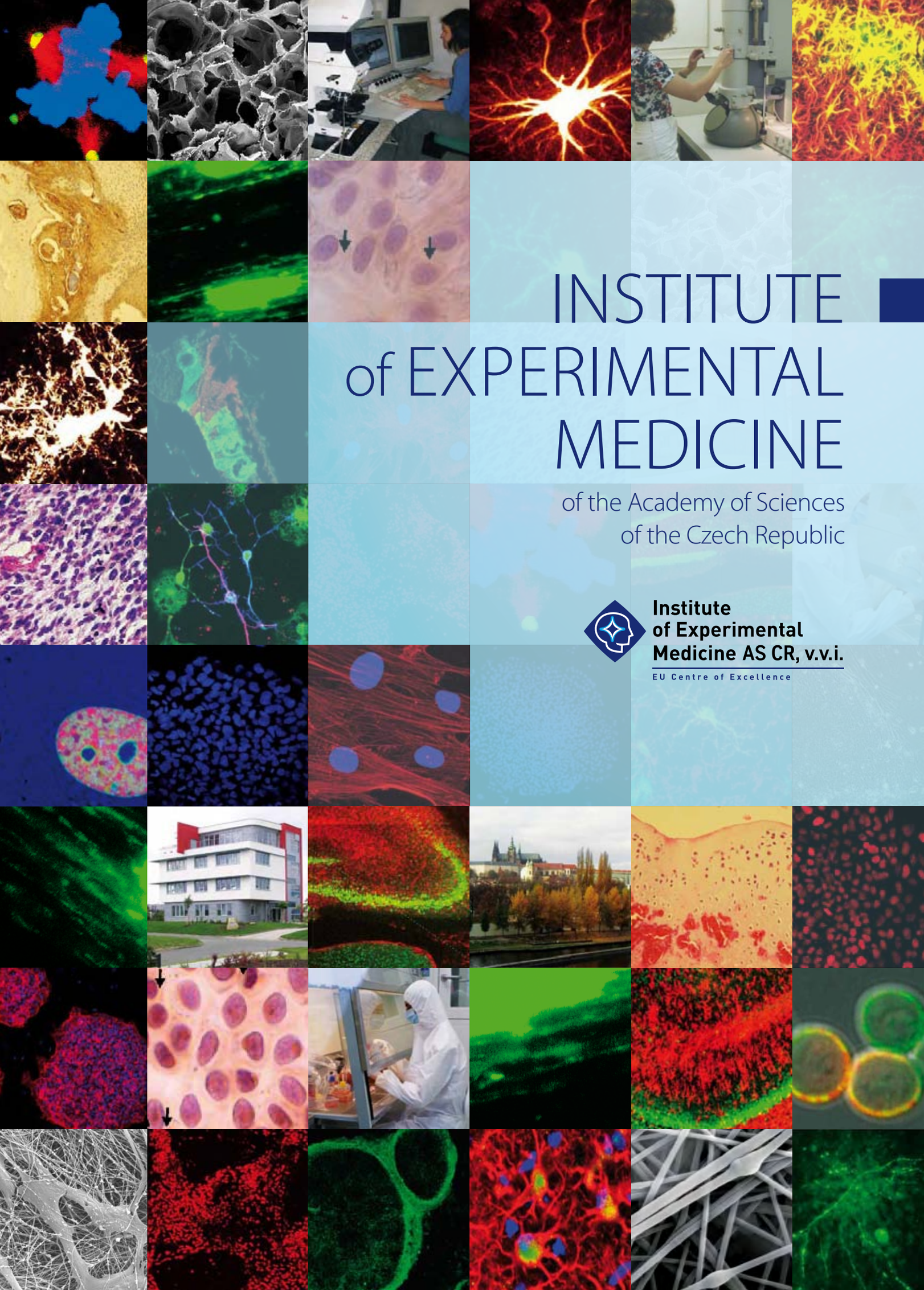

INSTITUTE of EXPERIMENTAL MEDICINE

of the Academy of Sciences of the Czech Republic

Institute of Experimental Medicine AS CR, v.v.i.

EU Centre of Excellence

Institute of Experimental Medicine AS CR, v.v.i.

EU Centre of Excellence

PREFACE

The Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, is the leading institution in the Czech Republic for biomedical research, particularly in cell biology and pathology, neurobiology, neurophysiology, neuropathology, developmental toxicology and teratology, molecular pharmacology, immunopharmacology, molecular embryology, stem cells and nervous tissue regeneration. The Institute is an internationally recognized center in these fields, and as such it was selected as an EU Center of Excellence (MEDIPRA).

The institute's already-established position is documented by the extensive collaboration that exists between Institute scientists and their colleagues in Europe, North America, Asia and Australia. Recently, a number of foreign Ph. D. students, postdoctoral fellows, as well as senior scientists have been working in our departments, financed from our institutional budget as well as from a number of projects of the European Commission's 6th and 7th Framework Programs. The Institute regularly organizes a number of international workshops, symposia and conferences as well as summer schools for young researchers.

We are proud to collaborate with leading Czech institutions, namely with Charles University's First, Second and Third Medical Faculties, the Faculty of Natural Sciences of Mendel University in Brno, the Institute for Clinical and Experimental Medicine (IKEM) of the Ministry of Health, the Regional Institute for Hygiene of Central Bohemia, the Institute of Macromolecular Chemistry ASCR, and the Institute of Animal Physiology and Genetics ASCR. Scientists of the Institute are involved in both the pregraduate and postgraduate training of students. These ties have been strengthened by the founding of two joint organizations: with the Institute of Neuroscience of the Second Medical Faculty, Charles University and the national research Center for Cell Therapy and Tissue Repair, Charles University and with the Institute of Hematology and Blood Transfusion.

Currently the Institute of Experimental Medicine consists of 10 departments and 2 independent laboratories. There are about 217 employees, including 88 university graduates and 44 foreign students or visitors, working in the Institute. The publication activity of the Institute is growing every year, and most of its publications appear in journals with high impact factors including Physiological Reviews, Nature, Cell, Trends in Neuroscience, Trends in Pharmacology, The Journal of Cell Biology, NeuroImage, Journal of Physiology, Journal of Cell Science, Molecular Pharmacology, Biophysical Journal, Carcinogenesis, Glia, Journal of Cerebral Blood Flow and Metabolism, Journal of Dental Research, Hippocampus, Leukemia and Journal of Leukocyte Biology.

The current research areas in the Institute of Experimental Medicine are a result of its history. It was officially founded in 1975 by combining four medical research laboratories that had been organized twenty years before. Three of the laboratories had been affiliated with clinical departments of Charles University, i. e., the Department of Plastic Surgery, the Department of Ophthalmology, and the Department of Otorhinolaryngology. The fourth laboratory was closely connected with the Department of Histology of the First Medical Faculty and was oriented toward cell and tissue ultrastructure. Under the leadership of the renowned professors Burian, Kurz, Přecechtěl and Wolf, the laboratories established themselves in the world of medicine and contributed significantly to the international recognition of Czechoslovak medical research. The four laboratories, although intellectually strong and reasonably well-equipped, suffered from physical isolation and lack of collaboration. Therefore, it was considered proper to join the laboratories and to establish an Institute under the Czechoslovak Academy of Sciences. An otolaryngologist, Professor Vlastimil Kusák, was appointed as the first director (1975–1984). The research spectrum was extended by inviting to the Institute a group of immunologists (Dr. Jiří Franěk, Dr. Karel Nouza), and by establishing a laboratory to investigate the health effects of mycotoxins in Eastern Bohemia (Olešnice, Eagle Mountains).

In the seventies and eighties the profile of the Institute crystallized, particularly in the period when most of the laboratories were transferred to a building on Legerova street and subsequently when Professor Jiří Elis was appointed director (1984–1990). Research areas broadened to include the electron microscopic investigation of the cell nucleus and nucleolus, particularly in blood cells; the morphological tracing of nucleic acids; the morphology and immunocytochemistry of the thyroid gland and pancreas; mechanisms of local immunity, cancer immunity and graft-versus-host reaction; biochemistry and histochemistry of the eye; corneal pathology and the testing of contact lenses; the morphology of the inner ear and its changes under the influence of noise; the electrophysiology of the central auditory system; the basics of genotoxicity and teratology; the mechanisms and epidemiology of craniofacial malformations; and the testing of mycotoxins. While several groups and individuals succeeded in reaching a high standard of scientific work, the Institute as a whole suffered from scattered topics, a lack of internal communication and many other obstacles characteristic of life in the seventies and eighties.

In the beginning of the nineties, several parallel processes led to the harmonizing of the scientific orientation of the Institute as well as of its human capital. These processes comprised not only the change in the political situation in the country but also a significant rejuvenation of the Institute. In 1990, Professor Jelínek, Head of the Laboratory of Teratology, was appointed director of the Institute (1990–1994). The structure of the Institute was reorganized on the basis of a free competition of internal projects and further strengthened by its success rate in the competition for grants from the Grant Agency of the Academy of Sciences. The involvement of members of the Institute in both the teaching of medical students and in ecologically oriented research increased, particularly concerning the adverse effects of exogenous factors on the organism.

Important for the formation of the improved profile of the Institute was the entrance of two new strong scientific groups in 1991 – the Laboratory of Cellular Neurophysiology from the Institute of Physiological Regulations, headed by Professor Eva Syková, and the Laboratory of Genetic Ecotoxicology, headed by Dr. Radim Šrám (a joint laboratory with the Regional Hygiene Station of Central Bohemia). Clinically oriented groups ceased to exist in the Institute or were transferred to clinics. In 1993 the Institute moved to a new building in Prague-Krč, where several other biomedical institutes of the Academy of Sciences are located. In 1994 Professor Josef Syka was appointed director (1994–2001). In the same year the Institute passed successfully through the evaluation process within the Academy of Sciences. Important changes in the organization of the Institute that have taken place since that time have focused its orientation and improved its scientific profile.

In 2001 Professor Eva Syková, was appointed as director. In 2002 the Institute's research program further increased through the establishment of four new groups, growing to its current size. The reason for this change was the affiliation of the former Institute of Pharmacology ASCR and the Department of Molecular Embryology from the Institute of Animal Physiology and Genetics ASCR.

At present, the Institute of Experimental Medicine belongs to the biomedical group of research institutes of the Academy of Sciences of the Czech Republic and is the only institute in the Czech Republic engaged in a comprehensive medical research program encompassing a number of diverse fields, based on the projects documented in this brochure.

Eva Syková, MD, DSc
Professor of Physiology
Director

Prague, July 2009

ORGANIZATIONAL SCHEME OF THE INSTITUTE

DIRECTOR

- Board of the Institute
- Supervisory Board
- Board of Directors
- Vice Director
- Commissions (attestation, ethical committee)
- Scientific Secretary

Management Units	The Innovation Biomedicine Center	Research Units	Associated Research Centers
Secretarial Units	Center of Support for Competitiveness in Biomedicine	Department of Neuroscience	Department of Neuroscience, 2nd Medical Faculty, Charles University
Accounting and Economic Department	Center of Applied Research in Biomedicine	Department of Auditory Neuroscience	Department of Biology, Masaryk University, Brno
Scientific Documentation Unit	Business Incubator	Department of Cell Neurophysiology	Center for Cell Therapy and Tissue Repair, Charles University
Microscopy Unit		Department of Teratology	
Department of Technology Transfer		Department of Genetic Ecotoxicology	
		Department of the Molecular Biology of Cancer	
		Department of Molecular Embryology	
		Department of Pharmacology	
		Laboratory of Tissue Engeneering	
		Laboratory of Cell Biology	

MANAGEMENT

Director:
Prof. Eva Syková, MD, DSc

Vice Director:
Assoc. Prof. Alexandr Chvátal, DSc, MBA

Chairman of the Board of the Institute:
Prof. Eva Syková, MD, DSc

Chairman of the Supervisory Board:
Jan Hrušák, PhD

ADDRESS AND CONTACT

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INSTITUTE PROFILE

BOARD OF THE INSTITUTE

The Board is the major management body of the Institute. It shares responsibilities for the operation of the Institute with the director. The Act on Public Research Institutions stipulates the statutory duties of the Board. The Board observes the basic objectives of the Institute as stated in its statute, sets the basic scientific orientation of research at the Institute and its development strategy, and approves the Institute's budget as well as its annual report. The Board consists of both internal and external members.

INTERNAL MEMBERS:

- Prof. Eva Syková, MD, DSc
  (president of the Board and Director of the Institute)
- Radim J. Šrám, MD, DSc.
  (vice-president of the Board)
- Prof. Josef Syka, MD, DSc
- Assoc. Prof. Miroslav Peterka, MD, DSc
- Assoc. Prof. Alexandr Chvátal, PhD, DSc, MBA
- Zdeněk Zídek, PhD, DSc
- Assoc. Prof. Aleš Hampl, DVM, PhD
- Pavla Jendelova, PhD
- Pavel Vodička, MD, PhD

EXTERNAL MEMBERS:

- Milan Hájek, DSc
- Prof. Rastislav Druga, MD, DSc
- Prof. Miroslav Ryska, MD, PhD
- Štefan Vítko, MD, PhD

SUPERVISORY BOARD OF THE INSTITUTE

The Supervisory Board of the Institute is another obligatory body of the Institute mandated by law. It exercises supervisory responsibilities regarding the operation and management of the Institute and gives prior consent to intended legal actions of the Institute as defined by law (e. g., the sale or purchase of a property, the establishment of a company or other legal person and/or the holding of shares in such a company, the signing of an occupational lease etc.). The Supervisory Board shall meet at least two times a year.

THE CURRENT BOARD HAS FIVE MEMBERS:

- Jan Hrušák, PhD
  (Academy of Sciences of the CR)
- Petr Bažant, PhD, MBA
  (Institute of Experimental Medicine AS CR)
- Prof. Miloš Grim, MD, DSc
  (Charles University in Prague, 1st Faculty of Medicine)
- Prof. Ivan Míšek, DVM, PhD
  (Institute of Animal Physiology and Genetics AS CR)
- Prof. Eduard Zvěřina, MD, DSc
  (Charles University in Prague, 1st and 3rd Faculties of Medicine)

The Institute of Experimental Medicine (IEM) is a renowned center of biomedical research, manifested by the fact that the Institute was awarded the status of an EU Center of Excellence – MEDIPRA, by the participation of the Institute's scientists in projects of the 6th and 7th EU Framework Programmes and by its extensive collaborations on both national and international levels. Both Czech and foreign Ph. D. students work in the Institute, funded by EU grants as well as by the Institute's core budget. The Institute supports young group leaders and is a partner in the EU project ENI-NET, bringing together leading European institutes in the field of neuroscience; it is the seat of the biomedical commission for awarding DSc. degrees; and it has the highest percentage of Ph. D. students among all the institutes of the Academy of Sciences. The outcomes of the Institute's research, which have already been applied in practice, include those in the fields of environmental protection, neuroscience, regenerative medicine, pharmacology and diagnostic methods. Currently the Institute consists of 10 departments and 2 independent laboratories.

DEPARTMENTS:

1. **DEPARTMENT OF NEUROSCIENCE**
2. **DEPARTMENT OF AUDITORY NEUROSCIENCE**
3. **DEPARTMENT OF CELLULAR NEUROPHYSIOLOGY**
4. **DEPARTMENT OF MOLECULAR EMBRYOLOGY**
5. **LABORATORY OF TISSUE ENGINEERING**
6. **DEPARTMENT OF TERATOLOGY**
7. **DEPARTMENT OF GENETIC ECOTOXICOLOGY**
8. **DEPARTMENT OF THE MOLECULAR BIOLOGY OF CANCER**
9. **LABORATORY OF CELL BIOLOGY**
10. **DEPARTMENT OF PHARMACOLOGY**
11. **MICROSCOPY UNIT**
12. **DEPARTMENT OF TECHNOLOGY TRANSFER / IBC**

Research workers of the Institute, Czech as well as foreign, also work in the following three research centers of the Ministry of Education:

CENTER FOR CELL THERAPY AND TISSUE REPAIR

(Head: Prof. Eva Syková, MD, DSc)

combines a number of sites. Important findings have been made in stem cell transplantation in models of pathologies of the central nervous system, pancreas, liver, epidermis, brain and spinal cord, the labelling of cells with superparamagnetic nanoparticles, the development of imaging methods and the development of materials for tissue repair.

CENTER FOR NEW ANTIVIROTICS AND ANTINEOPLASTICS

(Head: Zdeněk Zídek, PhD, DSc)

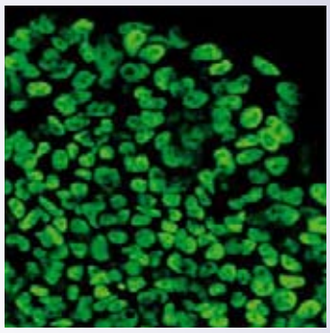
The Department of Pharmacology takes part in pharmacological research, for instance in the discovery of substances with immunomodulatory activity, significant in HIV therapy.

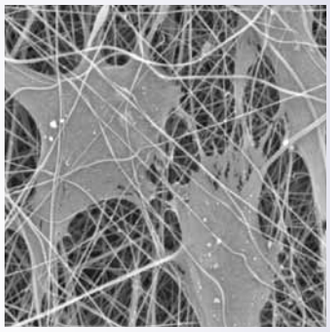
CENTER FOR NEUROSCIENCE

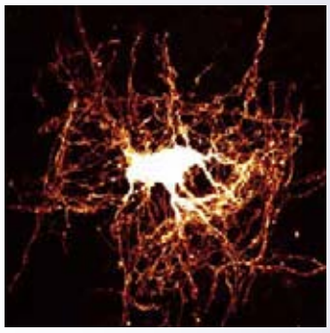
(Head: Prof. Josef Syka, MD, DSc)

joins together significant research sites in Prague in the field of neuroscience and takes part in research into the mechanisms of ionic channels, glial cells, synaptic and extrasynaptic transmission, the central mechanisms of hearing and pain, and the mechanisms underlying brain and spinal cord diseases.

DEPARTMENTS







DEPARTMENT OF NEUROSCIENCE

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LABORATORY OF TISSUE CULTURE AND STEM CELLS

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Takashi Amemori, DVM, PhD | Research Scientist
Šárka Kubinová, PharmD, PhD | Research Scientist
Nataliya Kozubenko, PhD | Research Scientist
Jiří Šedý, MD, PhD | Research Scientist
David Arboleda, MSc | PhD Student
Miroslava Kapcalova, MSc | PhD Student
Karolína Turnovcová, MD | PhD Student
Serhiy Forostyak, MD | PhD Student
Aleš Hejčl, MD | PhD Student
Petr Lesný, MD | PhD Student
Václav Vaněček, PharmD | PhD Student
Magdalena Kulijewicz, MSc | PhD Student
Dana Uhrova MSc | PhD Student
Pavlína Macková | Technician
Michal Douděra | Technician

LABORATORY OF DIFFUSION STUDIES AND IMAGING METHODS

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Assoc. Prof. Lýdia Vargová, MD, PhD | Research Scientist
Katarína Likavčanová-Mašínová, PhD | Research Scientist
Ivan Voříšek, PhD | Research Scientist
Aleš Homola, MD, PhD | Research Scientist
Lesia Dmytrenko, MSc | PhD Student
Michal Cicanič, MD | PhD Student
Oksana Forostyak, MD | PhD Student
Hana Hronová | Technician
Helena Pavlíková | Technician

LABORATORY OF EYE HISTOCHEMISTRY AND PHARMACOLOGY

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Assoc. Prof. Jitka Čejková, MD, DSc | Research Scientist
Taras Ardan, MD, PhD | Research Scientist
Čestmír Čejka, MSc | PhD Student

Laboratory of Tissue Culture and Stem Cells

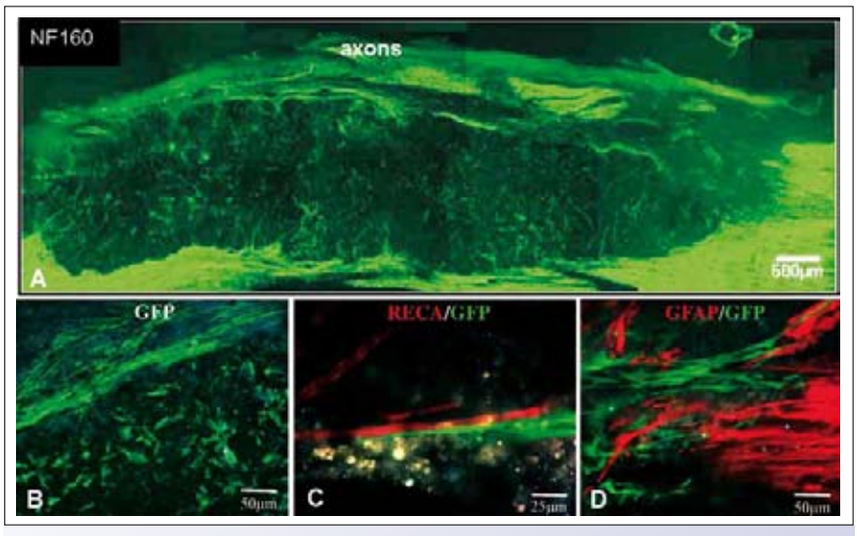
RESEARCH TOPICS

- Characterization of adult and embryonic stem cells *in vitro;*
- development of nanoparticles for cell labeling suitable for *in vivo* cell tracking;
- cultivation and differentiation of human embryonic stem cells into a neuronal phenotype;
- phenotyping of stem cells by means of flow cytometry;
- regeneration and repair of stroke lesions using human embryonic stem cells;
- regeneration and repair of injured spinal cord using stem cells and biomaterials;
- analysis of the growth factors and cytokines released from injured and tumor tissue and their role in the homing of MSCs to the lesions;
- cell-polymer constructs designed to bridge lesions of the central nervous tissue;
- nanofiber scaffolds for two- and three-dimensional cell cultivation.

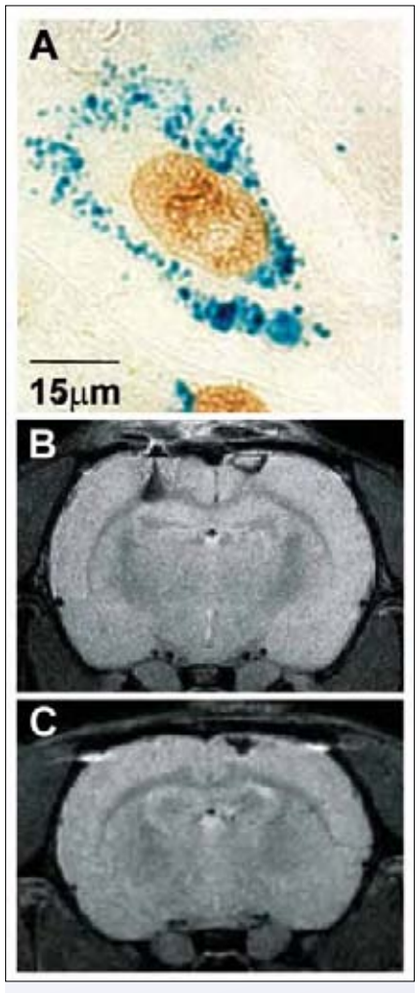
REGENERATION OF BRAIN AND SPINAL CORD INJURY USING STEM CELLS

Injury of the adult CNS, such as spinal cord trauma or stroke, invariably results in the loss of neurons and the loss of axonal processes involved in the lesion. This often results in severe functional impairment, due to the formation of complex scar tissue within the cavity as the result of cell death, inflammation and tissue degradation. Stem and progenitor cells from various sources are currently being investigated for their potential to treat CNS injury and numerous neurodegenerative diseases. Transplanted stem cells can either REPAIR damaged tissue by replacing missing populations of cells or RESCUE cells in the injured brain or spinal cord by the production of cytokines (interleukins) and/or neurotrophic factors that facilitate regeneration and/or revascularization.

In our projects we study adult stem cells (isolated from bone marrow, peripheral blood or fat tissue) as well as human embryonic stem cells and immortalized fetal spinal cord cells for the treatment of stroke and spinal cord injury. We use photochemical lesion and middle cerebral artery occlusion (MCAO) models to investigate stroke, while hemisection and balloon-induced compression lesions are employed as acute and chronic models of spinal cord injury, respectively. We evaluate the rescue



Bridging a chronic spinal cord lesion with biomaterials.
(A) Ingrowth of axons (staining for neurofilaments NF160) into a hydrogel implant that completely filled the post-traumatic cavity left after SCI.
(B) Cell/hydrogel implant 2 months after implantation. Mesenchymal stem cells survived in the implanted hydrogel and migrated towards the spinal cord stump.
(C) One month after implantation, MSCs facilitate the ingrowth of astrocytes into the implant by forming guiding strands towards the hydrogel.
(D) Similarly, blood vessels grow in close contact with MSCs.



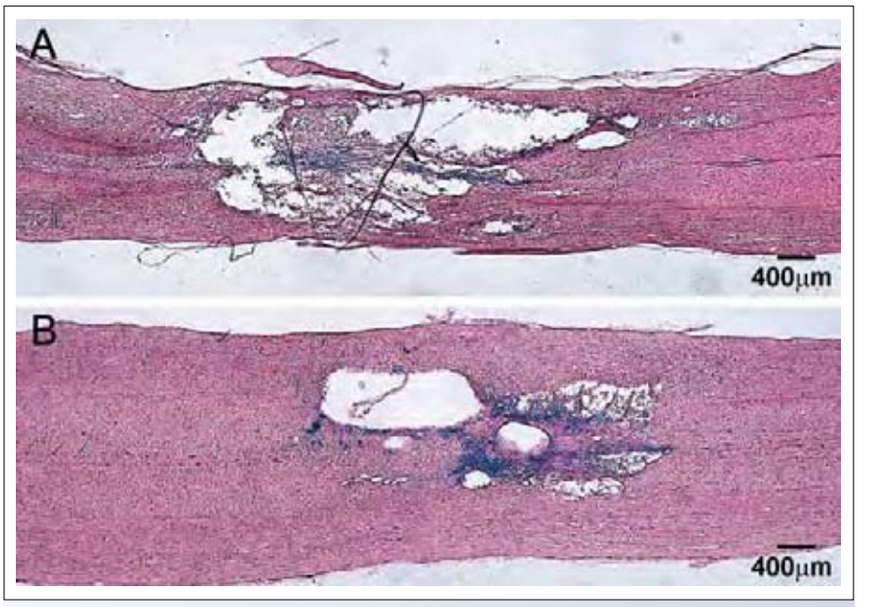
Labeling of stem cells with iron-oxide nanoparticles.
(A) A cell labeled with iron-oxide nanoparticles undergoing cell division (staining for BrdU), confirming that the incorporation of nanoparticles does not adversely affect cell viability.
(B) T-2 weighted image of a cortical photochemical lesion and mouse embryonic stem cells implanted into the contralateral hemisphere two weeks after implantation. The cell implant in the hemisphere contralateral to the lesion as well as the lesion itself are visible as hypointense areas.
(C) A hypointense signal in the lesion observed thirty days after the intravenous injection of MSCs labeled with nanoparticles.

as well as the repair effect of the cells used in the treatment by means of behavioral tests, histology and immunohistochemistry.

CELL LABELING

For the success of cell therapy it is important to monitor the fate of transplanted cells *in vivo*. One such approach involves the use of superparamagnetic iron oxide nanoparticles as labels for cell tracking.

