15 from 11.12.2025), Bulgarian Academy of Sciences (Bilateral project between the Bulgarian Academy of Sciences and the Romanian Academy, project IC-RO/04/2026-2028).

Evaluation of arterial system preparations in male rabbits after filling with silicone elastomer and acrylate plastic

Nikolay Tsandev[1*], Angel Vodenicharov[3], Pamela Sarova[2]

[1]*Trakia University, Faculty of Veterinary Medicine, Department of Veterinary Anatomy, Histology and Embryology, Stara Zagora, Bulgaria*

[2]*Trakia University, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria*

[3]*Medical University, Faculty of Veterinary Medicine, Department of Preclinical Sciences, Pleven, Bulgaria*

*Corresponding author e-mail: drcandev@abv.bg

Silicone elastomers and acrylate plastics have traditionally been used to study the angioarchitectonics of organs or the entire vascular system, as well as to obtain casts of cavities. There is currently no comparative data on the effectiveness of the two methods when applied to small domestic animals. A comparative study of the circulatory system of male rabbits filled with silicone elastomer and acrylic plastic was performed with the aim of critically assessing the effectiveness of the application of both materials for obtaining precise casts of the vascular system. After preliminary preparation, introduction of heparin and flushing with saline solution, silicone elastomer or acrylate plastic were introduced into the arterial system of male rabbits using a screw mechanism until the vessels were completely filled. For the removal of soft tissues, beetle larvae (*Dermestidae*) were used in the first, and by chemical maceration in the second method. The results obtained showed that both methods produce high-quality casts, filling even the finest branches. Silicone elastomer demonstrates high elasticity and resistance to deformation, which facilitates the manipulation and storage of the preparations. Acrylate plastic provides harder and more detailed casts, suitable for long-term storage and precise morphological analyses. As a disadvantage of both methods, the presence of a small artifacts from incompletely removed soft tissues is noted. The combined use of both methods for filling the arterial system of male rabbits is a successful approach, with sufficient efficiency and informativeness for a detailed study of vascular architectonics. The application of both materials allows the preparation of vascular preparations with complementary characteristics – elasticity and high structural stability. Further improvement of the technique for complete removal of artifacts would undoubtedly lead to an increase in the quality of the preparations of the casts.

Key words: angioarchitectonics, silicone elastomer, acrylate resin, rabbit

A rare case of mulberry second molar in a non-syphilitic patient

Zdravka Harizanova*, Ferihan Popova, Polina Ivanova, Zlatizara Todorova, Vyara Zaykova, Lilia Grozlekova, Atanas Baltadjiev

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

*Corresponding author e-mail: zdravka.harizanova@mu-plovdiv.bg

The ectoderm shares a common embryological origin with both the epidermis and the ameloblastic–odontogenic structures of the teeth, which may account for the coexistence of dermatological and dental developmental anomalies. Congenital syphilis has been classically associated with three principal dental manifestations: Hutchinson's incisors, bud molars, and mulberry molars, typically involving the permanent first molars. To the best of our knowledge, this report presents the first documented case of a mulberry-type second molar in a patient without clinical or historical evidence of syphilis. The occlusal surface of the mandibular second molar exhibited ten globular, projection-like structures. Based on the characteristic morphological features, the condition was diagnosed as a mulberry molarNo prosthetic intervention was deemed necessary. Preventive management included topical fluoride application, and the patient was scheduled for regular follow-up examinations. Increased awareness among gynecologists and pediatricians is essential regarding both local and systemic factors that may contribute to disturbances in dental development.

Key words: mulberry molars, congenital syphilis, developmental enamel diseases, Hutchinson's triad

Experimental model of rat with obesity to study the effects of green tea on metabolic syndrome

Sabri Sabri[1*], Nadya Penkova[1], Petar Hrischev[2], Penka Angelova[2], Katerina Georgieva[2], Yulia Nikolova[2], Snezhana Stoencheva[3] Nikol Kirchev[4], Yani Nikolov[4]

[1]*Department of Anatomy, Histology and Embryology, Medical University – Plovdiv, Bulgaria*
[2]*Department of Physiology, Medical University – Plovdiv, Bulgaria*
[3]*Department of Clinical Laboratory, Medical University – Plovdiv, Bulgaria*
[4]*Student, Medical University – Plovdiv, Bulgaria*

