Description of the main research directions investigated by the Institute

The Institute of Experimental Medicine (IEM) is a recognized center of basic research in the Czech Republic. It is primarily focused on basic and integral research in biomedicine. The IEM currently comprises of 12 departments with a staff of over 200 individuals (>150 full time equivalents), made up as follows: PhD-level scientists ~70, PhD students ~50, other graduate specialists ~40, technical staff ~20 and administrators and others ~30. During the evaluation period, two small departments (Department of Molecular Neurophysiology, Department of Pharmacology) have been terminated, and two new groups have recently been established (in 2018 and 2020). None of these will be subjected to the evaluation. The other departments will be evaluated individually as Teams 1-10.
The evaluated research teams vary in size from a few individuals to the largest one with over 20 individuals. The departments cover diverse areas comprising biochemistry, cell biology and pathology, molecular embryology, genetic toxicology and nanotoxicology, neurophysiology, neuropathology, stem cells, oncology, developmental biology and tissue engineering, along with the development and validation of analytical, diagnostic and therapeutic methods based on results of the basic research. The plan to further progress IEM is tightly connected with the actual knowledge in these disciplines, which is significantly contributed to by the scientists at IEM.
In general, the period 2015-2019 can be characterized as a period of enormous changes in IEM, which began in mid 2016 comprising of the forced abdication of the director and all members of the Board of the IEM; followed by the director and Board of the IEM re-election. In addition, six Heads of Departments were newly nominated. Despite these marked changes in the organizational structure of IEM, neither research directions nor sustained scientific production were affected.

IEM research directions
IEM deals with selected current problems of biomedicine with the potential for application in clinical medicine, focusing on four main directions of research: CNS injuries and neurodegeneration, cancerogenesis, the pathological impact of the environment and tissue regeneration. In general, these are problems associated with the aging of the human population and the growing impact of human activities on the environment. Therefore, the social relevance of research at IEM is high.
Regarding the specific projects solved in the evaluated period, in the field of neuroscience it was mainly topics related to the genesis of pathological conditions of the auditory system (Team 1), along with age related CNS disorders such as Alzheimer's disease, ALS, brain ischemia or corneal/macular degeneration, and CNS traumas, which include brain edema and spinal cord injuries (Teams 2, 3, 6, 8, 10). Our research in the field of cancer focuses mainly on the mechanisms of those selected, highly frequented, respectively: difficult-to-treat forms of tumors, identification of their early markers and the individual adaptation of treatment procedures (Team 7). This research direction is closely related to studies aimed at elucidation of the environmental impact, namely the production of potentially toxic nanoparticles on human health (Team 10). Finally, in the field of tissue regeneration we develop biodegradable scaffolds for tissue regeneration. Our projects focus on the repair of bone, cartilage, incisional hernia, and skin. In addition, the mechanisms of endogenous or induced regeneration of nervous tissue are studied (Teams 1-3, 8-10). Other research directions, which are equally important, namely developmental biology,

functional organization of biomembranes and pharmacology, also fit well into the IEM research activities. They focus on elucidating the genetic basis of selected human developmental defects (Team 4), the role of functional microdomains in biological membranes with respect to stress perception and adaptation, signaling and regulation of metabolic processes (Team 5), and on the identification of new anti-inflammatory drugs (former Dept. of Pharmacology).
Overall the researchers at IEM published 440 publications in peer-reviewed journals, with a summary impact factor of 1894.844, 110 book chapters and 21 non-impacted publications, in the evaluated period.

Selected consortial publications with IEM contribution (the authors from IEM are highlighted)

Childs, E.J., Mocci, E., Campa, D., Bracci, P.M., Gallinger, S., Goggins, M., Li, D., Neale, R.E., Olson, S.H., Scelo, G., Amundadottir, L.T., Bamlet, W.R., Bijlsma, M.F., Blackford, A., Borges, M., Brennan, P., Brenner, H., Bueno-de-Mesquita, H.B., Canzian, F., Capurso, G., Cavestro, G.M., Chaffee, K.G., Chanock, S.J., Cleary, S.P., Cotterchio, M., Foretová, L., Fuchs, C., Funel, N., Gazouli, M., Hassan, M., Herman, J.M., Holcatová, I., Holly, E.A., Hoover, R.N., Hung, R.J., Janout, V., Key, T.J., Kupcinskas, J., Kurtz, R.C., Landi, S., Lu, L., Malecka-Panas, E., Mambrini, A., Mohelniková-Duchonová, B., Neoptolemos, J.P., Oberg, A.L., Orlow, I., Pasquali, C., Pezzilli, R., Rizzato, C., Saldia, A., Scarpa, A., Stolzenberg-Solomon, R.Z., Strobel, O., Tavano, F., Vashist, Y.K., **Vodička, P.,** Wolpin, B.M., Yu, H., Petersen, G.M., Risch, H.A., Klein, A.P.: (2015) *Nat Genet.* 47(8): 911-916.

Newton, P.T., Li, L., Zhou, B., Schweingruber, C., **Hovořáková, M.,** Xie, M., Sun, X., Sandhow, L., Artemov, A.V., Ivashkin, E., Suter, S., Dyachuk, V., El Shahawy, M., Gritli-Linde, A., Bouderlique, T., Petersen, J., Mollbrink, A., Lundeberg, J., Enikolopov, G., Qian, H., Fried, K., Kasper, M., Hedlund, E., Adameyko, I., Sävendahl L., Chagin, A.S.: (2019) *Nature*. 567(7747): 234-238.

Abdelfattah, A.S., Kawashima, T., Singh, A., **Novák, O.,** Liu, H., Shuai, Y.C., Huang, Y.C., Campagnola, L., Seeman, S.C., Yu, J., Zheng, J.H., Grimm, J.B., Patel, R., Friedrich, J., Mensh, B.D., Paninski, L., Macklin, J.J., Murphy, G.J., Podgorski, K., Lin, B.J., Chen, T.W., Turner, G.C., Liu, Z., Koyama, M., Svoboda, K., Ahrens, M.B., Lavis, L.D., Schreiter, E.R.: (2019) *Science.* 365(6454): 699-704.

Thomas, A.M., Manghi, P., Asnicar, F., Pasolli, E., Armanini, F., Zolfo, M., Beghini, F., Manara, S., Karcher, N., Pozzi, C., Gandini, S., Serrano, D., Tarallo, S., Francavilla, A., Gallo, G., Trompetto, M., Ferrero, G., Mizutani, S., Shiroma, H., Shiba, S., Shibata, T., Yachida, S., Yamada, T., Wirbel, J., Schrotz-King, P., Ulrich, C.M., Brenner, H., Arumugam, M., Bork, P., Zeller, G., Cordero, F., Dias-Neto, E., Setubal, J.C., Tett, A., Pardini, B., Rescigno, M., Waldron, L., **Naccarati, A.,** Segata, N.: (2019) *Nature Medicine.* 25(4): 667-+

Wirbel, J., Pyl, P.T., Kartal, E., Zych, K., Kashani, A., Milanese, A., Fleck, J.S., Voigt, A.Y., Palleja, A., Ponnudurai, R., Sunagawa, S., Coelho, L.P., Schrotz-King, P., Vogtmann, E., Habermann, N., Nimeus, E., Thomas, A.M., Manghi, P., Gandini, S., Serrano, D., Mizutani, S., Shiroma, H., Shiba, S., Shibata, T., Yachida, S., Yamada, T., Waldron, L., **Naccarati, A.,** Segata, N., Sinha, R., Ulrich, C.M., Brenner, H., Arumugam, M., Bork, P., Zeller, G.: (2019) *Nature Medicine.* 25(4): 679-+.

Selected impacted publications - domestic cooperations with prevailing IEM contribution

Novotná, B., Turnovcová, K., Veverka, P., **Rössner, P. Jr., Bagryantsevá, Y., Herynek, V.,** Zvatora, P., Vosmanská, M., Klementová, M., **Syková, E., Jendelová, P.**: (2016) Nanotoxicology, 10(6): 662-670.

Valný, M., Honsa, P., Waloschková, E., Matušková, H., Kriška, J., Kirdajová, D., Androvič, P., Valihrach, L., Kubista, M., **Anděrová, M.**: (2018) Glia. 66 (5): 1068-1081.

Lukášová, V., Buzgo, M., Vocetková, K., Sovková, V., Doupnik, M., Himawan, E., **Staffa, A.,** Sedláček, R., Chlup, H., **Rustichelli, F., Amler, E., Rampichová, M.:** (2019) *Materials Science & Engineering C-Materials for Biological Applications.* 97: 567-575

Červená, K., Vodička, P., Vymetálková, V.: (2019) Mutation Research - Genetic Toxicology and Environmental Mutagenesis. 781: 100-129.

Holáň, V., Javorková, E., Vrbová, K., Večeřa, Z., Mikuška, P., Coufalík, P., Kulich, P., Skoupý, R., Machala, M., **Zajícová, A., Rössner, P.**: (2019) Nanotoxicology. 13(7): 952-963.

Selected impacted publications - international cooperations with prevailing IEM contribution

Ouda L., Burianová J., Balogová Z., Lu H. P., **Syka J.**: (2016) Brain Struct. Funct., 221(1): 617-629.

Choi, H., Song, W.M., Wang, M.H., **Dostál, M., Pastorková, A.,Líbalová, H.,Tulupová, E., Rössnerová, A., Rössner, P., Šrám, R.,** Zhang, B.: (2019) Environment International. 128: 218-232.

Vymetálková, V., Vodicka, P., Vodenková, S., Alonso, S., Schneider-Stock, R.: (2019) Molecular Aspects of Medicine. 69: 73-92.

Kárová, K., Wainwright, J.V., **Machová-Urdziková, L., Pisal, R.,** Schmidt, M., **Jendelová, P.,** Jhanwar-Uniyal, M.: (2019) Journal of Neuroinflammation. 16:12.

Řehořová, M., Vargová, I., Forostyak, S., Vacková, I., Turnovcová, K., Kupcová Skalníková, H., Vodička, P., **Kubinová, S., Syková, E., Jendelová, P.**: (2019) Stem Cells Translational Medicine. 8 (6): 535-547.

Selected outputs of applied research

"**Polysubstituted pyrimidines**" - Czech patent (308052) and submitted international patent application, the subject of which relates to particular polysubstituted pyrimidine compounds having the common structure, their use as medicaments and a pharmaceutical composition containing them. These compounds reduce the production of prostaglandin E2 and do not adversely affect cell viability. They are used for the treatment of inflammatory and / or cancer diseases as anti-angiogenic immunomodulatory, antiproliferative or antitumor agents. In cooperation with Institute of the organic Chemistry and Biochemistry, CAS.

"**Solution for storage and transportation of cells for up to 72 hours without the need of cryopreservation**" - Czech patent (306800) and submitted international patent application, the subject of which relates to a composition for the storage, transport and application of stem cells for therapeutic use, consisting of a buffered aqueous solution of trehalose. The composition is intended to store cells at a temperature of -4 ° C to 25 ° C, preferably 0 ° C to 8 ° C, most often 4 ° C to 6 ° C. Sufficient cell viability is maintained for at least 72 hours. The composition consists of pharmaceutically acceptable compounds and can be used directly to administer

the stem cells stored therein. The stem cell composition is intended for the treatment of inflammatory diseases, including the post-traumatic inflammatory response after injury, as well as for the treatment of degenerative and neurodegenerative diseases. The composition containing stem cells can also be used in the treatment of trauma, developmental defects, wound healing and skin burns, tissue replacement, treatment of diseases of the musculoskeletal system (tendons, joints, inflammatory diseases - arthritis, osteoarthritis), bone defects, diabetes, stroke, cardiac and oncological diseases. In cooperation with Bioinova company.

Outputs of the applied Research: An Overview

File designation Document number	**Name**	**applicant / owner**
PV 2015-606 308154	Nanoparticles for magnetic and fluorescent cell labelling, preparation and use	IEM Institute of Physics CAS Institute of Clinical and Experimental Medicine
PV 2015-698 306217	Low-temperature plasma source with possibility of both contact and contactless application and process for preparing sandwich structure for such a source	Institute of Physics CAS IEM
PUV 2015-30685 28419	Composite surgical net with nanofibrous layer	Motol University Hospital IEM Student Science, s.r.o. (Ltd.)
PUV 2015-31600 29159	Low-temperature plasma source with possibility of contact as well as contactless application	Institute of Physics CAS IEM
PUV 2015-31845 29236	Low-temperature plasma source, especially for plasma generation in the form of various voluminous formations	Institute of Physics CAS IEM
PV 2016-284 306800	A device for storage, transport and application of stem cells	IEM
PV 2016-644 307325	A composition containing stem cells for the treatment of post-traumatic inflammatory responses and a method of its manufacture	IEM
PUV 2016-32290 30686	A variable kit for cultivation of cell structures in cultivation plates	IEM
PUV 2016-32937 30239	A preparation containing stem cells for treatment of inflammatory diseases, post-traumatic reactions and degenerative diseases	IEM
PUV 2016-33078 30612	3D composite gels for controlled cell differentiation under in vitro conditions	IEM
PUV 2016-33101 30270	A means for storage, transportation and application of stem cells	IEM
PV 2017-84 307851	Medicinal product to prevent and treat inflammatory and degenerative diseases	IEM
PV 2017-293 308052	Polysubstituted pyrimidines	Institute of Organic Chemistry and Biochemistry CAS IEM
PUV 2017-33889 31206	A preparation for preserving human or animal cells at very low temperatures	Bioinova, s.r.o. (Ltd.) IEM
PUV 2017-33974 31034	A low-temperature plasma source, especially for plasma generation when used in medical bio-applications	Institute of Physics CAS IEM
PV 2018-381 307891	A method and the equipment for detecting particle concentration, particularly a nanoparticle	IEM

PUV 2018-34759 31773	A preparation for the treatment of inflammatory and degenerative eye diseases and eye drops adjusted in vials	IEM
PUV 2018-35218 32414	A device for detecting particle concentration, especially nanoparticles	IEM
PUV 2018-35244 32083	An audiometer for a measuring apparatus for a comprehensive hearing test and measuring apparatus for a comprehensive hearing test containing this audiometer	IEM
PUV 2018-35280 32813	A medical device for the application of a therapeutically active atmosphere in the treatment of eye diseases	IEM
PUV 2019-35874 32666	Lyophilized preparation for the treatment of inflammatory and degenerative diseases	IEM
PUV 2019-36581 33468	Toxicological incubator for exposing cell cultures to aerosol	Czech Technical University in Prague IEM Czech University of Life Sciences Prague
PUV 2019-36654 33331	Source of low temperature plasma, especially for use in the food industry and bioapplications	Institute of Physics CAS IEM

IEM financial resources

The structure of the IEM budget did not change significantly in the evaluated period 2015-2019. Only a small portion of it (less than 40%) consists of subsidies from the Czech Academy of Sciences, which is mainly used to cover wage costs. Research costs at IEM are provided mainly from extra-budgetary funds, i.e. project sources (~ 60%; GA CR (Czech Science Foundation), The Technology Agency of the Czech Republic, Ministry of Education, Youth and Sport, Ministry of Industry and Trade, Ministry of Health).

In recent years, IEM researchers have repeatedly managed to obtain financial support for a number of projects.

During the period 2015-2019 IEM participated in several research centers, namely the “Project of excellence in the field of neuroscience” (PEN, 2012-2018), Center for Studies on Toxicity of nanoparticles (CENATOX, 2012-2018), Center for Development of Original Drugs (CVOL, 2012-2018) and Center for Orofacial Development and Regeneration (2014-2018). These research centers covered four important research directions: neuroscience, nanotoxicology, pharmacology and orofacial development and regeneration. The PEN connected four research institutions (Institute of Physiology, The National Institute of Mental Health, Second Medical Faculty of the Charles University and IEM) with a common aim on the pathophysiological mechanisms underlying the development of neurodegenerative diseases, from genetic up to system level. The project not only enabled the creation of a network of high level scientific teams and promoted an interdisciplinary approach, but also provided a unique basis for PhD training in neuroscience. CENATOX was an interdisciplinary project that comprehensively addressed the issue of the possible side effects of nanomaterials. The project was coordinated by the Veterinary Research Institute and the partners were the Institute of Experimental Medicine of the CAS, the Institute of Animal Physiology and Genetics of the CAS, the Institute of Chemical Process Fundamentals of the CAS, the Institute of Analytical Chemistry of the CAS and the Faculty of Science, Charles University.

In addition, IEM was part of the Technology Agency of the Czech Republic Competence Center called the "Center for the Development of Original Medicines",

whose main recipient and coordinator was the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences. Other recipients besides IEM were Palacky University in Olomouc, the University of Chemistry and Technology in Prague and the Institute of Physiology of the Czech Academy of Sciences in Prague. The task of the IEM team was to analyze the biological, pharmacologically, promising properties of pyrimidine derivatives. In particular, their effect on the production of inflammatory mediators such as prostaglandin PGE2, nitric oxide (NO) and cytokines was studied. An important output of this center is the filing of an international patent application for the development of new anti-inflammatory drugs based on pyrimidines.
Within the Operational Program Research, Development and Education (OP RDE), which is a multi-annual thematic program under the auspices of the Ministry of Education, Youth and Sports, IEM is currently solving three projects: NEURORECON Center for Reconstructive Neuroscience (2017-2021) and Healthy Aging in Industrial Environment HAIE (2018-2022). At the end of 2019, we also managed to obtain funding for the Capacity Development project of the Institute of Experimental Medicine of the CAS; the outcome will be winning the prestigious HR award. By obtaining this award, we will join more than 1,300 institutions from the research and university environment that have previously received this award. Above all, however, human resources processes and conditions will be set up to demonstrate compliance with the European Charter for Researchers and the Code of Conduct for the Recruitment of Researchers.