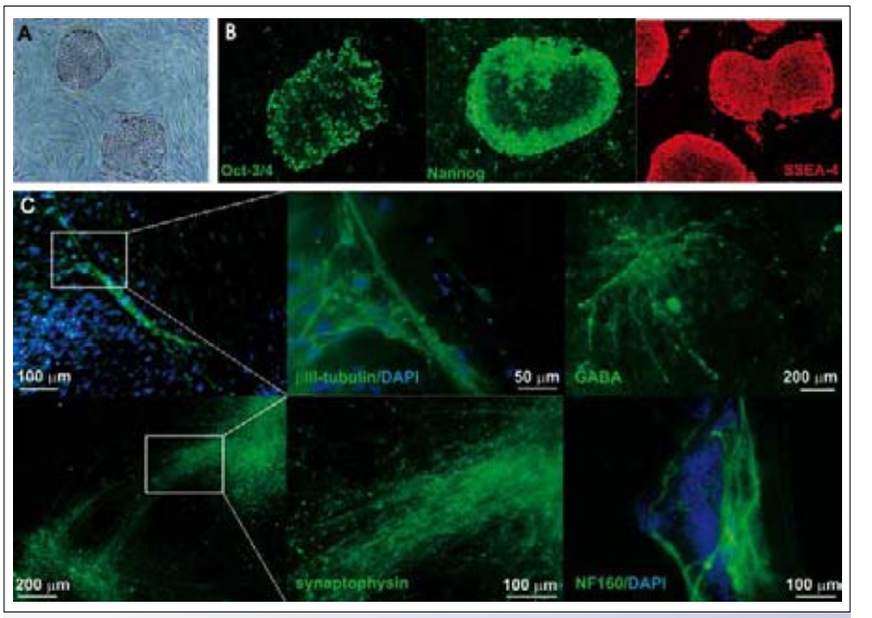
In collaboration with the Institute of Macromolecular Chemistry ASCR, we have developed and patented several types of iron oxide nanoparticles with modified coatings that can be used for cell labeling and *in vivo* tracking by MR imaging. Cells labeled with these nanoparticles exhibit better viability and improved labeling efficiency in combination with a lower concentration of iron within the cells when compared with commercial contrast agents, such as Endorem, (Guerbet, France).



MSCs labeled with nanoparticles implanted into rats with a spinal cord compression lesion.
(A) Prussian blue staining of a spinal cord compression lesion. Only a few weakly stained Prussian blue-positive cells are found in the area of a spinal cord lesion without cell implantation. (B) Prussian blue staining of a spinal cord lesion with intravenously injected nanoparticle-labeled MSCs. The lesion is populated with Prussian blue-positive cells. Note the smaller lesion size in implanted animals than in controls.

CELL MIGRATION

Though mesenchymal stem cells (MSCs) have a positive effect on functional outcome after spinal cord injury (SCI), it is still not clear what is the mechanism of action of MSCs in the lesion, what mechanisms are involved in attracting the MSCs into the site of SCI and why the cells migrate towards the lesion. Therefore, we study the mechanisms underlying the migration and engraftment of MSCs into a spinal cord injury from the point of view of chemotactic migration and cytokine expression. In collaboration with the University of Bergen, we also investigate the ability of MSCs to migrate to tumor tissue and the possibilities for their use in anti-cancer therapy.



In vitro differentiation of human embryonic stem cells (hESCs).
(A) Colony of undifferentiated hESCs growing on a feeder layer. (B) Set of surface markers typical of undifferentiated hESCs. (C) Two to four weeks after the induction of neuroectodermal differentiation in vitro, the cells express markers of differentiated neurons.

SCAFFOLDS AND POLYMER CONSTRUCTS

Poly(2-hydroxyethyl methacrylate) (pHEMA) and poly N-(2-hydroxypropyl)-methacrylamide hydrogels (pHPMA) belong to a group of synthetic, highly biocompatible polymers. In SCI repair they serve as a bridge for axonal growth across the lesion cavities. They also prevent scarring and thus create a permissive environment for tissue regeneration. In our Laboratory we investigate these hydrogels in combination with stem cells and/or scar-degrading enzymes and/or growth factors as

bridges and cell carriers that support and facilitate regeneration after SCI. Another type of scaffold consists of electrospun nanofibers that can be used for cell culturing and for the transfer of cells into the host organism.

CLINICAL STUDIES

Based on recent experimental studies, autologous bone marrow cell (BMC) implantation is used in our Phase I/II clinical trial in patients (n = 33) with a traumatic spinal cord lesion at Motol Hospital in Prague.

Another clinical study involves the use of BMCs and MSCs in the treatment of patients with a lower limb ischemic disease. The study is performed in collaboration with the Institute for Clinical and Experimental Medicine in Prague.

CURRENT GRANT SUPPORT

GA CR, 203/09/1242, Surface-modified magnetic nanoparticles for cell labeling and in vivo and in vitro diagnostics, 2009–2011.

GA AS CR, IAA500390902, The use of stem cells and biomaterials for spinal cord injury repair, 2009–2012.

Ministry of Education, 1M0538, Center for cell therapy and tissue repair, 2005–2009.

GA AS CR, KAN,201110651, Combined contrast agents for molecular MR imaging, 2006–2010.

GA AS CR, KAN,200520804, Biocompatible nanofibers for application of biologicaly and pharmacologicaly active substances, 2008–2012.

GA CR, 304/07/1129, Polarised cultures of hepatocytes and mesenchymal cells on nanofiber membranes in an experimental bioreactor, 2007–2011.

EU 6th FP, LSHC-CT-2004-504743, Targeting-Tumour-Vascular/Matrix Interactions, ANGIOTARGETING, 2004–2009.

EU 6th FP, CA LSHB-CT-2005-518233, From stem cell technology to functional restoration after spinal cord injury, RESCUE, 2005–2009.

EU 6th FP, LSHB-CT-2006-037328, STREP, Pre-clinical evaluation of stem cell therapy in stroke, STEMS, 2006–2010.

EU 6th FP, LSHB-CT-2005-512146, Diagnostic Molecular Imaging: A Network of Excellence for Identification of NEW Molecular Imaging Markers for Diagnostic Purposes, DiMI, 2005–2010.

EU 6th FP, MSCF-CT-2006-046102, Spring School on Regenerative Medicine – how to use neuronal stem cells for science and business, RegMedTeach, 2006–2009.

EU 6th FP, MEST-CT-2005-019729, EST: Cooperation in research and training for European excellence in neuroscience, CORTEX, 2006–2009.

EU 6th FP, LSHM-CT-2005-019063: Network of European neuroscience institutes, ENINET, 2005–2009.

EU 7th FP, Programme PITN-GA-2008-214003, Axonal regeneration, plasticity & stem cells, AXREGEN, 2008–2012.

SELECTED RECENT PUBLICATIONS

1. Jendelová P, Herynek V, Urdzíková L, Glogarová K, Kroupová J, Bryja V, Andersson B, Burian M, Hájek M, Syková E. (2004) MR tracking of transplanted bone marrow and embryonic stem cells labeled by iron oxide nanoparticles in rat brain and spinal cord. J Neurosci Res 76: 232–243.

2. Jendelova P, Herynek V, Urdzíková L, Glogarová K, Rahmatová S, Fales I, Andresson B, Procháka P, Zámečník J, Eckschlager T, Kobylka P, Hájek M, Syková E. (2005) MR Tracking of CD34+ progenitor cells separated by means of immunomagnetic selection and transplanted into injured rat brain. Cell Transplant 14: 173–182.

3. Syková E, Jendelová P, Urdzíková L, Lesný P, Hejčl A. (2006) Bone marrow stem cells and polymer hydrogels-two strategies for spinal cord injury repair Cell Mol Neurobiol 26(7–8): 1113–1129.

4. Syková E, Homola A, Mazanec R, Lachmann H, Konrádová SL, Kobylka P, Pádr R, Neuwirth J, Komrska V, Vávra V, Stulík J, Bojar M. (2006) Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. Cell Transplant 15(8–9): 675–687.

5. Syková E, Jendelová P. (2007) Migration, fate and *in vivo* imaging of adult stem cells in the CNS. Cell Death Differ 14(7): 1336–1342.

6. Hejčl A, Urdzíková L, Šedý J, Lesný P, Přádný M, Michálek J, Burian M, Hájek M, Zámečník J, Jendelová P, Syková E. (2008) Acute and delayed implantation of positively charged HEMA scaffolds in spinal cord injury in the rat. J Neurosurg–Spine 8 (1): 67–73.

7. Babič M, Horák D, Trchová M, Jendelová P, Glogarová K, Lesný P, Herynek V, Hájek M, Syková E. (2008) Poly(l-lysine)-Modified Iron Oxide Nanoparticles for Stem Cell Labeling. Bioconjug Chem 19(3): 740–750.

Laboratory of Diffusion Studies and Imaging Methods

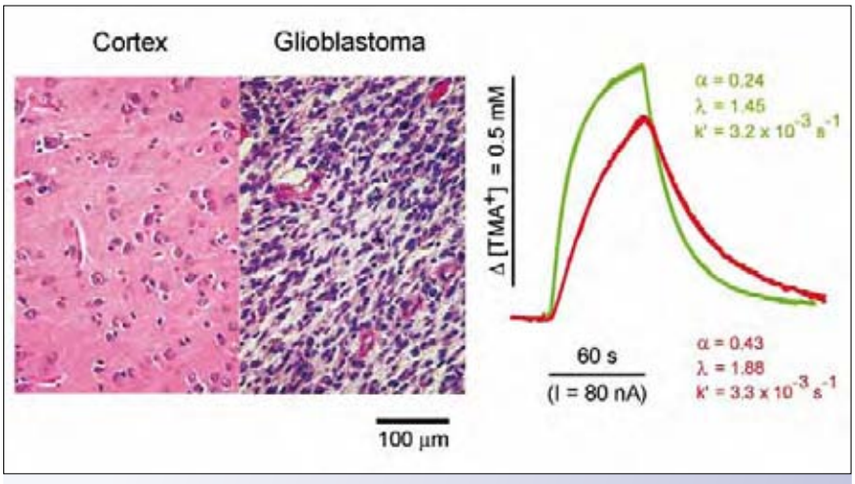
RESEARCH TOPICS

The Laboratory of Diffusion Studies and Imaging Methods studies the changes in the extracellular space diffusion parameters and extrasynaptic (volume) transmission that occur during physiological and pathological states.

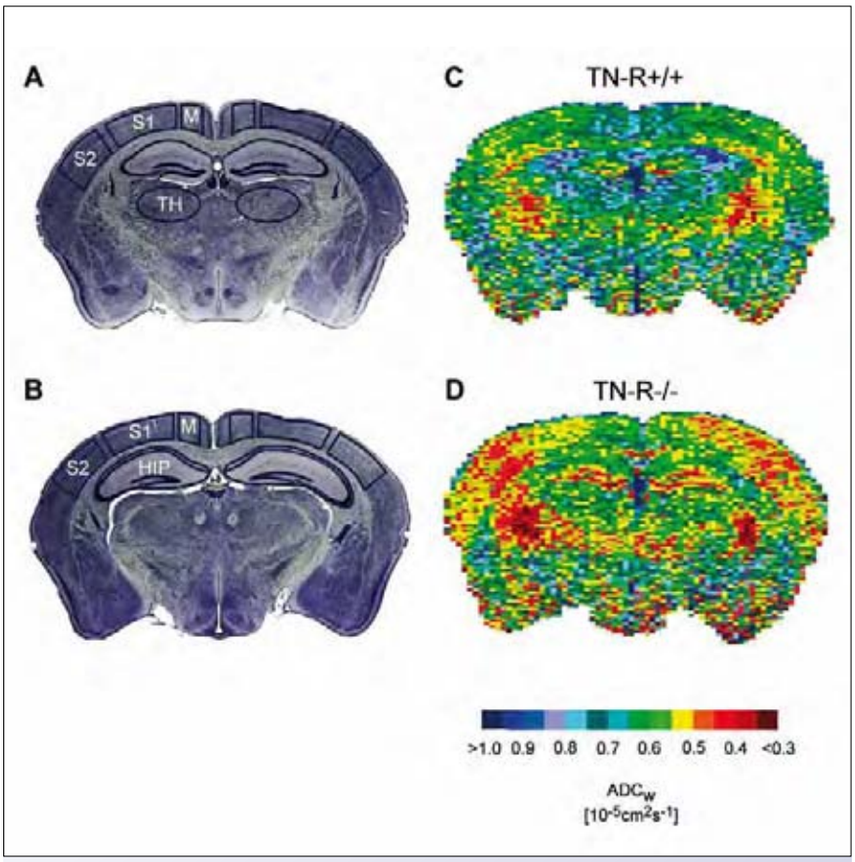
Several animal models of pathological states and diseases attacking the CNS are used, e. g., models of chronic pain, ischemia and ischemic lesions, perinatal and early postnatal anoxia, brain edema, hydrocephalus, multiple sclerosis, Parkinson's disease, Alzheimer's disease, tumors, epilepsy, developmental disorders, aging, and brain and spinal cord injury, as well as models of CNS damage evoked by chemical or physical factors such as neurotoxins or X-irradiation. The research aims are the improvement of therapy and diagnostic methods for CNS diseases and the prevention of CNS damage.

THE CURRENT RESEARCH FOCUSES ON:

- The origin, mechanisms and pathophysiological significance of ionic changes in the extracellular space;
- diffusion in the extracellular space: the underlying mechanism of extrasynaptic („volume") transmission;
- diffusion studies using the real-time TMA^+ iontophoretic method;
- diffusion studies using diffusion-weighted MR to measure the apparent diffusion coefficient of water;
- extracellular space volume and geometry – factors affecting diffusion in the CNS in health and disease;
- studies using models of pathological states, including transgenic animals;



Diffusion parameters in a glioblastoma.
Hematoxylin-Eosin staining of the human brain cortex and a glioblastoma (WHO grade IV) and representative TMA⁺ diffusion curves with the corresponding values of the ECS diffusion parameters α, λ and k´. In comparison with healthy tissue, the ECS volume fraction (α) is almost doubled and the tortuosity (λ) is significantly increased in a highly malignant glioblastoma.



Typical apparent diffusion coefficient of water (ADC_w) maps in tenascin TN-R+/+ and TN-R–/– mice.
ADC_w was calculated in five selected areas: the motor cortex (M), the primary somatosensory cortex (S1), the secondary somatosensory cortex (S2), the hippocampus (HIP) and the thalamus (TH). (A and B) The areas are outlined in the microphotographs of Cresyl Violet-stained slices. (C and D) The images show ADC_w maps of TN-R+/+ and TN-R–/– mice; both images are from the same coronal plane as shown in (B). The scale at the bottom of the figure shows the relation between the intervals of ADC_w values and the colors used for visualization. Note the lower ADC_w throughout the whole slice from the TN-R–/– mouse when compared with the TN-R+/+ control.

– magnetic resonance imaging and spectroscopy.

Studies at the Laboratory are aimed at understanding the maintenance of ionic and volume homeostasis in the CNS, the extracellular space as a communication channel, the diffusion parameters of the extracellular space, extrasynaptic „volume" transmission and the role of glia in signal transmission, behavior and regeneration.

CURRENT GRANT SUPPORT

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Ministry of Education, 1M0538, Research center: Center for Cell Therapy and Tissue Repair, 2005–2009.

Ministry of Education, LC554, Research center: Center of Neuroscience, 2005–2009.

GA CR, 309/09/1597, Role of the extracelullar matrix in the extracelullar space diffusion parameter changes during aging and the metabolic syndrome, 2009–2013.

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EU 6th FP, LSHB-CT-2005-512146, Diagnostic Molecular Imaging: A Network of Excellence for Identification of NEW Molecular Imaging Markers for Diagnostic Purposes, DiMI, 2005–2010.

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3. Likavčanová K, Urdzíková L, Hájek M, Syková E. (2008) Metabolic changes in the thalamus after spinal cord injury followed by proton magnetic resonance spectroscopy. Magn Reson Med 59(3): 499–506.

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parameters of the extracellular space. Neurochem Int 52(1–2): 5–13.

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6. Syková E, Nicholson C. (2008) Diffusion in brain extracellular space. Physiol Rev 88: 1277–1340.

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Laboratory of Eye Histochemistry and Pharmacology

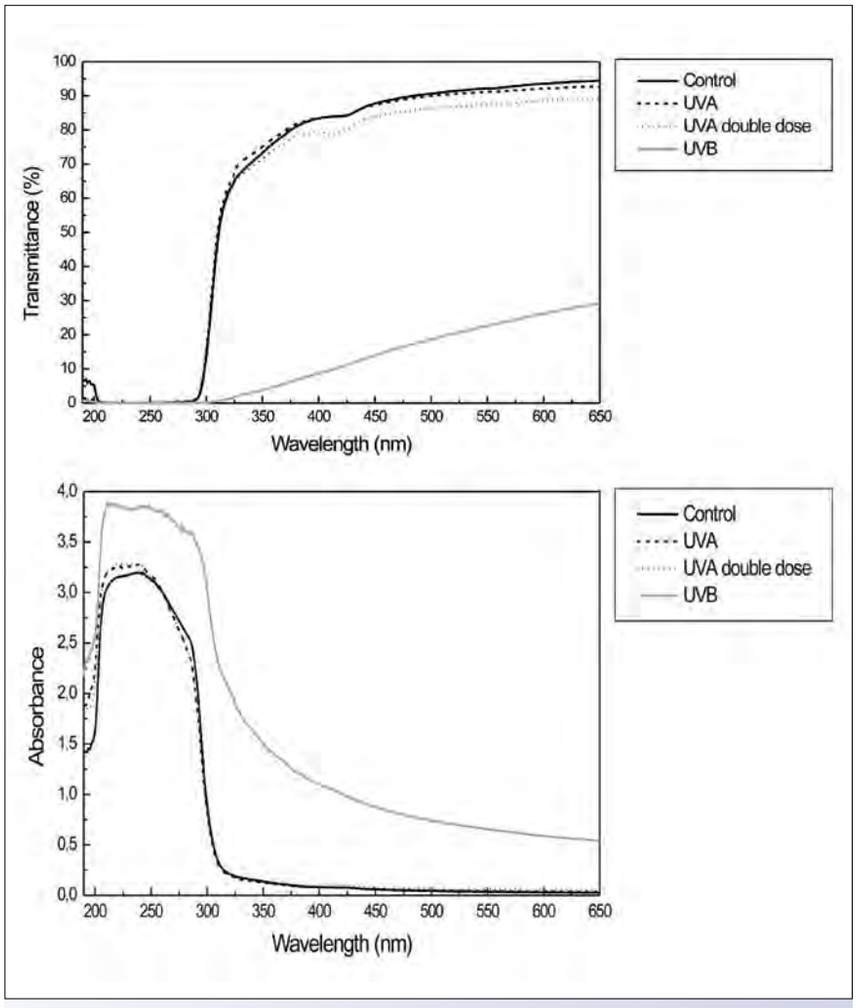
Research in the Laboratory of Eye Histochemistry and Pharmacology is focused on the metabolic profile of the anterior eye segment and alterations evoked by various diseases or ocular injuries, such as the irradiation of the eye with UV rays and thermal or chemical burns. These severe disorders may result in corneal epithelial limbal stem cell deficiency leading to corneal conjunctivalization (ingrowth of the conjunctival epithelium) and permanent loss of vision. Recently, attempts at corneal regeneration have been started in an animal model (rabbit eye) using mesenchymal stem cell and/or corneal epithelial limbal stem cell transplantation with the aim of vision rehabilitation. In corneal healing processes, attention is devoted to conditions leading to the development of extensive intracorneal or intraocular inflammation. To affect these processes and achieve positive healing, various drugs are employed, particularly specific inhibitors of destructive proteases and scavengers of toxic oxygen products.

Great effort has been devoted to the possibilities of protecting the eye against the damaging effect of UVB rays, known to induce the generation of reactive oxygen species, using UV filters (in cooperation with Laboratoires Thea, Clermont-Ferrand, France).

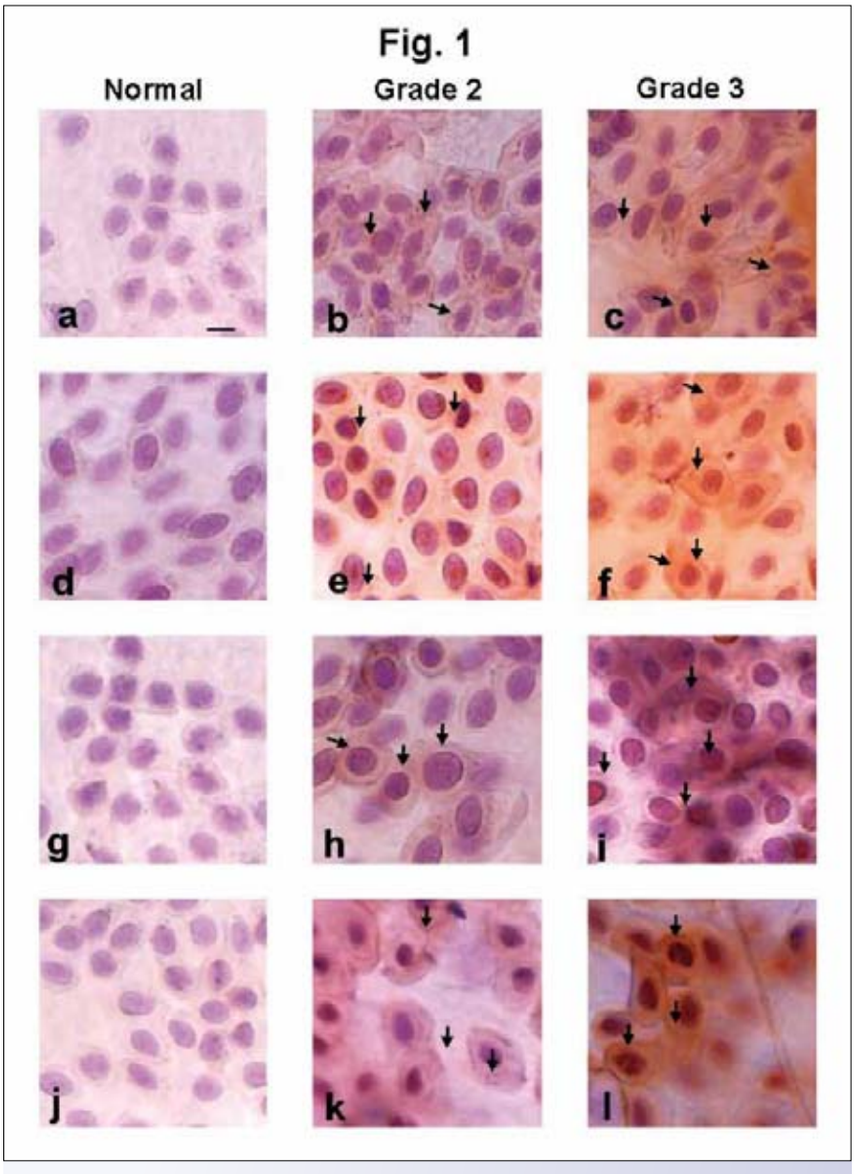
For evaluating the local toxicity of various drugs, a special method has been developed using the rabbit cornea, and a patent application based on this approach has been submitted (PV 2009–190).

Differences between UVB and UVA rays in terms of corneal light absorption have been distinguished. The same doses of UVB or UVA rays were compared (1. 01 J/cm^2 and also UVA at a two-fold larger dose, 2. 02 J/cm^2). The results showed that UVB rays are strongly absorbed by the cornea, whereas UVA rays are absorbed by the cornea only in small amounts (Čejka et al., 2007, 2008).

Our recent findings show that in some diseases of the ocular surface (particularly of an autoimmune character), oxidative injuries of the ocular surface appear in parallel with clinically observed



Spectrophotometry results *of the of the corneal center, expressed either as the spectrum of transmittance T = T (λ) (A) or absorbance A = A (λ) (B). The spectral curves are means from measurements of 14 normal corneas, 7 irradiated with UVA rays (1. 1 J/cm^2, once a day for 5 days), 6 irradiated with a double dose of UVA (2. 2 J/cm^2, once a day for 5 days), and 7 irradiated with UVB (1. 1 J/cm^2, once a day for 5 days). Note that for wavelengths shorter than about 300 nm, the spectra show the instrumental stray light error rather than the corneal optical properties.*
The corneas repeatedly irradiated with UVB rays (daily dose of 1. 01 J/cm^2 for five days) absorb more light throughout the whole measurable spectral range than do normal corneas. In contrast, no significant differences between normal corneas and corneas irradiated with UVA rays (daily dose of 1. 01 J/cm^2 or a double dose, for five days) were found (tested at 320, 380, and 550 nm by one-way ANOVA with Dunnett´s post-test).



Immunohistochemical staining of pro-inflammatory cytokines *(IL-1, IL-6, IL-8, THF-a) in the human conjunctival epithelium of autoimmune dry eye disease (Sjögren´s syndrome, SS). Scale bar: 10 µM. The severity of pro-inflammatory cytokine expression parallels the severity of the symptoms of dryness and the slit-lamp findings. Normal conjunctival cytology samples revealed no or very weak cytokine staining (mature interleukin-1beta (IL 1-b), Fig. 1 a; interleukin 6 (IL-6), Fig. 1 d; interleukin 8 (IL-8), Fig. 1 g; tumor necrosis factor alpha (TNF a), Fig. 1 j). Only nuclei were stained with haematoxylin. However, all cytokines studied were already expressed in the conjunctival epithelium of dry eye (SS) grade 2, moderate symptoms of dryness with reversible slit-lamp findings (IL1-b, Fig. 1 b; IL-6, Fig. 1 e; IL-8, Fig. 1 h; TNF-a Fig. 1 k), and their expression increased in the conjunctival epithelium of dry eye (SS) grade 3, severe symptoms of dryness with irreversible slit lamp findings (IL-1 b Fig. 1 c; IL-6, Fig. 1 f; IL-8, Fig. 1 i; TNF-a, Fig. 1 l). Arrows point to cytokine expression.*

slit lamp findings. The majority of injuries is evoked by the elevated expression of pro-inflammatory cytokines, which induce the increased expression as well as activity of enzymatic systems that generate reactive oxidative and nitrosative species. In contrast, enzymatic scavengers of toxic oxygen products are decreased (Čejková et al., Histol Histopathol. 22,997–1003, 2007; Čejková et al., Nitric Oxide 17, 10–7, 2007; Čejková et al., Histol Histopathol 23,1477–83, 2008, Čejková et al., Histol Histopathol 2009, *in press*).

CURRENT GRANT SUPPORT

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Laboratoires Thea, Clermont-Ferrand, France, 0801000013 and CAC08FRO0001123, 2008–2010.

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DEPARTMENT OF AUDITORY NEUROSCIENCE

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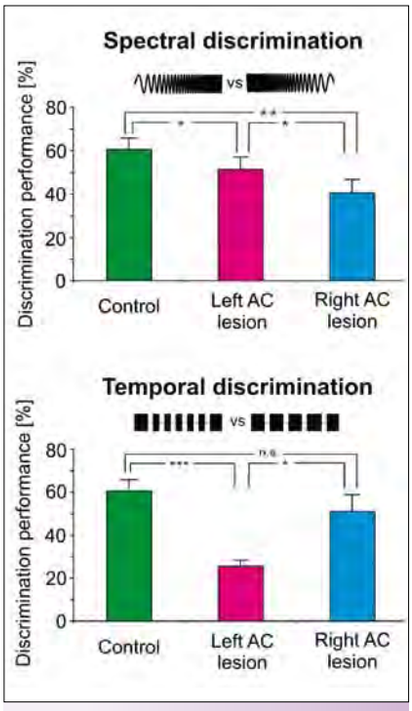
RESEARCH TOPICS

The Department of Auditory Neuroscience has existed since the foundation of the Institute of Experimental Medicine in 1975. The main research aims of the department are oriented towards investigations of the structure and function of the auditory system in animals and man under normal and pathological conditions and during ontogeny and ageing.