*Corresponding author e-mail: sabri.sabri@mu-plovdiv.bg

The etiology of metabolic syndrome is multifactorial. Low-grade chronic inflammation plays a key role in its pathogenesis. Some of these inflammatory reactions are endotoxin-mediated. They are triggered by the translocation of bacterial lipopolysaccharides, which act as endotoxins, from the intestinal lumen into the blood vessels of the intestinal wall. The aim of our study is to establish an experimental model of metabolic syndrome in rats that will allow us to investigate the effects of green tea on the permeability of the intestinal mucosal barrier and low-grade chronic inflammation. 40 mature male rats were fed a high-fat high-carbohydrate diet (HFHCD) for 12 weeks according to the protocol of the Department of Physiology at the Medical University of Plovdiv. They were divided into four groups of 10 animals each: 1. Controls; 2. Controls consuming green tea; 3. Rats on HFHCD; 4. Rats on HFHCD and consuming green tea. During the dietary feeding period, the experimental animals undergo biometric examinations and at the end of the experiment, blood is collected for clinical laboratory tests. Following humane euthanasia of the rats according to a protocol approved by the Bulgarian Food Safety Agency (BFSA), their internal organs will undergo histological and immunohistochemical examinations. The data from our studies will reveal whether green tea reduces the permeability of the intestinal mucosal barrier and its potential protective role regarding obesity and metabolic syndrome.

Key words: obesity, metabolic syndrome, green tea

Investigation of the external carotid artery and its terminal branches in sheep using latex and corrosion cast methods

Zafer Enes Güçlü, Semine Dalga*

Kafkas Universty, Faculty of Veterinary Medicine, Department of Anatomy, Kars, Türkiye

*Corresponding author e-mail: seminedalga@kafkas.edu.tr

Sheep heads, which exhibit significant variation within the species and are easily accessible as cadavers in many systems at veterinary faculties, were employed in this investigation. The objective was to use both the latex method and the corrosion cast technique to investigate the branching of sheep's head arteries in more detail. Ten skulls in all were acquired. The pots were filled with corrosive substance and latex. The materials injected with latex were dissected. Both techniques were effective in revealing the external carotid artery's branches. While the corrosion method visualized even the smallest capillaries, the latex method only revealed the presence of the main branches. The course of the vessel and the branching segments were consistent with the literature. When it comes to convenience of use, the latex approach can be utilized for a longer amount of time, but the corrosion method can cause the materials to deform during transportation and the vessels to break quickly. As a result, this study describes cranial arteries and contrasts the benefits and drawbacks of the approaches.

Key words: external carotid artery, latex, corrosion cast, sheep, skull

Secular changes on basic head dimensions in contemporary young generation in Western Rhodope region, Bulgaria

Silviya Mladenova*, Emiliya Andreenko

Plovdiv University „Paisii Hilendarski", Faculty of Biology, Department of Human Anatomy and Physiology

*Corresponding author e-mail: silviamladenova.sm@uni-plovdiv.bg

The objective of this study was to investigate secular changes in basic head dimensions among children and adolescents aged 7 to 17 years from the Western Rhodope region, Bulgaria, over the period 1998–2020. A total of 2138 subjects (1028 girls and 1110 boys) were divided into two cross-sectional samples. The first sample, measured between 1998 and 2001, comprised 595 girls and 629 boys, while the second sample, measured transversally in period 2016–2020, included 428 girls and 481 boys. Two basic dimensions—head length and head breadth were measured using the classical methodology of Martin-Saller (1957). The cephalic index was calculated using standard formulas. Secular changes were assessed by comparing data from the two periods. Mean values and standard deviations were calculated for each characteristic, age, and sex. The results show that contemporary boys (2016–2020) have a larger cephalic index than girls. Regarding secular changes, a clear tendency toward decreasing cephalic index values is evident in both sexes. In conclusion, over the past two decades (1998–2020), children from the Rhodope region exhibit a statistically significant secular trend toward dolichocephalization (reduction of the cephalic index) in both sexes, with the trend being more pronounced and occurring earlier in girls. Its maximum expression is observed in late adolescence (15–17 years). In conclusion our results were consistent with the process of debrachycephalization in Bulgarians previously documented by R. Stoev, which began with generations born after 1935–1940, and demonstrate that the process of debrachycephalization continues during the first two decades of the 21st century.

Key words: schoolchildren, head dimensions, cephalic index, debrachycephalization

Acknowledgments: The present study was performed by partial financial support of Fund "Scientific Researches" of Plovdiv University "Paisii Hilendarski", within contracts number FP 25 – FS 003.