Research activity and characterization of the main scientific results

I) We studied the role of transcription factors Islet1, Sox2 and Neurod1 in the development, maturation, and maintenance of the mammalian inner ear. We used transgenic mice with an altered expression of these factors. We found that an over-expression of Islet1 impairs the function of hair cells of the organ of Corti, resulting in an age-related decline in otoacoustic emissions and elevated hearing thresholds. The deletion of Sox2 disrupted the proper differentiation of hair cells and caused increased apoptosis of untargeted neurons from the spiral ganglion. The deletion of Neurod1 caused a disorganized primary cochleotopic map, which altered the tuning properties of structures in the ascending auditory pathway. Together these results indicated essential regulatory roles of the transcription factors in the early developmental stages of development of the cochlea.
The project was designed and carried out jointly at the Department of Auditory Neuroscience and in a collaborating laboratory at the Institute of Biotechnology. Our team members were responsible for all electrophysiological experiments, provided some of the immunohistochemistry data, and contributed to the writing of the manuscript.

References:
1. Chumak T, , Bohuslavová R, Mácová I, Dodd N, Buckiová D, Fritzsch B, Syka J, Pavlínková G, Deterioration of the Medial Olivocochlear Efferent System Accelerates Age-Related Hearing Loss in Pax2-Isl1 Transgenic Mice. Molecular Neurobiology. 2016, 53(4): 2368-2383.
2. Dvořáková M, Jahan I, Mácová I, Chumak T, Bohuslavová R, Syka J, Fritzsch B, Pavlínková G, Incomplete and delayed Sox2 deletion defines residual ear neurosensory development and maintenance. Scientific Reports. 2016, 6(Dec 5), 38253.
3. Macova I, Pysanenko K, Chumak T, Dvorakova M, Bohuslavova R, Syka J, Fritzsch B, Pavlinkova G. Neurod1 is essential for the primary tonotopic organization and related auditory information processing in the midbrain. Journal of Neuroscience, 2018, 10.1523

II) We investigated proteins which were specifically interacting with the inhibitory GABAB receptors that are widely distributed in the mammalian auditory system. With the use of heterologous expression systems we showed how KCTD proteins, auxiliary subunits of inhibitory GABAB receptors, regulate G-protein signaling of the receptor. Our data demonstrate that the simultaneous assembly of distinct KCTDs at the receptor increases the molecular and functional repertoire of native GABAB receptors, and modulates their physiological responses in the brain. We then reported a novel mechanism of neuronal trafficking of GABAB receptors. We used proteomic and functional analyses to reveal synaptic proteins that link the receptor in cargo vesicles to the axonal trafficking motor. The protein complexes also include the precursor proteins of Aβ, a component of senile plaques in Alzheimer's disease patients. Our findings support that dysfunctional axonal trafficking of GABAB receptor in Alzheimer's

disease increases Aβ formation. Finally, we have shown that the corticofugal pathways control the activity of GABAergic inhibitory circuits in the subcortical auditory nuclei.
These projects were fully designed and carried out at the Department of Auditory Neuroscience (ref. #3) or jointly with the cooperating laboratory at the Department of Biomedicine, University of Basel (refs. #1,2). We were responsible for the electrophysiology parts in all projects. The co-first author of the article (ref. #1) is from the Department of Auditory Neuroscience and has done a substantial part of the experimental work.

References:

1. Fritzius T., Tureček R, Seddik R, Kobayashi H, Tiao J, Rem PD, Metz M, Králíková M, Bouvier M, Gassmann M, Bettler B. KCTD Hetero-oligomers Confer Unique Kinetic Properties on Hippocampal GABA(B) Receptor-Induced K+ Currents. Journal of Neuroscience. 2017, 37(5): 1162-1175.
2. Dinamarca MC, Raveh A, Schneider A, Fritzius T, Früh S., Rem PD, Stawarski M, Lalanne T, Tureček R, Choo M., Besseyrias V, Bildl W, Bentrop D, Staufenbiel M, Gassmann M, Fakler B, Schwenk J, Bettler B. Complex formation of APP with GABA(B) receptors links axonal trafficking to amyloidogenic processing. Nature Communications. 2019, 10(mar), 1331.
3. Popelář, Jiří, Šuta, Daniel, Lindovský, Jiří, Bureš, Zbyněk, Pysaněnko, Kateryna, Chumak, Tetyana, Syka, Josef. Cooling of the auditory cortex modifies neuronal activity in the inferior colliculus in rats. Hearing Research.2016, 332(feb), 7-16. ISSN 0378-5955 doi: 10.1016/j.heares.2015.10.021

III) We studied the effects of early postnatal noise exposure on the hearing function of rats. We found that brief noise exposure during the critical period of postnatal development altered the development of hearing abilities in young animals, and resulted in anomalous processing of acoustic stimuli in the adult auditory system. Functional changes were accompanied by the altered morphology of central auditory structures. We found significantly decreased numbers of inner hair cell ribbon synapses and an abnormal morphology of principal neurons in the inferior colliculus, medial geniculate body and auditory cortex. Short exposure to noise also led to changes in sound-evoked behavioral responses of adult rats, indicating anomalies in intensity coding and volume perception. The project was fully carried out at the Department of Auditory Neuroscience by its members.

References:

1. Rybalko N, Chumak T, Bureš Z, Popelář J, Šuta D, Syka J. Development of the acoustic startle response in rats and its change after early acoustic trauma. Behavioural Brain Research. 2015, 286: 212-221.Šuta D, Rybalko N, Shen DW, Popelář J, Poon PWF, Syka J. Frequency discrimination in rats exposed to noise as juveniles. Physiology and Behavior. 2015, 144: 60-65.

2. Rybalko N, Mitrovič DM, Šuta D, Bureš Z, Popelář J, Syka J. Behavioral evaluation of auditory function abnormalities in adult rats with normal hearing thresholds that were exposed to noise during early development. Physiology and Behavior. 2019, 210, 112620.
3. Ouda L, , Burianová J, Balogová Z, Lu HP, Syka J. Structural changes in the adult rat auditory system induced by brief postnatal noise exposure. Brain Structure and Function. 2016, 221: 617-629.

IV) We tested the effect of physiological stimulation of early postnatal cochlear activity by complex sounds on the functional properties of neurons in the rat auditory pathway. We found that the stimulation lowered the hearing thresholds, enhanced frequency selectivity and magnitudes of sound-evoked responses, and increased spontaneous activity in the inferior colliculus and the auditory cortex of adult rats. These findings suggest that, unlike exposure to harmful noise, the presence of an acoustically enriched environment during the critical period of postnatal development, improves the ability of auditory structures to detect and distinguish sounds. Accordingly, we have also found that normal spontaneous and sound-induced cochlear activity is necessary to properly shape the neural circuits in the auditory brainstem. Therefore our results assist with understanding the activity-dependent mechanisms of developmental plasticity in the mammalian auditory system. The project was fully carried out at the Department of Auditory Neuroscience by its members.

References:

1. Pysanenko K, Bureš Z, Lindovský J, Syka J. The Effect of Complex Acoustic Environment during Early Development on the Responses of Auditory Cortex Neurons in Rats. Neuroscience. 2018, 371(feb 10), 221-228.
2. Bureš Z, Pysanenko K, Lindovský J, Syka, Josef. Acoustical Enrichment during Early Development Improves Response Reliability in the Adult Auditory Cortex of the Rat. Neural Plasticity, 2018, 5903720.
3. Hruskova B, Trojanova J, Kralikova M, Melichar A, Suchankova S, Bartosova J, Burianova JS, Popelar J, Syka J and Turecek R (2019) Cochlear ablation in neonatal rats disrupts inhibitory transmission in the medial nucleus of the trapezoid body. Neurosci. Lett. 699,145-150. doi: 10.1016/j.neulet.2019.01.058.

V) We examined changes in the aging rat auditory system by using quantitative morphometric and biochemical methods. We reported how the number, representation and structural components of neurons in the auditory pathway change as they age, and how these changes are related to presbycusis, age-related hearing loss. In addition, we identified Fischer 344 rats as a metabolic (strial) type of presbycusis in humans. We have found that this rat strain exhibits significantly accelerated age-related changes in hearing thresholds, auditory brainstem response amplitudes, and amplitudes of distortion product otoacoustic emissions.

Other projects focused on characterizing changes in the function of auditory subcortical centers and auditory cortex in older human subjects, using audiometry and functional MRI. The results indicated a strong dependence of the ability to comprehend speech in elderly patients (especially those with tinnitus) on temporal parameters of sound stimuli, especially in noisy environments. We also demonstrated a greater degree of cortical activation in the elderly with asymmetry towards the right side, and suggested the presence of a compensation mechanism for the impaired processing of sensory information associated with presbycusis. The project was designed and carried out by members of the Department of Auditory Neuroscience. Part of the fMRI experiments was performed using a specialized research infrastructure at a collaborating clinical department (ref. #3).

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2. Balogová Z, Popelář J, Chiumenti F, Chumak T, Burianová J, Rybalko N, Syka J. Age Related Differences in Hearing Function and Cochlear Morphology between Male and Female Fischer 344 Rats. Frontiers in Aging Neuroscience. 2018, 9(jan 4), 428.
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4. Bureš Z, Profant O, Svobodová V, Tóthová D, Vencovský V, Syka J. Speech Comprehension and Its Relation to Other Auditory Parameters in Elderly Patients With Tinnitus. Frontiers in Aging Neuroscience. 2019, 11(aug.), 219.
5. Profant O, Jílek M, Bureš Z, Vencovský V, Kuchárová D, Svobodová V, Korynta J, Syka J. Functional Age-Related Changes Within the Human Auditory System Studied by Audiometric Examination. Frontiers in Aging Neuroscience. 2019, 11(feb.), 26.

VI) We have been involved in the development of new genetically encoded, fluorescent dyes that can be used as Ca^{2+} or voltage sensors for in vivo imaging of neuronal activity in the mouse auditory cortex. This is a very ambitious research project, led by a world-renowned team based in top US laboratories. Our team members participated in it by providing surgical procedures, viral transductions of Voltron and in vivo imaging using two-photon microscopy (refs. #1, 2).
We designed and manufactured a new audiometer for monitoring the hearing function of patients. The device allows us to perform more audiometric tasks with greater accuracy than the current audiometers, and has been successfully tested in clinical practice. The equipment has been fully designed and manufactured by the Department of Auditory Neuroscience (ref. #3).

References:

1. Hod, D., Novák, Ondřej, Guardado-Montesino, M., Fransen, J.W., Hu, A., Borghuis, B.G., Guo, C., Kim, D.S., Svoboda, K. Thy1 transgenic mice expressing the red fluorescent calcium indicator jRGECO1a for neuronal population imaging in vivo. PLoS ONE 2018, 13(10), e0205444. doi: 10.1371/journal.pone.0205444
2. Abdelfattah, A.S., Kawashima, T., Singh, A., Novák, Ondřej, Liu, H., Shuai, Y.C., Huang, V.C., Campagnola, L., Seeman, S., Yu, J.N., Zheng, J., Grimm, J.B., Patel, R., Friedrich, J., Mensh, B.D., Paninski, L., Macklin, J.J., Murphy, G.J., Podgorski, K., Lin, B.J., Chen, T.W., Turner, G.C., Liu, Z., Koyama, M., Svoboda, K., Ahrens, M.B., Lavis, L.D., Schreiter, E.R. Bright and photostable chemigenetic indicators for extended in vivo voltage imaging. Science 2019, 365(6454), 699-704. doi: 10.1126/science.aav6416
3. Bureš, Zbyněk. Audiometr pro měřicí aparaturu pro komplexní vyšetření sluchu a měřicí aparatura pro komplexní vyšetření sluchu obsahující tento audiometr. 2018. Praha 4: Ústav experimentální medicíny AV ČR, v.v.i., 18.09.2018.32083. http://isdv.upv.cz/doc/FullFiles/UtilityModels/FullDocuments/FDUM0032/uv032083.pdf

Research activity and characterisation of the main scientific results

The research activity in the evaluated period 2015-19 involved following main topics. If it is not stated otherwise, all contributed authors are employed in the Department of Cellular Neurophysiology, IEM.

1. Compromised functions of astroglia and changes in the brain diffusion in CNS ischemia/ageing pathologies.

1.1. Ion channels and transporters

In the context of brain edema formation we aimed to elucidate the role of $[Na^+]_i$ on volume regulated anion channels (VRACs) and showed that VRAC are depressed by high $[Na^+]_i$ in adult astrocytes. These results provide the first evidence that intracellular Na^+ dynamics can modulate astrocytic membrane conductance that controls functional processes linked to cell volume regulation and add further support to the concept that limiting astrocyte intracellular Na^+ accumulation might be a favourable strategy to counteract the development of brain edema.
Minieri, Laura, Pivoňková, Helena, Harantová, Lenka, Anděrová, Miroslava, Ferroni, Stefano. Intracellular Na^+ inhibits volume regulated anion channel in rat cortical astrocytes. Journal of Neurochemistry. 2015, 132(3), 286-300. ISSN 0022-3042 doi: 10.1111/jnc.12962 (Contribution: P. H., H.L., data acquisition and analysis, manuscript writing, A.M. data analysis, manuscript writing and funding; collaboration with Prof. S. Ferroni, University of Bologna)

Despite several efforts, a comprehensive analysis of the entire family of glutamate receptors and their subunits present in glial cells is still missing. In this study, we provided an overall picture of the gene expression of ionotropic (AMPA, kainate, NMDA) and the main metabotropic glutamate receptors in cortical glial cells isolated from GFAP/EGFP mice before and after focal cerebral ischemia. Employing single-cell RT-qPCR, we detected the expression of genes encoding subunits of glutamate receptors in GFAP/EGFP-positive (GFAP/EGFP$^+$) glial cells in the cortex of young adult mice. Most of the analyzed cells expressed mRNA for glutamate receptor subunits, the expression of which, in most cases, even increased after ischemic injury. Data analyses disclosed several classes of GFAP/EGFP$^+$ glial cells with respect to glutamate receptors and revealed in what manner their expression correlates with the expression of glial markers prior to and after ischemia. Immunohistochemical analyses of all seven NMDA receptor subunits provided direct evidence that the GluN3A subunit is present in GFAP/EGFP$^+$ glial cells and that its expression is increased after ischemia. In situ and in vitro Ca^{2+} imaging revealed that Ca^{2+} elevations evoked by the application of NMDA were diminished in GFAP/EGFP$^+$ glial cells following ischemia. Our results provide a comprehensive description of glutamate receptors in cortical GFAP/EGFP$^+$ glial cells and may serve as a basis for further research on glial cell physiology and pathophysiology.
Džamba, Dávid, Honsa, Pavel, Valný, Martin, Kriška, Ján, Valihrach, Lukáš, Novosadová, Vendula, Kubista, Mikael, Anděrová, Miroslava. Quantitative Analysis of Glutamate Receptors in Glial Cells from the Cortex of GFAP/EGFP Mice Following Ischemic Injury: Focus on NMDA Receptors. Cellular and Molecular Neurobiology. 2015, 35(8), 1187-1202. ISSN 0272-4340 doi: 10.1007/s10571-015-0212-8 (Contribution: D. D., H.P., V. M., K.J. data acquisition and analysis, manuscript writing, A.M. data analysis, manuscript writing and funding)

Since ageing is associated with cognitive disturbances that are partly attributable to metabolic deficiency leading to brain glycopenia, we investigated the early structural and functional alterations induced in astrocytes by a transient metabolic challenge consisting in glucose deprivation. Electrophysiological recordings of hippocampal astroglial cells of the stratum radiatum in situ revealed that shortage of glucose specifically increases astrocyte membrane capacitance and astrocyte volume. We also found that glucose deprivation decreases astrocytic gap junction-mediated coupling and increases their intracellular calcium levels during the slow depression of synaptic transmission. Our data indicate that astrocytes rapidly respond to metabolic dysfunctions and are therefore central to the neuroglial dialog at play in brain adaptation to glycopenia.
Lee, Chun-Yao, Dallérac, Glenn, Ezan, Pascal, Anděrová, Miroslava, Rouach, Nathalie. Glucose Tightly Controls Morphological and Functional Properties of Astrocytes. Frontiers in Aging Neuroscience. 2016, 8(85). ISSN 1663-4365 doi: 10.3389/fnagi.2016.00082 (Contribution: A. M. conception and experimental design, methodology and data acquisition, analysis and interpretation of data)