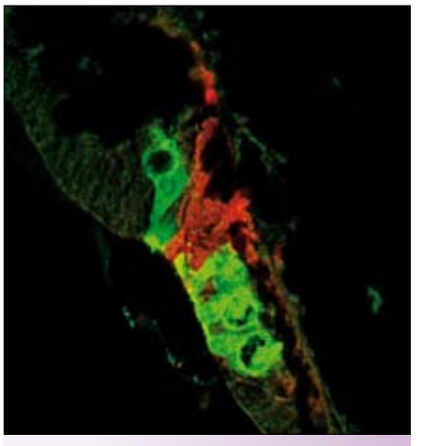
Laboratory of Auditory Physiology and Pathology

In the Laboratory of Auditory Physiology and Pathology, recordings of neuronal activity in individual auditory centers using multielectrodes have revealed the basic principles of the neuronal processing of simple tones as well as complex sounds such as artificially generated rippled noise or animal vocalizations. The development of the hearing organ during ontogeny and changes in the expression of calcium-binding proteins and other neuroactive substances are studied with immunostaining



Direction of frequency modulation discrimination *(Spectral discrimination) was more deteriorated after right auditory cortex (AC) lesion, whereas discrimination of gap repetition rate in noise (Temporal discrimination) was significantly worsened following left AC inactivation.*

methods and confocal microscopy analysis. Behavioral conditioning tests associated with permanent or pharmacologically-induced reversible lesioning of cortical structures are used to study the lateralization of auditory functions in the rat auditory cortex. Pathologies of the peripheral and central parts of the auditory system, appearing as a consequence of noise exposure or in conjunction with aging, are investigated in experimental animals and in human subjects. Among the methods used in the laboratory for this purpose are the recording of extracellular single neuron activity and auditory evoked responses, the assessment of hearing thresholds, measurements of psychoacoustic functions, the startle reaction, the recording of different types of otoacoustic emissions, as well as immunohistochemical and western blotting techniques used for evaluating changes in the expression of neuroactive proteins in the peripheral and central parts of the auditory system

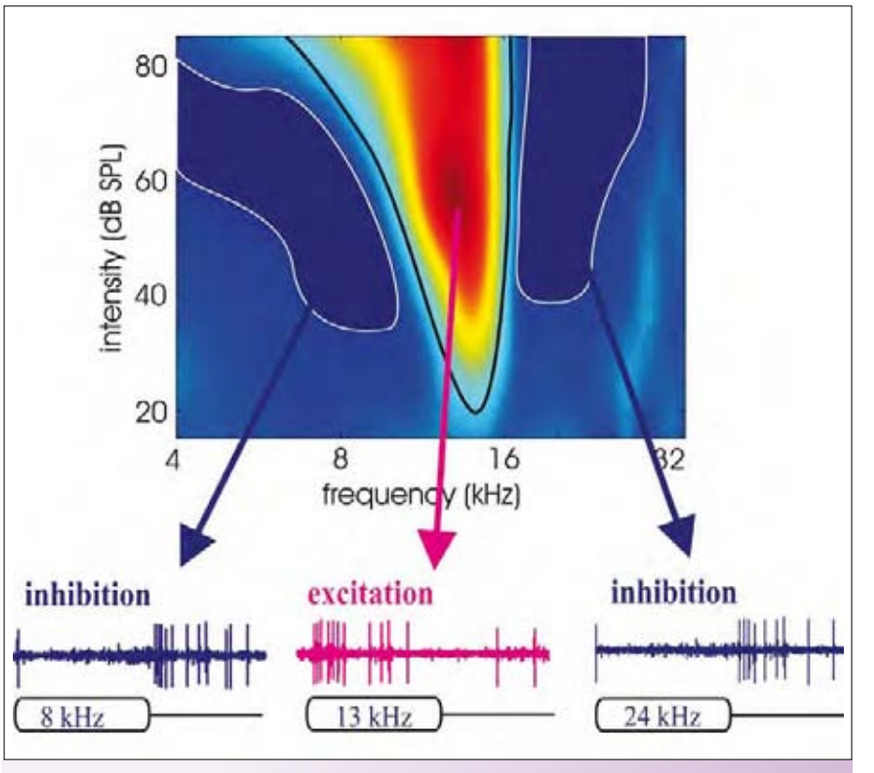


Calbindin (green) and S100 protein (red) in the organ of Corti *in mice at postnatal day 4.*

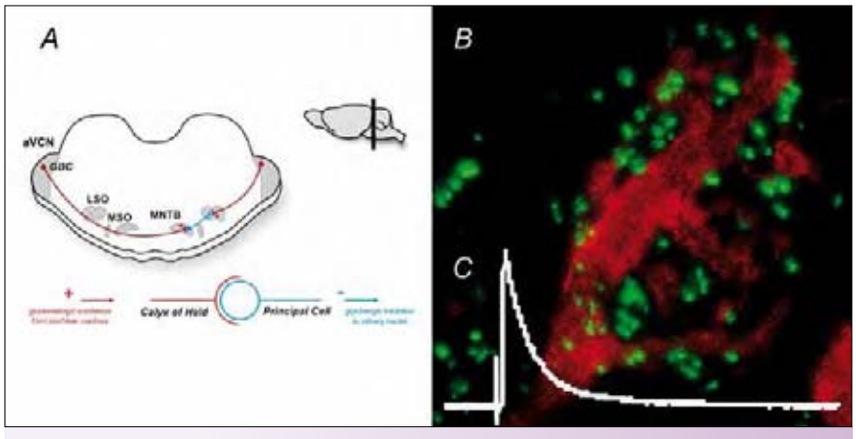
in experimental animals. Age-related changes of hearing function are investigated in special strains of rodents with accelerated aging (C57 mice or Fischer 344 rats). Special attention is given to the GABA inhibitory system in the central auditory pathway, since it is known that this system is vulnerable when animals are exposed to noise and during aging. Collaboration with ENT clinics is oriented towards investigations of hearing function in children and adolescents, the characterization of presbycusis and the genetic background of inherited deafness. Possible methods for the prevention or treatment of inner ear diseases by the application of biologically active drugs or genes to the cochlea are experimentally tested using nanoparticles as a targeted transporting tool.

Laboratory of Synaptic Physiology

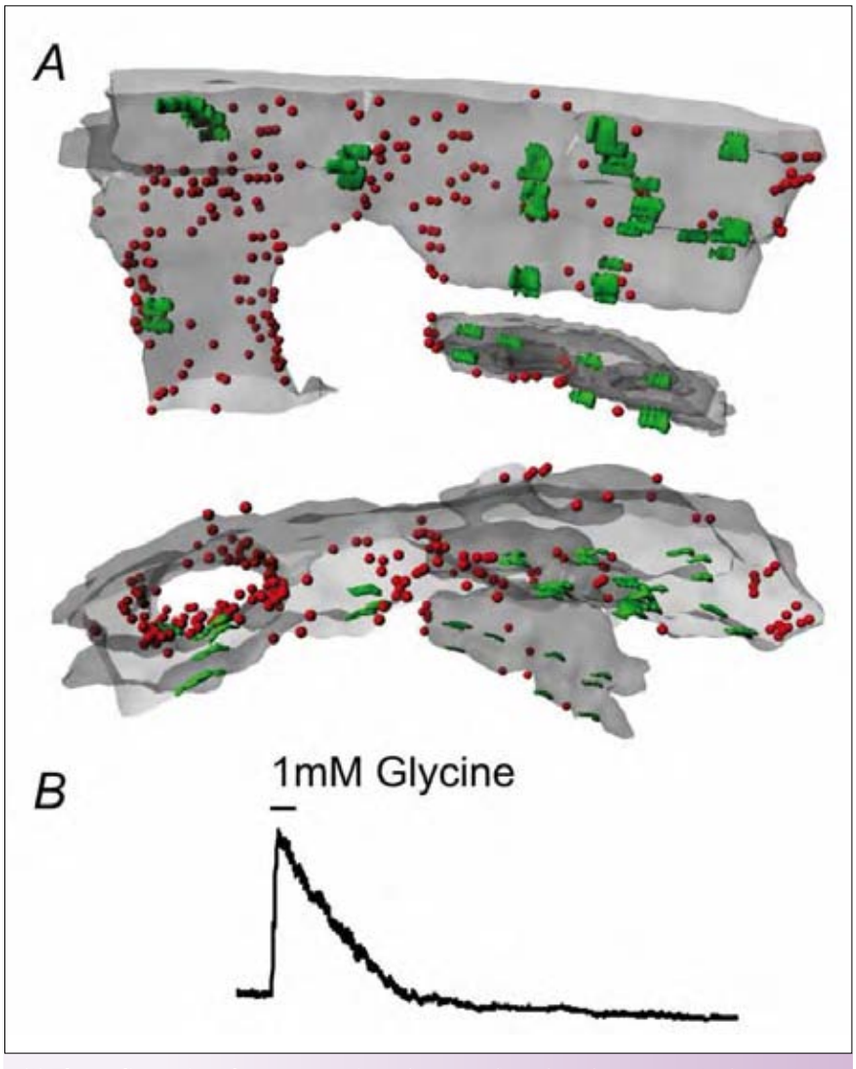
In the Laboratory of Synaptic Physiology the mechanisms underlying the plasticity of excitatory and inhibitory synaptic transmission are studied in rodent brain slices using electrophysiological and immunohistochemical techniques. The Calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB) is mostly used as a model of the central type of synapse due to its large size enabling direct examination by the patch-clamp technique. Recent projects in the lab are aimed at revealing the physiological roles of inhibitory transmitters, their receptors and uptake systems in the MNTB neurons.



Excitatory and inhibitory response areas *of a neuron recorded in the inferior colliculus in the rat to tones of variable frequencies.*



(A) A scheme representing a rat brainstem slice with auditory nuclei. Ventrally situated, the medial nucleus of the trapezoid body (MNTB) is composed of giant nerve terminals (calyx of Held) and principal cells.
(B) A confocal image of double fluorescence labeling. The calyx of Held, which is surrounding a principal cell was labeled with an antibody raised against calretinin (red). Note clusters of postsynaptic alpha 1 subunit-containing glycine receptors (green).
(C) Inhibitory postsynaptic current recorded from a MNTB principal cell evoked by stimulating small glycinergic terminals.



(A) ***Three dimensional reconstruction*** *of a part of a calyceal nerve terminal containing glycine receptor alpha 1 subunits (red dots) and glutamate release active zones (green stripes).*
(B) Current response of presynaptic glycine receptors evoked by the application of glycine on the calyx of Held.

Experimental work has provided evidence of the novel excitatory nature of the classical inhibitory transmitters GABA and glycine. The results show that chloride-permeable glycine receptors, G-protein coupled GABA-B receptors, N-type Ca^{2+} channels and calcium-activated potassium conductances work in concert to support the extremely high reliability of glutamatergic synaptic transmission at MNTB neurons.

CURRENT GRANT SUPPORT

Ministry of Education, LC 554,
Center of Neuroscience.

GA CR, 309/07/1336, Acoustical signal processing in the neuronal circuits of the auditory system.

GA CR, 309/06/1304, The role of GABA-B receptors in the mammalian MNTB.

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Wellcome Trust, No. 073966.

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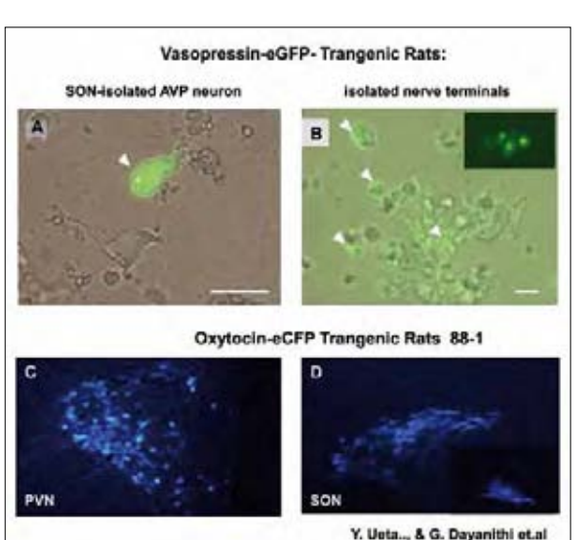
RESEARCH TOPICS

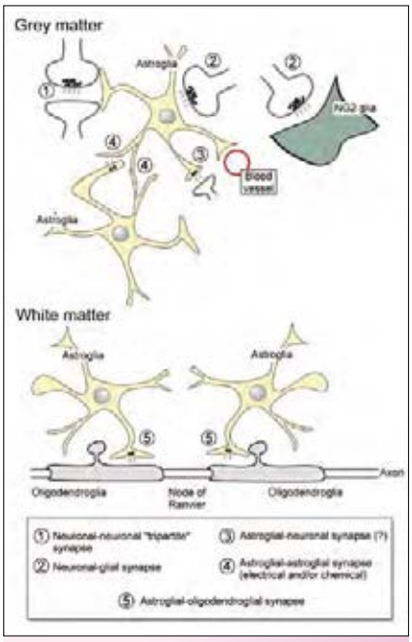
The identification of the cellular and molecular mechanisms of integration in neural networks, through the characterisation of intercellular signalling pathways within neuronal-glial circuits and intracellular signalling mechanisms in neurones and glia under physiological and pathological conditions:

– The role of glutamatergic and purinergic pathways in neuronal-glial signalling in the cortex, hippocampus and spinal cord with a specific emphasis on the glial NMDA and P2X receptors;

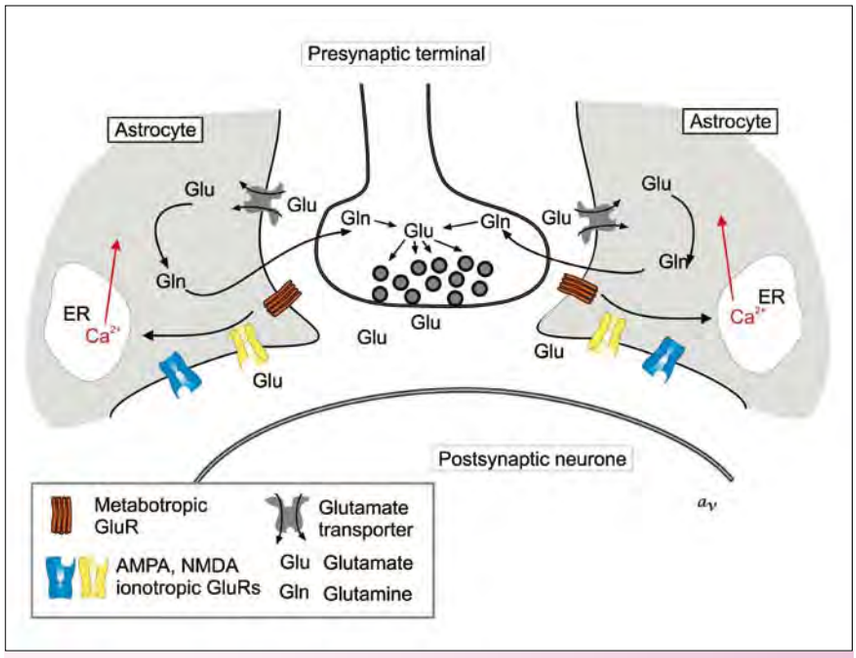
– purinoreceptor-mediated signalling in neurones and glia in the context of their role in sensory transduction and in acute and chronic pain;



Newly generated transgenic rats that express the vasopressin-enhanced green (eGFP) or an oxytocin-enhanced cyan fluorescent protein fusion genes in the HNS. AVP-eGFP fluorescence was observed in the isolated supraoptic nucleus (SON; A) neurons, nerve terminals (B); OT-eCFP in the paraventricular nucleus (PVN; C) and the SON sections (D). Inset is isolated OT neurons from SONs.



Diversity of synaptic contacts between neural cells.
In the grey matter synapses may include (1) classic "tripartite" neuronal-neuronal contacts, enwrapped by an astroglial membrane; (2) neuronal glial synapses (which have already been shown for neuronal-astroglial (Jabs and others 2005) or neuronal-NG2 cell contacts (Lin and Bergles, 2004); (3) astroglial-neuronal synapses (which are yet to be discovered); and (4) astroglial-astroglial synapses, which may exist as electrical/gap junctional or chemical contacts. In the white matter astrocytes may act as presynaptic elements in astroglial-oligodendroglial synapses (5).



Glutamate mediated neuronal-glial signalling.
Synaptically released glutamate activates glial ionotropic (AMPA and NMDA) and metabotropic receptors. Activation of group I metabotropic receptors initiates phospholipase C-dependent synthesis of InsP3, which, in turn, triggers Ca^{2+} release from the endoplasmic reticulum (ER) Ca^{2+} store. The majority (~ 80%) of glutamate released during synaptic transmission is taken up by astroglial Na^+/glutamate transporters; subsequently glutamate is converted into glutamine, which is transported back to neurones, where it acts as a main source of newly synthesised glutamate ("glutamate-glutamine shuttle").

– glial representation of TRP channels and their role in glial signalling;

– ion channels and Ca^{2+} signalling cascades in various types of neural cells at different stages of differentiation;

– *in vivo* imaging of neuronal-glial circuits under physiological and pathophysiological conditions;

– calcium signalling cascades in neurodegeneration and Alzheimer's disease, in particular; the morphology and physiology of glia during normal brain ageing.

Cellular, molecular and morphological changes in neurons and glial cells during pathological states:

– Characterization of events affecting ischemic brain damage, especially astrocytic swelling and the disturbance of Na^+, K^+, Cl^- and Ca^{2+} homeostasis;

– the role of chloride movement in regulatory volume processes in astrocytes during and after oxygen-glucose deprivation;

– characterization of the ischemia-induced time-dependent changes in Ca^{2+} entry carried by TRP channels, ionotropic glutamate and purinergic receptors in glial cells;

– correlation of ischemia-induced changes, such as the onset of reactive gliosis and glial proliferation or apoptosis, with the expression of Na^+ and K^+ ion channels;

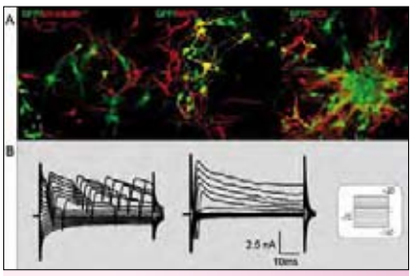
– identification of endogenous neural stem cell migration and differentiation during CNS regeneration – the role of morphogenes and growth factors;

– proliferation, migration and differentiation of region-specific neural stem/progenitor cells *in vitro* as well as after transplantation into the ischemic brain;

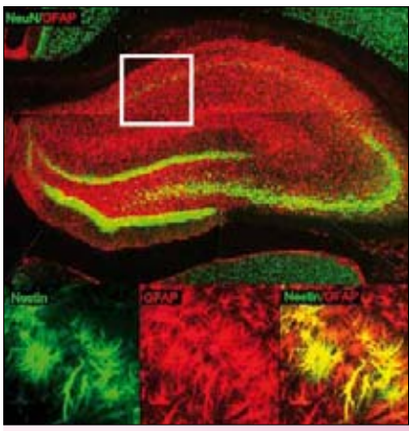
– morphometric measurements and three-dimensional reconstruction of morphological changes of neurons, glial cells and stem cells during pathological states and regeneration.

Laboratory of Molecular Neurophysiology

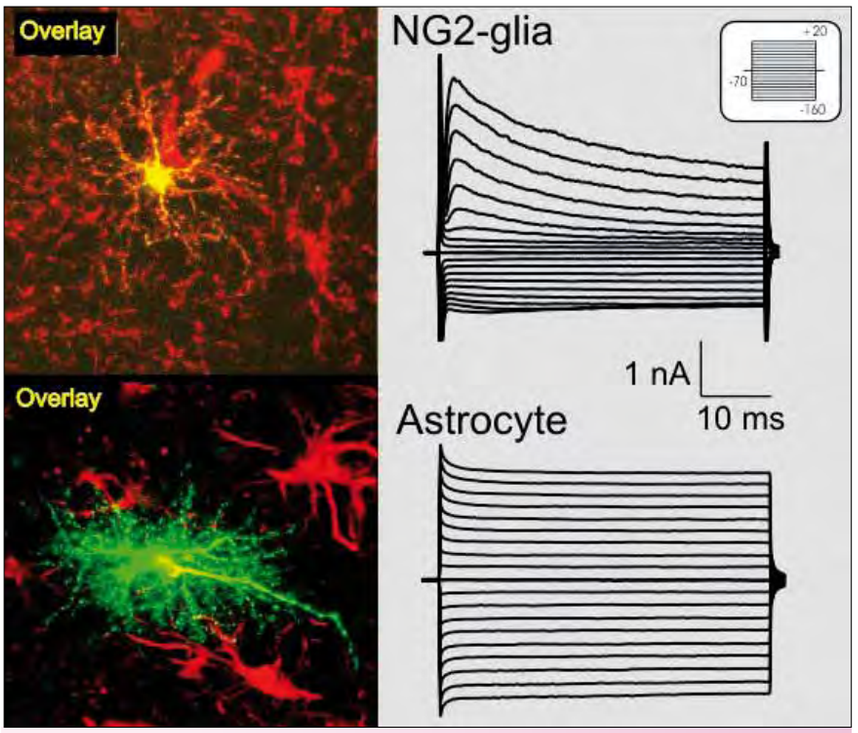
By employing a complex of electrophysiological, video-imaging and molecular biological techniques, we seek to identify the main receptors responsible for calcium signalling pathways and localise the intracellular signalling cascades. Further, we work to develop a complex understanding of information processing in neuronal-glial circuits, thus contributing to a more inclusive theory of brain function, which empha sizes a continuous interplay of discreet neuronal networks with the reticular and internally continuous astroglial web.



Membrane properties of GFP-labeled primary embryonic stem cells during differentiation.
(A) Green fluorescent protein (GFP)-labeled primary embryonic stem cells (D6/GFP) express the typical neuronal markers βIII-tubulin, MAP-2 and DCX (doublecortin), six days after in vitro differentiation. (B) A typical current pattern of D6/GFP-derived neuron-like cells.



Neuronal loss and astrogliosis in the hippocampus after global cerebral ischemia.
Coronal sections of the rat hippocampus 7 days after ischemia/reperfusion. The slices were stained with antibodies against glial fibrillary acidic protein (GFAP) and NeuN or nestin.



Membrane properties of astrocytes and NG2-glia after global cerebral ischemia.
Immunohistochemical identification and electrophysiological characterization of NG2-glia and astrocytes in the rat hippocampus (CA1 region) after bilateral carotid occlusion (left). Typical current patterns of NG2-glia and astrocytes in the rat hippocampus(CA1 region). NG2-glia express a typical complex current pattern of outwardly and inwardly rectifying K^+ currents; astrocytes display predominantly time- and voltage-independent passive K^+ currents (right).

Laboratory of Neurobiology

Research is focused on the cellular, molecular and morphological changes in neurons and glial cells during pathological states such as anoxia and ischemia and during nervous tissue regeneration. In addition, the morphological, electrophysiological and immunohistochemical properties of endogenous stem cells are studied to reveal their possible role in neuroregeneration after injury. Advanced electrophysiological, immunohistochemical and imaging techniques, as well as transgenic animals, are used to identify changes in membrane ionic channels and the expression of cell-type specific markers. Three-dimensional confocal morphometry is used to quantify morphological changes in neurons, glial cells and stem cells.

CURRENT GRANT SUPPORT

GA CR, 309/08/1381, Physiological and pathological potential of astroglial NMDA receptors, 2008–2012.

GA CR, 305/08/1384, Age-related changes in the structure and function of astrocytes, 2008–2010.

GA CR, 309/09/1696, Pathological potential of astroglia in Alzheimer disease, 2009–2011.

GA CR, 305/09/0717, Induction of neuro- and gliogenesis in rat brain after ischemia – the role of morphogenes and growth factors in nervous tissue regeneration, 2009–2011.

Ministry of Education, 1M0538, Research Center: Center of the cell therapy and tissue repair, 2005–2009.

Ministry of Education, LC554, Research Center: Center of Neuroscience, 2005–2009.

SELECTED RECENT PUBLICATIONS

1. Anděrová M, Kubinová S, Jelitai M, Neprašová H, Glogarová K, Prajerová I, Urdzíková L, Chvátal A, Syková E. (2006) Transplantation of embryonic neuroectodermal progenitor cells into the site of a photochemical lesion: immunohistochemical and electrophysiological analysis. J Neurobiol 66: 1084–1010.

2. Chvátal A, Anděrová M, Hock M, Prajerová I, Neprašová H, Chvátal V, Kirchhoff F, Syková E. (2007) Three-dimensional confocal morphometry reveals structural changes in astrocyte morphology in situ. J Neurosci Res 85: 260–271.

3. Neprašová H, Anděrová M, Petřík D, Vargová L, Kubinová Š, Chvátal A, Syková E. (2007) High extracellular K+ evokes changes in voltage-dependent K+ and Na+ currents and volume regulation in astrocytes. Pflugers Arch 453: 839–849.

4. Jelitai M, Anděrová M, Chvátal A, Madarász E. (2007) Electrophysiological characterization of neural stem/progenitor cells during *in vitro* differentiation: a study on an immortalized neuroectodermal cell line. J Neurosci Res 85(8): 1606–1617.

5. Verkhratsky A, Anděrová M, Chvátal A. (2009) Differential calcium signalling in neuronal-glial networks. Front Biosci 14: 2004–2016.

6. Benešova J, Hock M, Butenko O, Prajerová I, Anděrová M, Chvátal A. (2009) Quantification of astrocyte volume changes during ischemia in situ reveals two populations of astrocytes in the cortex of GFAP/EGFP mice. J Neurosci Res 87: 96–111.

7. Rodríguez JJ, Olabarria M, Chvátal A, Verkhratsky A. (2008) Astroglia in dementia and Alzheimer's disease. Cell Death Differ. 16: 378–385.

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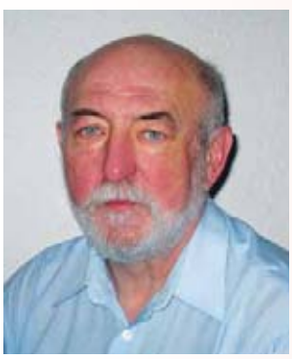

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RESEARCH TOPICS

The Department of Teratology focuses on experimental and clinical teratology with the aim of contributing to our knowledge of normal and pathological development, the ethiopathogenesis of developmental anomalies, and possibilities for their prevention. Only a small portion of inborn defects in man can be explained either by prenatal exposure to a harmful external factor (15 % of cases) or by genetic reasons (20 % of cases). Most developmental defects (65 %) are thought to result from prenatal exposure to the combined effect of several sub-threshold doses of external factors that act either simultaneously or sequentially; a genetic predisposition is presumed in some of these cases. In the Department, the causes and mechanisms that are responsible for the origin of developmental defects induced by environmental and/or genetic factors are investigated. In these studies, two experimental models are used (developing chick embryo and developing mouse dentition), as well as a clinical/epidemiological approach. The origin of external malformations, especially of orofacial clefts, is a pivotal research topic of the Laboratory of Embryogenesis (M. Peterka). The Laboratory of Odontogenesis (R. Peterkova) focuses on tooth development under normal, pathological, and experimental conditions.

Laboratory of Embryogenesis

RESEARCH TOPICS

– Investigation of orofacial clefts
– investigation of other developmental defects
– investigation of experimental and clinical/ epidemiological aspects.