Frequency of pterygospinous and pterygoalar osseous bridges in Bulgarians

Silviya Nikolova*, Diana Toneva

Department of Anthropology and Anatomy, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: sil_nikolova@abv.bg

The pterygospinous and pterygoalar bridges (PsB, PaB) are osseous bars located on the inferior surface of the sphenoid bone. These structures may form accessory foramina at the skull base and have potential clinical significance due to possible nerve entrapment, vascular compression, and obstruction of surgical access. This study aimed to determine the frequency of PsB and PaB in a Bulgarian population and to evaluate their bilateral distribution and sex-related differences. A retrospective analysis was conducted using head CT scans obtained for diagnostic purposes with a Toshiba Aquilion 64 CT system. The sample comprised 315 individuals (148 males and 167 females). Both PsB and PaB were classified as either complete or incomplete, based on whether they formed fully enclosed foramina, specifically the pterygospinous (Civinini's) foramen and the pterygoalar (porus crotaphitico-buccinatorius, Hyrtl's) foramen. The overall frequency of PsB (complete and incomplete) was 12.4%, with a higher frequency in males (16.9%) compared to females (8.4%). Complete PsB was identified in 4.3% of cases, while incomplete PsB occurred in 8.1%. No significant bilateral differences were observed; however, a statistically significant sex difference was noted for the left side. The total frequency of PaB was 2.4% (3.0% in males; 1.8% in females). Complete PaB was found in 1.4% of cases, and incomplete occurs in 1.0%. No significant bilateral or sex-related differences were identified for PaB. PsB demonstrates significant sex-related differences, particularly on the left side, while PaB shows no significant bilateral or sex differences. Although no correlations were found between different bridge types, both PsB and PaB exhibited significant positive associations between right- and left-side occurrence in both series.

Key words: pterygospinous bridge, pterygoalar bridge, Civinini's foramen, Hyrtl's foramen

Acknowledgement: This study was supported by the Bulgarian National Science Fund, Grant КП-06-Н51/4—11.11.2021.

Molecular mechanisms of negative effect of systemic lupus erythematosus on oogenesis and meiotic processes in oocytes

Stefka Delimitreva*, Ana Blagoeva, Irina Chakarova

Department of Biology, Medical University of Sofia, Bulgaria

*Corresponding author e-mail: s.delimitreva@medfac.mu-sofia.bg

Systemic lupus erythematosus is an autoimmune disease that mainly affects women of reproductive age. Its pathological manifestations directly and indirectly negatively affect the ovarian reserve, the quality of oocytes and the precise mechanisms of meiosis. This poster presents the molecular mechanisms by which lupus damages ovarian tissue and meiosis in oocytes. The role of chronic inflammation, impaired hormonal levels and the presence of specific autoantibodies are considered. The available data on how oocyte structures (meiotic spindle, actin cytoskeleton, membrane organelles and chromatin) are damaged by lupus symptoms are summarized.

Key words: oogenesis, oocyte, meiosis, systemic lupus erythematosus (SLE)

RET expression across autoimmune thyroid diseases, papillary thyroid carcinoma, and NIFTP: diagnostic and pathogenetic implications

Liana Gogiashvili[1,2], Tamuna Gvianishvili[1,3*], Elene Nikobadze[1], David Makaridze[2,4]

[1]*TSU, Al Natishvili Institute of Morphology, Tbilisi, Georgia*
[2]*David Tvildiani Medical University, Tbilisi, Georgia*
[3]*Tbilisi State Medical University, Tbilisi, Georgia*
[4]*Pathology Research Center, Tbilisi, Georgia*