In an invited review, we summarized current knowledge about ion channels/receptors or transporters in astrocytes, such as connexins, ionotropic glutamate receptors, AQP4, TRPV4 and chloride channels that might significantly contribute to astrocyte volume changes evoked by ischemia. Furthermore, we summarized currently known properties of reactive astrocytes, with emphasis on the expression and function of ion channels, transporters and neurotransmitter receptors; all of which possess the ability to change the functional state of astrocytes, such as the membrane equilibrium potentials for different ions. This may have major effects on the functioning of surviving neurons, consequently leading to changes in neuronal excitability and progression of secondary pathologies, such as epilepsy. Moreover, we provide possible clues for therapy, based on functional modulation of astrocytic ion transporting mechanisms.
Pivoňková, Helena, Anděrová, Miroslava. Altered Homeostatic Functions in Reactive Astrocytes and Their Potential as a Therapeutic Target After Brain Ischemic Injury. Current Pharmaceutical Design. 2017, 23(33), 5056-5074. ISSN 1381-6128 doi: 10.2174/1381612823666170710161858
*(*Contribution: A.M. and P.H. contributed equally in writing, A.M. supervision)

1. 1.2. NG2 glia and the role of sonic hedgehog

NG2 cells represent precursors of oligodendrocytes under physiological conditions; however, following cerebral ischemia they play an important role in glial scar formation. Here, we compared the expression profiles of oligodendroglial lineage cells, after focal cerebral ischemia (FCI) using transgenic mice, which enables genetic fate-mapping of Cspg4-positive NG2 cells and their progeny, based on the expression of red fluorescent protein tdTomato. We employed single cell RT-qPCR and analyzed the data using self-organizing Kohonen maps, which enable the division of NG2 cells and oligodendrocytes into subpopulations based on similarities in the expression profiles of individual cells. We identified three subpopulations of NG2 cells emerging after FCI: proliferative; astrocyte-like and oligodendrocyte-like NG2 cells (OL-NG2 cells); such phenotypes were further confirmed by immunohistochemistry. OL-NG2 cells were characterized by a high percentage of cells expressing oligodendrocyte-committed genes, such as and *Cldn11* and *Mbp*. This subpopulation displayed much higher expression of *Cldn11* and *Cnp*, and a decreased expression of Pdgfra when compared

to *bona fide*-NG2 cells, which points toward the phenotypical shift of OL-NG2 cells (Fig. 4B). Almost 40% of OL-NG2 cells started to express *Trpv4* (transient receptor potential cation channel subfamily V member 4), which was shown to be expressed by committed OLs precursors. Our results suggest that TRPV4 channels might play an important role in the NG2 cell transition to oligodendrocytes.
Valný, Martin, Honsa, Pavel, Waloschková, Eliška, Matušková, Hana, Kriška, Ján, Kirdajová, Denisa, Androvič, Peter, Valihrach, Lukáš, Kubista, Mikael, Anděrová, Miroslava. A single-cell analysis reveals multiple roles of oligodendroglial lineage cells during post-ischemic regeneration. Glia. 2018, 66(5), 1068-1081. ISSN 0894-1491 doi: 10.1002/glia.23301
Contribution: M.A. and M.V. designed and conceptualized all the experiments. P.H., M.V., J.K. and D.K. prepared all tissue samples. P.H., M.V., E.W. and H.M. and performed MCAo operations in this project. P.H., M.V., E.W. and H.M. performed immunohistochemical analysis. L.V. and M.K. performed and analyzed RT-qPCR experiments. M.V. and P.H. performed the overall analysis of results from these experiments. M.A. and M.V. wrote and edited the manuscript.

NG2 cells produce oligodendrocytes in the healthy nervous tissue, and display wide differentiation potential under pathological conditions, where they could give rise to reactive astrocytes. The factors that control the differentiation of NG2 cells after focal cerebral ischemia (FCI) are largely unknown. Here, we used transgenic Cspg4-cre/Esr1/ROSA26Sortm14(CAG-tdTomato) mice, in which tamoxifen administration triggers the expression of red fluorescent protein (tomato) specifically in NG2 cells and cells derived therefrom. Differentiation potential (in vitro and in vivo) of tomato-positive NG2 cells from control or post-ischemic brains was determined using the immunohistochemistry, single cell RT-qPCR and patch–clamp method. The ischemic injury was induced by middle cerebral artery occlusion, a model of FCI. Using genetic fate-mapping method, we identified sonic hedgehog (Shh) as an important factor that influences differentiation of NG2 cells into astrocytes in vitro. Shh signaling activation significantly increased the number of astrocytes derived from NG2 cells in the glial scar around the ischemic lesion, while Shh signaling inhibition caused the opposite effect. Since Shh signaling modifications did not change the proliferation rate of NG2 cells, we can conclude that Shh has a direct influence on the differentiation of NG2 cells and therefore, on the formation and composition of a glial scar, which consequently affects the degree of the brain damage.
Honsa, Pavel, Valný, Martin, Kriška, Ján, Matušková, Hana, Harantová, Lenka, Kirdajová, Denisa, Valihrach, Lukáš, Kubista, Mikael, Anděrová, Miroslava. Generation of Reactive Astrocytes from NG2 Cells is Regulated by Sonic Hedgehog. Glia. 2016, 64(9), 1518-1531. ISSN 0894-1491 doi: 10.1002/glia.23019 (Contribution: A.M., H.P. experiment design, data analysis, manuscript writing, H.L., K.J., K.D., V.M., M.H. data acquisition, A.M. funding)

In an invited review we described current knowledge about NG2 cell proliferation, their fate plasticity during embryogenesis as well as in postnatal CNS under physiological and pathological conditions. Here, we pointed out the possibility to modulate their multipotent phenotype and thus be useful approach to battle a wide range of CNS pathologies including TBI, SCI and ischemia. United and recent information in this review, can help scientists working in a similar field to achieve a better orientation in this problematic.

Valný, Martin, Honsa, Pavel, Kriška, Ján, Anděrová, Miroslava. Multipotency and therapeutic potential of NG2 cells. Biochemical Pharmacology. 2017, 141(SI), 42-55. ISSN 0006-2952 doi: 10.1016/j.bcp.2017.05.008
Contribution: all authors contributed equally in writing, A.M. review structure and supervision.

3. Role of AQP4 and TRPV4 channels in cell swelling
The involvement of TRPV4 channels and their functional cooperation with AQP4 channels in
astrocyte swelling and subsequent volume regulation was previously shown in astrocyte cultures
and retinal Muller glial cells. The aim of our study was to find out whether similar mechanisms of volume regulation take place also in astrocytes in their natural environment. For our experiments, we used trpv4-/- mice crossed with transgenic mice expressing EGFP under the control of the GFAP promoter, which leads to astrocyte visualization by EGFP expression in the mouse brain. For quantification of astrocyte volume changes in controls and in trpv4-/- mice, we used 2D and 3D morphometrical approaches and a newly applied quantification algorithm based on fluorescence intensity changes in astrocytes during their volume changes. Astrocytes in brain slices from control and trpv4-/- mice were exposed either to hypotonic solution or to oxygen-glucose deprivation conditions. In contrast to previous in vitro studies, we found no contribution of TRPV4 channels to volume regulation in astrocytes in situ in adult mice. On the other hand, adult trpv4-/- mice exposed to middle cerebral artery occlusion showed twice as much larger edema 1 day after the insult compared to controls, which was quantified by MRI method. These data suggest that TRPV4 channels are not involved in astrocyte volume regulation in situ, however, they substantially contribute to ischemia-induced brain edema formation.
Pivoňková, Helena, Heřmanová, Zuzana, Kirdajová, Denisa, Awadová, Thuraya, Malínský, Jan, Valihrach, Lukáš, Žucha, Daniel, Kubista, Mikael, Galisová, A., Jirák, D., Anděrová, Miroslava. The Contribution of TRPV4 Channels to Astrocyte Volume Regulation and Brain Edema Formation. Neuroscience. 2018, 394(dec), 127-143. ISSN 0306-4522 doi: 10.1016/j.neuroscience.2018.10.028 (Contribution: P.H., H.Z., K.D. data acquisition and analysis, P.H., A.M., experimental design and manuscript writing, A.M. funding)

The role of AQP4 and TRPV4 channels in changes of the ECS diffusion parameters was evaluated by the RTI method. Measurements were performed in somatosensory cortex of mice with genetic deficiency of AQP4 (AQP4-/-) or TRPV4 (TRPV4-/-) channel on GFAP/EGFP background and their controls (wild type (wt)). The basal value of α in AQP4-/- mice was significantly higher than in wt or TRPV4-/- with no difference in λ. The results obtained in three different models of cell swelling (with increasing severity: hypotonic stress, oxygen-glucose deprivation, terminal ischemia) showed that AQP4 and TRPV4 complex plays the most important role in the severe pathologies, while in milder pathological conditions other mechanisms of water transport may contribute to cell swelling.
Chmelová, Martina, Suchá, Petra, Bochin, Marcel, Voříšek, Ivan, Pivoňková, Helena, Heřmanová, Zuzana, Anděrová, Miroslava, Vargová, Lýdia. The role of aquaporin-4 and transient receptor potential vaniloid isoform 4 channels in the development of cytotoxic edema and associated extracellular diffusion parameter changes. Eur J Neurosci. 2019 Jul;50(1):1685-1699. doi: 10.1111/ejn.14338. Epub 2019 Feb 8.

Contribution: CH.M. , S.P., B.M. and V.I. performed the diffusion parameter measurements and analyses, V.L. and A.M. design of experiments, manuscript writing, P.H. and H.Z. slice preparation, crossbreeding and mouse genotyping, A.M. and V.L. funding.

2. Glial cell pathophysiology, protective role of extracellular matrix and changes in brain diffusion in aging and degenerative disorders

We performed a comprehensive study focused on age-related changes in astrocyte functioning, predominantly on the ability of astrocytes to regulate their volume in response to a pathological stimulus, namely extracellular 50 mM K^+ concentration. The aim was to identify changes in the expression and function of transport proteins in the astrocytic membrane and properties of the extracellular space, triggered by aging. We used three-dimensional confocal morphometry, gene expression profiling, immunohistochemical analysis, and diffusion measurement in the hippocampal slices from 3-, 9-, 12-, and 18-month-old mice, in which astrocytes are visualized by enhanced green fluorescent protein under the control of the promoter for human glial fibrillary acidic protein (GFAP/EGFP mice). Combining a pharmacological approach and the quantification of astrocyte volume changes evoked by hyperkalemia, we found that marked diversity in the extent of astrocyte swelling in the hippocampus during aging is due to the gradually declining participation of Na^+-K^+-Cl^- transporters, glutamate transporters (glutamate aspartate transporter and glutamate transporter 1), and volume-regulated anion channels. Interestingly, there was a redistribution of Na^+-K^+-Cl^- cotransporter and glutamate transporters from astrocytic soma to processes. In addition, immunohistochemical analysis confirmed an age-dependent decrease in the content of Na^+-K^+-Cl^- cotransporter in astrocytes. The overall extracellular volume changes revealed a similar age-dependent diversity during hyperkalemia as observed in astrocytes. In addition, the recovery of the extracellular space was markedly impaired in aged animals.

Koleničová, Denisa, Turečková, Jana, Pukajová, Barbora, Harantová, Lenka, Kriška, Ján, Kirdajová, Denisa, Voříšek, Ivan, Kamenická, Monika, Valihrach, Lukáš, Androvič, Peter, Kubista, Mikael, Vargová, Lýdia, Anděrová, Miroslava. High potassium exposure reveals the altered ability of astrocytes to regulate their volume in the aged hippocampus of GFAP/EGFP mice. Neurobiology of Aging. 2019. ISSN 0197-4580 doi: 10.1016/j.neurobiolaging. 2019.10.009 ASEP (ID 520735)

Contribution: K.D., T.J., P.B., H.L., astrocytes volume measurements and data analyses, T.J. and V.I. immunohistochemistry and its quantification, K.D., K.J. and K.D. astrocyte isolation, FACS., K. M. and V.L. diffusion parameter measurrements, data analyses, K.M., A.P. and V.L. SC RT-qPCR, data analyses, A.M. design of experiments, A.M.,V.L., T.J. and K.D. manuscript writing.

The recent findings regarding the role/participation of glial cells in Alzheimer's disease progression, focusing on changes in glia morphology and functions, and alterations in gene expression profiles and protein levels during the development of AD were summarized in an review. As for astrocytes and microglia, they are fundamental for the progression and outcome of AD either because they function as effector cells releasing cytokines that play a role in both neuroprotection and neurodegeneration, or because they fail to fulfill their homeostatic functions, such as glutamate uptake/glutamine release and glucose uptake/lactate release, ultimately leaving neurons to face excitotoxicity and oxidative stress

Džamba, Dávid, Harantová, Lenka, Butenko, Olena, Anděrová, Miroslava. Glial Cells - The Key Elements of Alzheimer's Disease. Current Alzheimer Research. 2016, 13(8), 894-911. ISSN 1567-2050 doi: 10.2174/1567205013666160129095924
Contribution: all authors contributed equally in writing, A.M. supervision.

Huntington's disease (HD) is an inherited neurodegenerative disorder with progressive impairment of motor, behavioral and cognitive functions. The clinical features of HD are closely related to the degeneration of the basal ganglia, predominantly the striatum. The main striatal output structure, the globus pallidus, strongly accumulates metalloprotein-bound iron, which was recently shown to influence the diffusion tensor scalar values. To test the hypothesis that this effect dominates in the iron-rich basal ganglia of HD patients, we examined the globus pallidus by measuring of magnetic resonance parameters T2 relaxation rate, fractional anisotropy and mean diffusivity. Our results indicate that especially magnetic resonance fractional anisotropy measurements in the globus pallidus of HD patients may be strongly affected by metalloprotein-bound iron accumulation. However, no correlation between MR and clinical parameters was found.
Syka, Michael, Keller, J., Klempíř, J., Rulseh, A. M., Roth, J., Jech, R., Voříšek, Ivan, Vymazal, J. Correlation between Relaxometry and Diffusion Tensor Imaging in the Globus Pallidus of Huntington's Disease Patients. PLoS ONE.2015, 10(3), e0118907. E-ISSN 1932-6203 doi: 10.1371/journal.pone.0118907 (Contribution of IEM: measurement and analysis of MR data (S. M., V.I.), manuscript writing (S.M., V. I.))

We further evaluated HD-related changes in an experimental R6/2 mouse model model (HD), comparing the values of the apparent diffusion coefficient of water (ADC_W) acquired by DW-MR and values of α and λ, measured by the real-time iontophoretic method (RTI). In HD animals, we detected an increase in ADC_W in all axes and larger α than in control mice. No significant difference between control and HD mice was found in the values of tortuosity and diffusion anisotropy was unaffected in HD. Compared to control, we found a weaker expression of the extracellular matrix (ECM), a decrease in neuron numbers, and astrogliosis-like changes in the morphology of astrocytic processes in HD. We conclude that in the R6/2 model of HD, a decrease in the number of neurons results in increased ADCW and α values. Values of λ were not changed as the increase of diffusion obstacles was compensated for by the extracellular matrix reduction.
Voříšek, Ivan, Syka, Michael, Vargová, Lýdia. Brain Diffusivity and Structural Changes in the R6/2 Mouse Model of Huntington Disease. Journal of Neuroscience Research. 2017, 95(7), 1474-1484. ISSN 0360-4012 doi: 10.1002/jnr.23965
Contribution: measurement and analysis of MR data (S. M., V.I.), manuscript writing (S.M., V. I.)