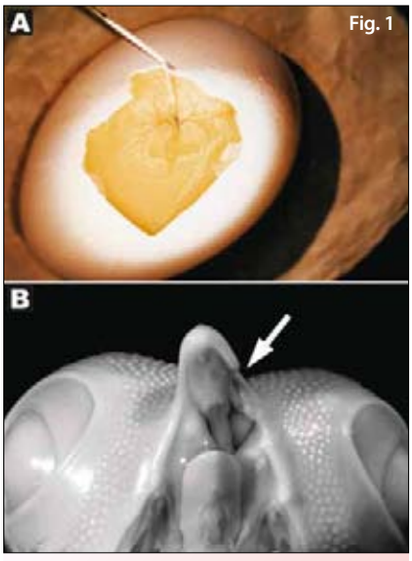
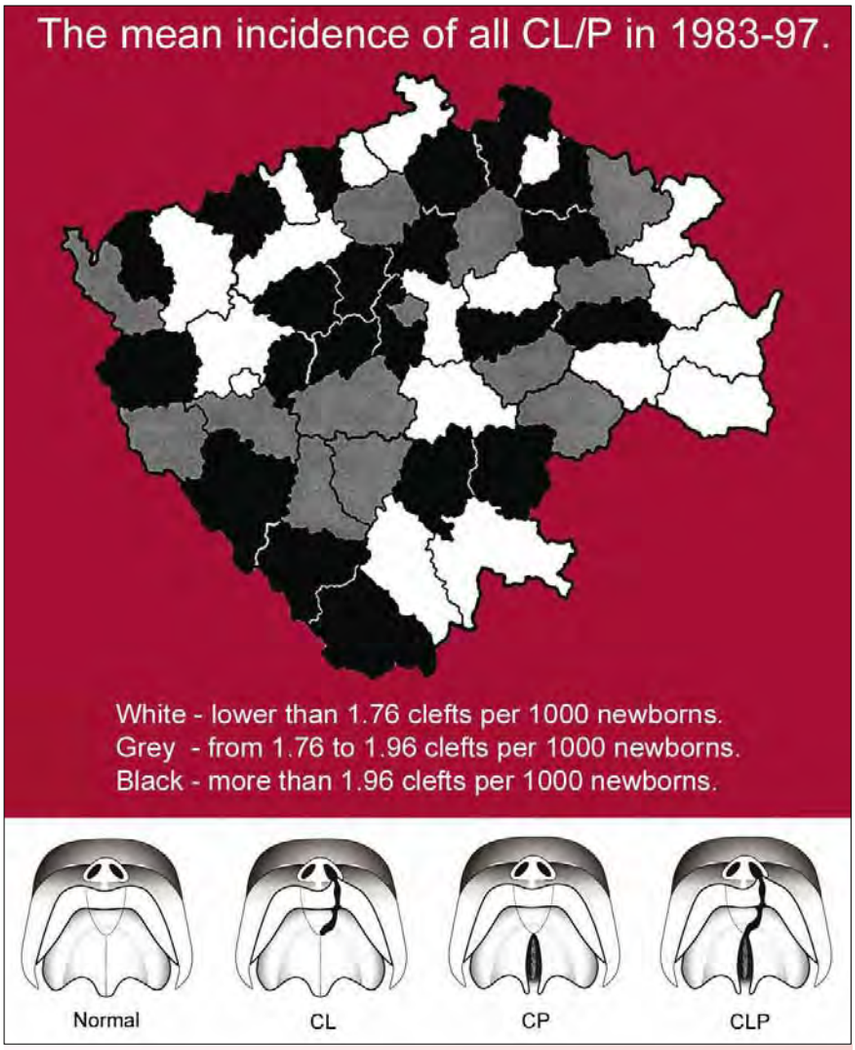


Fig. 1

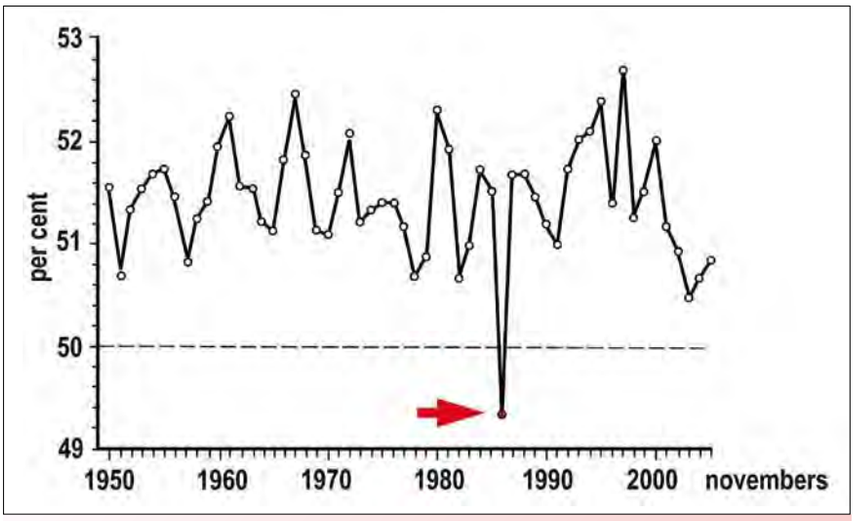
Experimental model – developing chick embryo.
(A) The injection of a test substance into the amniotic sac of a day 3 chick embryo in ovo. (B) Unilateral cleft beak in a day 9 chick embryo induced by the intra-amniotic injection of hydrocortisone on day 4 of incubation.

Harmful external factors and a genetic predisposition are sought in clinical/epidemiological studies of developmental defects. Suspected external factors are tested experimentally in an animal model – chick embryogenesis.

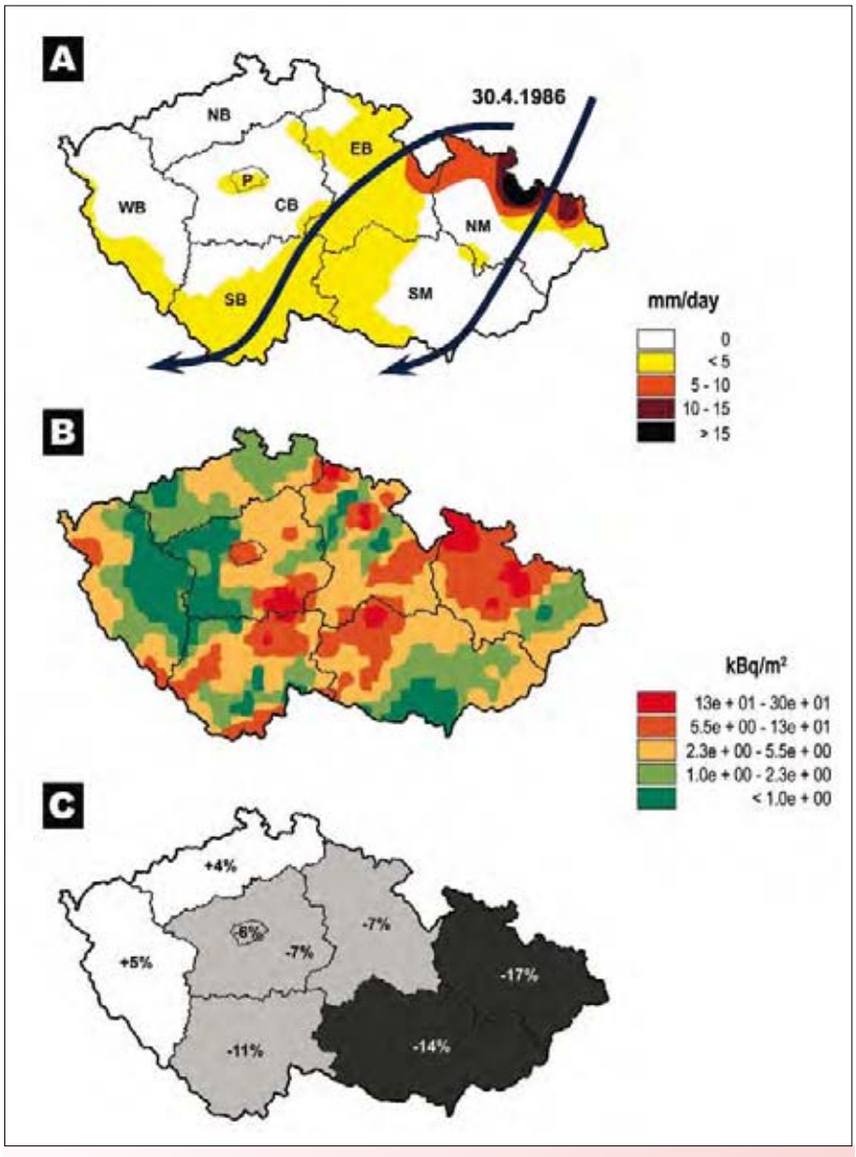
Previous investigations in the Department have detected significant differences in the incidence of orofacial clefts between Czech districts during the last 30 years. The analysis of natality data from the Czech Republic has revealed that the number of newborn boys was higher than that of girls in each month from 1950 to 2005. The only exception was November 1986, when the number of newborn boys was significantly reduced. This has been explained by a selective negative impact of the Chernobyl accident in April 1986 on male fetuses during the 3rd month of their prenatal development. The correlation between the numbers of missing boys with the radioactivity levels has suggested that I-131 probably played the most important role, being taken-up by the fetal thyroid gland during saturation by iodine at the onset of its function in the 3rd month of human prenatal develop-



Orofacial clefts in man.
(A) The mean incidence of orofacial clefts in the districts of the Czech Republic during 1983–1997. (B) Basic types of orofacial clefts in man. CL – cleft lip and jaw unilateral, CLP – cleft lip and palate unilateral, CP – isolated cleft palate. The CL and CLP can also affect both the right and left side - CL bilateral and CLP bilateral, respectively.



The percentage of boys among infants born in the Czech Republic in each November during 1950–2005.
Note the only exception – the percentage of newborn boys was less than 50 % in November 1986, indicating that fewer boys were born than girls.



Schematic maps of the Czech Republic showing the situation after the Chernobyl accident.

(A) Country regions are delineated: CB – Central Bohemia; EB - East Bohemia; NB - North Bohemia; NM - North Moravia; P - Prague; SB - South Bohemia; SM - South Moravia; WB - West Bohemia. The black arrows show the passage of the first radioactive cloud over the country on April 30, 1986. The colors indicate the intensity of the rainfall measured from 07: 00 hours on April 30 until 07: 00 hours on May 1.

(B) Distribution and levels of radiation represented by Cs-137; note that the highest radiation levels were in North and South Moravia, which reflects the areas of rainfall at the time the radioactive cloud passed over the country. The lowest radiation levels were recorded in the areas outside the passage of the radioactive cloud - in North and West Bohemia, where rain was absent or minimal.

(C) The percentage of missing boys in each region during November 1986.

ment. Experimental testing (see Fig. 1) of embryotoxic factors on the developing chick embryo) has determined the embryotoxicity ranges of more than 150 chemical substances and allowed for the estimation of embryotoxicity ranges for humans. We have shown that the upper second incisor originates from the fusion of two components in human embryos. These two components presumably do not fuse in patients with a jaw cleft; consequently, their upper lateral incisor can be duplicated, hypoplastic or missing.

PRESENT STUDIES

– Testing of harmful chemical and physical factors and estimation of their minimum embryotoxic doses using a chick embryotoxicity screening test;

– Mechanism of development of the cleft beak in chick embryos and its possible prevention and reparation;

– Clinical/epidemiological studies searching for the causes underlying the origin of orofacial clefts in humans, based on a critical analysis of case- and family-history data;

– Monitoring of the newborn sex ratio as a tool for detecting ecological accidents.

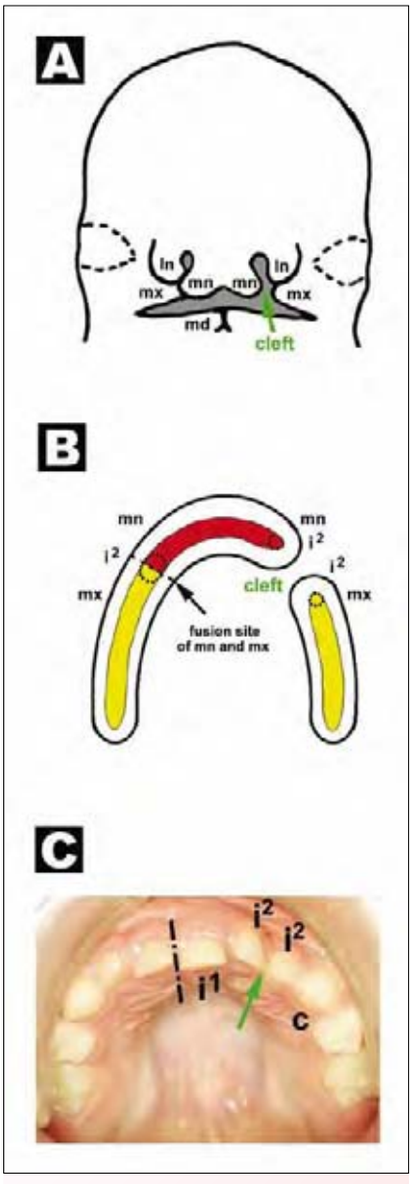
The studies bring new data about the ethiopathogenesis of developmental defects that help in the prevention of inborn anomalies in humans.

Laboratory of Odontogenesis

RESEARCH TOPICS

– investigation of tooth development under normal, pathological and experimental conditions

Findings on tooth development (odontogenesis) help in understanding the molecular control of organogenesis, the origin of tooth anomalies, and the evolution of an animal species. Recently, odontogenesis investigations have also focused on the possibilities for biological tooth replacements. To design such replacements, an understanding of the factors that promote or inhibit tooth development is essential. Previous studies of the Laboratory have revealed that the embryonic mouse dentition provides an ideal system for studying such factors, since it contains not only the germs of functional teeth, but also several types of rudimentary (vestigial) tooth primordia. These vestigial primordia are either incorporated into developing functional

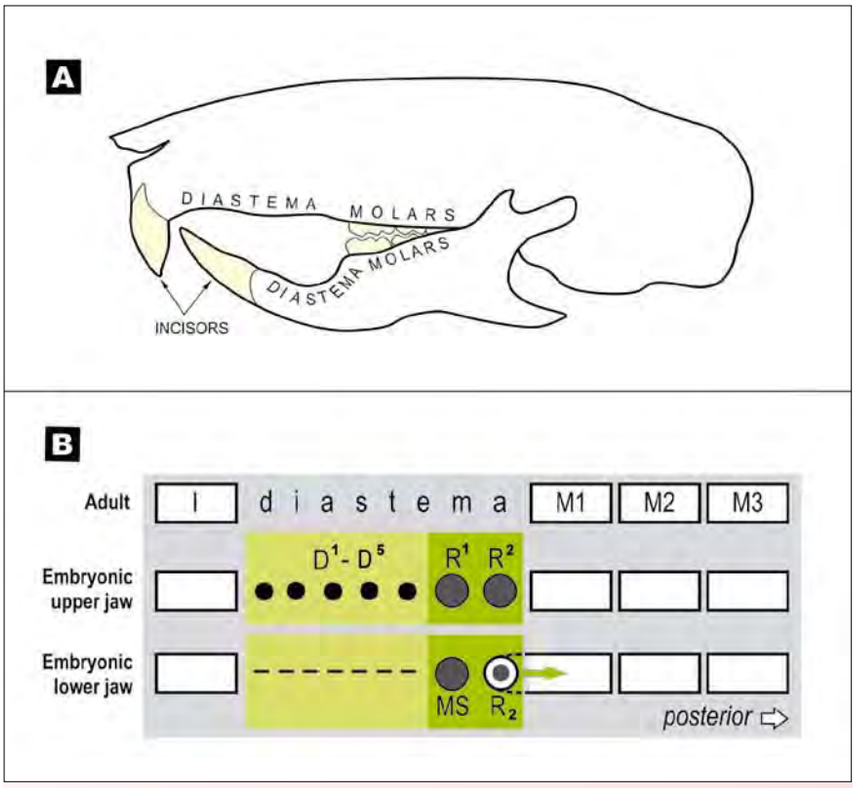


Origin of the double upper lateral incisor in humans.

(A) Scheme of the embryonic human face with a unilateral left-sided cleft of the lip and jaw (green arrow). The medial nasal (mn) and the maxillary (mx) facial processes are fused on the right and not fused on the left side. ln – lateral nasal process, md – mandibular process.

(B) Scheme of the human upper jaw arch viewed from the oral cavity. On the right site, the mn (red) and mx (yellow) fuse. At the fusion site, the lateral deciduous incisor (i^2) develops (dotted line), containing material from both facial processes. On the left side, non-fusion of the mn and mx results in a jaw cleft and the non-fusion of the dental epithelia, which leads to the formation of two i^2.

(C) Double deciduous lateral incisors i^2 (arrow) in a patient with a left-sided alveolar cleft after surgical treatment (from the archive of the Clinic of Plastic Surgery, Prague). The midline is shaded. i^1 – deciduous central incisor; c – deciduous canine.



Schematic of the tooth pattern of adult and embryonic mice.

(A) Adult mice have one incisor and three molars separated by a toothless diastema in each jaw quadrant.

(B) A schematic comparison of the tooth pattern in a jaw quadrant of adult and embryonic mice. In contrast to adult mice, we found that mouse embryos have rudimentary tooth primordia in the prospective diastema (green). In the anterior part of the diastema (light green), either rudimentary small placodes/buds (D^1-D^5) or an epithelial thickening (dashed line) develop in the maxilla or mandible, respectively. In the posterior part of diastema (dark green), two rudimentary buds are the most prominent primordia in the cheek region at early stages. Later on, D^1-D^5 disappear, R^1, R^2 and MS regress, while R^2 is incorporated into the first molar (M1). I - incisor; M2 and M3 – the second and third molars, respectively.

teeth or suppressed by epithelial apoptosis. We have interpreted some supernumerary teeth in mouse mutants as atavisms based on the revitalization of rudimental tooth anlagen. Vestigial odontogenous structures are also present in humans (see Fig. 8, page 25) and in other mammals. The inhibited tooth-forming capacity at specific loci of the mammalian dentition suggests that there might be a natural substrate responsive to the controlled stimulation of tooth regeneration.

PRESENT STUDIES

– Odontogenesis in wild type mice – model of the normal development of mammalian dentition;

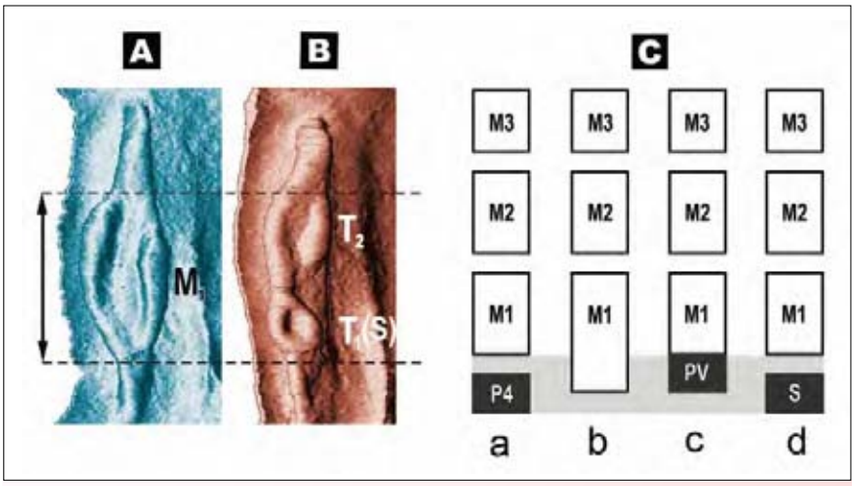
– Comparative odontogenesis studies;

– Development of tooth anomalies in mice with genetic alterations;

– Experimental odontogenesis studies – role of growth activating or inhibiting factors in primordial tooth organ cultures *in vitro*.

– Developmental dynamics of tooth development. Fluorescence transgenic mouse embryos and time lapse microscopy are used to study morphological and molecular events during tooth development in real time.

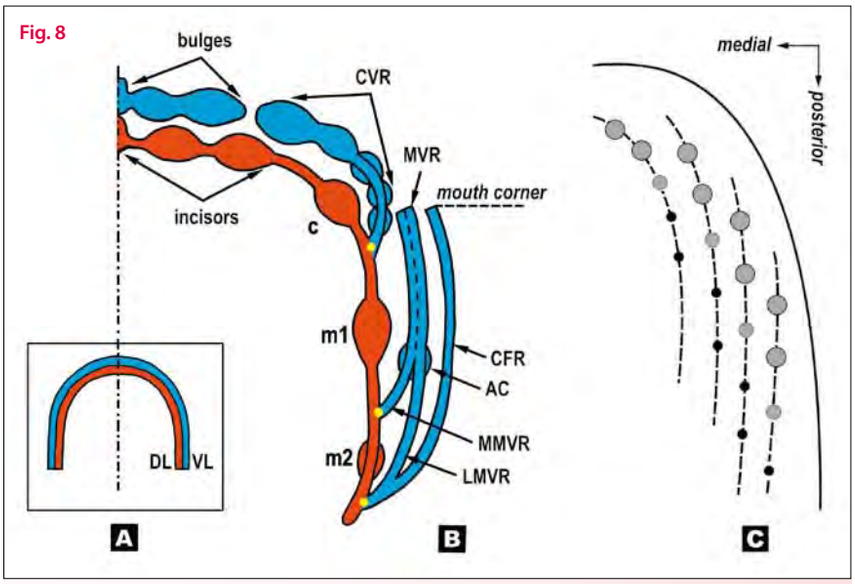
These studies are made in collaboration with H. Lesot (INSERM U-595, Strasbourg, France) and O. D. Klein (Departments of Orofacial Sciences and Pediatrics, UC, San Francisco, USA).



Tentative explanation of the supernumerary tooth in mouse mutants as an atavistic premolar.

The posterior part of the mouth cavity is at the top of each picture. 3D reconstructions show a similar antero-posterior length of the dental epithelium in wild type (A) and Tabby homo/hemizygous embryos (B) at ED 15. 5. However, the segmentation of the dental epithelium along the antero-posterior axis is different. At the level of one cap of the first molar (M_1) in the wild type embryo, we found two small caps (T_1, T_2) in the mutant. T_1 gives rise to the so-called supernumerary tooth (S), which thus corresponds to the anterior part of the M_1 in wild type mice. (C) We have suggested a developmental relationship between (a) the last premolar (P_4) of non-muroid rodents, (b) the anterior part of the adult M1, (c) the embryonic diastemal vestigial bud (PV) in normal mice, and (d) the supernumerary tooth (S) in mutants. The S in mutants can be considered as an atavism – the revitalization of a premolar suppressed during evolution.

Fig. 8



Schemes of the pattern of the dental and vestibular epithelium in human embryos and in the teeth of fishes.

(A) A textbook concept presenting two parallel U-shaped ridges in human embryos (e. g. Bhaskar, 1980): DL – dental lamina (giving rise to teeth) and VL – vestibular lamina or labio-gingival band (where the oral vestibule will form). (B) Our 3D reconstructions have documented that no continuous vestibular lamina exists, but rather a set of discontinuous epithelial structures (ridges and bulges) transiently occurs externally to the dental lamina. Red – dental epithelium; blue – vestibular epithelium. c, m1 and m2 – the deciduous canine, first and second molars. The yellow spot indicates the site of fusion between the dental lamina and the vestibular ridges. (For further explanations, see Hovorakova et al., 2005). (C) The schematic pattern of tooth rows ("Zahnreihen") in fish (according to data by Edmund, 1960). The empty rings and black spots indicate the older and younger teeth, respectively. New teeth are formed at the posterior end of each tooth row.

The results can help to elucidate the origin of tooth anomalies and to develop methods of tooth regeneration and engineering.

CURRENT GRANT SUPPORT

GA CR, 304/07/0223, Developmental anomalies of dentition in a phylogenetic context, 2007–2012.

GA CR, 304/09/1579, Development of incisor malformations in a mouse model, 2009–2013.

SELECTED RECENT PUBLICATIONS

1. Peterka M, Peterková R, Likovský Z. (2004) Chernobyl: prenatal loss of four hundred male fetuses in the Czech Republic. Reprod Toxicol 18: 75–79.

2. Peterková R, Lesot H, Viriot L, Peterka M. (2005) The „supernumerary" cheek tooth in the tabby/EDA mice – a reminiscence of the premolar in mouse ancestors. Arch Oral Biol 50: 219–225.

3. Hovořáková M, Lesot H, Peterková R, Peterka M. (2006) Origin of the deciduous upper lateral incisor and its clinical aspects. J Dent Res 85: 167–171.

4. Peterková R, Lesot H, Peterka M. (2006) Phylogenetic memory of developing mammalian dentition. J Exp Zoolog B 306: 234–250.

5. Hovořáková M, Lesot H, Vonesch J-L, Peterka M, Peterková R. (2007) Early development of the lower deciduous dentition and oral vestibule in human embryo. Eur J Oral Sci 115: 280–287.

6. Peterka M, Peterková R, Likovský Z. (2007) Chernobyl: Relationship between Number of Missing Newborn Boys and the Level of Radiation in the Czech Regions. Environ Health Perspect 115: 1801–1806.

7. Peterková R, Churavá S, Lesot H, Rothová M, Procházka J, Peterka M, Klein OD. (2009) Revitalization of a diastemal tooth primordium in Spry2 null mice results from increased proliferation and decreased apoptosis. J Exp Zoolog B 312: 292–308.

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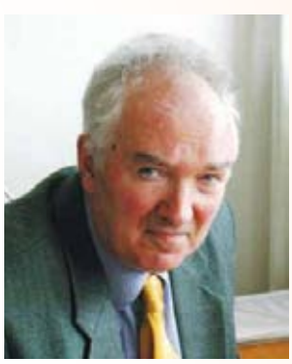

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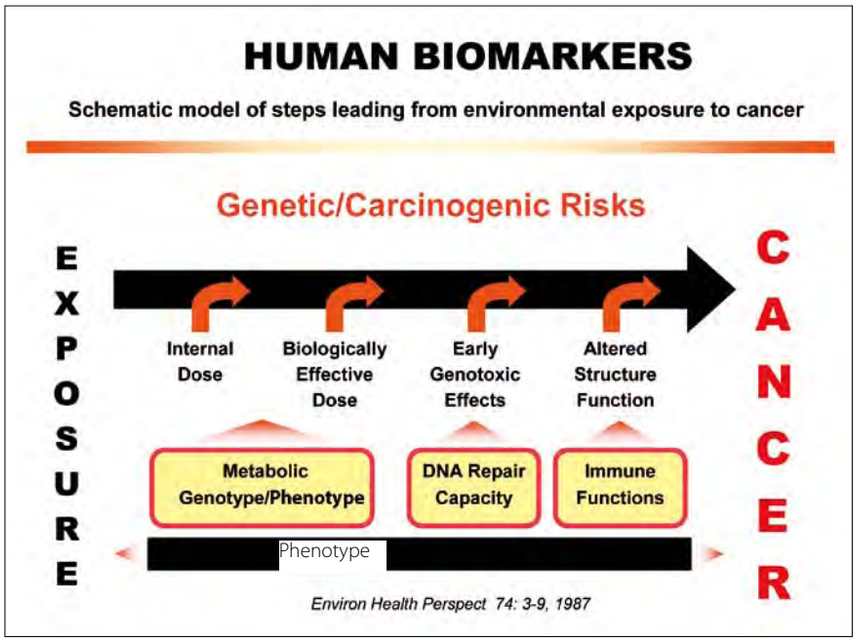
RESEARCH TOPICS

The Department of Genetic Ecotoxicology (DGE) was formed from the Laboratory of Genetic Ecotoxicology, which in turn was founded in 1991 as a joint venture of the Institute of Experimental Medicine AS CR and the Regional Institute of Health of Central Bohemia with the aim of coordinating the international Teplice Program (1991–1999). This program, which studied the effect of air pollution on the health of the population living in the coal basin of Northern Bohemia, was carried out in collaboration with the U. S. Environmental Protection Agency and was supported by the EC program PHARE. This international collaboration helped to establish molecular epidemiology methods and to use them to assess the risk of exposure to air pollution.

The major findings included the fact that carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) in the ambient air are responsible for most of the genotoxicity of complex mixtures and that exposure to c-PAHs in the early stages of pregnancy significantly increases intrauterine growth retardation (IUGR). Further, in polluted regions the relationship between c-PAHs exposure and DNA adduct levels, as well as the effect of genetic polymorphisms on DNA adducts, were studied.

The DGE participates in other international collaborative projects (EC, US EPA, HEI); among these, participation in the EC project EXPAH (Effects of PAHs in environmental pollution on exogenous and endogenous DNA damage, QLK4-CT-2000-00091) has been the most important.

Research in the DGE concentrates mostly on the effects of air pollution on genetic material, on the mechanisms of changes induced by environmental factors as well as modeling the relationships between individual factors (e. g. air pollution vs. life style), and the genetic damage caused by genotoxic and carcinogenic compounds, including polycyclic aromatic hydrocarbons, alkenes and other xenobiotics.



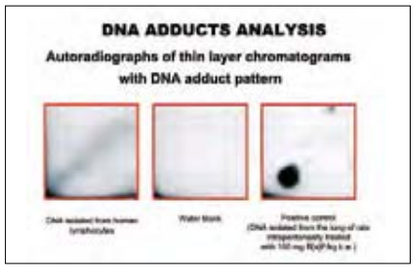
THE RESEARCH IS ORGANIZED IN SEVERAL LEVELS

- Model studies on human cell cultures;
- molecular-epidemiological studies on model populations using biomarkers of exposure, effect and susceptibility;
- reproductive epidemiology – the effect of the environment on pregnancy outcomes (the involvement of genetic material, genetic polymorphisms, gene expression, and oxidative stress);
- the effect of air pollution on upper respiratory diseases in children and the modulatory effects of genetic polymorphisms on childhood morbidity.

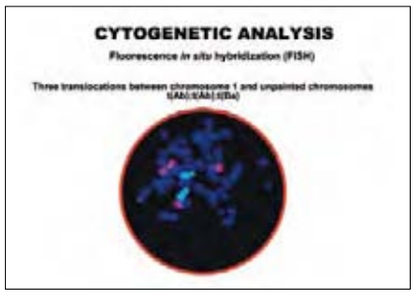
Currently, molecular-epidemiological studies are being conducted on volunteers in Prague and Ostrava, reproductive epidemiology studies in Prague and České Budějovice, and epidemiology studies on respiratory morbidity in children from the Teplice, Prachatice and Ostrava regions. As far as respirable particulate matter and c-PAHs are concerned, the Ostrava region is considered the most polluted EU area.