*Corresponding author e-mail: tamuna.gvianishvili8375@med.tsu.edu.ge

Chromosomal rearrangements involving the RET/PTC oncogene represent key molecular events in the pathogenesis of papillary thyroid carcinoma (PTC), leading to constitutive activation of tyrosine kinase signaling pathways and the development of characteristic nuclear features. While RET expression is well established in PTC, its role in autoimmune thyroid diseases and borderline thyroid neoplasms, such as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), remains insufficiently elucidated. The aim of this study is to evaluate RET expression using a monoclonal antibody in autoimmune thyroid diseases with non-invasive nuclear alterations and to compare these findings with PTC and NIFTP, in order to assess its diagnostic and pathogenetic significance. RET/PTC protein expression was assessed immunohistochemically using a monoclonal RET antibody (clone 421R25, USA) in thyroid tissue specimens, including Hashimoto's thyroiditis (HT), Riedel's thyroiditis (RT), PTC, and selected cases with morphological features consistent with NIFTP. Immunoreactivity was evaluated using a semi-quantitative scoring system (0–4), based on the proportion of positively stained cells and staining intensity. Strong RET expression was observed in PTC and showed a clear association with characteristic nuclear features, including nuclear irregularities and euchromatin predominance. Moderate RET immunoreactivity was detected in HT, suggesting possible activation of oncogenic signaling pathways in the setting of chronic inflammation. In contrast, RT demonstrated absent or minimal RET expression. NIFTP cases exhibited low to absent RET expression, further supporting their indolent biological behavior and molecular distinction from classical PTC. RET expression is strongly associated with PTC but lacks sufficient specificity as a standalone diagnostic marker. Its presence in HT may indicate a potential link between chronic inflammation and thyroid carcinogenesis. The low or absent expression in NIFTP supports its classification as a borderline neoplasm. Integration of RET immunoexpression with histopathological features may improve diagnostic accuracy and enhance understanding of thyroid tumor progression.

Key words: RET/PTC, PTC, HT, RT, NIFTP, immunohistochemistry

GP2 expression in colorectal cancer

Tsvetomira Ivanova[1,2*], Dorian Dikov[3], Tatiana Damyanova[1,2], Tatyana Todorova[1,2], Elitsa Tanova[1,2], Toma Tsvetanov[1,2], Victoria Sarafian[1,2]

[1]*Department of Medical Biology, Medical University-Plovdiv, Plovdiv, Bulgaria*
[2]*Research Institute at Medical University-Plovdiv, Plovdiv, Bulgaria*
[3]*Department of Pathology, Jossigny Hospital, Jossigny, France*

*Corresponding author e-mail: tsvetomira.ivanova@mu-plovdiv.bg

Colorectal cancer (CRC) remains a significant global health burden, ranking as the third most common malignancy and the second leading cause of cancer-related mortality worldwide. GP2 (Zymogen Granule Membrane Protein 2) is traditionally recognized as the most abundant protein within pancreatic zymogen granules. However, it is also expressed in intestinal M cells, where it functions as a critical immune receptor by capturing specific pathogenic bacteria. There is no available data on GP2 expression in colonic tissue. This glycoprotein has been identified as a metastatic marker in prostate cancer, but its specific role in the CRC landscape remains under-explored. This study aims to evaluate the tissue expression of GP2 across distinct tumor regions in CRC. We performed immunohistochemical analysis on 31 CRC patients, comparing tumorous samples with non-tumorous colonic tissue. GP2 expression levels were quantified across the tumor parenchyma, stroma, invasive front, and normal parenchyma. These levels were then correlated with clinicopathological characteristics, including tumor differentiation, lymphatic and vascular invasion, and tumor budding. Our findings reveal a strong expression of GP2 specifically at the invasive tumor front. This high expression correlated with low-grade, well-differentiated tumors; as notably, 93.5% of the patients in our cohort presented with G1/G2 grade tumors. We present novel data on GP2 expression and tumor budding in CRC suggesting its involvement in cancer cell aggressiveness.

Key words: CRC, 3D bioprinted models, ECT, therapeutic platform strategies

Acknowledgments: To MU-Plovdiv, project № HO-21/2025; by the Bulgarian National Science Fund- grant № KH-06-H-83/06.12.2024 and by the Next Generation EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C01.

Anthropometric study of the facial profile in young Bulgarians based on five angular dimensions