Protective role of extracellular matrix in aging was studied in Bral2 deficient mice in two studies. Bral2/Hapln4 is a link protein stabilizing the binding between lecticans and hyaluronan in perineuronal nets (PNNs) and axonal coats in specific brain regions. Using the real-time iontophoretic method (RTI) and diffusion-weighted magnetic resonance (DW-MRI), we determined the extracellular space (ECS) volume fraction (α), tortuosity (λ), and apparent diffusion coefficient of water (ADC_W) of young adult and aged Bral2-deficient (KO) mice and age-matched wild-type (wt) controls in the thalamic ventral posteromedial nucleus (VPM) and sensorimotor cortex (the first study) and in two acustic nuclei: 1. the inferior colliculus (IC) and 2. the medial nucleus of

trapezoid body (MNTB; the second study) . The results were correlated with an analysis of extracellular matrix composition. The cortex and IC, where Bral2 is not expressed, served as negative controls. In both studies, we observed typical age-related changes in VPM or MNTB only in KO animals, while changes in aged wt animals remained insignificant. Immunohistochemical analysis in aged wt animals showed a shift in the ECM composition in favor of Bral2/brevican-based PNNs. In KO mice, PNNs were not disrupted completely, as the role of Bral2 compensate the other link protein Crtl1. Our data suggest that aging is a critical point that reveals the effect of Bral2 deficiency on VPM diffusion and that a protective ability of Crtl1/brevican-based PNNs may be altered, which results in the more pronounced age-related changes in the aged KO mice.
Cicanič, Michal, Edamatsu, Midori, Bekku, Yoko, Voříšek, Ivan, Oohashi, Toshitaka, Vargová, Lýdia. A deficiency of the link protein Bral2 affects the size of the extracellular space in the thalamus of aged mice. Journal of Neuroscience Research. 2018, 96(2), 313-327. ISSN 0360-4012 doi: 10.1002/jnr.24136Aug 16. (Contribution: C. M., V.I., V.L.: diffusion measurements, data analysis, manuscript writing, V.L. project design and funding)

Suchá, Petra, Chmelová, Martina, Kamenická, Monika, Bochin, Marcel, Oohashi, Toshitaka, Vargová Lýdia. The Effect of Hapln4 Link Protein Deficiency on Extracellular Space Diffusion Parameters and Perineuronal Nets in the Auditory System During Aging. Neurochem Res. 2020 Jan;45(1):68-82. doi: 10.1007/s11064-019-02894-2. Epub 2019 Oct 29. (Contribution: S.P., Ch.M., K.M., B.M., V.L.: data measuremen and analysis, manuscript writing, V.L. project design and funding)

3. Wnt signaling
The canonical Wnt signaling pathway also plays an important role in the establishment of neurogenic niches. Using transgenic mouse models, we examined the impact of Wnt signaling activation or inhibition on proliferation or differentiation of NS/PC. Within differentiated cells, we identified three electrophysiologically and immunocytochemically distinct cell types, whose incidence was markedly affected by the Wnt signaling output. Activation of this pathway suppressed gliogenesis, and promoted neurogenesis while its inhibition via Dkk1 led to suppressed neurogenesis and increased counts of glial phenotype. Wnt signaling hyperactivation resulted in an increased incidence of cells expressing outwardly rectifying K+ currents, together with inwardly rectifying Na+ currents, a typical current pattern of immature neurons. We showed that the Wnt signaling pathway orchestrates neonatal NS/PCs differentiation towards cells with neuronal characteristics, which might be important for nervous tissue regeneration during central nervous system disorders.
Kriška, Ján, Honsa, Pavel, Džamba, Dávid, Butenko, Olena, Koleničová, Denisa, Janečková, Lucie, Nahácka, Zuzana, Anděra, Ladislav, Kozmík, Zbyněk, Taketo, Makoto Mark, Kořínek, Vladimír, Anděrová, Miroslava. Manipulating Wnt signaling at different subcellular levels affects the fate of neonatal neural stem/progenitor cells. Brain research. 2016, 1641(nov.), 73-87. ISSN 1872-6240 doi: 10.1016/j.brainres.2016.09.026 (Contribution: K.J., H.P., D. D., B. O., K. D. data acquisition and analysis, manuscript preparation, A.M. experimental design, data analysis, manuscript writing funding)

The Wnt pathway plays also a crucial role in self-renewal and differentiation of cells in the adult gut. In the present study, we revealed the functional consequences of

inhibition of canonical Wnt signaling in the intestinal epithelium. The study was based on generation of a novel transgenic mouse strain enabling inducible expression of an N-terminally truncated variant of nuclear Wnt effector T cell factor 4 (TCF4). The TCF4 variant acting as a dominant negative (dn) version of wild-type (wt) TCF4 protein decreased transcription of β-catenin-TCF4-responsive genes. Interestingly, suppression of Wnt/β-catenin signaling affected asymmetric division of intestinal stem cells (ISCs) rather than proliferation. ISCs expressing the transgene underwent several rounds of division but lost their clonogenic potential and migrated out of the crypt. Expression profiling of crypt cells revealed that besides ISC-specific markers, the dnTCF4 production downregulated expression levels of epithelial genes produced in other crypt cells including markers of Paneth cells. Additionally, in Apc conditional knockout mice, dnTCF activation efficiently suppressed growth of Apc-deficient tumors. In summary, the generated mouse strain represents a convenient tool to study cell-autonomous inhibition of β-catenin-Tcf-mediated transcription.
Janečková, Lucie, Fafílek, Bohumil, Krausová, Michaela, Horázná, Monika, Vojtěchová, Martina, Alberich-Jorda, Meritxell, Šloncová, Eva, Galušková, Kateřina, Sedláček, Radislav, Anděrová, Miroslava, Kořínek, Vladimír. Wnt signaling inhibition deprives small intestinal stem cells of clonogenic capacity. Genesis. 2016, 54(3), 101-114. ISSN 1526-954X doi: 10.1002/dvg.22922 (Contribution: A.M. data consulting and manuscript writing)

4. Methodological approaches to study glial cells

The tamoxifen-inducible Cre-loxP system is widely used to overcome gene targeting pre-adult lethality, to modify a specific cell population at desired time-points, and to visualize and trace cells in fate-mapping studies. In this study we focused on tamoxifen degradation kinetics and aimed to define the tamoxifen administration scheme, enabling the maximal recombination rate together with minimal animal mortality. We defined the optimal time window, allowing the complete degradation of tamoxifen and its metabolites, such as 4-hydroxytamoxifen, *N*-desmethyltamoxifen, endoxifen and norendoxifen, in the mouse brain after intraperitoneal tamoxifen injection. We determined the biological activity of these substances *in vitro,* as well as a minimal effective concentration of the most potent metabolite 4-hydroxytamoxifen causing recombination *in vivo.* For this purpose, we analyzed the recombination rate in double transgenic Cspg4-cre/Esr1/ROSA26Sortm14(CAG-tdTomato) mice, in which tamoxifen administration triggers the expression of red fluorescent protein in NG2-expressing cells, and employed a liquid chromatography, coupled with mass spectrometry, to determine the concentration of studied substances in the brain. We determined the degradation kinetics of these substances, and revealed that this process is influenced by mouse strains, age of animals, and dosage. Taken together, we showed that tamoxifen metabolism in mouse brains is age-,strain-and dose-dependent, and these factors should be taken into the account in the experimental design.
Valný, Martin, Honsa, Pavel, Kirdajová, Denisa, Kameník, Zdeněk, Anděrová, Miroslava. Tamoxifen in the Mouse Brain: Implications for Fate-Mapping Studies Using the Tamoxifen-Inducible Cre-loxP System. Frontiers in Cellular Neuroscience. 2016, 10(ost), 243. ISSN 1662-5102 doi: 10.3339/fncel.2016.00243. (Contribution: MA, MV and PH designed and conceptualized all the experiments. MV, PH and DK performed and analysed all the in vitro experiments, prepared all tissue samples and performed MCAo operations in this project. ZKperformed and analysed LC-MS experiments. MVand PH performed the overall analysis

of results from these experiments.MV,MA andPHwrote and edited the manuscript.

TRPV4 channels function as a Ca^{2+}-permeable, nonselective cationic ion channel and are activated by numerous stimuli, such as endogenous and exogenous small molecule ligands or temperature. Their activity is optimal at temperature higher than 27 °C, so in our experiments we have used temperature of 32°C, which resulted in much larger volume changes than those evoked at room temperature. Such large changes in EGFP-labeled astrocytes led to a decrease in fluorescence signal during their swelling, and consequently, also to loss of certain astrocytic structure. Since we used 3D-confocal morphometry, which relies on minimal fluorescence changes, the estimated volume changes in astrocytes exposed to hypoosmotic stress were markedly underestimated. Therefore, we compared three different approaches, which allow for estimations of the cell volume changes in biological samples containing individual fluorescently labeled cells either in culture or in the tissue context. Three- and two-dimensional morphometries assess the cell volume from the accurate localization of the border of fluorescently labeled cells. Consequently, their results strongly depend on the cell shape complexity and image sampling. In contrast, in fluorescence intensity-based measurement, the relative cell volume is calculated just in terms of reciprocal fluorescence intensity of the label. Therefore, the yield of such measurement depends rather on mobility of the used fluorescent marker and does not require any determination of the cell border. Volume changes induced by variations in the extracellular osmolality in murine fibroblasts and astrocytes either in the culture or in the acute brain slices were registered by the three approaches. Specific requirements, limitations and advantages are discussed.
Awadová, Thuraya, Pivoňková, Helena, Heřmanová, Zuzana, Kirdajová, Denisa, Anděrová, Miroslava, Malínský, Jan. Cell volume changes as revealed by fluorescence microscopy: Global vs local approaches. Journal of Neuroscience Methods. 2018, 306(aug), 38-44. ISSN 0165-0270 doi: 10.1016/j.jneumeth.2018.05.026 (Contribution: P.H., H. Z., K.D. data acquisition and analysis, A.M. experimental design, data analysis, manuscript writing)

Reverse transcription quantitative PCR (RT-qPCR) is already an established tool for mRNA detection and quantification. Since recently, this technique has been successfully employed for gene expression analyses, and also in individual cells (single cell RT-qPCR). Although the advantages of single cell measurements have been proven several times, a study correlating the expression measured on single cells, and in bulk samples consisting of a large number of cells, has been missing. Here, we collected a large data set to explore the relation between gene expression measured in single cells and in bulk samples, reflected by qPCR Cq values. We measured the expression of 95 genes in 12 bulk samples, each containing thousands of astrocytes, and also in 693 individual astrocytes. Combining the data, we described the relation between Cq values measured in bulk samples with either the percentage of the single cells that express the given genes, or the average expression of the genes across the single cells. We show that data obtained with single cell RT-qPCR are fully consistent with measurements in bulk samples. Our results further provide a base for quality control in single cell expression profiling, and bring new insights into the biological process of cellular expression.
Džamba, Dávid, Valihrach, Lukáš, Kubista, Mikael, Anděrová, Miroslava. The correlation between expression profiles measured in single cells and in traditional bulk samples. Scientific Reports. 2016, 6(nov), 37022. ISSN 2045-2322 doi:

10.1038/srep37022 (Contribution: D.D. data acquisition and analysis, A.M., manuscript writing, funding)

5. Membrane properties of stem cells

We analyzed calcium signals and membrane properties in rat adipose-derived stromal cells (ADSCs) and bone marrow stromal cells (BMSCs) in basal conditions, and then following a switch into medium that contains factors known to modify their character. Modified ADSCs (mADSCs) expressed L-type calcium channels whereas both L- and P/Q- channels were operational in mBMSCs. Both mADSCs and mBMSCs possessed functional endoplasmic reticulum calcium stores, expressed ryanodine receptor-1 and -3, and exhibited spontaneous oscillations in intracellular calcium concentration. Electrophysiological data revealed that passive ion currents dominated the membrane conductance in ADSCs and BMSCs. Medium modification led to a significant shift in the reversal potential of passive currents from -40 to -50mV in cells in basal to -80mV in modified cells. Hence membrane conductance was mediated by non-selective channels in cells in basal conditions, whereas in modified medium conditions, it was associated with K^+-selective channels. Our results indicate that modification of ADSCs and BMSCs by alteration in medium formulation is associated with significant changes in their Ca^{2+} signaling and membrane properties.

Forostyak, Oksana, Butenko, Olena, Anděrová, Miroslava, Forostyak, Serhiy, Syková, Eva, Verkhratsky, Alexander., Dayanithi, Govindan. Specific profiles of ion channels and ionotropic receptors define adipose- and bone marrow derived stromal cells. Stem Cell Research. 2016, 16(3), 622-634. ISSN 1873-5061 doi: 10.1016/j.scr.2016.03.010 (Contribution: B.O. – electrophysiological measurements and data analysis, A.M. data analysis, manuscript writing)

Research activity and characterisation of the main scientific results

The evaluation period for our department should be divided into two parts. Firstly, in years 2015-2017 the department was called Teratology and was led by Miroslav Peterka. At the beginning of 2018, a new PI Ondřej Machoň was selected. XXX.

Besides that new projects on craniofacial and neural development were imported to the laboratory as Ondřej Machoň moved from the Institute of Molecular Genetics to the Inst. of Experimental Medicine. Moving new transgenic mouse strains, methodology and expertise took almost one year. Since the late autumn 2019, new projects could be initiated and performed in reasonable pace.

Publication 1: Chodelková, O., Mašek, J., Kořínek, Z., Kozmik, Z., Machoň, Ondřej. Tcf7L2 is essential for neurogenesis in the developing mouse neocortex. Neural Development. 2018, 13 may 11, 8. ISSN 1749-8104 doi: 10.1186/s13064-018-0107-8

In the transition period of moving to DDB IEM, we characterized the role of transcription factors Tcf7L1 and Tcf7L2 during **embryonic development of the telencephalon** that gives rise to the frontal cortex and hippocampus. We generated double conditional mutants for Tcf7L1 and Tcf7L2 genes, crucial mediators of canonical Wnt signalling. For tissue specific deletion in the dorsal telencephalon, transgenic mouse strain D6-Cre was employed. Conditional knock-out analysis showed that Tcf7L2, but not Tcf7L1, is indispensable for corticogenesis and hippocampus formation. Tcf7L2 is the the principal Wnt mediator important for maintenance of progenitor cell identity in the ventricular zone. In the absence of Tcf7L2, the Wnt activity is reduced, ventricular zone markers Pax6 and Sox2 are downregulated and the neuroepithelial structure is severed due to the loss of apical adherens junctions. This resulted in decreased proliferation of radial glial cells, the reduced number of intermediate progenitors in the subventricular zone and hypoplastic forebrain. Our data showed that canonical Wnt signalling, which is essential for determining the neuroepithelial character of the neocortical ventricular zone, is mediated by Tcf7L2. 3/5 authors were from DDB IEM including the first and corresponding authors. These are underlined.

In autumn 2018, one PhD student Jaroslav Fábik and two MSc. Students Petr Nickl and Veronika Brežinová were hired. Jaroslav Fábik studies the role of transcription factors Meis1 and Meis2 during **development of neural crest cells**. He uses Wnt1Cre2 driver to inactivate Meis factors in the neural crest. This project follows on the previous work by Ondřej Machoň et al. Meis2 is essential for cranial and cardiac neural crest development. BMC Dev Biol. 2015 Nov 6;15:40. doi: 10.1186/s12861-015-0093-6. This work was done at the former Institute of Molecular Genetics. At present, Jaroslav Fábik completed a manuscript and submitted. It will be published during 2020.

MSc students P. Nickl and V. Březinová established **zebrafish model** in DDB and gene knock-out technology CRISPR/Cas9. Zebrafish knock-out strains for genes drMeis1a, drMeis1b, drMeis2a and drMeis2b were generated and we analyze the phenotype in these mutant fish lines with a special attention to craniofacial and heart development. The results are not published yet. P. Nickl graduated in July 2019 and his project was taken over by a new PhD student Viktorie Psutková in autumn 2019.

Projects on **tooth development** are supervised and managed by Mária Hovořáková. She supervised two MSc students and three PhD students in the period 2015-2019, including those from the former Teratology dept.

Publication 2: Lochovska K, Peterkova R, Pavlikova Z, Hovorakova M. Sprouty gene dosage influences temporal-spatial dynamics of primary enamel knot formation. BMC Dev Biol. 2015 Apr 22;15:21. doi: 10.1186/s12861-015-0070-0.

Mice lacking Sprouty genes develop supernumerary tooth in front of the lower M1 (first molar) primordium during embryogenesis. We focused on temporal-spatial dynamics of Sonic Hedgehog expression as a marker of early odontogenesis during supernumerary tooth development. Using mouse embryos with different dosages of Spry2 and Spry4 genes, we showed that during the normal development of M1 in the mandible the sooner appearing Shh signaling domain of the R2 bud transiently coexisted with the later appearing Shh expression domain in the early M1 primordium. Both domains subsequently fused together to form the typical signaling center representing primary enamel knot (pEK) of M1 germ at embryonic day (E) 14.5. However, in embryos with lower Spry2;Spry4 gene dosages, we observed a non-fusion of original R2 and M1 Shh signaling domains with consequent formation of a supernumerary tooth primordium from the isolated R2 bud.Our results bring new insight to the development of the first lower molar of mouse embryos and define simple tooth unit capable of individual development, as well as determine its influence on normal and abnormal development of the tooth row which reflect evolutionarily conserved tooth pattern. Our findings contribute significantly to existing knowledge about supernumerary tooth formation. 4/4 authors are from DDB (former Teratology Dept.)

Publication 3. Hovořáková, Mária, Lochovská, Kateřina, Zahradníček, Oldřich, Domonkosová Tibenská, K., Dornhoferová, M., Horáková Smrčková, Lucie, Bodoriková, S. One OdontogenicCell-Population Contributes to the Development of the Mouse Incisors and of the Oral Vestibule. PLoS ONE. 2016, 11(9), e0162523. E-ISSN 1932-6203 doi: 10.1371/journal.pone.0162523

The area of the oral vestibule is often a place where pathologies appear such as peripheral odontomas. In the present study, we traced a cell population expressing Sonic hedgehog (Shh) from the beginning of tooth development using Cre-LoxP system in the lower jaw. We focused on Shh expression in the area of the early appearing rudimentary incisor germs located anteriorly to the prospective incisors. The localization of the labelled cells in the incisor germs and also in the inner epithelial layer of the vestibular anlage showed that the first very early developmental events in the lower incisor area are common to the vestibulum oris and the prospective incisor primordia in mice. The location of labelled descendant cells with the early appearing Shh expression domain related to the rudimentary incisor anlage not only in the rudimentary and functional incisor germs but also in the externally located anlage of the oral vestibule. This documents the odontogenic potential of the vestibular epithelium and reveals a potential cause of odontomas in the vestibular area. 4/7 authors are from DDB (former Teratology Dept.) including the frist author.