Laboratory of Molecular Epidemiology

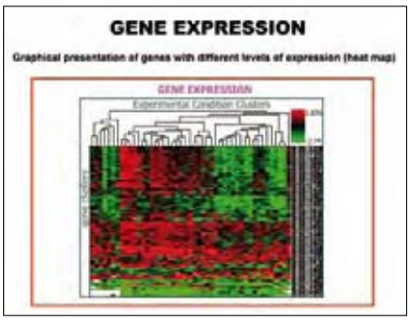
The Laboratory of Molecular Epidemiology conducts molecular epidemiological studies, including the risk assessment of mutagen and carcinogen exposure, using biomarkers of exposure, effect and susceptibility (DNA adducts,



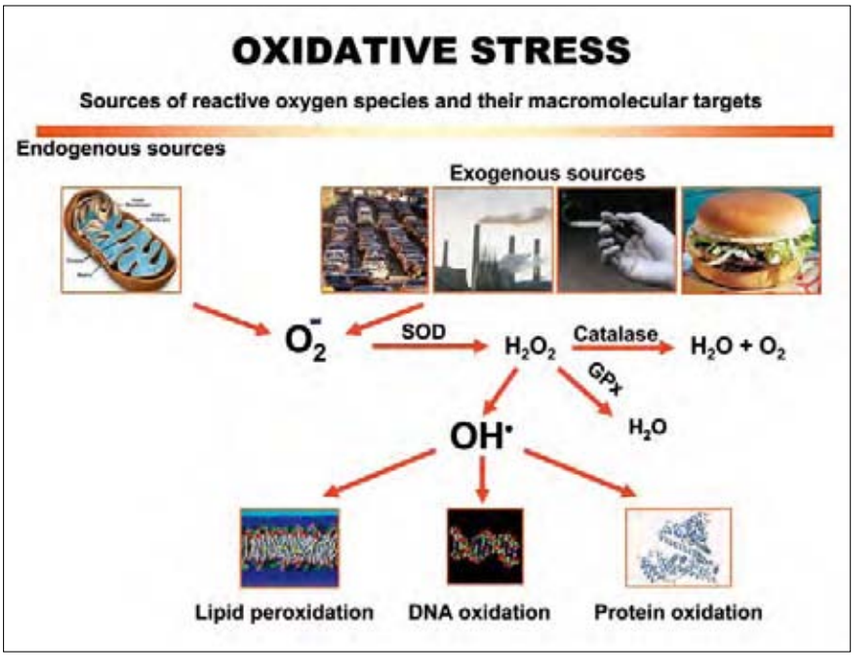
chromosomal aberrations,

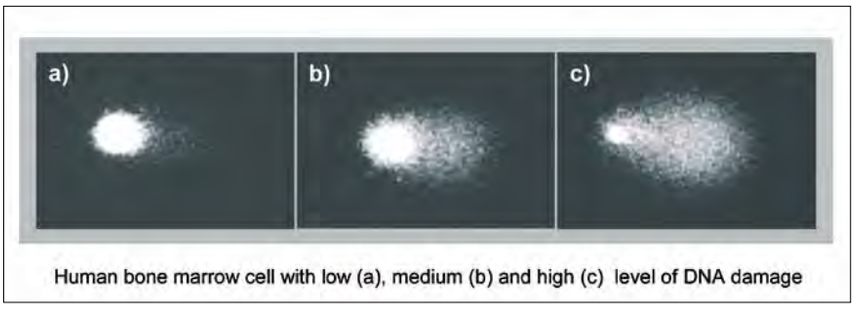


micronuclei, oxidative damage to DNA, proteins and lipids)



analyses of genetic polymorphisms and RNA expression profiles, studies of the effect of the environment on pregnancy outcomes, and the effect of the environment on children's health. DNA instability in patients with myelodysplastic syndrome is also studied.





Human bone marrow cell with low (a), medium (b) and high (c) level of DNA damage

Laboratory of Genetic Toxicology

The Laboratory of Genetic Toxicology concentrates on the genotoxic effects of xenobiotics and oxidative damage to DNA, proteins and lipids in cell cultures (the hepatoma cell line HepG2, human diploid embryonic fibroblasts). The laboratory also studies the effect of environmental pollutants on the mechanisms underlying prostate cancer induction and progression.

Laboratory of Genomics

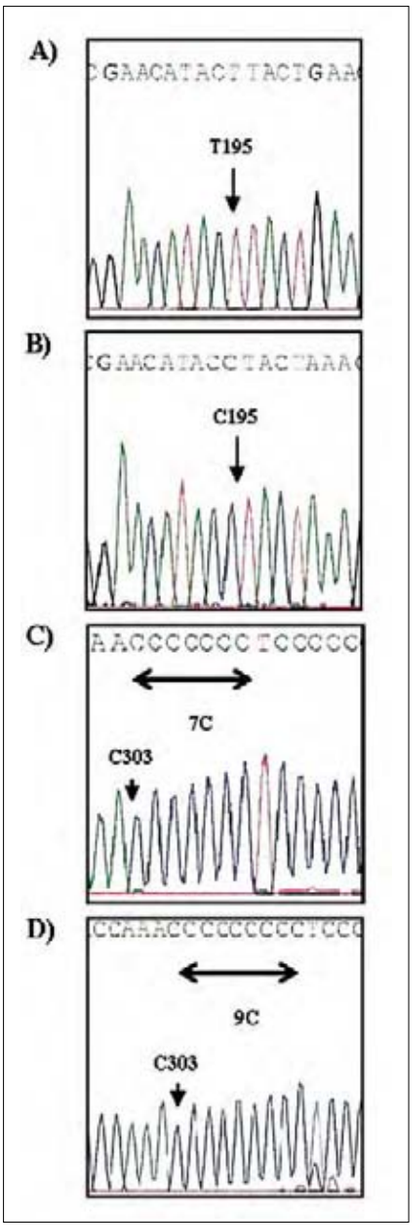
The Laboratory of Genomics studies gene expression profiles in populations exposed to air pollution, tobacco smoke and other factors. It also concentrates on the analysis of single nucleotide polymorphisms affecting the metabolism of xenobiotics, DNA repair, immune responses and other biological processes. The Laboratory of Genomics is a joint venture of the Institute of Experimental Medicine AS CR, v.v.i. and the Institute of Hematology and Blood Transfusion.

CURRENT GRANT SUPPORT

EU 6th FP, 044232, ENVIRISK, Assessing the risks of environmental stressors: contribution to the development of integrating metodology, 2008–2009.

EU 6th FP, 018385-2, INTARESE, Integrated assessment of health risks of environmental stressors in Europe, 2006–2009.

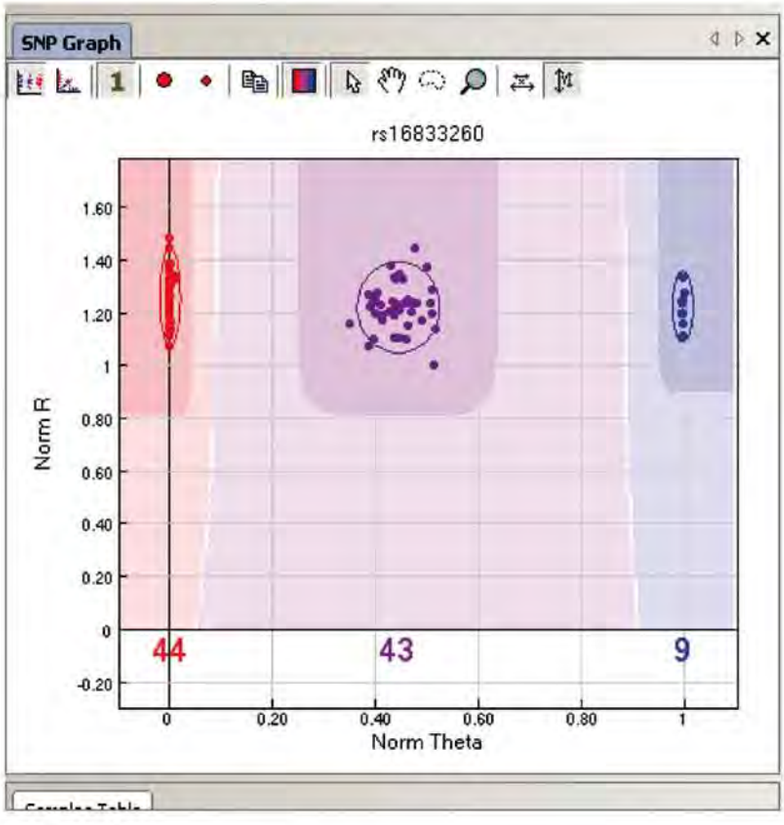
Ministry of Environment, SP/1b3/8/08, Study of the health consequences of polluted air in the Ostrava region with the use of genomics, AIRGEN, 2008–2010.

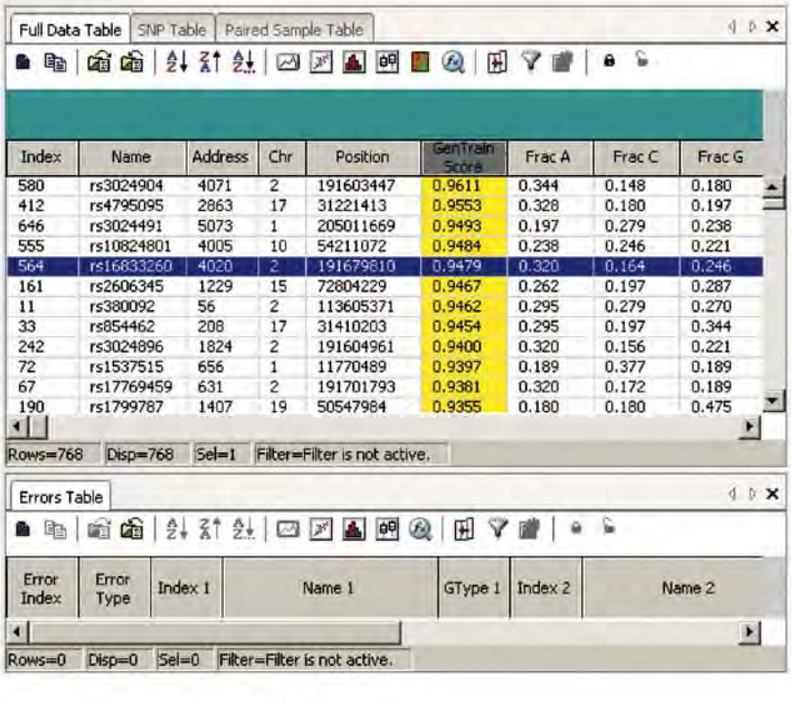


Single nucleotide polymorphisms of HVRII mtDNA in children associated with respiratory morbidity: T195 allele(A); C195 allele (B); 7C 303 allele (C); 9C 303 allele(D).

ANALYSIS OF GENETIC POLYMORPHISMS

Clusters representing samples with different combination of alleles





Ministry of Environment, SP/1b3/50/07, The effects of genome variability on the interaction between the human organism and the environment, ENVIRONGENOM, 2007–2011.

Ministry of Education, 2B08005, New approaches to study the toxicity of air pollution and their contribution to assess limits for selected pollutants,AIRTOX, 2008–2011.

Ministry of Education, 2B06088, Application of toxicogenomics to study mechanisms of the action of environmental pollutants on human health, ENVIRONGEN, 2006–2011.

Ministry of Education, 2B06150, Modulation of anti-PAH-antibody levels in relation to smoking and lung disease (cancer/noncancer) and the increase of PAH-resistance of the organism by immunization, 2006–2011.

GA CR, 310/07/0961, The role of environmental pollutants in the mechanisms regulating the development of prostate carcinoma, 2007–2010.

IGA of the Ministry of Health, NS9804-4/2008, Genetic databases, their structure and application, 2009–2011.

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1. Binková B, Šrám RJ. (2004) the genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM), on human diploid lung fibroblasts. Mutation Res (Fundam.) 547(1-2): 109–121.

2. Šrám RJ, Beskid O, Binková B, Chvátalová I, Lněničková Z, Milcová A, Solanský I, Tulupová E, Bavorová H, Očadlíková D, Farmer PB. (2007) Chromosomal aberrations in environmentally exposed population in relation to metabolic and gene polymorphisms. Mutation Res (Fundam.) 620: 22–33.

3. Binková B, Chvátalová I, Lněničková Z, Milcová A, Tulupová E, Farmer PB, Šrám RJ. (2007) PAH-DNA adducts in environmentally exposed population in relation to metabolic and DNA repair genes polymorphisms. Mutation Res (Fundam.) 620: 49–61.

4. Van Leeuwen DM, Pedersen M, Hendriksen PJM, Boorsma A, van Herwijnen MHM, Gottschalk RWH, Kirsch-Volders M, Knudsen LE, Šrám RJ, Bajak E, van Delft JHM, Kleinjans J. (2008) Genomic analysis suggests higher susceptibility of children to air pollution. Carcinogenesis 29: 977–983.

5. Švecová V, Rössner P Jr., Dostál M, Topinka J, Solanský I, Šrám RJ. (2009) Urinary 8-oxodeoxyguanosine levels in children exposed to air pollutants. Mutation Res (Fundam.) 662: 37–43.

6. Schmuczerová J, Brdička R, Dostál M, Šrám RJ, Topinka J. (2009) Genetic variability of HVRII mtDNA in cord blood and respiratory morbidity in children. Mutation Res (Fundam.) 666: 1–7.

DEPARTMENT OF THE MOLECULAR BIOLOGY OF CANCER

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LABORATORY OF THE GENETICS OF CANCER

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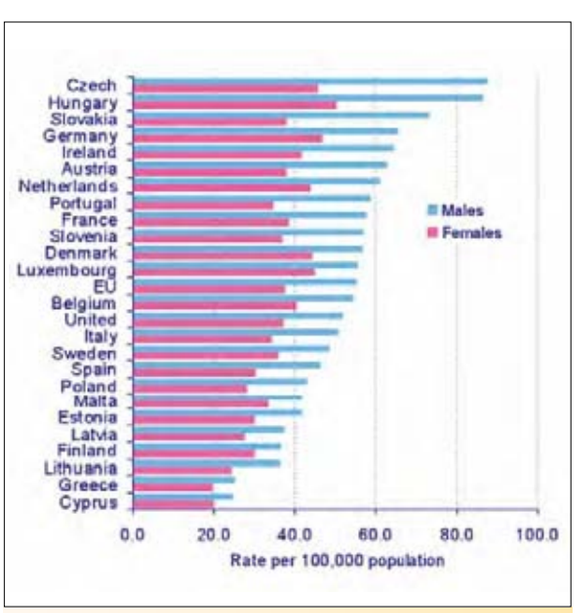
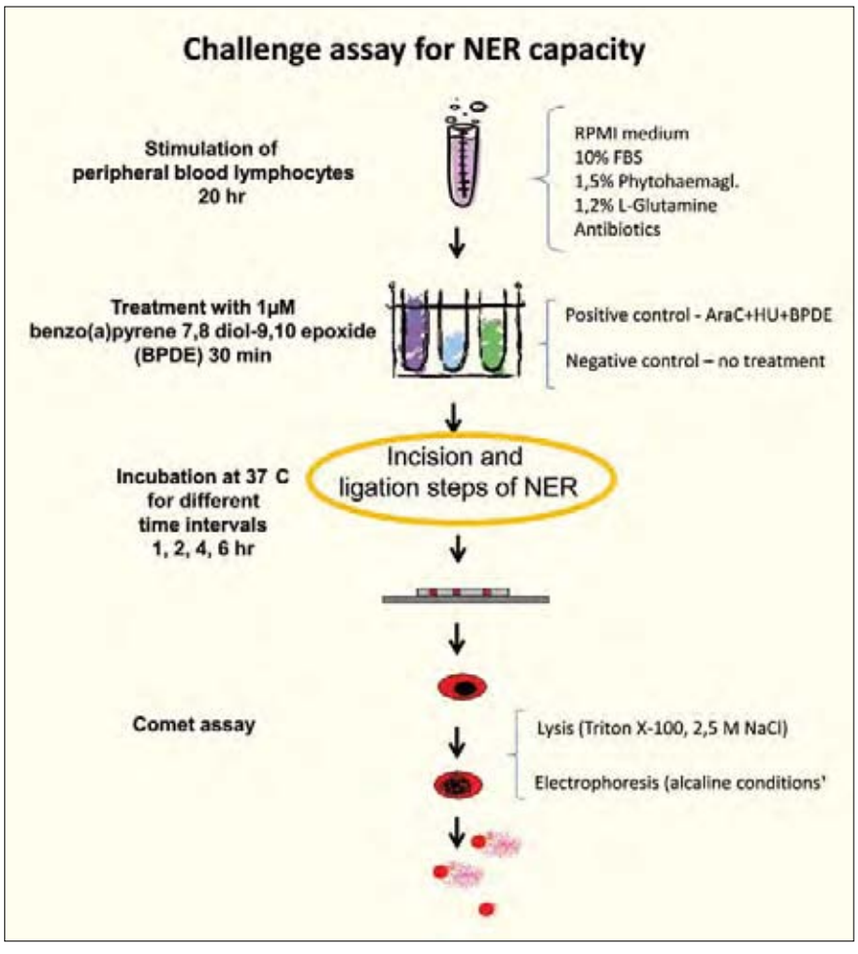
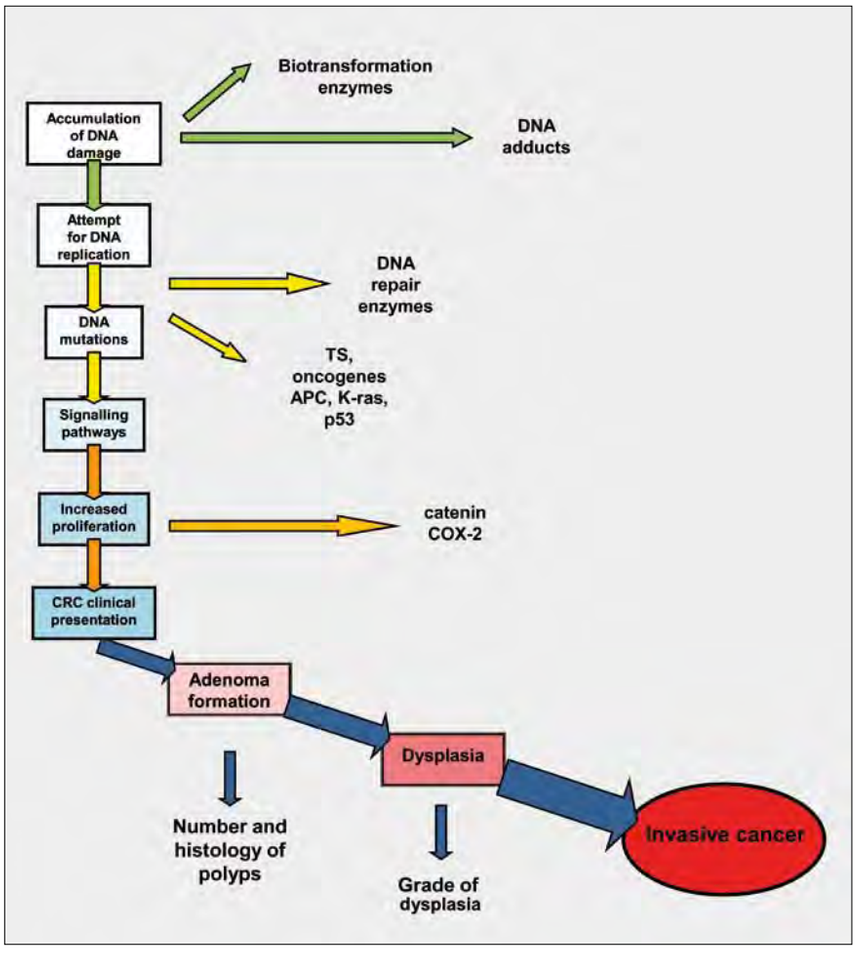
RESEARCH TOPICS

The department is interested in investigating the molecular events involved in the susceptibility to sporadic forms of cancer. Our particular interest is focused on sporadic colorectal cancer, which poses a serious health problem in the Czech Republic. As part of the identification of common genetic variants that predispose an individual to this cancer, candidate genes within various relevant pathways in colorectal carcinogenesis have been addressed (e. g. DNA repair, cell cycle, biotransformation). The Department has participated in whole genome association studies (GWAS), aimed at the identification of susceptibility loci in sporadic colorectal cancer, and will be involved in detailed investigations of the functional consequences of these loci.



Age standardized incidence rates, bowel cancer in EU countries (Modified from IARC, GLOBOCAN 2002).





Laboratory of the Genetics of Cancer

RESEARCH TOPICS

– Investigation of the role of transient markers (DNA and chromosomal damage) in carcinogenesis;

– relationship between transient markers of cancer and individual susceptibility (i. e., relevant candidate genes);

– identification of the mechanisms underlying chromosomal and genomic instability;

– investigation of the role of low-penetrance genes (cell cycle, DNA repair) in the risk of sporadic colorectal cancer and other types of cancer;

– the role of genetic variants in pre dicting anticancer therapy outcome and overall prognosis;

– investigation of the links between genotype and gene expression with implications for the proper/improper functioning of the cell;

– investigation of somatic mutations in oncogenes and microsatellite status in adenomatous polyposis coli.

Colorectal cancer (CRC) is a common neoplasia in both men and women (with an estimated risk incidence of 5 % worldwide) and ranks as the fourth most common cancer in the world, with approximately 875,000 new cases diagnosed each year. An increased incidence in Europe has been recorded over the past decade, with a particularly severe situation in the central European region. The incidence of CRC in the Czech Republic ranks third worldwide, while the incidence of rectal cancer, particularly in men, is the highest. Both environmental and genetic factors are involved in the onset of sporadic CRC, which represents the predominant form

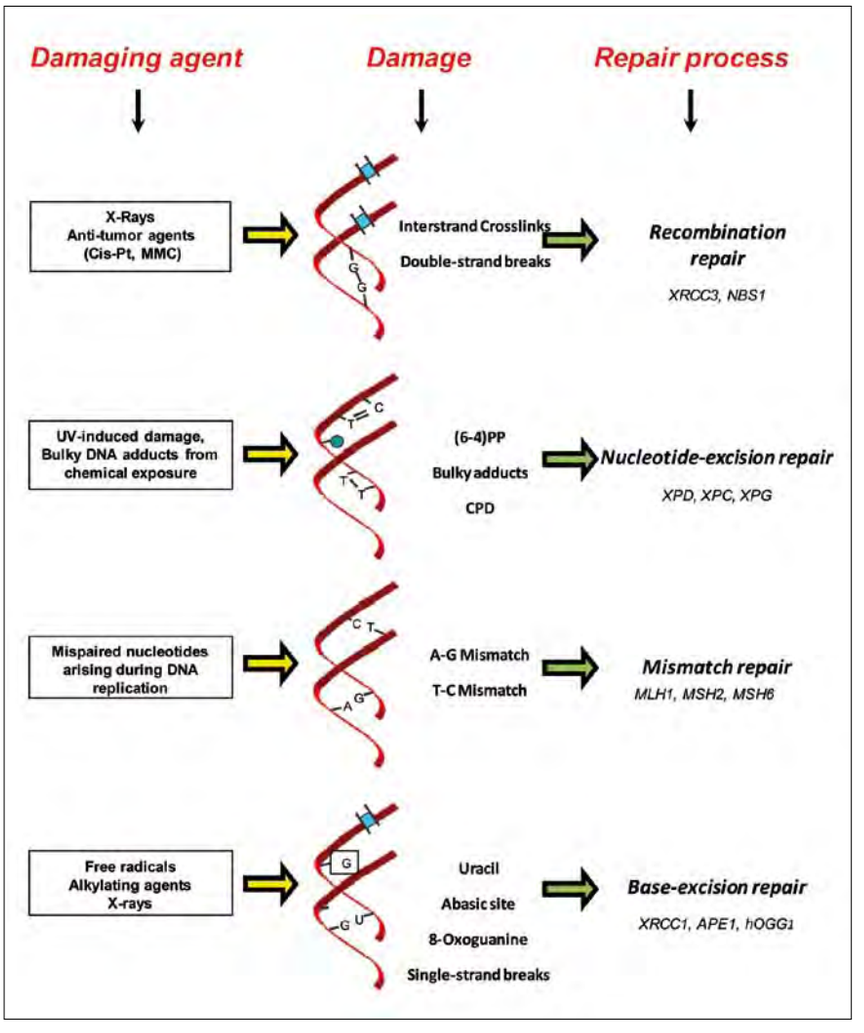
of this cancer (approx. 90% of all cases). It is believed that sporadic CRC involves multiple genes with moderate effects (low penetrance type) and progression occurs due to aggressive gene-environment interactions. The complex etiology of CRC and the observed high incidence in the Czech Republic stress the importance of a systematic approach, combining epidemiological and molecular biological methods, to understand the critical pathways in CRC tumorigenesis. Our recent interest has been oriented towards chemotherapy regimes, usually selected with the help of classical predictive markers such as TNM, which are not equally effective in all patients and which exert significant side effects. An assumption that the genetic profiles of patients could improve the prediction of an individual's response to standard chemotherapy regimes in CRC resulted in a selection of candidate genes, comprising the metabolic, transport, DNA repair and cell-cycle genes. Screening the loci in the above genes may have relevance in pharmacogenomics with the ultimate goal of individualized chemotherapy. Most recently, the participation of the Department in a multicenter study has resulted in the identification of several susceptibility loci of CRC using genome wide association (GWA). A detailed analysis of these loci, regarding their function in tumor cell biology, is ongoing.

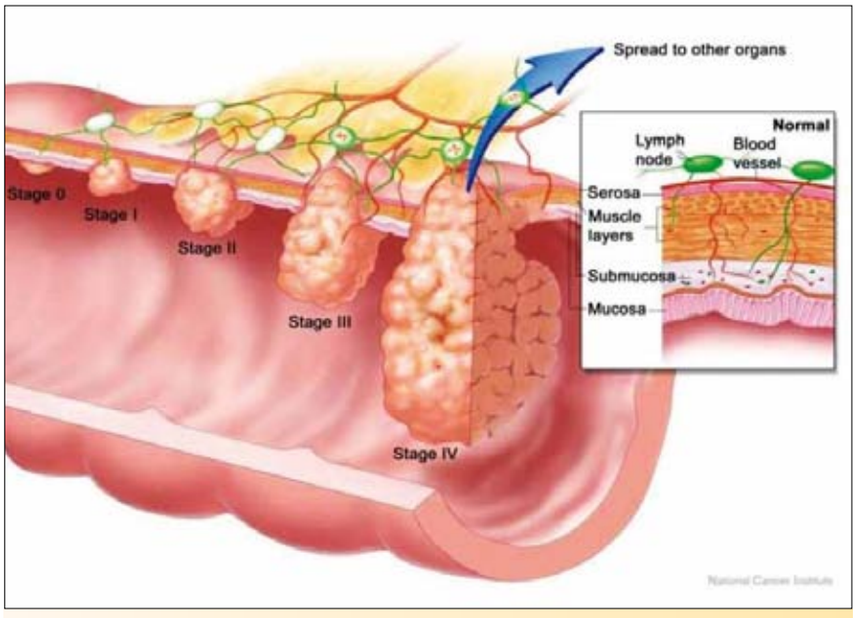
Laboratory of DNA Repair

RESEARCH TOPICS

- Investigation of the mechanisms involved in DNA repair pathways;
- functional tests for DNA repair capacity;
- expression of relevant DNA repair candidate genes;
- interest in genotype-phenotype interactions;
- the role of several DNA repair pathways in carcinogenesis.

Nuclear DNA is constantly exposed to DNA damaging agents from the environment and the diet. Normally, there is a dynamic equilibrium between DNA damage and its removal by effective and accurate cellular repair enzymes. If the steady state is disturbed, the damage measured will increase, but then increased repair activity (through normal enzyme kinetics, with possibly induction or activation in addition) will tend to restore the equilibrium. The steady state level depends on the intrinsic repair rate in the individual's cells, which may be in part genetically determined and in part affected by metabolic or environmental factors. To date, more than 150 human DNA repair genes have been identified, which can be categorized into 5 main pathways: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), double-strand break repair (DSB) and direct repair. The cell cycle and mitotic spindle checkpoints are also critical in this process to ensure that cell proliferation only follows the correct replication and organization of genetic material, respectively. Otherwise, if genetic material is altered, it can be repaired at the DNA level, enabling the cell to replicate. If the genetic damage is too excessive for repair, the cell avoids propagating the damaged DNA by undergoing apoptosis. Thus DNA repair plays an important role in close relationship with cell cycle control in cancer prevention, by removing potentially mutagenic lesions and maintaining chromosomal and genomic stability. DNA repair capacity measurement represents a complex marker inte-





Stages of colorectal cancer (source: National Cancer Institute).

grating polymorphisms, gene expression, the stability of the gene product, the effect of inhibitors/stimulators, environmental factors and lifestyle factors. In sporadic cancers, greatly affected by gene-environment interactions, the employment of new and pathway-specific DNA repair assays hallmarks cancer risk identification and the prediction of therapeutical outcome.