Tsvetanka Petleshkova*, Katerina Topalova, Kristian Argirov, Atanas Baltadjiev

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

*Corresponding author e-mail: tsvetanka.petleshkova@mu-plovdiv.bg

The aim of this study was to analyze the facial profile of young Bulgarians through the assessment of five angular anthropometric parameters. The study included 95 Bulgarian subjects (46 males and 49 females) aged 21-30 years. Subjects of different ethnicities, with a history of facial injury, craniofacial anomalies, or mental disorders were excluded from the study. Three-dimensional images were obtained using a hand-held laser scanner (FastSCAN Cobra, Polhemus Inc., Colchester VT). Nine anthropometric landmarks were placed on each of three-dimensional image: alare (alr, all), nasion (n), subnasale (sn), pronasale (prn), labiale superius (ls), labiale inferius (li), sublabiale (sl), and pogonion (pg). Based on these anthropometric landmarks, the following angular measurements were performed (in degrees): mentolabial angle (li-sl-pg), nasolabial angle (prn-sn-ls), nasal convexity angle (sn-n-prn), inter-alar angle (alr-prn-all), and nasal tip angle (n-prn-sn). Regarding angular measurements, the inter-alar angle values were significantly higher in males than in females, with a statistically significant difference ($p < 0.01$). For the remaining angular dimensions – nasal convexity, nasal tip angle, nasolabial, and mentolabial angles – no statistically significant differences were observed between the two sexes ($p>0.05$). The results suggest that the soft tissue facial profile of young Bulgarians is largely similar for both sexes in regard to angular measurements, with the main morphological difference located at the nasal base. Understanding the specific characteristics of facial profiles in different populations and gender groups is essential for achieving optimal results in plastic surgery and orthodontic practice.

Key words: angular measurements, facial profile, Bulgarians

Disrupted angiogenic signaling and inflammatory cell accumulation in hypertensive kidney disease progression

Tsvetko Obetsanov[1*], Lyubomir Gaydarski[1], Kristina Petrova[1], Nikola Stamenov[1], Tihomir Dikov[2], Vesela Ivanova[2], Boycho Landzhov[1], Alexandar Iliev[1], Stancho Stanchev[1]

[1]*Department of Anatomy, Histology and Embryology, Medical University of Sofia, Sofia, Bulgaria*
[2]*Department of General and Clinical Pathology, Medical University of Sofia, Sofia, Bulgaria*

*Corresponding author e-mail: tsv.obetsanov@medfac.mu-sofia.bg

Hypertensive chronic kidney disease is driven by progressive renal microvascular injury, capillary rarefaction, and dysregulated angiogenic signaling. In spontaneously hypertensive rats (SHRs), disease progression is accompanied by altered VEGF and neuronal nitric oxide synthase (nNOS) expression, together with accumulation of mast cells in the renal interstitium, suggesting a coordinated inflammatory-angiogenic response to chronic hypertensive damage. Kidney tissue from 6- and 12-month-old SHRs and age-matched normotensive Wistar rats was examined histologically and immunohistochemically. VEGF and nNOS expression were assessed semiquantitatively in the renal cortex and medulla, while mast cell number (MCN) was quantified in the renal cortex using tryptase and CD117 staining. Statistical comparisons were performed between age-matched groups and across disease stages. VEGF expression was highest in 6-month-old SHRs and declined markedly by 12 months, with strong early cortical and medullary staining giving way to predominantly low-positive or negative expression in older animals. In contrast, nNOS showed low expression in 6-month-old SHRs, especially in the cortex, but increased with age in the cortex and remained detectable in the medulla of 12-month-old SHRs. Mast cells were significantly increased in hypertensive kidneys, with cortical MCN rising from 0.72 to 1.85 per high-power field by tryptase staining and from 0.69 to 1.79 by CD117 staining between 6 and 12 months (both $p<0.001$). This increase paralleled the progression of renal injury and the decline in VEGF. Progressive hypertensive kidney injury in SHRs is characterized by increasing mast cell infiltration, age-related loss of VEGF expression, and a compensatory rise in nNOS. Together, these changes suggest that early hypertensive nephropathy may involve an attempted pro-angiogenic response, which becomes insufficient as disease advances, contributing to microvascular rarefaction and chronic kidney damage.

Key words: vascular endothelial growth factor (VEGF), neuronal nitric oxide synthase (nNOS), hypertensive nephropathy, capillary rarefaction, renal fibrosis

Role of chitinase-like proteins YKL-40 and YKL-39 in colorectal cancer

Tsvetomira Ivanova[1,2*], Maria Kazakova[1,2], Dorian Dikov[3], Angel M. Dzhambov[4], Nikolay Belev[5], Boyko Atanasov[6], Elitsa Tanova[1,2], Toma Cvetanov[1,2], Victoria Sarafian[1,2]

[1]*Department of Medical Biology, Medical University-Plovdiv, Plovdiv-4000, Bulgaria*

[2]*Research Institute at Medical University-Plovdiv, Plovdiv-4000, Bulgaria*

[3]*Department of Pathology, Jossigny Hospital, Jossigny, France*

[4]*Environmental Health Division, Research Institute at Medical University of Plovdiv, Plovdiv, Bulgaria*