Publication 4. Sadier, A., Twarogowska, M., Steklíková, Klára, Hayden, L., Lambert, A., Schneider, P., Laudet, V., Hovořáková, Mária, Calvez, V., Pantalacci, S. Modeling

Edar expression reveals the hidden dynamics of tooth signaling center patterning. PLoS Biology. 2019, 17(2), e3000064. E-ISSN 1545-7885 doi: 10.1371/journal.pbio.3000064

When patterns are set during embryogenesis, it is expected that they are straightly established rather than subsequently modified. The patterning of the three mouse molars is, however, far from straight, likely as a result of mouse evolutionary history. The first-formed tooth signalling centers, called MS and R2, disappear before driving tooth formation and are thought to be vestiges of the premolars found in mouse ancestors. Moreover, the mature signalling center of the first molar (M1) is formed from the fusion of two signaling centers (R2 and early M1). Here, we report that broad activation of Edar expression precedes its spatial restriction to tooth signaling centers. This reveals a hidden two-step patterning process for tooth signaling centers, which was modeled with a single activator-inhibitor pair subject to reaction-diffusion (RD). The study of Edar expression also unveiled successive phases of signaling center formation, erasing, recovering, and fusion. Our model, in which R2 signaling center is not intrinsically defective but erased by the broad activation preceding M1 signaling center formation, predicted the surprising rescue of R2 in Edar mutant mice, where activation is reduced. The importance of this R2-M1 interaction was confirmed by ex vivo cultures showing that R2 is capable of forming a tooth. Finally, by introducing chemotaxis as a secondary process to RD, we recapitulated in silico different conditions in which R2 and M1 centers fuse or not. In conclusion, pattern formation in the mouse molar field relies on basic mechanisms whose dynamics produce embryonic patterns that are plastic objects rather than fixed end points. 2/10 authors are from DDB. PhD student Klára Steklíková performed the experiment shown in Fig. 5. Tooth primordium organ culture was maintained in vitro for 6 days and signalling centers were flowed using Shh-GFP transgenic mouse reporter. Centers R2 and M1 were manually separated and resulted in growth of an extra molar.

Paper 5. Newton, P.T., Li, L., Zhou, B.Y., Schweingruber, C., Hovořáková, Mária, Xie, M., Sun, X.Y., Sandhow, L., Artemov, A.V., Ivashkin, E., Suter, S., Dyachuk, V., El Shahawy, M., Gritli-Linde, A., Bouderlique, T., Petersen, J., Mollbrink, A., Lundeberg, J., Enikolopov, G., Qian, H., Fried, K., Kasper, M., Hedlund, E., Adameyko, I., Sävendahl, L., Chagin, A.S. A radical switch in clonality reveals a stem cell niche in the epiphyseal growth plate. Nature. 2019, 567(7747), 234-238. ISSN 0028-0836 doi: 10.1038/s41586-019-0989-6

Longitudinal bone growth in children is sustained by growth plates, narrow discs of cartilage that provide a continuous supply of chondrocytes for endochondral ossification[1]. However, it remains unknown how this supply is maintained throughout childhood growth. Chondroprogenitors in the resting zone are thought to be gradually consumed as they supply cells for longitudinal growth[1,2], but this model has never been proved. Here, using clonal genetic tracing with multicolour reporters and functional perturbations, we demonstrate that longitudinal growth during the fetal and neonatal periods involves depletion of chondroprogenitors, whereas later in life, coinciding with the formation of the secondary ossification centre, chondroprogenitors acquire the capacity for self-renewal, resulting in the formation of large, stable monoclonal columns of chondrocytes. Simultaneously, chondroprogenitors begin to express stem cell markers and undergo symmetric cell division. Regulation of the pool of self-renewing progenitors involves the hedgehog and mammalian target of rapamycin complex 1

(mTORC1) signalling pathways. Our findings indicate that a stem cell niche develops postnatally in the epiphyseal growth plate, which provides a continuous supply of chondrocytes over a prolonged period. Only 1/27 is from DDB. M. Hovořáková provided long bone material from postnatal stages of Shh-GFP reporter mouse strain and participated in FACS analysis of bone marrow and endosteal region.

Research activity and characterization of the main scientific results

Team 5 is being evaluated for the first time. It has been established in 2006 as a service unit consisting of one scientist (J. Malinsky) and a technician in order to take care of the shared microscopic systems at IEM and to provide an expert methodological support for other research teams. Besides these activities, J. Malinsky was allowed to apply for his own projects and together with his students hired later on, he was able to continuously publish competitive results in established peer-reviewed journals. During organizational changes at IEM in fall 2016, the Microscopy unit become one of the research teams, keeping however its service duties. In November 2019, the Department of Microscopy was renamed to Department of Functional Organization of Biomembranes to better describe the scientific focus of the J. Malinsky group.

During the evaluated period, research activities of Team 5 reflected the two main topics in the team scope:

- microdomain structure of the yeast plasma membrane and its engagement in the cell metabolism and pathogenicity regulation (results published in 5 original papers and 3 invited reviews)
- role of membrane microdomains in the regulation of phosphatidylglycerol (PG) distribution within the cellular architecture, with the emphasis on induced respiration deficiency (3 original papers)

Besides this, our continued methodological support provided to other teams resulted in developing new methods of quantitative analysis of the fluorescence microscopy data (3 original papers). The main scientific results are described below.

Eukaryotic mRNA decay is regulated at the plasma membrane

We discovered a novel mechanism of the regulation of mRNA degradation. We found that a widely evolutionarily conserved 5'-3' exoribonuclease Xrn1, the key enzyme of eukaryotic mRNA decay, is sequestered from the rest of the mRNA decay machinery in yeast under conditions of chronic lack of fermentable carbon sources. Quantitative PCR analysis revealed that this sequestration reversibly inactivates Xrn1, which could be beneficiary upon nutrient emergence.

Our data documented that the described modulation of Xrn1 exoribonuclease activity takes place at a specialized lateral microdomain of the plasma membrane (MCC/eisosome). In cells lacking the microdomain, we detected mRNA decay misregulation. Two aspects of the observed phenomenon could be of particular importance: i) clear stratification of the yeast colony in terms of Xrn1 localization/activity could be detected, suggesting the functional significance of this regulatory step in multicellular organisms; ii) similar tendency of Xrn1 homologue to form local accumulations upon nutritional stimuli has been observed by others in rat hippocampal neurons, suggesting wide conservation of the described mechanism among eukaryotes from yeast to mammals.

Grousl T, ***Opekarová M, Stradalova V****, Hasek J,* ***Malinsky J****. Evolutionarily Conserved 5'-3' Exoribonuclease Xrn1 Accumulates at Plasma Membrane-Associated Eisosomes in Post-Diauxic Yeast. PLOS ONE 10(3):e0122770. (2015).* (A study generated in the cooperation of Team 5 and the group of J. Hasek at the Institute of

Microbiology CAS. 3/5 authors, including the corresponding one, are Team 5 members. All the fluorescence microscopy data were generated at IEM by Team 5.)

Vaskovicova K, Awadova T, Vesela P, *Balazova M,* ***Opekarová M, Malinsky J****. mRNA decay is regulated via sequestration of the conserved 5'-3' exoribonuclease Xrn1 at eisosome in yeast. Eur J Cell Biol 96(6):591-599 (2017).* (A study primarily generated by Team 5 - 5/6 authors, including the first and the corresponding one, are Team 5 members. The idea, experimental design and manuscript preparation have been delivered by the Team 5. Slovak co-author contributed by the quantitative PCR analysis.)

Lipid droplet protein controls the subcellular distribution of phosphatidylglycerol

In a cooperation with M. Balazova group from the Institute of Animal Biochemistry and Genetics (Centre of Biosciences, Slovak Academy of Sciences) we described a new yeast model of Barth syndrome, a serious hereditary mitochondrial disorder characterized by the defect in cardiolipin remodelation. Similar to human patients, the newly described model not only fails in this last step of cardiolipin biosynthesis, but also accumulates excess phosphatidylglycerol (PG). We showed that elevated PG levels are responsible for specific aberrations in mitochondrial morphology and function.

Using a combination of fluorescence microscopy approaches, we were able to uncover the mechanism by which the levels of PG, anionic phospholipid synthesized in the inner mitochondrial membrane, are kept low in other membranes within the cellular architecture. We showed that this is achieved through the action of Pgc1, a specific phospholipase localized at the surface of lipid droplets (LD). We showed that while LD store the vast majority of the enzyme, Pgc1 activity takes place mainly at the endoplasmic reticulum (ER), where also a massive Pgc1 degradation occurs, however. Rapid Pgc1 exchange between LD and ER revealed by FRAP analysis indicated a high dynamics of this PG regulation. Described regulatory mechanism represents another example of a cellular regulation, in which spatial sequestration of an inactivated key enzyme at a specialized membrane microdomain (in this case, the ER-connected LD surface) plays a primordial role.

Pokorna L, Cermakova P, Horvath A, Baile MG, Claypool SM, Griac P, ***Malinsky J****, Balazova M. Specific degradation of phosphatidylglycerol is necessary for proper mitochondrial morphology and function. Biochim Biophys Acta (Bioenergetics) 1857(1):34-45 (2016).* (Only one of eight authors of this paper is a Team 5 member. However, the team contribution to this paper was fundamental, as all the fluorescence microscopy analyses, including the GFP tagging of the protein of interest, fluorescence imaging and quantitative analyses were performed by Team 5.)

Kubalová D, Káňovičová P, ***Veselá P, Awadová T****, Džugasová V, Daum G,* ***Malínský J,*** *Balážová M. The lipid droplet protein Pgc1 controls the subcellular distribution of phosphatidylglycerol. FEMS Yeast Res. 9(5): pii: foz045. doi: 10.1093/femsyr/foz045 (2019).* (Collaborative study of four groups - besides Team 5, two Slovak groups (Inst. Anim. Biochem. and Genetics, Slovak Academy of Sciences, and Commenius University, Bratislava) and one Austrian group (Graz Univ. of Technology) were involved. 3/8 authors are Team 5 members, which reflects a crucial importance of fluorescence microscopy analyses of Pgc1 localization and dynamics performed by

Team 5. Based on these we were able to draw a new model of Pgc1 action in the cellular architecture.)

Membrane potential governs lateral distribution of membrane components

In 2014, in a cooperation with prof. Herman group from the Faculty of Mathematics and Physics, Charles University (Prague), we documented the existence of sphingolipid-enriched, highly ordered, gel-like microdomains in the plasma membrane of living cells. In a subsequent study, using time-resolved fluorescence spectroscopy we showed that the abundance of these sphingolipid-enriched microdomains strongly depends on the actual membrane potential. Independent on the mechanism of plasma membrane depolarization, the amount of highly ordered membrane areas was drastically reduced with the loss of transmembrane voltage.

We suggested that this potential-induced reorganization of the lateral plasma membrane structure could significantly contribute to maintaining membrane integrity during fast response to stress, pathogen attack and other challenges involving partial depolarization of the plasma membrane. It could also be responsible for lower susceptibility to detergents and lower sensitivity to antibiotics or antimycotics treatment of cells (incl. bacteria) lacking energy.

*Vecer J, **Vesela P**, **Malinsky J**, Herman P. Sphingolipid levels crucially modulate lateral microdomain organization of plasma membrane in living yeast. FEBS Lett. 588(3):443-9. doi: 10.1016/j.febslet.2013.11.038. (2014).* (An earlier study – not a subject of evaluation.)

*Herman P, Vecer J, **Opekarova M, Vesela P**, Jancikova I, **Zahumensky J, Malinsky J**. Depolarization affects the lateral microdomain structure of yeast plasma membrane. FEBS J. 282(3):419-34 (2015).* (Collaborative study of two groups: Team 5 and the group from the Faculty of Mathematics and Physics, Charles University (Prague, CZ). 4/7 authors, including the corresponding author, are Team 5 members. The original idea came from Team 5. Both groups conceived, designed and performed the experiments.)

***Malinsky J**, Tanner W, **Opekarova M**. Transmembrane voltage: potential to induce lateral microdomains. Biochim Biophys Acta 1861:806-11 (2016).* (A study primarily generated by Team 5 - 2/3 authors, including the first and the corresponding one.)

Research activity and characterisation of the main scientific results

Here we demonstrate selected major scientific results of the team 6, with major contribution of the members of the team 6 as demonstrated by prevailing authorship in papers resulted from this research:

Important results in 2019

1. **Benzo[a]pyrene is associated with dysregulated myelo-lymphoid hematopoiesis in asthmatic children**

Exposure to B[a]P might contribute to concurrent suppression of pro-inflammatory (e.g. NF-κB mediated Natural Killer T cells), and activation of anti-inflammatory pathways (e.g. IL10-secreting CD8+ T cells) in the urban asthmatic children. In addition, B[a]P appears to elevate heme biosynthesis, which in turn, promotes neutrophilic metamyelocyte expansion and reduction of CD71+ erythroid cells.
Choi, H., Song, WM., Wang, M., Dostal, M., Pastorkova, A., Libalova, H., Tulupova, E., Rossnerova, A., Rossner, P. Jr., Sram, RJ., Zhang, B. (2019): Benzo[a]pyrene is associated with dysregulated myelo-lymphoid hematopoiesis in asthmatic children. Environ Int. 128: 218-232. doi: 10.1016/j.envint.2019.04.052. IF=7.943

2. **The biological effects of complete gasoline engine emissions exposure in a 3D human airway model (MucilAirTM) and in human bronchial epithelial cells (BEAS-2B)**

The aim of this study was real-time exposure of complete combustion emissions in two cell models. The models used were the BEAS-2B lung epithelial cell line and the MucilAirTM 3D lung model. To allow direct contact of combustion emissions and cells, both models were cultured on porous membranes. A unique exposure system has been created to allow real time direct expose to diluted exhaust gases. One-day and five-day repeated exposure showed higher tolerance and more realistic results in complex 3D models than commonly used lung cells.

Rossner, P., Jr., Cervena, T., Vojtisek-Lom, M., Vrbova, K., Ambroz, A., Novakova, Z., Elzeinova, F., Margaryan, H., Beranek, V., Pechout, M., Macoun, D., Klema, J., Rossnerova, A., Ciganek, M., Topinka, J.: (2019) The Biological Effects of Complete Gasoline Engine Emissions Exposure in a 3D Human Airway Model (MucilAirTM) and in Human Bronchial Epithelial Cells (BEAS-2B). International Journal of Molecular Sciences. 20(22). pii: E5710. doi: 10.3390/ijms20225710. IF=4.331

3. **Molecular responses in THP-1 macrophage-like cells exposed to diverse nanoparticles**

Nanomaterials (NM), as foreign structures, are recognized by immune cells, especially by macrophages. In this study, we compared the effects of diverse NM (TiO2, ZnO, SiO2, Ag) on THP-1 macrophage-like cells. While no significant effects were observed in cells exposed to TiO2, the other NM induced production of proinflammatory molecules, with Ag exhibiting also immunosupressive effects. The obtained results contribute to the understanding of the molecular mechanisms of NM toxicity, emphasing the role of immune cells and differences among various types of NM.

Brzicova, T., Javorkova, E., Vrbova, K., Zajicova, A., Holan, V., Pinkas, D., Philimonenko, V., Sikorova, J., Klema, J., Topinka, J., Rossner, P., Jr.: (2019) Molecular Responses in THP-1 Macrophage-Like Cells Exposed to Diverse Nanoparticles. Nanomaterials (Basel). 9(5). pii: E687. doi: 10.3390/nano9050687. IF=3.811

Important results in 2018

1. Transcriptional response to organic compounds from diverse gasoline and biogasoline fuel emissions in human lung cells

We collected and characterized gasoline exhaust particles (GEPs) produced by Gasoline Direct Injection (GDI) engine running neat gasoline fuel (E0) and its blends with 15% ethanol (E15), 25% n-butanol (n- But25) and 25% isobutanol (i-But25). To study the toxic effects of organic compounds extracted from GEPs, we analyzed gene expression profiles in human lung BEAS-2B cells. Our results indicate that i-But25 extract possessed the weakest genotoxic potency possibly due to the low PAH content.

Libalova, H., Rossner, P. Jr., Vrbova, K., Brzicova, T., Sikorova, J., Vojtisek-Lom, M., Beranek, V., Klema, J., Ciganek, M., Machala, M., Topinka, J.: (2018) Transcriptional response to organic compounds from diverse gasoline and biogasoline fuel emissions in human lung cells. Toxicology in Vitro. 48: 329-341. doi: 10.1016/j.tiv.2018.02.002. IF=3.067

2. Gene expression profiling in healthy newborns from diverse localities of the Czech Republic

We analyzed whole genome expression in cord blood leukocytes of 202 newborns from the Czech Republic, differing localities and levels of air pollution. We aimed to identify differentially expressed genes (DEGs) and pathways in relation to locality. The highest number of DEGs was found in samples from Karvina. A pathway analysis revealed a deregulation of processes associated with cell growth, apoptosis or cellular homeostasis, immune response-related processes or oxidative stress response.