CURRENT GRANT SUPPORT

GA CR, 310/07/1430, Molecular and genetic characteristics of sporadic colorectal cancer in the Czech Republic, 2007–2011.

IGA NR, 8563-5/2005, Genetic profile of xenobiotic metabolising and DNA repair genes in cancer patients and control individuals in the Czech Republic, 2005–2009.

IGA NR, 9423-3/2007, XME, DNA repair and cell cycle regulation genes in head and neck cancer prediction, 2007–2009.

IGA NR, 9422-3/2007, The influence of environmental and genetic factors of pancreatic cancer (genetic profile), 2007–2009.

GA AS CR, IAA 500 200 917, Genetic and immunity in early stages of colorectal adenocarcinoma: inflammatory environment in conventional vs germ-free animal models, and in human samples, 2009–2013.

EEA-researchfund, A/CZ0046/2/0012, Quality and safety of food in relation to colorectal cancer predisposition. A pilot study. 2009–2010.

GA AS CR, IAA 500 390 806, The determination of expression levels of DNA repair and cell cycle genes in peripheral blood lymphocytes in styrene exposed individuals, 2008–2010.

SELECTED RECENT PUBLICATIONS

1. Vodička P, Kumar R, Štětina R, Sanyal S, Souček P, Haufroid V, Dusinska M, Kuricová M, Zámečníková M, Musak L, Buchancová J, Norppa H, Hirvonen A, Vodičková L, Naccarati A, Matoušů Z, Hemminki K. (2004) Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. Carcinogenesis 25(5): 757–763.

2. Vodička P, Tuimala J, Štětina R, Kumar R, Manini P, Naccarati A, Maestri L, Vodičková L, Kuricová M, Järventaus H, Majvaldová Z, Hirvonen A, Imbriani M, Mutti A, Migliore L, Norppa H, Hemminki K. (2004) Cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in styrene-exposed lamination workers. Environ Health Perspect 112(8): 867–871.

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6. Pittman AM, Webb E, Carvajal-Carmona L, Howarth K, Di Bernardo MC, Broderick P, Spain S, Walther A, Price A, Sullivan K, Twiss P, Fielding S, Rowan A, Jaeger E, Vijayakrishnan J, Chandler I, Penegar S, Qureshi M, Lubbe S, Domingo E, Kemp Z, Barclay E, Wood W, Martin L, Gorman M, Thomas H, Peto J, Bishop T, Gray R, Maher ER, Lucassen A, Kerr D, Evans GR, CORGI Consortium, van Wezel T, Morreau H, Wijnen JT, Hopper JL, Southey MC, Giles GG, Severi G, Castellví-Bel S, Ruiz-Ponte C, Carracedo A, Castells A, EPICOLON Consortium, Försti A, Hemminki K, Vodička P, Naccarati A, Lipton L, Ho J. W, Cheng KK, Sham PC, Luk J, Agúndez JA, Ladero JM, de la Hoya M, Caldés T, Niittymäki I, Tuupanen S, Karhu A, Aaltonen LA, Cazier JB, Tomlinson IP, Houlston RS. (2008) Refinement of the basis and impact of common 11q23. 1 variation to the risk of developing colorectal cancer. Hum Mol Genet 17(23): 3720–3727.

7. Poláková V, Pardini B, Naccarati A, Landi S, Slyšková J, Novotný J, Vodičková L, Bermejo JL, Hánová M, Šmerhovský Z, Tulupová E, Kumar R, Hemminki K, Vodička P. (2009) Genotype and haplotype analysis of cell cycle genes in sporadic colorectal cancer in the Czech Republic. Hum Mutat 30(4): 661–668.

DEPARTMENT OF PHARMACOLOGY

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Darina Korečková, MSc | PhD Student
Petra Melkusová, MSc | PhD Student
Dana Uhrová, MSc | PhD Student
Vladimír Kameník | Undergraduate Student
Vlasta Krejčová | Technician
Jana Křížková, MA | Research Assistant

RESEARCH TOPICS

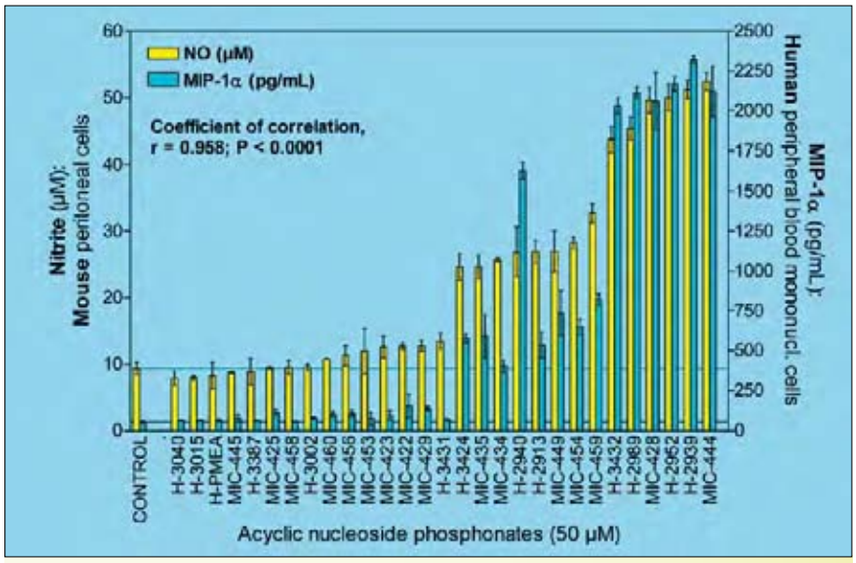
Manipulation of the cytokine network is a central paradigm for successful immunotherapy. The search for new drugs that would directionally modulate immune system activity has become a permanent challenge for pharmacological research. We investigate possibilities for the pharmacological modulation of immune factors such as cytokines, chemokines, interferons, and nitric oxide. On one side, these factors play critical roles in the defence of organisms against infections and cancer. On the other side, their long-term overproduction is often associated with the etiopathogenesis of many diseases such as asthma, chronic obstructive pulmonary disease, rheumatoid arthritis, etc. Therefore, novel agents that would contribute to both cytokine and anti-cytokine therapies are urgently needed in clinical practice.

Toward this point, the department pays major attention to antiviral acyclic nucleoside phosphonates, to modulators of intracellular calcium (inhibitors of sarco/endoplasmic Ca^{2+}-ATPase, i. e. SERCA inhibitors), natural compounds such as sesquiterpene lactones, and probiotics.

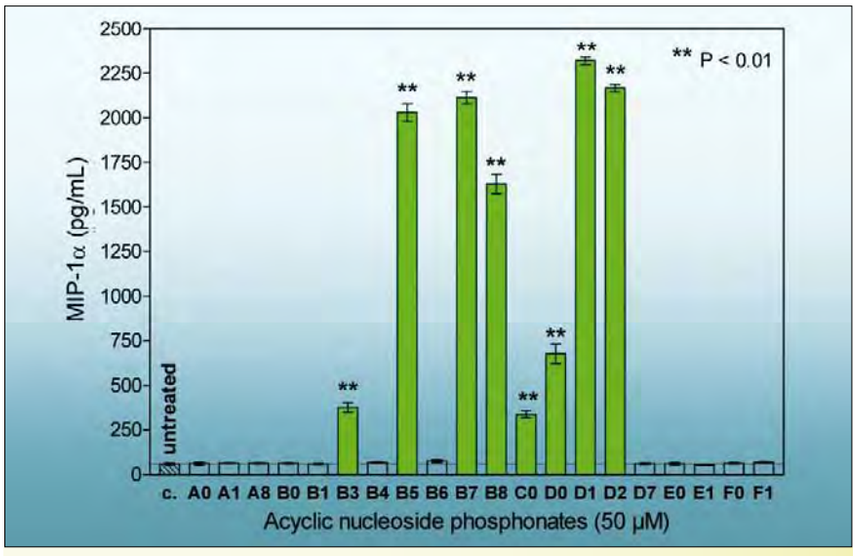
In order to test the cytokine-modulatory activities of both synthetic and natural agents, we have developed a nitric oxide-based, moderate-throughput screening bioassay, allowing for the reliable and inexpensive screening of a drug's potential to activate cytokine secretion. The data thus obtained in animal cell cultures can be employed to predict the immunomodulatory effects of drugs in cells of human origin.

We have found that many acyclic nucleoside phosphonates activate the production of cytokines interfering with virus replication and chemokines (e. g. RANTES, MIP-1α, MCP-1) that inhibit the penetration of HIV into cells. SERCA inhibitors have been found to be potent inducers of the Th1-type cytokine interferon-gamma (IFN-γ), which plays a crucial role in antiviral activity.

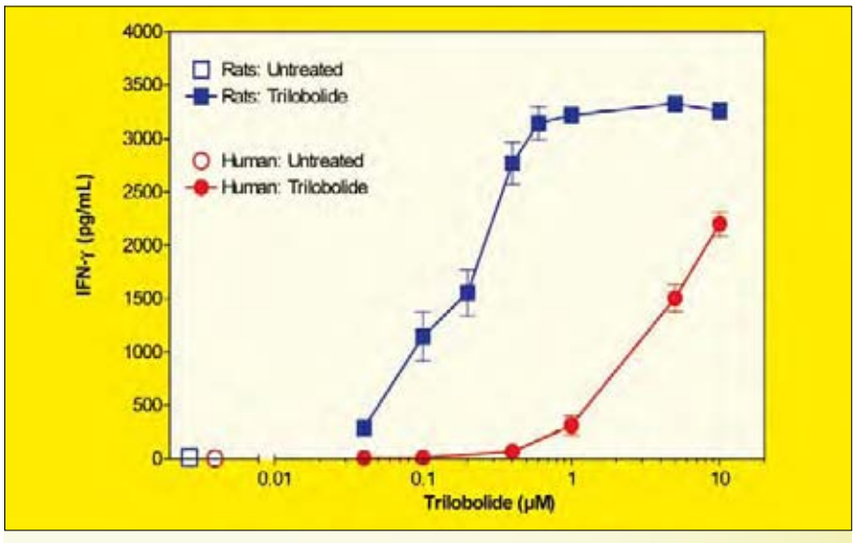
The methods employed to characterize the immunobiological activity of compounds include cell cultures, ELISA, multiplex analysis systems (LUMINEX), RT-PCR, etc. In order to understand the mechanisms of interference of agents with the immune system, the underlying signaling pathways and the expression of transcription factors are analyzed.



The production of nitric oxide by mouse peritoneal cells can be used as a biomarker predicting the cytokine-stimulatory effects of drugs in human cells.



Some acyclic nucleoside phosphonates (A0 – F1) are potent stimulators of chemokine MIP-1α in human cells (MIP-1α inhibits the entry of HIV-1 into cells).



Sesquiterpene lactone trilobolide, an inhibitor of Ca^{2+}ATP-ase, activates the secretion of interferon-g (IFN-γ) in rat and human immunocompetent cells.

CURRENT GRANT SUPPORT

Ministry of Education,1M0508, Center for New Antivitals and Antineoplastics, 2005–2011.

GA CR, 305/07/0061, Immunopharmacological potential of endoplasmic Ca^{2+}-ATPase SERCA, 2007–2011.

GA CR, 305/08/0535, Interference of probiotics with factors determining pharmacokinetics of drugs, 2008–2011.

SELECTED RECENT PUBLICATIONS

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2. Kmoníčková E, Potměšil P, Holý A, Zídek Z. (2006) Purine P1 receptor-dependent mmunostimulatory effects of antiviral acyclic analogues of adenine and 2,6-diaminopurine. Eur J Pharmacol 530(1–2): 179–187.

3. Potměšil P, Holý A, Kmoníčková E, Křížková J, Zídek Z. (2007) Acyclic nucleoside hosphonate antivirals activate gene expression of monocyte chemotactic protein 1 and 3. J Biomed Sci 14: 59–66.

4. Zídek Z, Kmoníčková E, Holý A. (2007) Secretion of antiretroviral chemokines by human cells cultured with acyclic nucleoside phosphonates. Eur J Pharmacol 574: 77–84.

5. Kmoníčková E, Melkusová P, Farghali H, Holý A, Zídek Z. (2007) Nitric oxide production in mouse and rat macrophages: A rapid and efficient assay for screening of drugs immunostimulatory effects in human cells. Nitric Oxide 17: 160–169.

6. Kmoníčková E, Melkusová P, Harmatha J, Vokáč K, Farghali H, Zídek Z. (2008) Inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPase thapsigargin stimulates production of nitric oxide and secretion of interferon-gamma. Eur J Pharmacol 588(1): 85–92.

7. Zídek Z, Anzenbacher P, Kmoníčková E. (2009) Current status and challenges of cytokine pharmacology (Review). Br J Pharmacol 157: 342–361.

DEPARTMENT OF MOLECULAR EMBRYOLOGY

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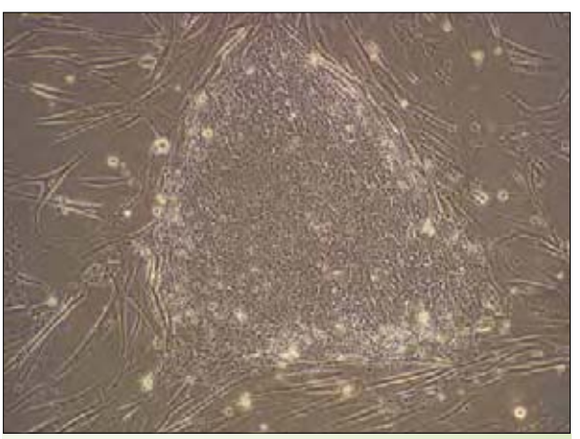
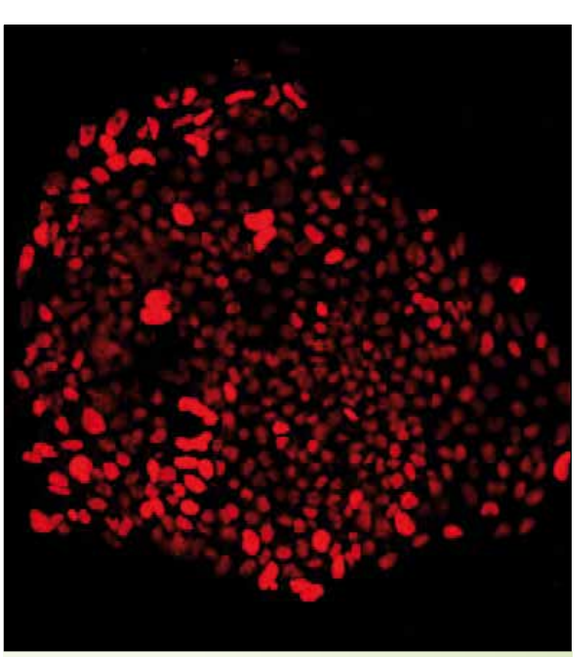
RESEARCH TOPICS

Historically, research in the department has focused on studying cells with pluripotent properties, including developing gametes and cells of embryonal origin – embryonal carcinoma cells and embryonic stem cells. The department is the first and only laboratory in the Czech Republic where cell lines (embryonic stem cells – ESC) were established from human blastocyst-stage embryos in 2003.



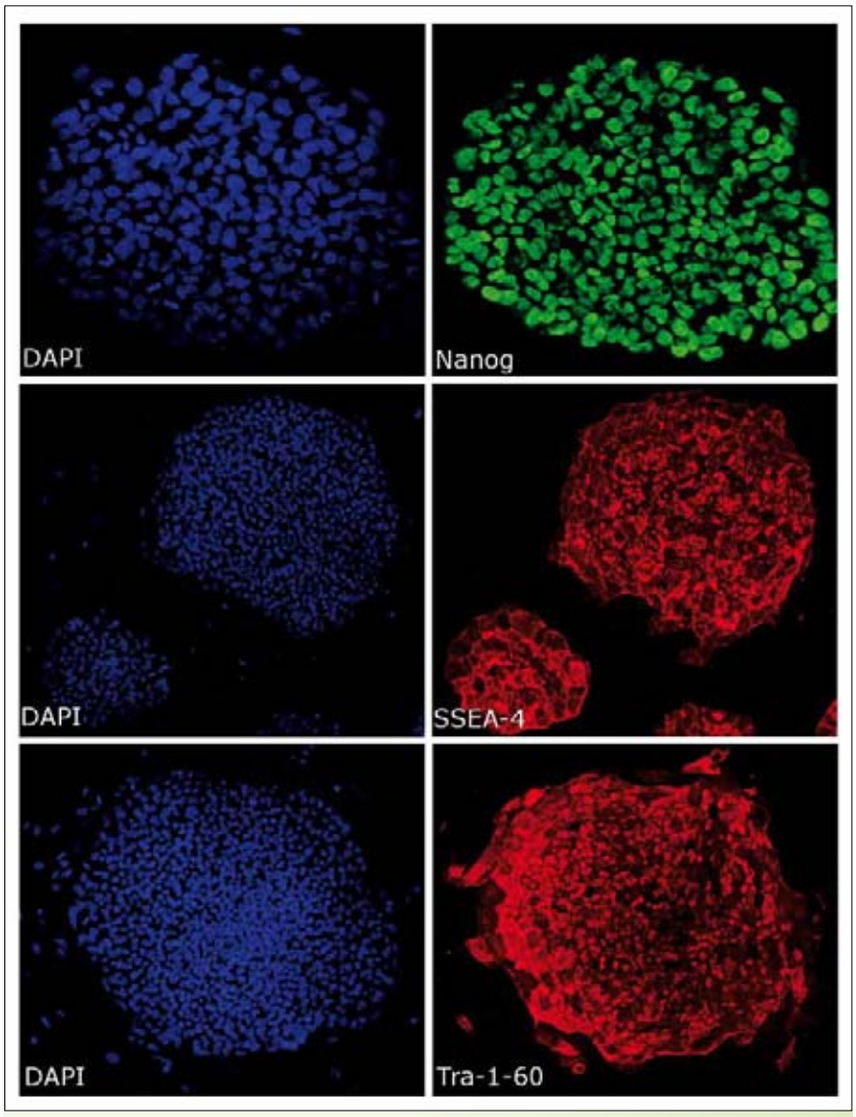
Colony of undifferentiated human embryonic stem cells *(line CCTL14 established in the department) growing on a feeder layer of mouse embryonic fibroblasts.*

Since then, the major focus of the department has been on various aspects of the biology of these unique primitive cells, which, because of their potential to differentiate into all specialized cell types of the adult body, represent unprecedented promise for new-age medicine.

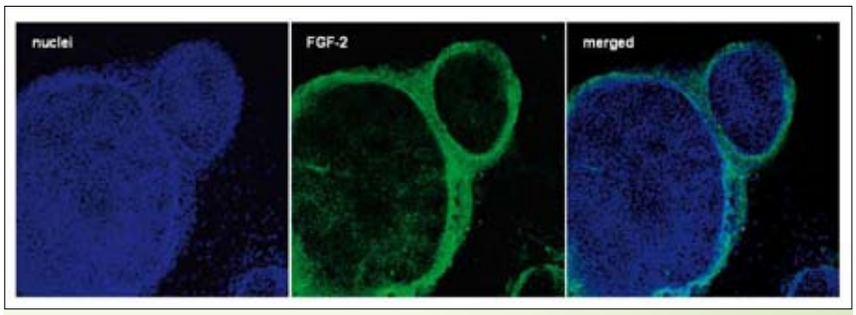


Tumor suppressor *protein p53 is highly expressed in the nuclei of some cells in a colony of human embryonic stem cells cultured in vitro. The red color visualizes p53 protein inside the cell nuclei.*

Most importantly, we investigate i) the functioning of cell cycle regulatory molecules, CDKs, cyclins, and CKIs, and their significance for the undifferentiated growth of human ESC (hESC), ii) the molecular pathways that are employed by hESC to transmit signals produced by fibroblast growth factor (FGF-2) and their biological role in this cell type, and iii) the molecular mechanisms that underlie the genetic alterations occurring in hESC propagated in culture and the phenotypical changes produced by such damage to the hESC genome. Besides the research focused on the molecular and biological properties of hESC, we also conduct experiments that target more clinically relevant issues, which include mainly the development of new protocols and conditions for the propagation of undifferentiated hESC and/or their differentiation into specific functional cell types. We have established fruitful collaborations with several renowned laboratories around the world and participate in various international studies aimed at exploiting the potential of hESC. Among them, a global comparative study lead by Prof. Peter Andrews (University of Sheffield) called the International Stem Cell Initiative is the most significant.



The presence of pluripotency markers *(transcription factor Nanog; cell membrane glycoproteins SSEA-4, TRA-1-60) in undifferentiated human embryonic stem cells as visualized by indirect immunofluorescence followed by confocal microscopy. Blue – cell nuclei, green/red – markers.*



Expression of fibroblast growth factor (FGF-2) in human embryonic stem cells. *The expression is higher in differentiating cells at the periphery of the cell colonies. Blue – cell nuclei, green – FGF-2.*

CURRENT GRANT SUPPORT

EU 6th FP, 018739, Platforms for biomedical discovery with human ES cells, ESTOOLS.

Ministry of Education, 1M0538, Center of Cell Therapy and Tissue Repair.

GA CR, 204/09/2044, and MRC International Stem Cell Initiative II.

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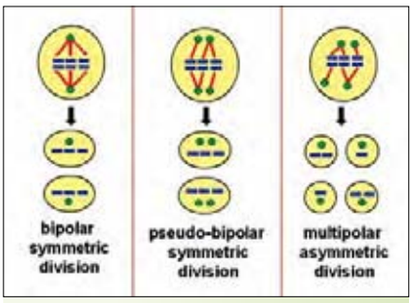
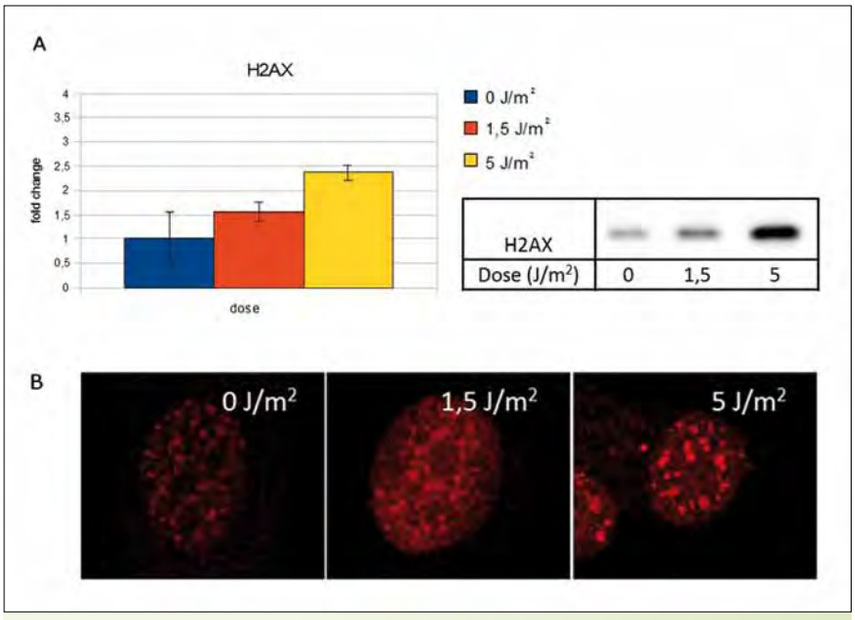


Schematic of the potential outcomes of overamplification of centrosomes.

Hyllner J, Imreh MP, Itskovitz-Eldor J, Jackson J, Johnson JL, Jones M, Kee K, King BL, Knowles BB, Lako M, Lebrin F, Mallon BS, Manning D, Mayshar Y, McKay RD, Michalska AE, Mikkola M, Mileikovsky M, Minger SL, Moore HD, Mummery CL, Nagy A, Nakatsuji N, O'Brien CM, Oh SK, Olsson C, Otonkoski T, Park KY, Passier R, Patel H, Patel M, Pedersen R, Pera MF, Piekarczyk MS, Pera RA, Reubinoff BE, Robins AJ, Rossant J, Rugg-Gunn P, Schulz TC, Semb H, Sherrer ES, Siemen H, Stacey GN, Stojkovic M, Suemori H, Szatkiewicz J, Turetsky T, Tuuri T, van den Brink S, Vintersten K, Vuoristo S, Ward D, Weaver TA, Young LA, Zhang W. (2007) Characterization of human embryonic stem cell lines by the > International Stem Cell Initiative. Nat Biotechnol 25(7): 803–816.

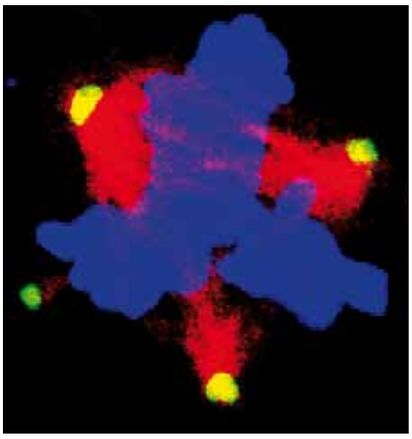
6. Bryja V, Pacherník J, Vondráček J, Souček K, Čajánek L, Horvath V, Holubcová Z, Dvořák P, Hampl A. (2008) Lineage specific composition of cyclin D-CDK4/CDK6-p27 complexes reveals distinct functions of CDK4, CDK6 and individual D-type cyclins in differentiating cells of embryonal origin. Cell Prolif 41: 875–893.

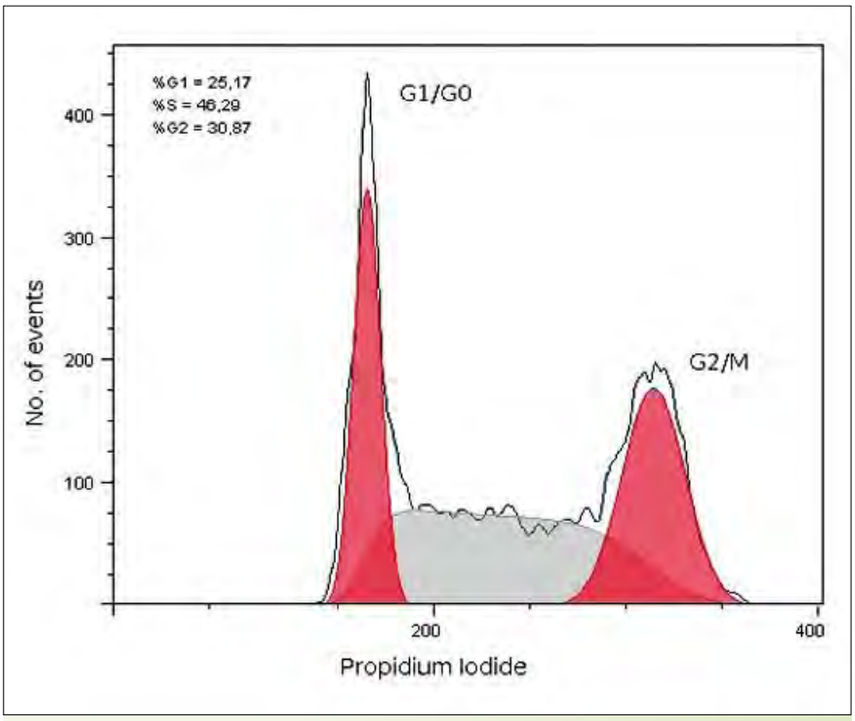


Expression of H2AX protein in neural precursors *differentiated from human embryonic stem cells upon damage to their DNA caused by UVC irradiation. A – quantity of H2AX as determined by western blot. B – visualization of H2AX foci (red) inside the cell nuclei.*

Multipolar mitosis in a human embryonic stem cell *suffering from overamplified centrosomes as visualized by indirect immunofluorescence followed by confocal microscopy. Blue – chromosomes, red – microtubules of the mitotic spindle, green – centrosomes.*

→





Distribution of undifferentiated human embryonic stem cells *in the phases of the cell cycle as determined by flow cytometry upon visualization of DNA content by propidium iodide staining (A)*

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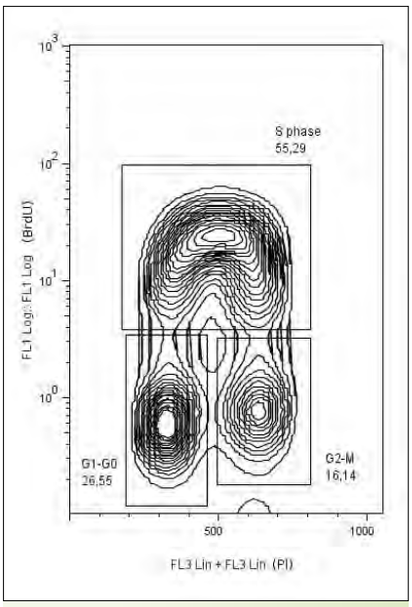

and upon metabolic labeling of DNA with BrdU combined with propidium iodide staining (B).