[5]*Department of Propaedeutics of Surgical Diseases, Medical University-Plovdiv; University Hospital Eurohospital, Plovdiv, Bulgaria*

[6]*Department of Propaedeutics of Surgical Diseases, Medical University-Plovdiv; Unihospital, Panagyurishte, Bulgaria*

*Corresponding author email: tsvetomira.ivanova@mu-plovdiv.bg

YKL-40 and YKL-39 chitinases-like proteins (CLPs) are secreted glycoproteins involved in inflammation, macrophage polarization, and carcinogenesis. Their expression is significantly upregulated in various inflammatory and immunological conditions, including several cancers, suggesting a role as potential diagnostic markers. Colorectal cancer (CRC) remains a significant global health concern, with a continued need for reliable biomarkers to stratify patients and predict therapy response. In this study, we assessed tissue, plasma, and transcript levels of both CLPs in CRC. We found a strong association between their tissue expression and tumor budding. Notably, plasma YKL-39 levels were lower in CRC patients than in controls, while YKL-40 concentrations were higher in the patient group. Gene expression analysis for both CLPs in white blood cells (WBCs) did not reveal statistical significance between CRC patients and controls. These findings enhance our understanding of the clinical relevance of these molecular signatures and support their potential application as biomarkers in CRC stratification.

Key words: YKL-39, YKL-40, CRC, tumor budding

Acknowledgements: The results had been published in Cells 2026; doi: 10.3390/cells15030263. Funding: this study is financed by the Bulgarian National Science Fund - grant project № КП-06-561 Н63/8 from 13.12.2022 (CHI3L1 reagents) and by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004- 563 0007-C03 (CHI3L2 reagents).

Development of experimental in vivo-models, suggesting the protective reaction of the organism against increased creatinine. A pilot study

Vera Kolyovska, Iskra Sainova, Veselin Nanev*

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: veselinnanev@gmail.com

The main goal was to be investigated the processes in the organism of patients with increased creatinine blood levels. In serum probes from patient and healthy control blood samples, the titers of IgG antibodies against gangliosides GM1, GM3 and from the general pool were assessed by Enzyme-Linked Immuno Sorbent Assay (ELISA). Subsequently, aliquot of the patient's serum was mixed with total homogenate from healthy experimental mouse kidneys. The titers of the same gangliosides in the prepared mixture, but also of the IgG antibodies against them in the healthy mouse kidneys extract were determined by appropriate ELISA modifications. In the patient with high creatinine levels, reaction was established in the anti-GM1 antibodies at dilution 1:40, where they were significantly higher compared to the healthy control. In the mixed sample between serum from the patient and healthy mouse kidneys extract, a reaction by ganglioside GM3 was detected. This result was explained with the protective role of this ganglioside against inflammatory process, available in the patient with high creatinine levels. In support of this hypothesis was the approximately equal titer of the anti-GM3 IgG antibodies in the healthy mouse kidneys extract. Here again was suggested a possibility about antibodies production by non-lymphoid cells, tissues and organs as a protective reaction, but also the role of gangliosides in the control of their function. The assessed biochemical data were confirmed by morphological differences between kidneys histochemical slides from experimental rat in vivo-model of early kidney disorder stage and of healthy control rat.

Key words: gangliosides and anti-ganglioside antibodies, creatinine, kidneys, morphological/histological features, experimental models in vivo models

Tissue expression of chitinase-like proteins in glioblastoma

Maria Kazakova[1,2], Dorian Dikov[3], Ilian Koev[4], Yordan Sbirkov[1,2], Azad Ozanian[4], Tatyana Damyanova[1,2], Victoria Sarafian[1,2*]

[1]*Department of Medical Biology, Medical University – Plovdiv, Plovdiv, Bulgaria*
[2]*Research Institute at Medical University – Plovdiv, Plovdiv, Bulgaria*
[3]*Department of Pathology, Jossigny Hospital, Jossigny, France*
[4]*Department of Neurosurgery, University Hospital Pulmed, Plovdiv, Bulgaria*