Honkova, K., Rossnerova, A., Pavlikova, J., Svecova, V., Klema, J., Topinka, J., Milcova, A., Libalova, H., Choi, H., Veleminsky, M., Sram, R.J., Rossner, P. Jr.: (2018) Gene Expression Profiling in Healthy Newborns from Diverse Localities of the Czech Republic. Environmental and Molecular Mutagenesis.59: 401-415; doi: 10.1002/em.22184. IF=2.528

3. Inhalation of ZnO nanoparticles: splice junction expression and alternative splicing in mice

Biological effects of ZnO nanoparticles inhalation were investigated in mice. The inhalation affected splice junctions expression in genes participating in oxidative stress, apoptosis, immune response, inflammation and DNA repair. Further, alternative splicing in oxidative stress and inflammation-related genes was affected. In summary, ZnO nanoparticles have a potential negative effect on biological systems.

Rössner, Jr.P., Vrbová, K., Strapáčová, S., Rössnerová, A., Ambrož, A., Brzicová, T., Libalová, H., Javorková, E., Kulich, P., Vecera, Z., Mikuska, P., Coufalik, P., Krumal, K., Capka, L., Docekal, B., Moravec, P., Sery, O., Misek, I., Fictum, P., Fiser, K.,

Machala, M., and Topinka, J.: (2018) Inhalation of ZnO nanoparticles: splice junction expression and alternative splicing in mice. Toxicological Sciences. 168(1): 190-200. doi: 10.1093/toxsci/kfy288. IF=3.654

Important results in 2017

1. Ultrafine and Fine Particles and Hospital Admissions in Central Europe. Results from the UFIREG Study

We investigated effects of ultrafine and fine particulate matter on cause-specific hospital admissions in Central and Eastern European cities. We found that a 2,750 particles/cm^3 increase of ultrafine particles caused an increase in the risk of respiratory hospital admissions. We also found increases in the risk of cardiovascular and respiratory admissions associated with exposure to particulate matter of aerodynamic diameter < 2.5 μm.

Lanzinger S, Schneider A, Breitner S, Stafoggia M, Erzen I, Dostal M, Pastorkova A, Bastian S, Cyrys J, Zscheppang A, Kolodnitska T, Peters A; UFIREG study group: (2016) Ultrafine and Fine Particles and Hospital Admissions in Central Europe. Results from the UFIREG Study. *Am J Respir Crit Care Med.*194(10):1233-1241. IF 13.204

2. Adaptation of the human population to the environment: Current knowledge, clues from Czech cytogenetic and "omics" biomonitoring studies and possible mechanisms

The main aim of this review was to provide a comprehensive overview of the last decade of Czech biomonitoring research, concerning the effect of various levels of air pollution and radiation, on the differently exposed population groups. A detailed analysis of data leads to a hypothesis of the versatile mechanism of adaptation to environmental stressors via DNA methylation settings which may even originate in prenatal development, and help to reduce the resulting DNA damage levels.

Rössnerová, A., Pokorná, M., Švecová, V., Šrám, R., Topinka, J., Zölzer, F., Rössner, P.: (2017) Adaptation of the human population to the environment: Current knowledge, clues from Czech cytogenetic and "omics" biomonitoring studies and possible mechanisms. *Mutation Research-Reviews in Mutation Research.* 773: 188-203. IF=5.5. doi: 10.1016/j.mrrev.2017.07.002. Epub 2017 Jul 12. Review.

3. Blends of butanol and hydrotreated vegetable oils as drop-in replacement for diesel engines: Effects on combustion and emissions

We investigated the performance of a mix of two emerging biofuels, butanol and hydrotreated vegetable oil (HVO), in an Iveco Tector diesel engine. HVO, a possible drop-in fuel for diesel engines features excellent combustion and emissions characteristics. All biofuels exhibited, relative to diesel fuel, a decrease in the concentration of particulate PAHs and n-alkanes in emissions, while the concentrations of hopanes and steranes originated from lubricating oil were comparable across all fuels. Cold starts yielded about 15% higher concentrations of both particulate matter and particulate organic compounds in emissions than hot startsparticulate matter and particulate organic compounds in emissions than hot starts.

Vojtíšek-Lom, M., Beránek, V., Mikuška, P., Křůmal, K., Coufalík, P., Sikorová, J., Topinka, J.: (2017) Blends of butanol and hydrotreated vegetable oils as drop-in replacement for diesel engines: Effects on combustion and emissions. *Fuel*, 197: 407-421. IF 4,601

Important results in 2016

1. Comparative Analysis of Toxic Responses of Organic Extracts from Diesel and Selected Alternative Fuels Engine Emissions in Human Lung BEAS-2B Cells

Exhaust particles from four different diesel/biodiesel fuels were collected, organic compounds were extracted and human lung cells were exposed to each extract. Chemical analysis revealed that extract from neat hydrotreated vegetable oil NexBTL contained the lowest content of carcinogenic PAHs and gene expression profiling identified deregulation of numerous genes and processes common for all extract as well as specific for each extract. Despite subtle differences in overall toxic effects induced by different diesel or alternative fuels, NexBTL extract exhibited the weakest toxicity comparing to others.

Helena Libalova, Pavel Rossner Jr., Kristyna Vrbova, Tana Brzicova, Jitka Sikorova, Michal Vojtisek-Lom, Vit Beranek, Jiri Klema, Miroslav Ciganek, Jiri Neca, Katerina Pencikova, Miroslav Machala, Jan Topinka:(2016) Comparative Analysis of Toxic Responses of Organic Extracts from Diesel and Selected Alternative Fuels Engine Emissions in Human Lung BEAS-2B Cells. *International Journal of Molecular Sciences. IF 3.257*

2. Urinary 8-oxo-7,8-dihydro-2‘-deoxyguanosine analysis by an improved ELISA: An inter-laboratory comparison study

We performed an inter-laboratory comparison of 8-oxo-7,8-dihydro-2‘-deoxyguanosine by ELISA using standardized conditions, compared the ELISA results with high-performance liquid chromatography-tandem mass spectrometry and performed identification of compounds that may contribute to the discrepancy between methods. Assay standardization significantly improved inter-laboratory agreement, although some variability caused by aromatic and heterocyclic compounds and saccharides is still observed.

Rössner, P., Orhan, H., Koppen, G., Sakai, K., Santella, R.M., Ambrož, A., Rossnerová, A., Šrám, R.,Ciganek, M., Neca, J., Arzuk, E., Mutlu, N., Cooke, M.S.: (2016) Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine analysis by an improved ELISA: An inter-laboratory comparison study. *Free Radic. Biol. Med.,* 22;95: 169-179. IF 5.784

3. The impact of silica encapsulated cobalt zinc ferrite nanoparticles on DNA, lipids and proteins of rat bone marrow mesenchymal stem cells

Cobalt-zinc-ferrite nanoparticles (NPs) encapsulated by amorphous silica were tested to assess their effect on DNA, lipids and proteins in rat mesenchymal stem cells. No significant cytotoxic effects were observed, except for the highest dose of CZF-NPs, that slowed down cell proliferation and induced damage to DNA, lipids and proteins.

The results suggest that the silica-coated CZF-NPs, when applied at a non-toxic dose, represent a promising contrast agent for cell labeling for magnetic resonance analysis.

Novotná, B., Turnovcová, K., Veverka, P., Rössner, P. Jr., Bagryantsevá, Y., Herynek, V., Zvatora, P., Vosmanská, M., Klementová, M., Syková, E., Jendelová, P.: (2016)The impact of silica encapsulated cobalt zinc ferrite nanoparticles on DNA, lipids and proteins of rat bone marrow mesenchymal stem cells. *Nanotoxicology,* 10(6): 662-670. IF 7.913

Important results in 2015

1. Analysis of genetic damage in lymphocytes of former uranium processing workers

The frequency of cells containing micronuclei (MN) and the presence of centromeres in the MN were analyzed in lymphocytes of men from Southern Bohemia (former workers of a former uranium processing plant „MAPE Mydlovary“ and controls). No differences were found between formerly exposed workers and the control group. Moreover former workers with X-ray examination had significantly lower level of DNA damage than the control group. Possible reason of this results may be adaptation of the organism.

Zölzer, F., Havránková, R., Freitinger Skalická, Z., Rössnerová, A., Šrám, R.J.: (2015) Analysis of Genetic Damage in Lymphocytes of Former Uranium Processing Workers. Cytogenet Genome Res. 2015; 147 (1): 17-23

2. Day-to-day variability of toxic events induced by organic compounds bound to size segregated atmospheric aerosol

The temporal variability of size-segregated aerosol mass collected on a daily basis during the winter period in highly polluted districts of Ostrava city as well as toxic effects of organic compounds extracted from each size fraction were quantified. We revealed that upper accumulation mode fraction (0.5 < dae < 1 μm) comprises most of the aerosol mass and contained 44% of total c-PAHs while ultrafine fraction (< 0.17 μm) contained only 11% of c-PAHs. DNA adduct levels and dioxin-like activity strongly correlated with both aerosol mass and c-PAH concentrations suggesting genotoxicity as a major toxic effect of organic compounds bound to size-segregated aerosol.

Topinka, J., Rossner, P. Jr., Milcová, A., Schmuczerová, J., Pěnčíková, K., Rossnerová, A., Ambrož, A., Štolcpartová, J., Bendl, J., Hovorka, J., Machala, M.: (2015) Day-to-day variability of toxic events induced by organic compounds bound to size segregated atmospheric aerosol. Environ. Pollut. 202: 135-145.

3. Reduced gene expression levels after chronic exposure to high concenrations of air pollutants

We analyzed the effect of air pollution on gene expression in subjects living in Prague and in the polluted Ostrava region. We expected to observe changes in expression of DNA repair genes in subjects from the Ostrava region. Unexpectedly, the changes were found in Prague subjects, particularly in genes associated with immune response and neurodegenerative diseases. The results suggest that chronic exposure to air pollution may result in adaptation of the organism with possible negative health effects.

Rossner, Jr. P., Tulupová, E., Rossnerová, A., Líbalová, H., Hoňková, K., Gmuender, H., Pastorková, A., Švecová, V., Topinka, J., Šrám, R.J.: (2015) Reduced gene expression levels after chronic exposure to high concentrations of air pollutants. Mutat Res. 780:60-70.

Research activity and characterisation of the main scientific results

We are implementing a concept of non-invasive liquid biopsy, thus above biomarkers are being sought in urine and stool of the patients and control subjects. Our studies are regularly complemented by functional *in vitro* studies on cell lines. Our outputs may be classified into following areas: A) Genetic landscape of above malignant diseases; B) Mutational hallmarks and epigenetics of malignant diseases; C) DNA and chromosomal damage and DNA repair in malignant diseases; D) Other findings in molecular oncology with our participation. In order to classify the outputs, the publications since 2015 are listed under the particular category (see above).

A) Genetic landscape of above malignant diseases: 1. Campa et al. TERT gene harbors multiple variants associated with pancreatic cancer susceptibility. *Int J Cancer* 2015 137(9):2175-2183; 2. Childs et al. Common variation at 2p13.3, 3q29, 7p13 and 17q25.1 associated with susceptibility to pancreatic cancer. *Nature Genet.* 2015 47(8):911-916; 3. Vymetalkova et al. Genotype and haplotype analyses of TP53 gene in breast cancer patients: association with risk and clinical outcome. *Plos One* 2015 10(7):e0134463; 4. Campa et al. Functional single nucleotide polymorphisms within the cyclin-dependent kinase inhibitor 2A/2B region affect pancreatic cancer risk. *Oncotarget*. 2016 Aug 30;7(35):57011-57020; 5. Zhang et al. Three new pancreatic cancer susceptibility signals identified on chromosomes 1q32.1, 5p15.33 and 8q24.21. *Oncotarget*. 2016 Oct 11;7(41):66328-66343; 6. Mohelnikova-Duchonova et al. SLC22A3 polymorphisms do not modify pancreatic cancer risk, but may influence overall patient survival. *Sci Rep*. 2017 4:43812; 7. Schubert et al. Evidence for genetic association between chromosome 1q loci and predisposition to colorectal neoplasia. *Br J Cancer*. 2017 117(6):876-884; 8. Campa et al. Do pancreatic cancer and chronic pancreatitis share the same genetic risk factors? A PANcreatic Disease ReseArch (PANDoRA) consortium investigation. *Int J Cancer*. 2018 142(2): 290-296; 9. Barontini et al. Association between polymorphisms of TAS2R16 and susceptibility to colorectal cancer. *BMC Gastroenterol*. 2017 17(1):104; 10. Catalano et al. Investigation of single and synergic effects of NLRC5 and PD-L1 variants on the risk of colorectal cancer. *PLoS One*. 2018 13(2):e0192385; 11. Klein et al. Genome-wide meta-analysis identifies five new susceptibility loci for pancreatic cancer. *Nature Commun*. 2018 9(1):556; 12. Huhn et al. Coding variants in NOD-like receptors: An association study on risk and survival of colorectal cancer. *PLoS One*. 2018 13(6),e0199350; 13. Campa et al. Genetic determinants of telomere length and risk of pancreatic cancer: a PANDoRA study. *Int J Cancer*. 2019 144(6):1275-1283; 14. Huyghe et al. Discovery of common and rare genetic risk variants for colorectal cancer. *Nature Genet*. 2019 51(1):76-87; 15. Obazee et al. Germline *BRCA2* K3326X and *CHEK2* I157T mutations increase risk for sporadic pancreatic ductal adenocarcinoma. *Int J Cancer*. 2019 Aug 1;145(3):686-693; 16. Jiraskova et al. Functional polymorphisms in DNA repair genes are associated with sporadic colorectal cancer susceptibility and clinical outcome. *Int J Mol Sci* 2018 20(1):97; 17. Gentiluomo et al. Genetic variability of the ABCC2 gene and clinical outcomes in pancreatic cancer patients. *Carcinogenesis*. 2019 Jun 10; 40(4):544-550; 18. Thomsen et al. Genome-wide association study on monoclonal gammopathy of unknown significance (MGUS): comparison with multiple myeloma. *Leukemia* 2019 Jul;33(7):1817-1821; 19. Lu et al. Single nucleotide polymorphisms within Mucin-type O-glycan genes are associated with colorectal cancer survival. *Plos One* 2019 14(5):e0216666.

B) Mutational hallmarks and epigenetics of malignant diseases: 1. Aherne et al. Circulating miRNAs miR-34a and mi-R 150 associated with colorectal cancer progression. *BMC Cancer* 2015 15(1):329; 2. Vymetalkova et al. Epigenome-wide analysis of DNA methylation reveals a rectal cancer-specific epigenomic signature. *Epigenomics*. 2016 Sep;8(9):1193-1207; 3. Vodicka et al. Polymorphisms in Non-coding RNA Genes and Their Targets Sites as Risk Factors of Sporadic Colorectal Cancer. *Adv Exp Med Biol*. 2016;937:123-149; 4. Vymetalkova et al. Polymorphisms in microRNA binding sites of mucin genes as predictors of clinical outcome in colorectal cancer patients. *Carcinogenesis*. 2017 38(1):28-39; 5. Schneiderova et al. MicroRNA-binding site polymorphisms in genes involved in colorectal cancer etiopathogenesis and their impact on disease prognosis. *Mutagenesis*. 2017 Oct 17;32(5):533-542; 6. Thiele et al. lncRNAs in Non-Malignant Tissue Have Prognostic Value in Colorectal Cancer. *Int J Mol Sci*. 2018 19(9):2672; 7. Kral et al. Expression profile of miR-17/92 cluster is predictive of treatment response in rectal cancer. *Carcinogenesis*. 2018 39(1):1359-1367; 8. Vymetalkova et al. Circulating Cell-Free DNA and Colorectal Cancer: A Systematic Review. *Int J Mol Sci*. 2018 Oct 26;19(11). pii: E3356; 9. Vymetalkova et al. DNA methylation and chromatin modifiers in colorectal cancer. *Mol Aspects Med* 2019 69:73-92.

C) DNA and chromosomal damage and DNA repair in malignant diseases: 1. Vodicka et al. Interactions of DNA repair gene variants modulate chromosomal aberrations in healthy subjects. *Carcinogenesis* 2015 36(11):1299-1306; 2. Naccarati et al. Double-strand break repair and colorectal cancer: gene variants within 3' UTRs and microRNAs binding as modulators of cancer risk and clinical outcome. *Oncotarget* 2016 7(17):23156-23169; 3. Försti et al. Genetic variation in the major mitotic checkpoint genes associated with chromosomal aberrations in healthy humans. *Cancer Lett*. 2016 380(2):442-446; 4. Vodicka et al. DNA and chromosomal damage in medical workers exposed to anaesthetic gases assessed by the lymphocyte cytokinesis-block micronucleus (CBMN) assay. A critical review. *Mutat Res*. 2016 770(Pt A):26-34; 5. Kroupa et al. Bleomycin-induced chromosomal damage and shortening of telomeres in peripheral blood lymphocytes of incident cancer patients. *Genes Chromosomes Cancer*. 2018 57(2):61-69; 6. Vodenkova et al. Base excision repair capacity as a determinant of prognosis and therapy response in colon cancer patients. *DNA Repair (Amst)*. 2018 72:77-85; 7. Niazi et al. Genetic variation associated with chromosomal aberration frequency: A genome-wide association study. *Environ Mol Mutagen*. 2019 60(1):17-28; 8. Vodicka et al. Genetic variation of acquired structural chromosomal aberrations. Mutat Res. 2018 836:13-21; 9. Pardini et al. DNA repair and cancer in colon and rectum: novel players in genetic susceptibility. *Int J Cancer*. 2020 in press.