LABORATORY OF TISSUE ENGINEERING

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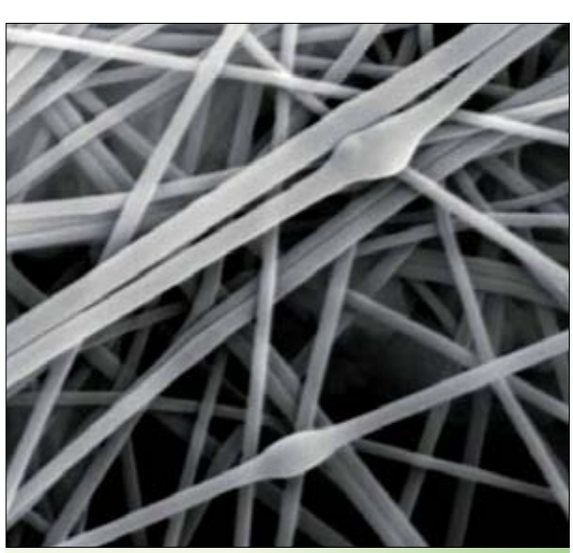
Jarmila Greplová | Undergraduate Student

The Laboratory of Tissue Engineering was established in the year 2005 after the research team moved from the Institute of Physiology of the AS CR. In conjunction with the relocation, the main research effort was focused on tissue engineering. Currently, three main research topics are investigated in the laboratory: tissue engineering, controlled drug delivery and protein engineering. The laboratory closely collaborates with the Department of Biophysics, Charles University in Prague, the 2nd Faculty of Medicine and the Department of Nonwovens, Faculty of Textile Engineering, Technical University of Liberec.

RESEARCH TOPICS

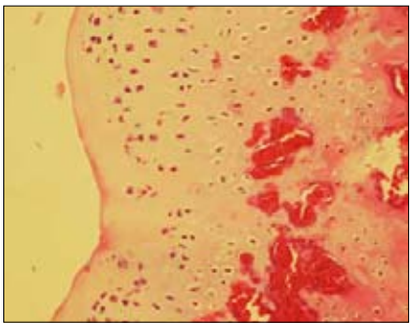
The research is concentrated on the development of novel three-dimensional scaffolds utilizing biodegradable materials. Textiles, both woven and nonwoven, as well as composite scaffolds are generated mainly employing a nanofiber-based approach and

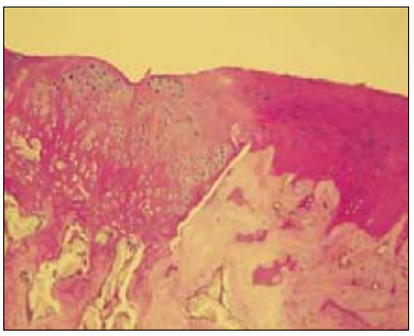


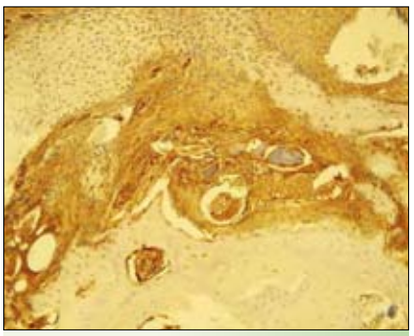
Controlled drug delivery.
Liposome-enriched nanofibers.

applied separately or in combination with an isotropic gel. Grafts based on autologous chondrocytes and mesenchymal stem cells are used for tissue defect regeneration (namely cartilage and bone). A special technique for the rapid evaluation of biomechanical properties in miniature tissue pieces was developed.

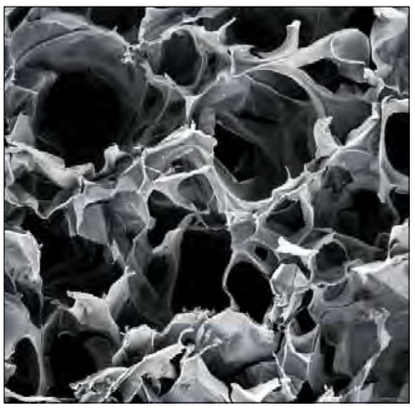
A liposome-based controlled drug delivery and nutrient supply system to deliver bioactive substances directly into defects was developed. The application of the novel technology of coaxial spinning for the production of smart nanofibers is intensively studied, especially in combination with liposomes, with the aim of developing suitable systems for controlled drug delivery. This advanced drug delivery system is mediated with liposome- and immunoliposome-enriched nanofibers and controlled by ultrasound sonication and shock-waves.

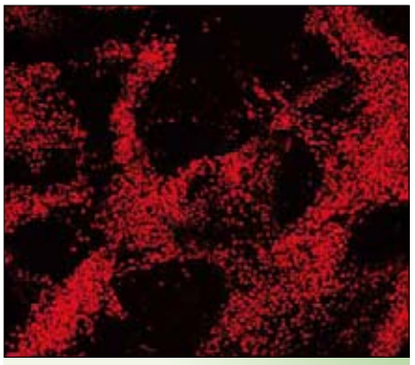






Histology of an osteochondral defect 6 weeks after implantation demonstrates the capability of a composite hyaluronan/type I collagen/fibrin scaffold to regenerate rabbit-knee cartilage.
Hematoxylin–eosin staining. Glycosaminoglycan detection by Alcian blue staining and PAS reaction. Immunohistochemical detection of type II collagen.

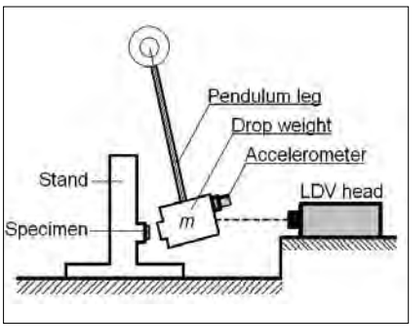


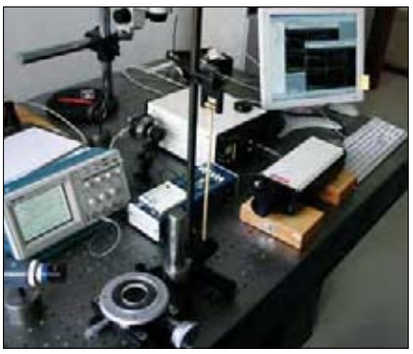


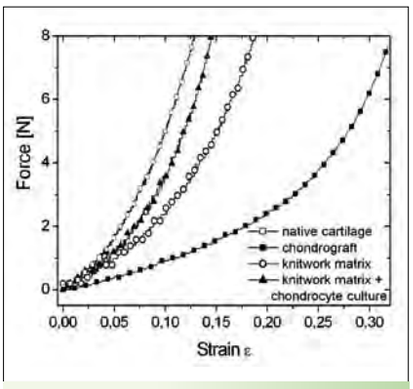
Scaffolds and cell seeding.
Electron microscopy of a cross-linked gelatin scaffold and confocal microscopy of a chondrocyte-seeded scaffold.

Artificial tissue implantation is another research topic. A novel approach was found to improve chondrocyte proliferation, nutrition and re-differentiation capacity, at the same time providing appropriate mechanical stability. The constructed scaffolds seeded with autologous chondrocytes can successfully heal osteochondral defects in experimental animals (rabbits and miniature pigs). Pre-clinical studies, following Good Laboratory Practice, are currently in progress.

The modern approach of computer modeling is applied for predicting the structural properties of cells and tissues, including protein dynamics.



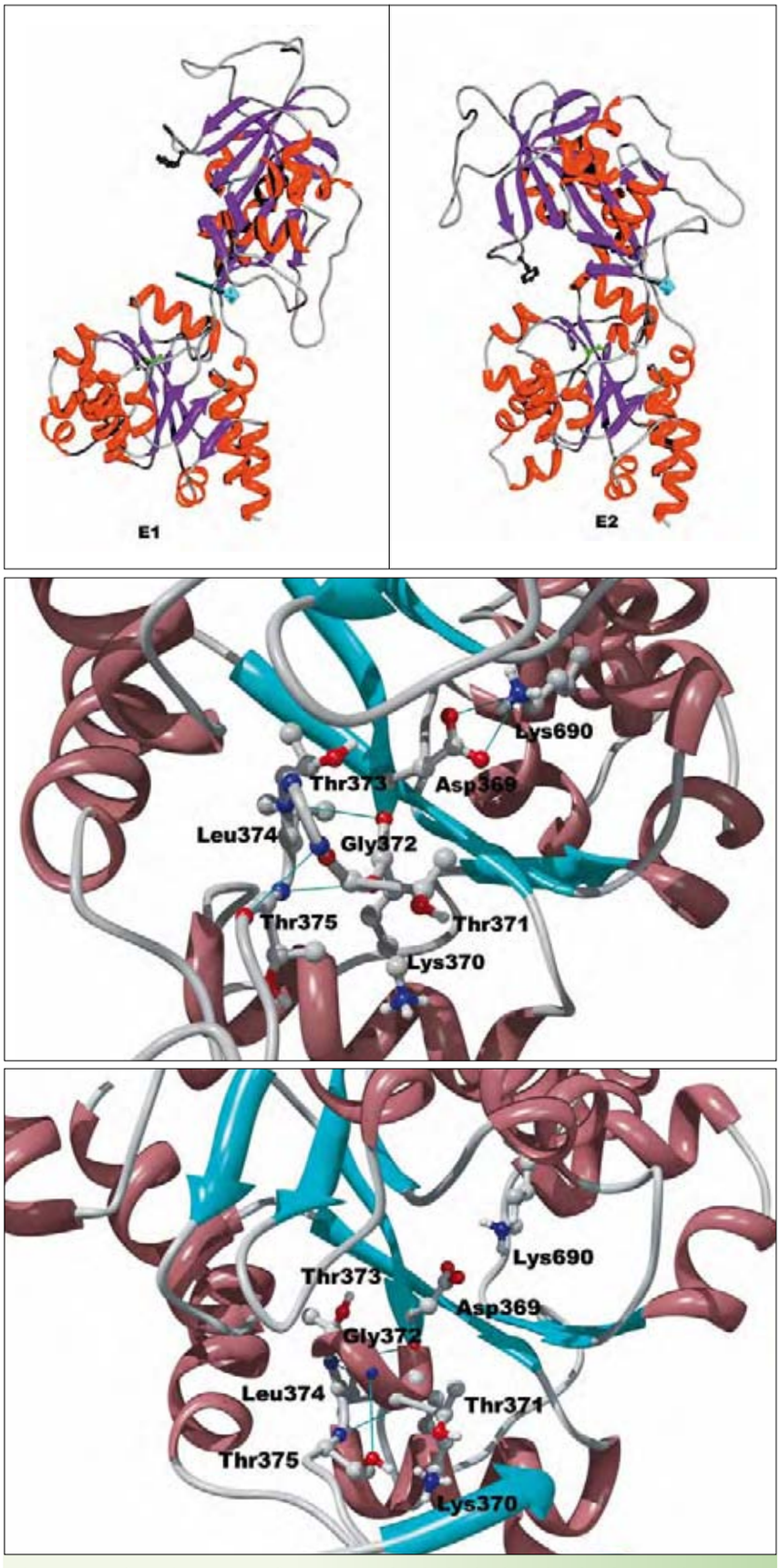




Biomechanical testing.
Scheme and apparatus for impact loading measurement. Loading curves of native cartilage and some of the materials tested.

Computer modeling, based on homology and similarity with proteins of known structure, is focused on protein structure determination and molecular dynamics simulation. Advanced studies of the molecular mechanism of Na^+/K^+-ATPase phosphorylation and of the structure and dynamics of the ATP-binding site on Na^+/K^+-ATPase are carried out. The relation between Na^+/K^+-ATPase structure, function and diseases (specifically familial hemiplegic migraine – FHM2) is also investigated.

High on our priority list is also the accelerated transfer of newly developed technologies and know-how into clinical practice.



Structure of Na^+/K^+-ATPase from mouse brain (α_2 isoform) in both E_1 and E_2 conformations.

Structure of the H_4-H_5 loop of Na^+/K^+-ATPase. The N-domain bends toward the P-domain by 64.8°. Detailed structure of the phosphorylation site in the E_1 and E_2 conformations. A hydrogen bond between the O atom of Asp^{369} and the N atom of Lys^{690} detected in the E_1 conformation disappears in the E_2 conformation, accompanied by the appearance of the short π-helix.

CURRENT GRANT SUPPORT

GA AS CR, IAA500390702, Scaffolds from liposome nanofibers for tissue engineering, 2007–2011.

Ministry of Education, NPV II 2B06130, Synthesis of new biomaterials and preparation of stem cell derived cells, and their applications in for the treatment of diseases affecting human tissues derived from mesoderm: cartilage, bone, ligament and meniscus, 2006–2011.

EU 7th FP, ID number 214539, Bioscent – Bioactive highly porous and injectable scaffolds controlling stem cells recruitment, proliferation and differenciation and enabling angiogenesis for cardiovascular engineered tissues, 2009–2013.

GA AS CR, Institutional research plan AV0Z50390512, Molecular, cellular and systemic mechanisms of major diseases of the human organism, their diagnosis, therapy and pharmacotherapy, 2005–2011.

GA AS CR, Institutional research plan AV0Z50390703, New biotechnologies, nanomaterials and stem cells for use in regenerative medicine, 2007–2013.

PATENT

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5. Tejral G, Koláčná L, Schoner W, Amler E. (2008). The π-helix formation between Asp^{369} and Thr^{375} as a key factor in E^1-E^2 conformational change of Na^+/K^+-ATPase. Physiol Res *in press*

6. Filová E, Jelínek F, Handl M, Lytvynets A, Rampichová M, Varga F, Činátl J, Soukup T, Trč T, Amler E. (2008) Novel composite hyaluronan/type I collagen/fibrin scaffold enhances repair of osteochondral defect in rabbit knee. J Biomed Mater Res B Appl Biomater 87B(2): 415–424.

LABORATORY OF CELL BIOLOGY

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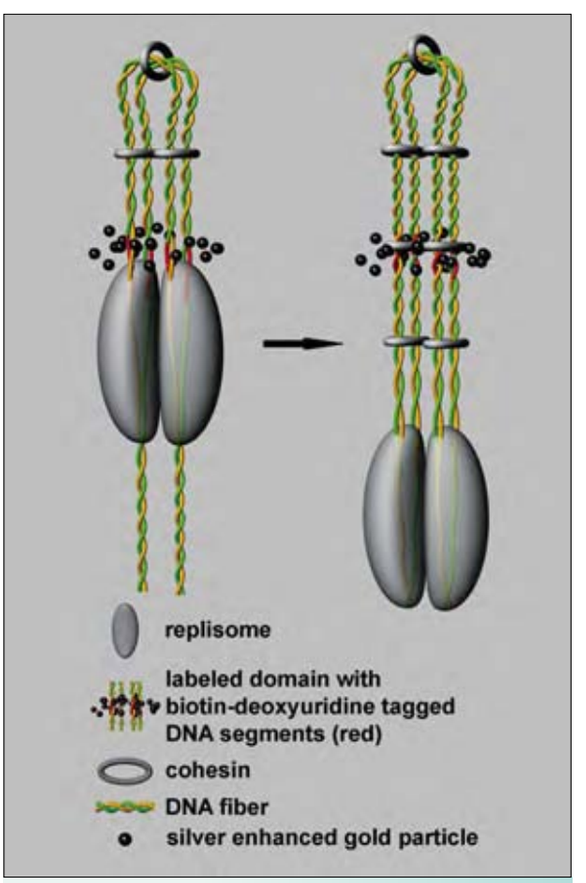
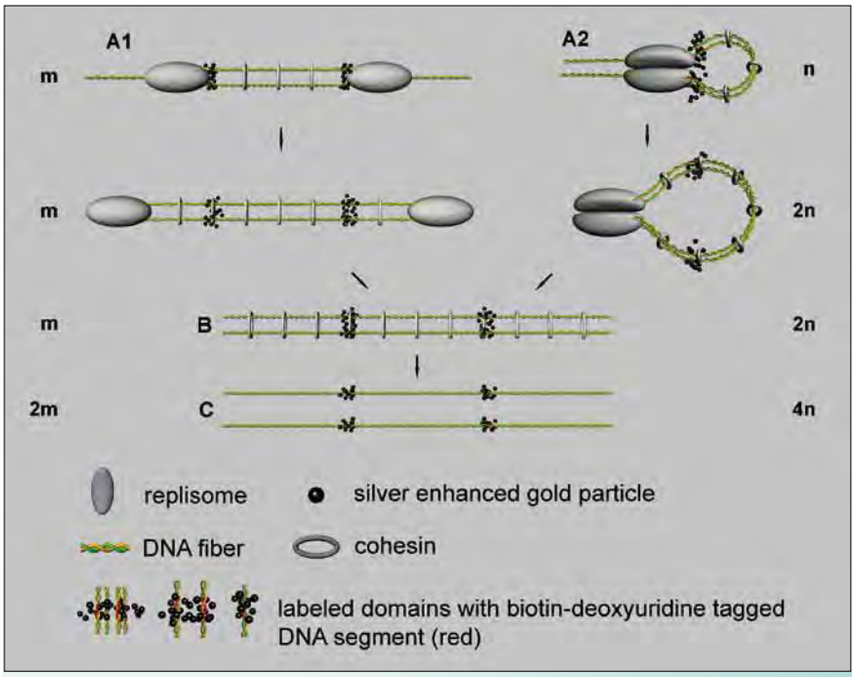
RESEARCH TOPICS

Research in the Laboratory of Cell Biology focuses on DNA replication and the organization of human chromatin. DNA replication of the two template strands at DNA replication forks is a highly coordinated process comprising the functioning of many factors that ensures accurate and efficient duplication of the eukaryotic genome. According to the general paradigm, proper DNA duplication from each replication origin is ensured by two protein complexes termed replisomes. In this respect, our comparative analysis of short segments of replicons labeled in pulse-chase experiments of various lengths showed that replisomes in HeLa cells are organized into couples during DNA replication.



The model of zipping loops.
Zipping of a DNA loop is shown. During replication, replisome couples produce a loop with the associated (zipped) arms probably in the form of four tightly associated 30 nm fibers. According to this model, "sister" pairs of biotin-dU-tagged segments of chromatids do not separate before the termination of the DNA synthesis of the replicon and the relaxation of the zipped arms. Immediately after labeling, the four tagged segments are present in one labeled domain (the left part of the image). Such an organization of the tagged segments persists during the synthesis of the whole replicon (the right part of the image). Although the mutual changes of the replisome position between the left and right parts of the Figure can result in the impression of movement of the replisome along the DNA, this model does not reflect whether the DNA or the replisome complex is moving during replication.

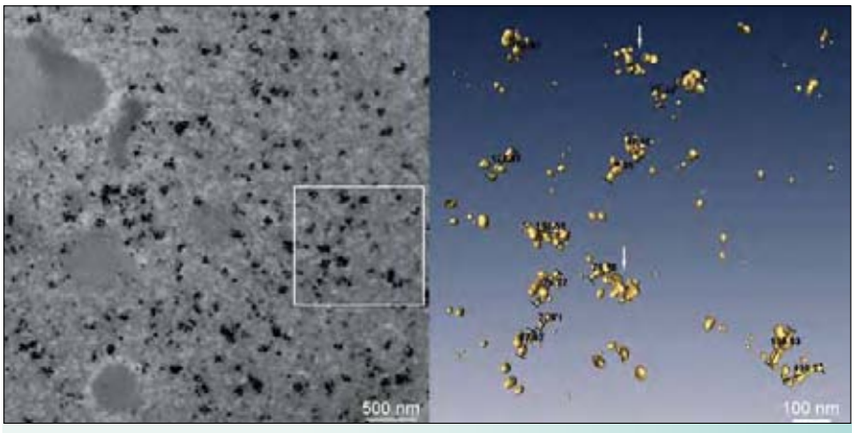


Two models of the arrangement of "sister" replisomes in HeLa cells and the effect of different organizations of biotin-dU-tagged segments on the number of labeled domains during various pulse-chase experiments.
The scheme shows the expected results of the consecutive mapping (indicated by arrows) of segments tagged during a short pulse of biotin-dUTP in the early S phase followed by chase periods of different lengths from the time immediately after the pulse (the upper part of the scheme) to the complete mitotic segregation of the sister chromatids (the lower part of the scheme).

Moreover, our data suggested a new model of the organization of replicated DNA. According to this model, replisome couples produce a loop with the associated arms in the form of four tightly associated 30 nm fibers.

The Laboratory's research is also focused on changes in chromatin arrangement during the cell cycle, the timing of DNA replication, epigenetically inherited chromatin remodeling, and the large scale organization of chromosomal territories.

Concurrently, the Laboratory participates in a project focused on the development of new techniques for the delivery of bioactive molecules into human cells. Two basic research lines are followed in this respect: the development of methods based on the use of viral kinases for the efficient phosphorylation of chosen nucleosides and the development of methods based on the modification of nucleotide precursors.



3D reconstruction of the labeled domains.
The original image of a 200 nm-thick section of a cell nucleus is shown on the left (Scale bar: 500 nm), whereas a 3D reconstruction of the labeled domains reconstructed from the insert is shown on the right (Scale bar: 100 nm). The length measurement is demonstrated. The arrows indicate labeled domains traversing the section faces.

Techniques that are employed include cell and bacterial cultivation, affinity cytochemistry involving immunocytochemical and in situ hybridization techniques, light and confocal microscopy, electron microscopy including electron tomography, stereology approaches, protein, RNA and DNA purification and analysis, immunoprecipitation and GFP technology.

CURRENT GRANTS SUPPORT

GA AS CR, KAN200520801, Targeted expression and transport of bioactive molecules, 2008–2012.

GA CR, 206/07/0233, Biochemical, ultrastructural and partial proteomic analysis of the penetration apparatus of bird Schistosoma cercariae, 2007–2010.

GA CR, 305/08/0535, Organization of human nuclear chromatin, 2009–2011.

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2. Li J, Santoro R, Koberna K, Grummt I. (2005) the chromatin remodeling complex NoRC controls replication timing of rRNA genes. EMBO J 24: 120–127.

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5. Ligasová A, Raška I, Koberna K. (2009) Organization of human replicon: singles or zipping couples. J Struct Biol 165: 204–213.

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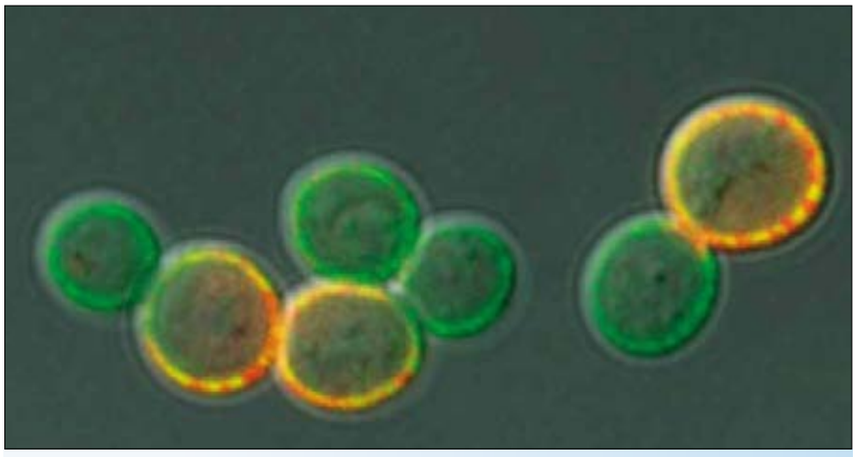
Jitka Eisensteinová | Secretary

RESEARCH TOPICS

We study the assembly and maintenance of cellular structures not bound by a membrane. These highly dynamic, steady state organelles engaged in specific functions communicate with their surroundings directly by diffusion, and the mechanisms controlling their structure and function are still poorly understood. Many of these compartments, such as the nucleolus or Cajal body in the cell nucleus, are easily seen under a microscope and have been known for a long time. Others have been described only recently – for instance lipid raft-based compartments in membranes. Morphological changes or the disintegration of these cellular structures often accompany pathologic phenotypes. Knowledge about the composition, formation and maintenance of these structures is limited. Thus, a great deal is open for studies using contemporary microscopy techniques, capable not only of exactly localizing cellular components, but also of detecting their movement and potential interactions at the molecular level as well.

Zeiss LSM 5 DUO confocal microscope.
We are well equipped for a variety of fluorescence and electron microscopy approaches. Besides our own research, methodological support for other groups is provided by the Unit staff.

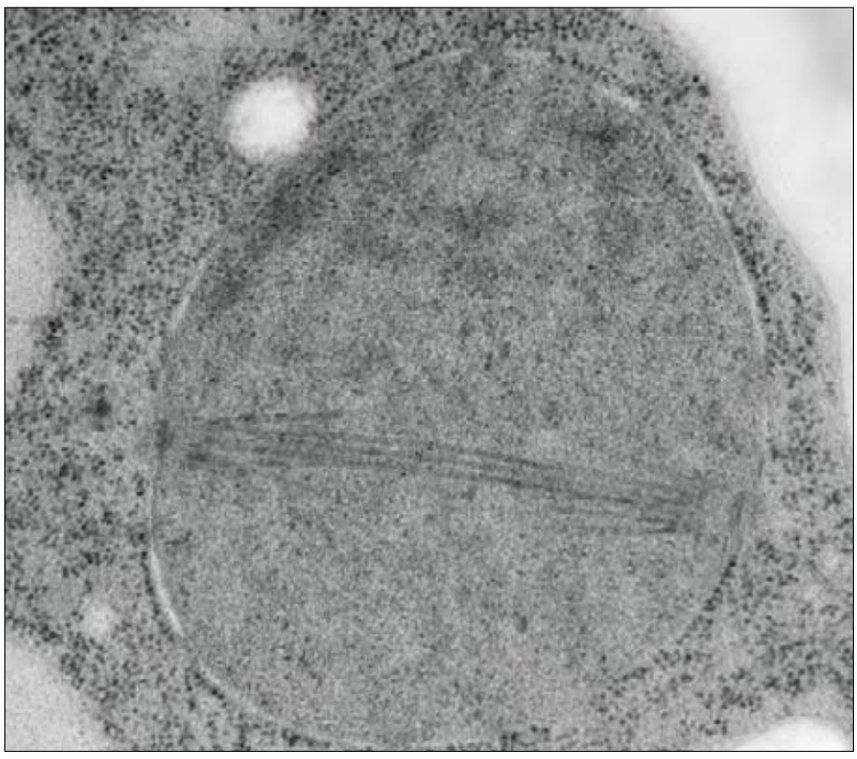
We were able to distinguish distinct domains in the plasma membrane of the yeast S. cerevisiae, the Membrane Compartments MCC (containing can1) and MCP (with Pma1). These domains are large enough to be resolved by fluorescence microscopy (Malinska et al., MBC14: 4427-36, 2003). We showed that MCC consists of stable, uniform, isolated patches within the plasma membrane. To date, twenty integral and soluble proteins have been shown to co-localize with the MCC pattern. The composition of the MCC patches depends on the membrane potential. We have hypothesized a specific role for them in protein turnover. Using various approaches of electron microscopy as well



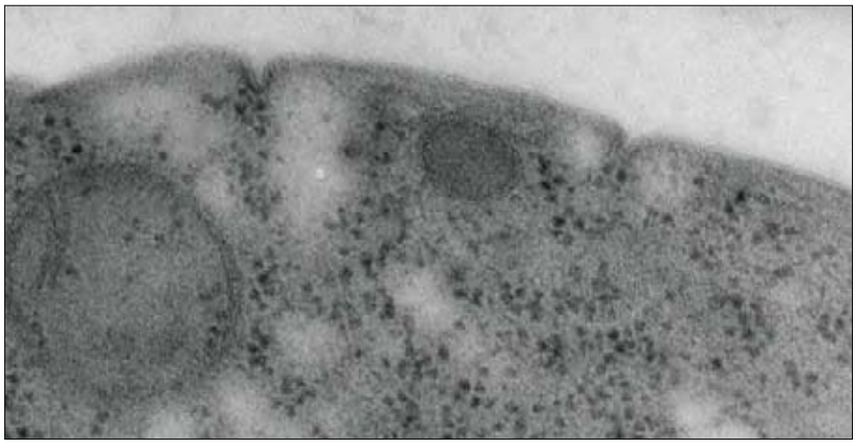
Plasma membrane domains in S. cerevisiae.
Transversal confocal section of living cells co-expressing the plasma membrane proton ATP-ase Pma1p-ds (red) and the proton-arginine symporter Can1-GFP (green) combined with a DIC image (grayscale). Pma1 and Can1 are laterally segregated within the plasma membrane; Can1 is confined to isolated patches, while Pma1 fills the surrounding area. Note the absence of a red signal in the growing buds caused by the slow maturation of the dsRed molecule.

as goal-directed mutant yeast strains, we are now attempting to describe the fine structure of MCC and to localize its protein components with a resolution of 20 nm. The specific lipid composition of MCC reported in fluorescence microscopy studies is indicative of the specific ultrastructure of this organelle.