*Corresponding author e-mail: sarafian@abv.bg

The chitinase-like proteins, CHI3L1 and CHI3L2, share similar structures but have different functions as they are implicated in diverse pathologic conditions. CHI3L1 has proinflammatory and proangiogenic potential and is overexpressed in various tumors, while CHI3L2 is believed to be involved in crosstalk with the tumor microenvironment in some cancers. The studies on these molecules in gliobastoma are still limited. The short survival time and the resistance to therapy of malignant glioma imposes the search of novel biomarkers for better stratification and prognosis. The aim of the present investigation is to follow the parallel expression of both chitinase-like proteins in tissues of patients with gliobastoma. The immunohistochemical analysis revealed presence of chitinase-like proteins in malignant cells with CHI3L2 being expressed with higher intensity. It was positive in large number of tumor cells while CHI3L1 was detected basically in multi-nucleated monstruous cancer cells. In conclusion, CHI3L1 and CHI3L2 demonstrate differential tissue expression in gliobastoma suggesting distinct involvement in the pathogenesis of this malignancy.

Key words: chitinase-like proteins, gliobastoma multiforme, CHI3L1, CHI3L2
Acknowledgments: Project №HO-06/2026 at Medical University - Plovdiv

Oleic acid promotes cardiomyocyte maturation via the mTOR-Znf384-SDH pathway

Volodymyr Balatskyi*, Pawel Dobrzyn

Laboratory of Molecular Medical Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland

*Corresponding author e-mail: v.balatskyi@nencki.edu.pl

The maturation of cardiomyocytes (CMs) is the final stage of their differentiation, which occurs after birth. During this process, neonatal CMs acquire the functional and metabolic characteristics of adult CMs, enabling efficient blood circulation. Fatty acids (FAs) are the main energy source in the adult heart; however, knowledge regarding the signaling functions is limited. We investigated the role of saturated and monounsaturated long-chain FAs in regulating CM maturation. Neonatal CMs were treated with palmitic, stearic, palmitoleic, and oleic acids. Oleic acid proved to be the most effective in the induction of CM maturation, shifting CM metabolism toward a more oxidative state, increasing sarcomere length, and improving calcium handling and contractile properties. Activation of the oxidative phosphorylation complex II (SDH) was associated with oleic acid-induced CM maturation. From a mechanistic point, oleic acid inhibited the mTOR pathway, leading to nuclear translocation of the Znf384 protein, which promoted the transcription of genes involved in SDH assembly.

Key words: cardiomyocyte maturation, fatty acids, oleate

Acknowledgements: This research was funded by the National Science Centre, Poland (UMO-2022/47/B/NZ4/01311)

Altered hemispheric lateralization of Heschl's gyrus in auditory verbal hallucinations: a resting-state fMRI study

Vyara Zaykova[1*], Ferihan Popova[1], Sevdalina Kandilarova[2], Drozdstoy Stoyanov[2]

[1]*Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

[2]*Department of Psychiatry and Medical Psychology, Research Institute and SRIPD-MUP, Translational and Computation Neuroscience Group, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

*Corresponding author e-mail: vyara.zaykova@mu-plovdiv.bg

Hemispheric lateralization has been demonstrated to play a crucial role in language and auditory processing, typically manifesting as left-hemispheric dominance. It has been hypothesized that alterations in this organization are implicated in the aetiology of schizophrenia, particularly in relation to auditory verbal hallucinations. The objective of the present study was to examine the hemispheric lateralization and resting-state functional connectivity of the Heschl's gyrus bilaterally in patients with schizophrenia experiencing auditory verbal hallucinations. The sample comprised 105 subjects, including 42 individuals diagnosed with schizophrenia who experienced severe auditory verbal hallucinations and 63 age- and sex-matched healthy controls. The acquisition of resting-state fMRI data was conducted on a 3T scanner, followed by analysis employing the CONN toolbox and SPM12. A series of seed-based connectivity analyses were conducted for the bilateral Heschl's gyrus. In comparison with the control group, patients demonstrated enhanced resting-state functional connectivity between the right Heschl's gyrus and the right postcentral gyrus, as well as between the left Heschl's gyrus and the right lateral occipital gyrus. The results obtained suggest that there has been an alteration in the intrinsic connectivity of the auditory cortical regions in individuals diagnosed with schizophrenia. These aberrations might be indicative of disrupted functional lateralization, which is associated with the experience of hallucinations.

Key words: schizophrenia, auditory verbal hallucinations, resting-state, functional magnetic resonance imaging, Heschl's gyrus

Acknowledgments: S.K. and D.S. have been funded by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C03.