D) Other findings in molecular oncology with our participation: 1. Elsnerova et al. Gene expression of membrane transporters: Importance for prognosis and progression of ovarian carcinoma. *Oncol Rep*. 2016 35(4):2159-2170; 2. Korenkova et al. The focus on sample quality: Influence of colon tissue collection on reliability of qPCR data. *Sci Rep*. 2016 6:29023; 3. Kunicka et al. Molecular profile of 5-fluorouracil pathway genes in colorectal carcinoma. *BMC Cancer*. 2016 16(1):795; 4. De Santi et al. Mesothelin promoter variants are associated with increased soluble mesothelin-related peptide levels in asbestos-exposed individuals. *Occup Environ Med*. 2017 Jun;74(6):456-463; 5. Hubackova et al. Interferon-regulated suprabasin is essential for stress-induced stem-like cell conversion and therapy resistance of human malignancies. *Molecular*

Oncology 2019 13(7):1467-1489; 6. Cervena et al. Diagnostic and prognostic impact of cell-free DNA in human cancers: Systematic Review. *Mutation Research-Reviews in Mutat Res* 2019 781:100-129; 7. Kroupa et al. Relationship of telomere length in colorectal cancer patients with cancer phenotype and patient prognosis. *Br J Cancer* 2019 121(4):344-350.

Research activity and characterisation of the main scientific results

The use of stem cells in spinal cord injury repair

The use of stem cells in human medicine requires good understanding the mechanisms of action, otherwise the expectations will not meet the reality. We therefore compared three types of stem cells (mesenchymal stem cells and 2 different neural progenitors) from different sources in spinal cord injury. We studied the survival, paracrine effect and differentiation potential and functional outcome. From our results we can say that iPS-NPs, were the most suitable candidate for treatment of SCI due to their robust survival, tissue sparing, reduction of glial scarring, and increased axonal sprouting.

We further studied if intrathecal application of neural progenitors is comparably efficient as intraspinal and we found that there is a positive effect on SCI, but is less robust than after intraspinal application.

We studied the immunomodulatory properties of neural stem cells in SCI and found reduction of activity of proinflammatory pathway NFkB after stem cell application, which led to reduced levels of TNFa and lesser astrogliosis (In collaboration with N.Y. Medical College – cytokine analysis).

Also the use of induced pluripotent stem cell derived neural progenitors require protocols that can be easily translated to GMP grade, so we in collaboration with stem cell bank in Valencia developed simple protocols for NP differentiation and expansion.

Finally, we tested in collaboration with IMC (hydrogel preparation) a polymer hydrogel as cell carrier for iPS-NPs in chronic model of SCI and we found a good graft survival, reduced cavitation, increased number of TH+ fibers in a lesion and trend in functional outcome improvement.

The major advantage of hMSC use is its less invasive route of administration coupled with a strong anti-inflammatory effect, with repeated applications potentially overcoming its transiency. We therefore tested the dose and repeated application of MSC isolated from Wharton Jelly and their secretome in SCI and found that the 3 times applied high dose is the most effective and cell secretome has comparative effect as cell application. (In collaboration with team 2 –in vitro results, our team 8 all in vivo work and in vivo data interpretation).

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Tackling neuroinflammation in spinal cord injury

Affecting early immune response after SCI may lead to decelerating cavity formation processes and thus resulting in preservation of spinal tracts and decreased locomotor deficits after SCI. We therefore studied anti-inflammatory drugs (curcumin, EGCG, nanocurcumin, combination of curcumin and EGCG, curcumin and stem cells) and photobiomodulation with dual pulse laser) in SCI treatment. We found that curcumin and EGCG reduce pro-inflammatory NFkB pathway activity, reduce inflammatory cytokines and glial scar. Laser treatment resulted in robust tissue sparing, less apoptosis and shift in M1/M2 macrophage phenotype towards M2 pro regenerative one. All animals with anti-inflammatory treatment had better functional outcome, however, no clear synergistic effect was observed in combined treatments. All experiments were done by team 8, cytokine evaluation in collaboration with N.Y. Medical College.

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enhances neuroregeneration after spinal cord injury by altering levels of inflammatory cytokines. Neuropharmacology. 126: 213-223.
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Spinal cord injury repair using hydrogels.

We evaluated polymer hydrogels based on methacrylate with modified surface as suitable implants for bridging spinal cord injury. We assessed the dynamics of tissue ingrowth into the implant, different peptide modifications (RGD, SIKVAV, Fibronectin) facilitating cell adhesion and axonal growth. We found that natural hydrogels are more suitable for axonal ingrowth, since they mimic extracellular matrix and contain bioactive molecules, however, their drawback is fast degradation and macrophage accumulation in the lesion.

All polymer hydrogels were developed in collaboration with Institute of Macromolecular Chemistry, where the polymer matrices were prepared. All in vitro and in vivo experiments were performed in IEM often in collaboration with team 2, who was responsible for hydrogels based on natural polymers (decellularized matrix and hyaluronic acid). Our team 8 was responsible for surgery and implantation, functional evaluation and tissue analysis.

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Stem cells in the treatment of neurodegenerative diseases

To unravel the mechanisms of action of applied stem cells in neurodegenerative diseases, we performed preclinical studies in animal model of **Alzheimer's disease** and **ALS**. Transplantation of human mesenchymal stem cells (hMSC) prepared in GMP conditions into the lateral ventricle of a triple transgenic mouse model of **Alzheimer´s disease (3xTg-AD**) at the age of 8 months led to the preservation of working memory in MSC-treated 3xTg-AD mice, suggesting that such preservation might be due to the protective effect of MSCs on Glutamine synthase levels and the considerable downregulation of A*β**56 levels in the entorhinal cortex. These changes were observed 6 months after transplantation, accompanied by clusters of proliferating cells in the SVZ.

Since the MSCs applied intrathecally do not survive for more than 2 weeks, we tested the effect of repeated application of MSCs in **ALS rat model**. The cells were applied into spinal canal, into muscle or into both and we assessed the effect of cell therapy on overal survival, number of motoneurons, 3 major cell death pathways and neuromuscular junctions. We found that a combination of repeated intrathecal and intramuscular hMSC applications protects motor neurons and neuromuscular junctions, not only through a reduction of apoptosis and autophagy but also through the necroptosis pathway, which is significantly involved in cell death in rodent model of ALS.

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mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: Results of Phase I/IIa clinical trial. Cell Transplant.

Generation of new induced pluripotent (iPS) cell lines and their use in hereditary diseases.

In collaboration with Stem cell bank and Research Centre in Valencia, we collaborate with dr Slaven Erceg and his team. Dr Erceg is working part time in IEM in our team 8 and in Spain. This give us an opportunity to participate on generation of iPS cell lines from rare hereditary diseases. We have generated different iPS cell lines for 2D as well as organoid 3D cultures to study and correct impaired genes.

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The use of magnetic nanoparticles in regenerative medicine and cancer treatment

In 2015-2019 we have continued our collaboration **on development** of different **magnetic nanoparticles** (based in iron oxide or ferrites) for not only in vivo imaging, but also for drug delivery and cancer treatment. Such particles showed a higher labeling efficiency and theranostic properties when compared with commercial products. Therefore, we patented our new method of particle surface modification (patent No: 308154 granted in 2019). All the prepared nanoparticles were tested from the point of view of genotoxicity, cell viability, proliferation, as well as ability of differentiation using mesenchymal stem cells (MSCs) and neural progenitors derived from iPS. We also compared different types of nanoparticles and their suitability for cell labeling. We tested the influence of labeled cells on brain tissue. The ferrite particles did not cause oxidative damage, but were not suitable for neural progenitor labeling, since they impaired neuronal differentiation. Iron oxide nanoparticles carrying doxorubicin were effective in glioblastoma treatments, since they reduce tumor cell growth at lower concentrations (10nM) than free drug and did not affect normal healthy tissue. The biological part of this research was performed at IEM (collaboration of team 8 and 6), nanoparticles were prepared at Academy Institutes of Macromolecular Chemistry or Institute of Physics.

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5. Novotná, B., Herynek, V., Rössner ml., P., **Turnovcová, K., Jendelová**, P.: (2017) The effects of grafted mesenchymal stem cells labeled with iron oxide or cobalt-zinc-iron nanoparticles on the biological macromolecules of rat brain tissue extracts. International Journal of Nanomedicine. 12: 4519-4526.
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7. **Herynek, V**., **Turnovcová,K.,** Gálisová,A., Kaman,O., **Mareková,D**., Koktan, J., Vosmanská, M., Kosinová, L., **Jendelová, P.** Manganese-zinc ferrites as safe and efficient nanolabels for cell imaging and tracking in vivo. ChemistryOpen. 2019 Jan 23;8(2):155-165. doi: 10.1002/open.201800261.
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Axon regeneration

In 2017 part of our team became a member of Centre for reconstructive neurosciences led by prof James Fawcett from Cambridge University, where he retired and recently he works part time in Prague. This gave us an opportunity to share know how in new different field of regenerative medicine. Prof Fawcett and Jessica Kwok, who joined our team, are unravelling the role of extracellular matrix and chondroitin sulfate proteoglycans in axonal regeneration and plasticity. They also use gene delivery to incorporate into axons missing signals and receptors that enables axonal growth in development, but are missing in adulthood. They showed the importance of different sulfation position of chondroitin sulfate proteoglycans for plasticity and how it changes during aging, they recognized important transport molecules and underlying mechanisms responsible for axonal transport and regeneration (Rab11 EFA6) and describe the role of perineuronal nets in neural plasticity under physiological as well as pathological conditions (AD, SCI, memory formation). Part of our team 8 joined this topic, focused on sensory regeneration. We developed a test for measurement of sensitivity in perineal area. Finally, our postdoc Forostyak spend 6 weeks training in Cambridge University and he collaborated on two projects. Part of the experiments were done in IEM. They found an important molecule secreted by injured neurons that drive the astrocytes into protective mode but is impaired in ALS conditions. The second project was studying the expression and localization of hepcidin (iron metabolism regulator) in brain tissue.

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2. Foscarin, S., Raha-Chowdhury, R., **Fawcett, J., Kwok**, .: (2017) Brain ageing changes proteoglycan sulfation, rendering perineuronal nets more inhibitory. Aging. 9 (6): 1607-1622.
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6. Irvine, S.F., **Kwok, J**.: (2018) Perineuronal Nets in Spinal Motoneurones: Chondroitin Sulphate Proteoglycan around Alpha Motoneurones. International Journal of Molecular Sciences. 19 (4):1172.
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8. **Fawcett**, J.W. Oohashi, T., Pizzorusso, T.: (2019) The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nature Reviews Neuroscience*. 20 (8): 451-465.
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Research activity and characterisation of the main scientific results

The results significantly contributed to the development in the field of nanotechnology and regenerative medicine. We published high quality publications, established cooperation with many international laboratories. We developed novel technologies to produce nanofibers, mainly core-shell nanofibres, needleless electrospinning, alternating current (AC) spinning. Materials were further tested as scaffolds for cells and for the delivery of growth factors, bioactive substances, and/or proteins. These systems have broad applications in tissue engineering applications, for the delivery of proteins, drugs, chemical substances. We proved the ability of the scaffold to bind platelets and their derivatives, release growth factors in a controlled manner and stimulate growth of different cell types and effect MSC differentiation. We have tested the systems for the regeneration of bone and osteochondral defects in rabbits.
Mainly platelets and their derivatives are planned for their use in clinical practice for their simple preparation and autologous source. Moreover, we developed three-dimensional scaffolds based on foams for regeneration of osteochondral defects, scaffolds prepared by melt-blown methods and a 3D printing, which have a great potential for use in clinical practice. We prepared nanostructured titanium surfaces with improved properties for implant osseointegration. Some materials were also tested in animal models – nanofibers, three-dimensional scaffolds for their use in healing of skin, bone and cartilage.

Main scientific results:

1.Vojtová, L., Michlovská, L., Valová, K., Zboncak, M., Trunec, M., Částková, K., Krtička, M., Pavliňáková, V., Poláček, P., Dzurov, M., Lukášová, Věra, Rampichová, Michala, Suchý, Tomáš, Sedláček, R., Ginebra, M.P., Montufar, E.B. The Effect of the Thermosensitive Biodegradable PLGA-PEG-PLGA Copolymer on the Rheological, Structural and Mechanical Properties of Thixotropic Self-Hardening Tricalcium Phosphate Cement. International Journal of Molecular Sciences. 2019, 20(2), 391. E-ISSN 1422-0067 doi: 10.3390/ijms20020391

Novel calcium phosphate cements with a copolymer PLGA–PEG–PLGA were prepared. The setting times of α-TCP mixed with aqueous solutions of PLGA–PEG–PLGA determined by time-sweep curves revealed a lag phase during the dissolution of the α-TCP particles. The copolymer demonstrates the pseudoplastic rheological behaviour with a small decrease in shear stress and the rapid recovery of the viscous state once the shear is removed, preventing cement phase separation and providing good cohesion.
Two authors of total 16 authors are from IEM.

2. Daňková, Jana, Buzgo, Matej, Vejpravová, Jana, Kubíčková, Simona, Sovková, Věra, Vysloužilová, L., Mantlíková, Alice, Nečas, A., Amler, Evžen. Highly efficient mesenchymal stem cell proliferation on poly-epsilon-caprolactone nanofibers with embedded magnetic nanoparticles. International Journal of Nanomedicine. 2015, 10(2015), 7307-7317. ISSN 1176-9114 doi: 10.2147/IJN.S93670

The first/correspondence author is from IEM. Our data indicate that due to the synergic effect of the poly-ε-caprolactone nanofibers and magnetic particles, cell adhesion and proliferation of MSCs is enhanced and osteogenic differentiation is supported. The results are very promising for the acceleration of bone regeneration. Highly cited article.
4 authors of total 9 authors are from IEM. J. Daňková, V. Sovková have two affiliations, and E. Amler, M. Buzgo have three affiliations because of different methodologies and projects financing the work.

3. Plencner, Martin, Prosecká, Eva, Rampichová, Michala, East, B., Buzgo, Matej, Vysloužilová, L., Hoch, J., Amler, Evžen. Significant improvement of biocompatibility of polypropylene mesh for incisional hernia repair by using poly-epsilon-caprolactone nanofibers functionalized with thrombocyte-rich solution. International Journal of Nanomedicine. 2015, 10(2015), 2635-2646. E-ISSN 1178-2013 doi: 10.2147/IJN.S77816

The first/correspondence author is from IEM.
Incisional hernia affects up to 20% of patients after abdominal surgery. The aim of the study was to develop a functionalized scaffold for ventral hernia regeneration, based on on a polypropylene surgical mesh functionalized with poly-epsilon-caprolactone (PCL) nanofibers and adhered thrombocytes. The composite mesh showed better biocompatibility than polypropylene mesh and was improved with thrombocytes.
5 authors of total 8 authors are from IEM. M. Plencner, E. Prosecká, M. Buzgo and M. Rampichová have two affiliations, and E. Amler has three affiliations because of different methodologies and projects financing the work.

4. Vysloužilová, L., Buzgo, Matej, Pokorný, P., Chvojka, J., Míčková, Andrea, Rampichová, Michala, Kula, J., Pejchar, K., Bílek, M., Lukáš, D., Amler, Evžen. Needleless coaxial electrospinning: A novel approach to mass production of coaxial nanofibers. International Journal of Pharmaceutics. 2017, 516(1-2), 293-300. ISSN 0378-5173 doi: 10.1016/j.ijpharm.2016.11.034

We describe a simple spinneret setup for needleless coaxial electrospinning that exceeds the limited production capacity of current approaches. This approach leads to the formation of coaxial nanofibers with higher and uniform shell/core ratio, which results in the possibility of better tuning of the degradation rate. The throughput and quality could increase the broader application of coaxial nanofibers. Highly cited article.
4 authors of 11 authors are from IEM. M. Rampichová and A. Míčková have three affiliations because of different methodologies and co-financing of other projects.

5. Rampichová, Michala, Buzgo, M., Míčková, Andrea, Vocetková, Karolína, Sovková, Věra, Lukášová, Věra, Filová, Eva, Rustichelli, Franco, Amler, Evžen. Platelet-functionalized three-dimensional poly-e-epsilon-caprolactone fibrous scaffold prepared using centrifugal spinning for delivery of growth factors. International Journal of Nanomedicine. 2017, 12(2017), 347-361. E-ISSN 1178-2013 doi: 10.2147/IJN.S120206

The first /correspondence author is from IEM. Centrifugal spinning can produce 3D fibrous scaffolds with large and interconnected pores. We introduced a simple composite scaffold based on platelet adhesion to poly-e-caprolactone 3D fibres. After adhesion, platelets bioavailability was prolonged. MG-63 model showed improved metabolic activity, proliferation and alkaline phosphatase activity in comparison to non-functionalized scaffold. Highly cited article.
The first /correspondence author is from IEM.
7 authors of 9 authors are from IEM. M. Rampichová and A. Míčková have two affiliations because of different methodologies and co-financing from other projects.