A 3D bioprinted model of colorectal cancer brain metastasis

Yordan Sbirkov[1,2*], Tatyana Todorova[1,2], Tatyana Damyanova[1,2], Tsvetomira Ivanova[1,2], Toma Tsvetanov[2], Azad Ozanian[3], Ilian Koev[3], Maria Kazakova[1,2], Victoria Sarafian[1,2]

[1]*Department of Medical Biology, Faculty of Medicine, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*
[2]*Research Institute at Medical University of Plovdiv, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*
[3]*Department of Neurosurgery, University Hospital Pulmed, Plovdiv, Bulgaria*

*Corresponding author e-mail: yordan.sbirkov@mu-plovdiv.bg

Colorectal cancer (CRC) metastases to the brain account for 1-3% of all metastatic CRC (mCRC) cases. Importantly, overall survival of patients with brain mCRC remains very poor (~5 months). Therefore, deeper understanding of basic cellular and molecular mechanisms that underly this malignancy and improved drug development strategies are urgently needed. The aim of this work is to design and validate a model of brain metastases from CRC. Extrusion-based 3D bioprinting was carried out with a BioX 3D bioprinter (Cellink, Sweden). Cellink RGD hydrogel was mixed with HCT-116 CRC cells and HMC3 microglial cells and 2-layer discs with diameter of 5mm were bioprinted. Bioprinted brain mCRC models were then cultured for >3 weeks while cell growth and morphology were assessed at several timepoints. Live/dead staining with calcein AM/ PI and histomorphological evaluation with H&E staining were carried out. CRC cells showed sustained proliferation in the 3D bioprinted microglia tumor microenvironment for at least 3 weeks. Within 7 days of culturing, visible clusters of cancer cells could be observed (>100µm) that increased in numbers and size (>300 µm) over time. In conclusion, we present the first 3D bioprinted *in vitro* experimental platform that may provide a suitable model of CRC early metastasis development in the brain microenvironment. This innovative approach could be used to study fundamental processes of tumorigenesis and to assess novel therapeutic strategies.

Key words: colorectal cancer, brain metastasis, 3D bioprinting, microglia, HCT-116

Acknowledgments: BNSF КП-06-Н83/9 from 06.12.2024; NUCBPI-BBMRI.BG contract no. DO1-257/15.12.2025; MU-Plovdiv project № NO-06/2026

Discriminant functions analysis of facial skull sexual dimorphism in a contemporary sample of the population of South Bulgaria

Zlatizara Todorova[1]*, Ferihan Popova[1], Irina Angelova[2], Iva Naydenova[3], Zdravka Harizanova[1]

[1]*Department of Anatomy, Histology and Embriology, Faculty of Medicine, Medical University of Plovdiv,Plovdiv, Bulgaria*

[2]*Department of Imaging Diagnostics, Dental Allergology and Physiotherapy, Faculty of Dental Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

[3]*Department of Mathematics, Physics, Chemistry, Technical University of Sofia, Branch Plovdiv, Plovdiv, Bulgaria*

*Corresponding author e-mail: zlatizara.todorova@mu-plovdiv.bg

Modern people show sexual dimorphism which is characterized by systematic differences between males and females of the same population. Cranial morphometric indicators have proved to be reliable and effective way in order to acknowledge human biological diversity and to provide new data from a contemporary reference sample in distinguishing sex. The purpose of this research is to generate a set of discriminant functions in order to establish the morphological characteristics that best distinguish between male and female sexes in South Bulgarian population. The study included CT acquired images of 120 valid cases (55 males and 65 females) and subsequent reconstruction of three-dimensional skull models. A total of 14 variables related to skull measurements were assessed. All 14 variables showed statistical significance ($p < 0.05$) in distinguishing between men and women. The most discriminating indicators were Bizygomatic width (Wilks' $\lambda = .389$, $F = 185.591$), Bigonial width ($F = 96.846$) and Morphological facial height ($F = 88.142$). Stepwise Fisher method indicated classification accuracy of the model 91.7%, where 90.9% of the men and 92.3% of the women were correctly classified. The discriminant analysis showed that the selected morphological indicators can successfully distinguish between male and female sexes with high accuracy. The resulting function can be used for future classifications of unknown cases which makes it a valuable tool in scientific and applied disciplines that require sex diagnosis based on skeletal material.

Key words: discriminant analysis, facial skull, sexual dimorphism, South Bulgaria, CT images

Acknowledgments: This study was supported by the Medical University of Plovdiv, Bulgaria, as part of scientific project DPDP-09/2025.