6. Paino, F., Noce, M.L., Giuliani, A., de Rosa, A., Mazzoni, F., Laino, L., Amler, Evžen, Papaccio, G., Desiderio, V., Tirino, V. Human DPSCs fabricate vascularized woven bone tissue: A new tool in bone tissue engineering. Clinical science. 2017, 131(8), 699-713. ISSN 0143-5221 doi: 10.1042/CS20170047
Authors are from different international laboratories. The article describes novel source of stem cells - human dental pulp stem cells, their growth and differentiation potential into osteoblasts in vitro and in vivo. These cells showed an osteogenic potential. The results are promising for their use in tissue engineering. Highly cited article.
One author from IEM (Evzen Amler). An interdisciplinary work which was done in different laboratories. E, Amler has 2 affiliations because of different methodology used in both laboratories.

7. Gregor, A., Filová, Eva, Novák, M., Kronek, J., Chlup, H., Buzgo, M., Blahnová, Veronika, Lukášová, Věra, Bartoš, M., Nečas, A., Hošek, J. Designing of PLA scaffolds for bone tissue replacement fabricated by ordinary commercial 3D printer. Journal of Biological Engineering. 2017, 11(oct), 31. ISSN 1754-1611 doi: 10.1186/s13036-017-0074-3
Rapid Prototyping enables preparation of scaffolds of precise structure, shape and size. In the study, we tested commercially available 3D printer. Structures from polylactic acid with different geometry were prepared and tested with osteosarcomas cells. Porosity lower than 90% did not affect cell proliferation, and scaffolds demonstrated better biomechanical properties. Our work showed possibilities and limitations of Rapid Prototyping. Highly cited article (36 citations since Oct 2017).
3 authors of 11 authors are from IEM. Filova and Blahnová have the second afilliation of 2 Faculty of Medicine - the work was partially financed from other projects and the faculty is covering the PhD study of Mrs. Blahnova. Mrs. Lukasova has a part time affiliation at two institutions as the special methods are used at the second institute.

8. Zarone, M.R., Misso, G., Grimaldi, A., Zappavigna, S., Russo, M., Amler, Evžen, Di Martino, M.T., Amodio, N., Tagliaferri, P., Tassone, P., Caraglia, M. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Scientific Reports. 2017, 7(dec), 17949. ISSN 2045-2322 doi: 10.1038/s41598-017-18186-0

The work was done within an international cooperation. The article describes a novel method for the treatment of multiple myeloma cancer using miR-34a and γ-secretase inhibitors. It has a potential for its use in clinical practice.

One author (E. Amler) of all 11 authors from IEM. He has two affiliations because of different methodology and project support.

9. Rampichová, Michala, Kuželová Košťáková, E., Filová, Eva, Chvojka, J., Šafka, J., Pelcl, M., Daňková, Jana, Prosecká, Eva, Buzgo, Matej, Plencner, Martin, Lukáš, D., Amler, Evžen. Composite 3D printed scaffold with structured electrospun nanofibers promotes chondrocyte adhesion and infiltration. Cell Adhesion and Migration. 2018, 12(3), 271-285. ISSN 1933-6918 doi: 10.1080/19336918.2017.1385713

The first/correspondence author is from IEM. Additive manufacturing, also called 3D printing, produces scaffolds with defined structure. Its disadvantage is the excessive smoothness of the fibres. In the study, a 3D printed scaffold was combined with electrospun classic or structured nanofibers. Electrospun layers were connected to 3D printed fibres by gluing. We showed excellent chondrocyte infiltration, viability, and good proliferation. However, partial chondrocyte dedifferentiation was shown.
6 of 12 authors are from IEM. M. Rampichova and E. Amler have the affiliations at 2 institutes because of different methodologies and projects supporting the work.

10. East, B., Plencner, Martin, Královič, Martin, Rampichová, Michala, Sovková, Věra, Vocetková, Karolína, Otáhal, M., Tonar, Z., Kolinko, Y., Amler, Evžen, Hoch, J. A polypropylene mesh modified with polye-epsilon-caprolactone nanofibers in hernia repair: large animal experiment. International Journal of Nanomedicine. 2018, 13(2018), 3129-3143. E-ISSN 1178-2013 doi: 10.2147/IJN.S159480

For incisional hernia repair, a composite scaffold assembled out of a standard polypropylene hernia mesh and poly-epsilon-caprolactone nanofibers was tested in minipigs. The layer of nanofibers led to tissue overgrowth and the formation of a thick fibrous plate around the implant. Collagen maturation was accelerated, and the final scar was more flexible and elastic than a scar found under a standard mesh. However, tissues with composite scaffold were less resistant to distracting forces than a standard mesh.
6 authors of 11 authors are form IEM. Plencner, Kralovic, Sovkova, Vocetkova, Amler have three affiliations beacuse of different methodologies and projects and a present PhD study (Kralovic, Sovkova, Vocetkova) at the Charles University.

11. Alaia, C., Boccellino, M., Zappavigna, S., Amler, Evžen, Quagliuolo, L., Rossetti, S., Facchini, G., Caraglia, M. Ipilimumab for the treatment of metastatic prostate cancer. Expert Opinion on Biological Therapy. 2018, 18(2), 205-213. ISSN 1471-2598 doi: 10.1080/14712598.2018.1420777

Ipilimumab, a fully humanized monoclonal antibody blocking CTLA4 activity, was approved by FDA for the treatment of malignant melanoma and is currently being studied in metastatic castration-resistant prostate cancer. The review collates the most significant preclinical and clinical studies and proposes strategies for the future. A possible strategy is to combine it with standard anti-cancer therapeutics, e.g. vaccines, PDL1 inhibitors, antiandrogen drugs, and chemotherapy. Highly cited article.
1 author of 8 authors is from IEM. International team.

12. Burglová, K., Rylová, G., Markoš, A., Přichystalová, H., Soural, M., Petrácek, M., Medvědiková, M., Tejral, Gracian, Sopko, Bruno, Hradil, P., Džubák, P., Hajduch, M., Hlaváč, J. Identification of Eukaryotic Translation Elongation Factor 1-α 1 Gamendazole-Binding Site for Binding of 3-Hydroxy-4(1 H)-quinolinones as Novel Ligands with Anticancer Activity. Journal of Medicinal Chemistry. 2018, 61(7), 3027-3036. ISSN 0022-2623 doi: 10.1021/acs.jmedchem.8b00078

A multidisciplinary work describes the interaction site of contraceptive drug gamendazol. We have successfully identified the interaction site of the contraceptive drug gamendazole using computational modelling. The drug was previously described as a ligand for eukaryotic translation elongation factor 1-α 1 (eEF1A1) and found to be a potential target site for derivatives of 2-phenyl-3-hydroxy-4(1H)-quinolinones (3-HQs), which exhibit anticancer activity. The interaction of this class of derivatives of 3HQs with eEF1A1 inside cancer cells was confirmed via pull-down assay. We have designed and synthesized a new family of 3-HQs and subsequently applied isothermal titration calorimetry to prove that these compounds strongly bind to eEF1A1. Moreover, we found that some of these derivatives possess significant in vitro anticancer activity. Two authors of 13 authors are rom IEM. Tejral has 3 affiliations and Sopko has 2 affiliations because of different methodology used in different institutes.

13. Patent No. 308053 3 D collagen porous composite carriers for accelerated bone regeneration (3 D kolagenové porézní kompozitní nosiče pro akcelerovanou regeneraci kostí). Industrial Property Office (Czech Patent and Trademark Office); 08.11.2017; Filing date: 30.12.2013 Authors: Evžen Amler, Eva Prosecká, Michala Rampichová, Matej Buzgo, Mgr. Andrea Míčková, Eva Filová, Martin Plencner, Andrej Litvinec, Lucy Vojtová, Josef Jančář, Alois Nečas.

Research activity and characterisation of the main scientific results

Stem cell-based therapy of ocular surface damages

Corneal injuries or diseases represent one of the main causes of a decreased quality of vision or even blindness worldwide. If the corneal damage is extensive and involves the limbal region, where limbal stem cells (LSCs) reside, the cornea cannot heal properly from the reason of stem cell deficiency. The only way to tread this deficiency is transplantation of limbal tissue or a transfer of LSCs. However, if the limbus is destroyed bilaterally, autologous LSCs are not available and alternative sources of stem cells have to be tested. We have shown previously that autologous mesenchymal stem cells (MSCs) when transferred onto damaged ocular surface, can support healing and improve characteristics of the cornea. The analysis of mechanisms of therapeutic action of MSCs showed antiinflammatory activity, production of numerous cytokines and growth factors by MSCs and a possible differentiation of MSCs into cornea-like cells (Čejka et al. 2016a). In this respect we showed that insulin-like growth factor-I, which is produced by corneal cells after injury, significantly enhances differentiation of MSCs into cells expressing cornea cell specific markers (Trosan et al. 2016). In the following study we demonstrated that therapeutic effect of MSCs for corneal healing can be further potentiated by co-application of MSCs and immunosuppressive drug cyclosporine A (Cejka et al. 2016b). For the transfer of MSCs onto damaged ocular surface we used various biocompatible nanofiber scaffolds. Using a rabbit model of alkali damaged ocular surface and nanofiber scaffolds loaded with LSCs, adipose tissue-derived MSCs or bone marrow-derived MSCs we demonstrated that bone marrow-derived MSCs can effectively replace tissue-specific LSCs, and thus can be used as autologous stem cells for ocular surface regeneration in cases when autologous LSCs are destroyed or unfunctional (Holan et al. 2015)

Cejka C., Holan V., Trosan P., Zajicova A., Javorkova E., Cejkova J.: The favorable effect of mesenchymal stem cell treatment on the antioxidant protective mechanism in the corneal epithelium and renewal of corneal optical properties changed after alkali burns. Oxid. Med. Cell. Longev., 2016; doi: 10.1155/2016/5843809, 2016a.
Cejka C., Cejkova J., Trosan P., Zajicova A., Sykova E., Holan V.: Transfer of mesenchymal stem cells and cyclosporine A on alkalli injured rabbit cornea using nanofiber scaffolds strongly reduces corneal neovascularization and scar formation. Histol. Histopathol. 31, 969-980, 2016b.
Holan V., Trosan P., Cejka C., Javorkova E., Zajicova A., Hermankova B., Chudickova M, Cejkova J.: A comparative study of the therapeutic potential of mesenchymal stem cells and limbal epithelial stem cells for ocular surface reconstruction. Stem Cells Translat. Med. 4, 1052-1063, 2015.
Trosan P., Javorkova E., Zajicova A., Hajkova M., Hermankova B., Kossl J., Krulova M., Holan V.: The supportive role of insulin-like growth factor-I in the differentiation of murine mesenchymal stem cells into corneal-like cells. Stem Cells Dev. 25, 874-881, 2016.

Immunoregulatory properties of MSCs

Experiments *in vivo* suggested that one of the main mechanisms of therapeutic effect of MSCs is a suppression of a local inflammatory reaction, which regularly occurs in the site of injury and have deleterious effect on tissue healing. Analysis of mechanisms of this therapeutic effect showed that multiple factors contribute to the effects of MSCs and that their therapeutic action depends on cytokine environment in the vicinity of the injured tissue (Holan et al. 2016). Analysis of the mechanism of action of MSCs on B cell population revealed a novel cell contact-dependent cyclooxygenase-2 pathway which is upregulated and highly effective on IFN-γ–treated MSCs (Hermankova et al.

2016). Knowledge of techniques for study of immunoregulatory properties of MSCs enabled us to perform study focused on comparison of immunoregulatory properties of bone marrow-derived MSCs isolated from patients with amyotrophic lateral sclerosis (ALS) and from healthy donors (Javorkova et al. 2019). The results suggested that MSCs isolated from patients with ALS retain similar phenotypic and immunoregulatory properties as have MSCs from healthy donors and thus can be used as autologous stem cells for cell-based therapy of this currently incurable diseases.

Hermankova B., Zajicova A., Javorkova E., Chudickova M., Trosan P., Hajkova M., Krulova M., Holan V.: Suppression of IL-10 production by activated B cells via a cell contact-dependent cyclooxygenase-2 pathway upregulated in IFN-γ-treated mesenchymal stem cells. Immunobiology 221, 129-136, 2016.
Holan V., Hermankova B., Bohacova P., Kossl J., Chudickova M., Hajkova M., Krulova M., Zajicova A., Javorkova E.: Distinct immunoregulatory mechanisms in mesenchymal stem cells: Role of the cytokine environment. Stem Cels Rev. Rep. 12, 654-663, 2016.
Javorkova E., Matejckova N., Zajicova A., Hermankova B., Hajkova M., Bohacova P., Kossl J., Krulova M., Holan V.: Immunomodulatory properties of bone marrow mesenchymal stem cells of patients with amyotrophic lateral sclerosis and healthy donors. J. Neuroimnune Pharmacol. 14, 215-225, 2019.

Impacts of opioids and nanoparticles on stem cells and cells of the immune system

The established immunological methods and the experience with study of stem cells enabled us to test impacts of opioids and nanoparticles on stem cells. Using human bone marrow-derived MSCs and opioid drug morphine we described negative effects of morphine on phenotypic and function properties of MSCs. Morphine inhibited in dose-dependent manner proliferation of MSCs, decreased their secretion of cytokines and growth factors and attenuated their differential potential (Holan et al. 2018). We concluded that these negative impacts of opioids on stem cells might be at least one of the mechanisms of slow down and aberrant healing and regeneration of injured or damaged tissues observed in opioid users or patients treated with these analgesic drugs. In another study, we tested, in collaboration with colleagues from the Department of Genetic Toxicology and Nanotoxicology, the effect of the long-term continuous inhalation of CuO nanoparticles on composition of populations and reactivity of cells of the immune system. We observed that the exposure of mice to nanoparticles significantly altered the composition of cells of the innate immune system and influenced reactivity of cells of adaptive immunity. The proliferation and production of cytokines by T and B lymphocytes were significantly changed in mice exposed to nanoparticles, but the impacts of nanoparticles depended on the time of exposure (Holan et al. 2019). This study stressed danger of nanoparticle inhalation for the functions of cells of the immune system and for human health.

In the study using morphine, our partner from Institute of Physiology provided the drug and performed experiments focused on detection of opioid receptor expression on stem cells. All other results were obtained in our Department. In the study of the effect of inhalation of nanoparticles on the immune system, the model of particle inhalation was established by our collaborating partners and we performed experiments with characterization of immune cell populations and testing reactivity of cells of the immune system of mice exposed to particles.

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Interactions of stem cells and immunosuppressive drugs

Since, in some clinical settings, the stem cell-based therapy can be running simultaneously with immunosuppressive therapy, or immunosuppressive drugs are administered to attenuate immune response associated with the use of allogeneic stem cell therapy, we tested effects of conventional immunosuppressive drugs on stem cells. Using a panel of selected immunosuppressive drugs we tested effects of these drug on phenotype and function properties of human MSCs. We found significant differences in the effects of individual drugs on characteristics of MSCs (Javorkova et al. 2018). In other study, we tested influence of MSCs on the inhibitory effects of immunosuppressive drugs on functions of distinct T cell subpopulations. In these studies we observed that MSCs selectively attenuated adverse effects of immunosuppressive drugs (Hajkova et al. 2017). Nevertheless, combined treatment of recipients with MSCs and cyclosporine A after skin transplantation was more effective than monotherapy with MSCs or cyclosporine A, and the inhibition of activity of proinflammatory Th17 cells was proved as the main mechanism of suppression in this combined therapy (Hajkova et al. 2019).

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Perspectives of stem cell therapy for the treatment of retinal degenerative diseases

Retinal degenerative diseases, such as age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa or glaucoma, represent the main cause of a decreased quality of vision or even blindness worldwide. All these disorders are associated with a loss of retinal cells and with a local inflammatory reaction. However, for all these diseases are not available treatment protocols to prevent, stop or even treat these disorders. We proposed that MSCs by their differentiation potential, immunosuppressive properties or by a production of growth factors could replace missing retinal cells, inhibit undesirable inflammatory reaction and support surviving of remaining retinal cells (Holan et al. 2017). We demonstrated that MSCs can differentiate into cells expressing markers and characteristics of retinal cells. For the differentiation we established a novel protocol involving retinal cell extracts and supernatant from activated spleen T cells to mimic inflamatous environment of diseased retina. In this protocol, we identified IFN-γ as the most important cytokine supporting retinal differentiation of murine MSCs (Hermankova et al. 2017). To test therapeutic potential of MSCs for retinal diseases, we established a model of retinal inflammation by the intravitreal administration of proinflammatory cytokines. Using this model we showed that intravitreally delivered MSCs inhibited infiltration with immune cells and suppressed local inflammatory reaction (Hermankova et al. 2019). In

following studies we demonstrated the ability of MSCs to inhibit pharmacologically induced retinal cell degeneration. All these observations supporting the idea of the perspective use of MSCs for the treatment of retinal diseases were summarized in the paper Holan et al. (2019).

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