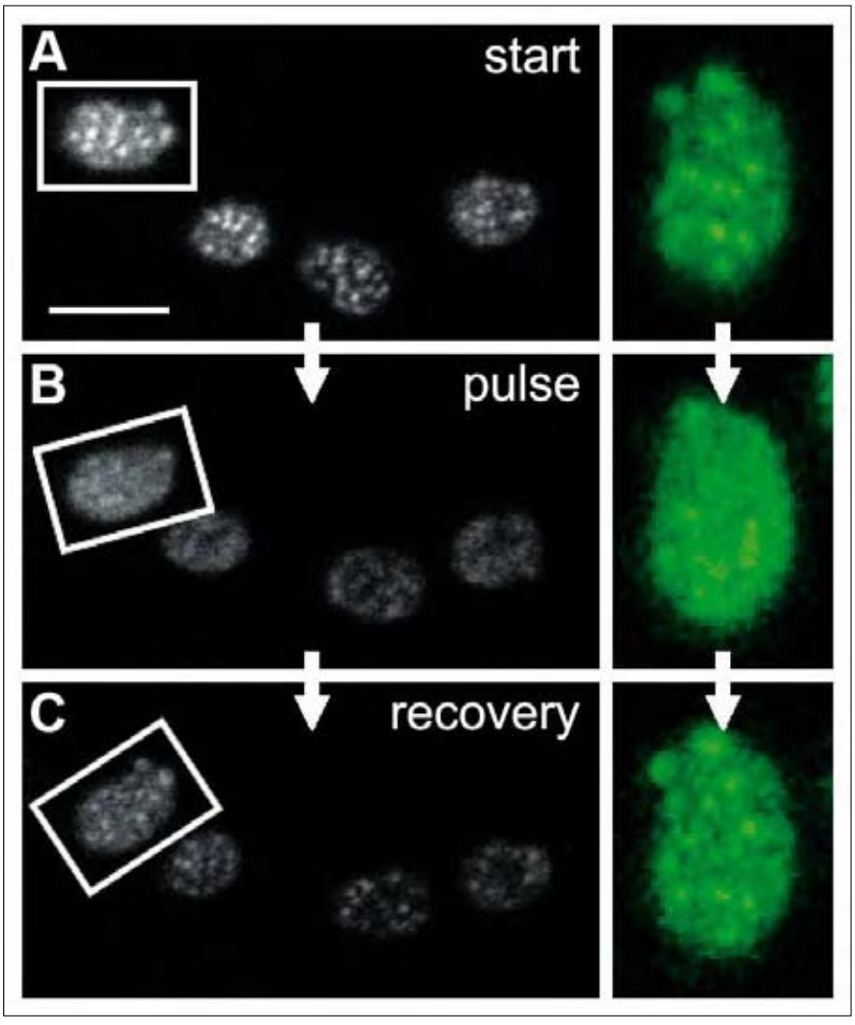
We also focus on the formation, distribution and dynamics of structures coupled to chromatin function – DNA replication sites, nucleoli etc. Using fluorescently-labeled components of the replication machinery, we visualize the dynamics of DNA replication. We also focus on the formation, distribution and dynamics of structures coupled to chromatin function – DNA replication sites, nucleoli etc. Using fluorescently-labeled components of the replication machinery, we visualize the dynamics of DNA replication. By kinetic analysis of the continuous exchange of small nuclear ribonucleoprotein particles between the nucleoplasm and Cajal bodies, we contribute to the understanding of mRNA splicing. Our aim is to formulate mathematical models describing the behavior of various nuclear compart-ments in general.



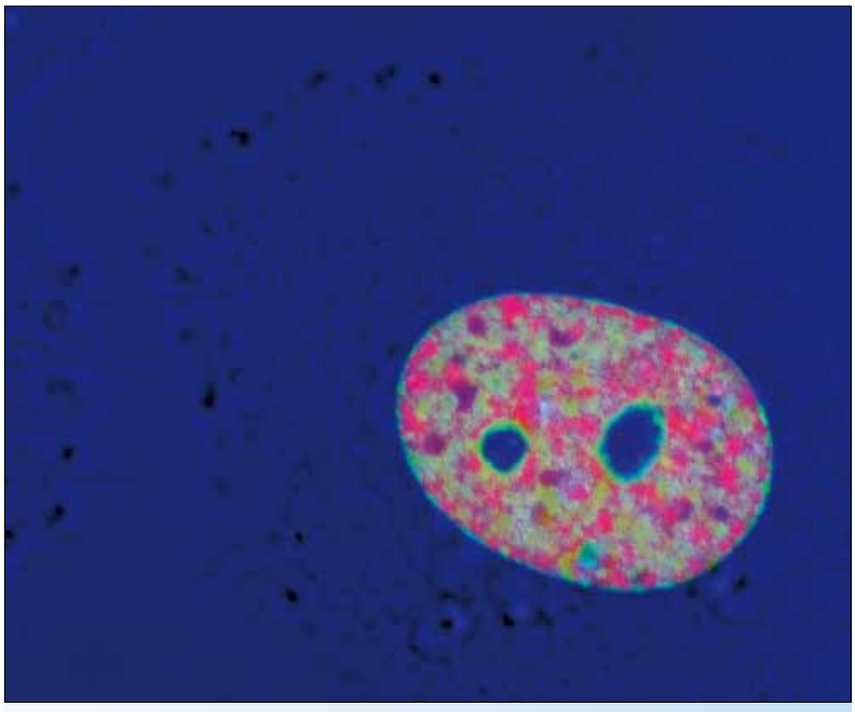
Yeast cell nucleus during mitosis.
During endomitosis, the nuclear envelope remains intact. Within the nucleus, the mitotic spindle with spindle pole bodies immersed in the nuclear envelope can be clearly recognized. A living yeast cell was frozen under high pressure, embedded into a synthetic resin and sectioned. High contrast of protein-rich structures (ribosomes, microtubuli) was achieved by the uranylacetate stain.



Curved membranes in yeast.
Various biological membranes, including the invaginated plasma membrane and the outer and inner membranes of mitochondria, were visualized on an ultrathin section of a yeast cell. The cytoplasm is filled with dark ribosomes and lucent round-shaped aggregates of glycogen.



Transient re-distribution of plasma membrane components upon membrane depolarization.
Some proteins are able to leave the membrane compartment of Can1 in a reversible manner after membrane depolarization. If heterologously expressed in S. cerevisiae, the glucose transporter HUP1 of C. kessleri is one of these proteins. A tangential section of living cells in three consecutive snapshots is presented. A pulse of an external electric field was applied prior to (B), while a 20 minute recovery preceded (C). Note that the patches in (C) are re-forming in their original positions (if compared to A).



Localization of replicating chromatin.
The DNA replication marker PCNA-mRFP (red) was co-localized with the histone H3. A confocal section of a mid-to-late S-phase cell, combined with a DIC image (blue), is shown. Note the spatial separation of the condensed chromatin and the active process of DNA replication.

CURRENT GRANT SUPPORT

GA CR, 204/07/0133, Self organization principles of nonmembrane-bound organels in eukaryotic cells.

GA CR, 204/08 J024), Lateral membrane compartments: Formation, functional relevance and genomics.

GA AS CR, KAN200520801, Targeted expression and transport of bioactive molecules.

SELECTED RECENT PUBLICATIONS

1. Mašata M, Malínský J, Fidlerová H, Smirnov E, Raška I. (2005) Dynamics of replication foci in early S phase as visualized by cross-correlation function. J Struct Biol 151(1): 61–68.

2. Smirnov E, Kalmarova M, Koberna K, Zemanová Z, Malínský J, Mašata M, Cvačková Z, Michalová K, Raška I. (2006) NORs and Their Transcription Competence during the Cell Cycle. Folia Biol. -Prague 52: 59–70.

3. Grossmann G, Opekarová M, Malínský J, Weig-Meckl I, Tanner W. (2007) Membrane potential governs lateral segregation of plasma membrane proteins and lipids in yeast. EMBO J 26(1): 1–8.

4. Staněk D, Přidalová J, Novotný I, Huranová M, Blažíková M, Wen X, Sapra AK, Neugebauer KM. (2008) Spliceosomal snRNPs repeatedly cycle through Cajal bodies. Mol Biol Cell 19(6): 2534–2543.

5. Střádalová V, Gaplovská-Kyselá K, Hozák P. (2008) Ultrastructural and nuclear antigen preservation after high-pressure freezing/freeze-substitution and low-temperature LR White embedding of HeLa cells. Histochem Cell Biol 130(5): 1047–1052.

6. Grossmann G, Malínský J, Loibl M, Stahlschmidt W, Weig-Meckl I, Frommer WB, Opekarová M, Tanner W. (2008) Plasma Membrane Microdomains Regulate Turnover of Transport Proteins in Yeast. J Cell Biol 183(6): 1075–1088.

DEPARTMENT OF TECHNOLOGY TRANSFER

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The crucial role of the department is to provide assistance to the research departments and laboratories of the Institute in their efforts to translate the innovative ideas and outcomes of their research work into business and clinical practice. The collaboration of public research centers with private companies and other application partners (e. g. hospitals) has been a worldwide trend. Technology transfer has become even more important in light of the research and innovation reform adopted by the Czech government in 2008.

The Institute has taken advantage of the opportunity to use available European Union structural funds for its technology transfer projects. The completed Center for the Support of Competitiveness in Biomedicine project focuses on developing human resources and raising public awareness of biomedicine in general and stem cell therapy in particular. A number of research workshops, conferences and tailor-made training activities have been realized within the framework of this project, focused both on experts and also on the general public.

To facilitate the desired cooperation of the private sector with public research organizations in biomedicine, the Institute proposed the construction of an Innovation Center for Biomedicine and eventually succeeded in the project competition to obtain financing for the Center, the only business incubator facility in the Czech Republic equipped with class A clean rooms. This technology, featuring constant monitoring of the air quality, enables the development of stem cell products for the treatment of various diseases and disorders, e. g. spinal cord injuries, articular cartilage, diabetic leg etc. Several new spin-off companies have already begun operations in the incubator, opened by Prime Minister Mirek Topolanek in October 2008.

Training of the Institute's researchers in biomedical technology transfer is another recently started EU-funded project, planned to last two years.

Its objective is to improve the capabilities of researchers by providing them with training in communication and presentation skills, intellectual property right protection and legislation, mainly in the field of biotechnology. The other part of the training programme will focus on pre-clinical development and clinical trials, including governing legislation and practical examples (case studies), both national and international, and further on biotechnology product security and quality management.

OPERAČNÍ PROGRAM
PRAHA ADAPTABILITA

IBC

INNOVATION BIOMEDICAL CENTER

View of the Innovation Biomedical Center.

The management of the IEM decided to establish the Innovation Biomedical Center (IBC) on the basis of medical market demand and in an effort to enhance the innovative potential of the IEM´s scientific outputs and the effectiveness of their transfer to medical practice. The innovation process is essentially wedded to entrepreneurship; therefore the IBC´s activities are oriented towards the support and development of small spin-off firms.

The Innovation Biomedical Center is a newly built facility located in the close vicinity of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, in Prague-Krč. It was constructed from August 2007 to March 2008. The project was financed with contributions from the EU and the city of Prague.

The Science and Technology Parks Association of the CR awarded the Innovation Biomedicine Center accreditation in October 2008.

The goal of the IBC project is to build the complex infrastructure necessary for technology transfer from the IEM through applied research and innovative entrepreneurship to the medical market place.

The vision of the IBC is to become the key center in the field of cell-based therapies and their transfer to medical practice in the Czech Republic, and to take part in world networks in this field.

The mission of the IBC is to stimulate research outputs and their commercialization by providing favorable conditions for the emergence and development of spin-off firms with innovative potential in the field of biomedicine.

The IBC promotes the use of IEM technology to benefit the IEM through licensing inventions to spin-off companies capable of successfully commercializing them. It supports the research mission of the IEM by finding industrial partners for spin-off firms, sponsoring research and generating licensing income that supports future research. The IBC earns a fair return and increases the recognition of the IEM and the inventors, thus contributing to the growth of the spin-off firms and the development of the IEM. The IBC helps move technologies from the IEM's laboratories to the marketplace by developing and managing an array of partnerships with the private sector. Successful technology transfer involves a number of steps, beginning with the invention of new technology. These inventions are evaluated, then if appropriate, intellectual property protection (patents or copyright) is sought. Collaborative research with industry may further develop the technology, which may then be promoted and, hopefully, licensed.

THE INNOVATION BIOMEDICINE CENTER HAS THREE COMPONENTS

Center of Support for Competitiveness in Biomedicine – provides offices and a small conference room for activities focused on training, consultation and legal support in biomedicine.

Center of Applied Research in Biomedicine – offers laboratories for applied research and scale-up technologies, focused on regenerative medicine, cell therapy, the development of biomaterials and pharmaceuticals as well as the design of clinical studies.

Business Incubator (office area, GMP-certified clean rooms) for spin-off companies. The companies housed in the incubator benefit from shared consultation, patent, tax and other services and take advantage of opportunities to participate in applied research projects run in the IBC facility.

Work in the clean rooms.

THE FIRST SPIN-OFF COMPANIES HAVE ALREADY MOVED THEIR OFFICES INTO THE IBC

BIOINOVA, s. r. o.

BioInova, s. r. o. is a subsidiary of the IEM, charged with the transfer of the IEM's intellectual property into practice. It mainly focuses on the operation of the new Innovation Biomedical Center (IBC) facilities and its Business Incubator for spin-off companies planning to develop innovative medical products based on current advances in cell therapy. The IBC is positioned in the high-end market as a specialized Business Incubator equipped with cutting edge clean rooms. Moreover, BioInova provides all necessary Good Manufacturing Practice (GMP) services for various companies and institutions for the development of modern medical products.

The mission of BioInova is to stimulate the startup, dynamic development and commercial success of innovative spin-off companies commercializing the scientific outputs of the IEM.

CHONDROS, s. r. o.

Chondros, s. r. o. provides complex solutions in the field of the biomedical treatment of locomotor injuries and cartilege replacement implants.

TATAA MOLECULAR DIAGNOSTICS, s. r. o.

TATAA Molecular Diagnostics, s. r. o. is a TATAA diagnostics facility providing diagnostic detection and analysis of circulating tumor cells in the blood of breast cancer patients.

STUDENT SCIENCE, s. r. o.

Student Science, s. r. o. is a spin-off company that emerged from the scientific output of the IEM in the field of autologous cartilage replacement therapy. The company's know-how includes methods for the cultivation of autologous cartilage cells and the production of innovative cartilage replacement implants.

CB BIO, s. r. o.

CB Bio, s. r. o. performs contract research under GLP requirements on small laboratory animals and is well established among Czech CRO's and small pharmaceutical companies.

MRSUPORT, s. r. o.

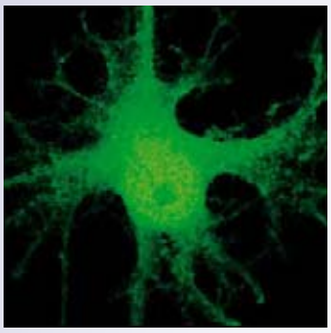
MRsuport, s. r. o. provides magnetic resonance software for specialized image enhancing, suitable, e. g., for visualizing cells labeled with superparamagnetic iron oxide nanoparticles in a living organism.

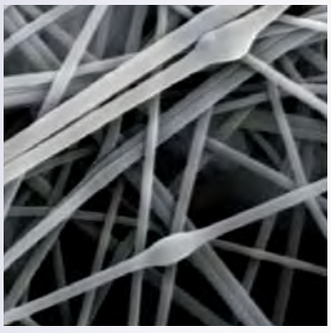
CELLNOVA, s. r. o.

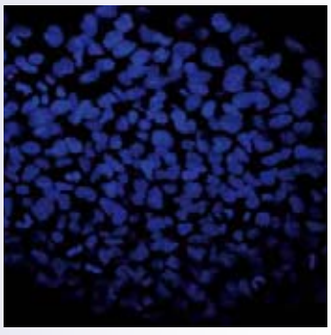
CellNova, s. r. o. is a spin-off company that emerged from the IEM's scientific output in the field of mesenchymal stem cell therapy. The company's know-how extends to methods for the cultivation of mesenchymal stem cells and their therapeutic application.


Společnost vědeckotechnických parků ČR
Science and Technology Parks Association of the CR
uděluje
Inovačnímu biomedicínskému centru
ÚEM AV ČR, Praha 4
AKREDITACI
ACCREDITATION
v rámci 8. etapy akreditace VTP v ČR dle stavu k 31. 10. 2008
na období do 31. 12. 2009
Doc. Ing. Pavel Šrejda, CSc., FEng.

The Science and Technology Parks (STP) Association of the CR has accredited IBC to join the CR National Network of STP according to these criteria: sorted out issues of the owner-founder-operator, incubator of small and medium innovation companies, technology transfer, education in innovative entrepreneurship, technical and consulting services of good quality, active part of the innovative infrastructure.







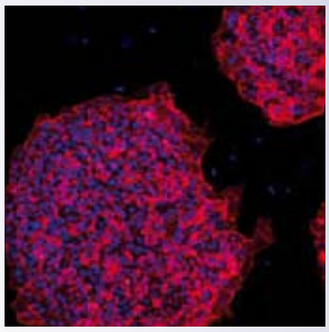
INTERNATIONAL GRANT PROJECTS

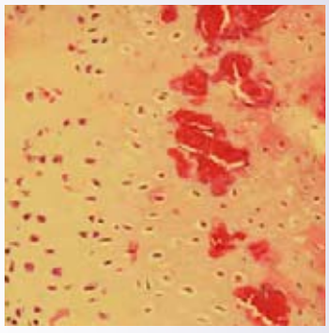
INTERNATIONAL GRANT PROJECTS OF THE INSTITUTE OF EXPERIMENTAL MEDICINE – INTERNATIONAL RESEARCH COOPERATION

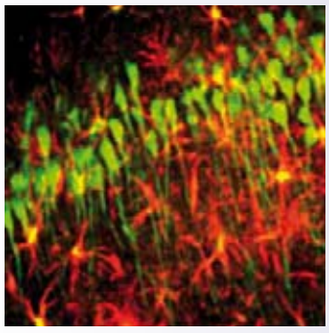
Number	Funding Agency	Type of project	Name of project	Coordinator	Head of the project at the IEM ASCR and the number of participants	Other participating countries	Activity
1	EU	NOE	DIMI: A network of excellence for indentification of NEW molecular imaging markers for diagnostic purposes	Prof. Andreas Jacobs, University of Cologne, Germany	Prof. Eva Syková, MD, DSc (53)	Italy, Belgium, France Sweden Germany Netherlands	Molecular imaging in diagnostics: Network of Excellence for the identification of new molecular imaging markers for diagnostic purposes
2	EU	IP	ANGIOTARGETING Targeting-Tumour-Vascular/ Matrix Interactions	Prof. Rolf Bjerkvig, University of Bergen, Norway	Prof. Eva Syková, MD, DSc (14)	Italy, Sweden, Netherlands, Germany, United Kingdom Finland, Luxembourg	Research focusing on interaction between tumor, vessels and extracellular matrix
3	EU	STREP	RESCUE From stem cell technology to functional restoration after spinal cord injury	Prof. Alain Privat, Institute of Neuroscience, Montpellier, France	Prof. Eva Syková, MD, DSc (9)	Belgium, France, Spain, Germany, United Kingdom	Research focusing on using stem cell technology for functional recovery of the spinal cord
4	EU	CA	ENINET Network of European neurosciense institutes	Prof. Erwin Neher, Max-Planck-Gesellschaft zur Forderung der Wissenschaften, Goettingen, Germany	Prof. MUDr. Prof. Eva Syková, MD, DSc (14)	Germany, France, United Kingdom, Spain, Norway, Sweden, Switzerland	Network of European Neuroscience Institutes
5	EU	EST	CORTEX Cooperation in research and training for European excellence in the neurosciences	Prof. Ulrich Dirnagl, Charité Universitatsmedizin Berlin, Germany	Prof. Eva Syková, MD, DSc (8)	Germany, Finland, United Kingdom, Norway, Sweden, Switzerland	Cooperation in research and the education of PhD students in neuroscience in Europe
6	EU	STREP	STEMS Pre-clinical evaluation of stem cell therapy in stroke	Dr. Brigitte Onteniente, Institut National de la Sante et de la Recherche Medicale, Paris, France	Prof. Eva Syková, MD, DSc (9)	Sweden, Denmark, France, Germany	Preclinical evaluation of stroke therapy with the use of stem cells
7	EU	IP	NANOEAR Nanotechnology based targeted drug delivery using the inner ear as a model target organ	Prof. Illmari Pyykkö, University of Tampere, Finland	Prof. Josef Syka, MD, DSc (25)	Finland, Sweden Italy, Germany, France, Austria, Switzerland, Greece, United Kingdom	Research of active substances application into the inner ear with the use of nanoparticles
8	EU	IP	EU SYNAPSE From molecules to networks: understanding synaptic physiology and pathology in the brain through mouse models	Prof. R. Jahn, MPI Goettingen, Germany	Rostislav Tureček, PhD (20)	Italy, Germany, Israel, France, Switzerland, United Kingdom	Basic research of synaptic plasticide mechanisms with the use of GMOs
9	EU	NoE	INTARESE	D. Briggs, Imperial College, London, U. K.	Radim Šrám, MD, DSc. (33)	United Kingdom, Netherlands, Italy, Finland, France, Greece, Germany, Sweden, Spain, Belgium, Serbia, Slovakia	Integrated assessment of health risk of environmental stressors in Europe

Number	Funding Agency	Type of project	Name of project	Coordinator	Head of the project at the IEM ASCR and the number of participants	Other participating countries	Activity
10	EU	IP	ENVIRISK	A. Bartonova, NILU, Kjeller, Norway	Radim Šrám, MD, DSc (8)	Norway, Slovakia	Assessing the risks of environmental stressors: Contribution to the development of integrating methodology
11	EU	IP	ESTOOLS Platforms for biomedical discovery with human ES cells	Prof. Peter Andrews, University of Sheffield, UK	Assoc. Prof. Petr Dvořák, PhD, Assoc. Prof. Aleš Hampl, DVM, PhD (20)	United Kingdom, Germany, Sweden, Italy, Finland, Israel, Spain	Biomedical research focused on stem cells
12	EU	ITN	AXREGEN Axonal regeneration, plasticity & stem cells	Prof. Joe Herbert, Cambridge University, UK	Prof. Eva Syková, MD, DSc (11)	UK, Sweden, Italy, Netherlands, Switzerland, Spain, France, Germany, Poland	International network of education focused on axonal regeneration and stem cells
13	EU	ITN	EdU-GLIA Innovative techniques and models to study glia-neuron interactions	Prof. Andreas Reichenbach, Leipzig University, Germany	Prof. Eva Syková, MD, DSc (10)	Germany, United Kingdom, Israel, France, Sweden, Slovenia	Education and research focused on neuron-glia interaction
14	EU	Collaborative project (CP)	BIOSCENT BIOactive highly porous and injectable Scaffolds controlling stem cell recruitment, proliferation and differentiation and enabling angiogenesis for Cardiovascular ENgineered Tissues	Prof. Paolo Giusti, University of Pisa, Italy	Evžen Amler, PhD (13)	Italy, United Kingdom, Germany, France, Netherlands, Denmark, Romania	Development of new bioactive polymeric scaffolds enabling formation of autologic cardiovascular engineered tissue from dissociated stem cells
15	ISCF	International Stem Cell Forum	International Stem Cell Initiative	Prof. Peter Andrews, University of Sheffield/ Univerzita Sheffield	Assoc. Prof. Petr Dvořák, PhD (11)	Australia, Finland, Israel, Japan, Canada, Singapore, Spain, Sweden, USA, United Kingdom	Worldwide international activity with the aim to compare biological and molecular properties of human embryonic stem cells lines
16	GA CR, DFG	Bilateral project	Lateral membrane compartments: formation, functional relevance and genomics	Jan Malínský/ Widmar Tanner	Jan Malínský (2)	Germany	Study of the formation, functional relevance and genomics of lateral membrane compartments

SELECTED POSTERS







Differentiation of human embryonic stem cells (CCTL14) into a neural phenotype

Kozubenko, N[1], Kapcalova, M[1], Turnovcova, K[1], Butenko, O[1], Anderova, M[1], Hampl, A[1,2,3], Jendelova, P[1,2], Sykova, E[1,2]

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Introduction

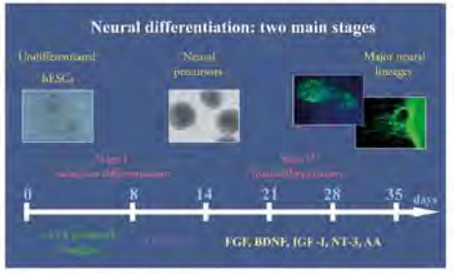
The isolation and culture of human embryonic stem cells (hESCs) have opened up new opportunities for the study of basic stem cell biology, early human embryonic development, and regenerative medicine. hESCs can be expanded to large numbers while retaining their differentiation potential. This is particularly important for transplantation therapies, because hESCs promise an almost unlimited supply of specific cell types. However, directing hESC differentiation along specific lineage pathways remains a challenge. The clinical application of hESCs requires the generation of highly purified donor cells for specific tissues or organs. For neural differentiation of hESCs, most protocols available so far are based on the initial formation of embryoid bodies (EBs) in the presence of serum/serum replacements or the coculture of hESCs with particular stromal cell lines. Here, we demonstrate the derivation of highly enriched and expandable populations of proliferating neural precursors from hESCs. Furthermore, these neural precursors could differentiate in vitro into mature neurons.

Methods

hESC culture
Human ES cells (CCTL14 line derived by the Department of Molecular Embryology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic. Complete information on characterization of hESCs is available at http://www.isscr.org/science/sclines.htm) were cultured on a feeder layer of mouse embryonic fibroblasts. Culture medium (CM) was D-MEM/F-12 supplemented with FBS, β -mercaptoethanol, nonessential amino acids, L-glutamine, penicillin, streptomycin and hrFGF 4 ng/ml. Colonies of hESC were mechanically passaged every 4-7 days.

Karyotype analysis
Chromosome number and size were scored using G-banding by the Institute of Biology and Medical Genetics, Prague, Czech Republic. The karyotype was stable during the whole experiment.

Neural progenitor generation, propagation and differentiation
To induce neural differentiation, clumps of undifferentiated hESCs were plated in agarose-coated tissue culture dishes with CM without FGF for 4 days, then with CM supplemented with Noggin for the next 4 days. At this time, 70%-90% of the colonies formed spherical bodies or embryoid bodies (EBs). Aggregates, whose diameter exceeded 0.5 mm, were dissected into smaller clumps with 20 G surgical blades, then replaced in serum-free medium consisting of DMEM/F12, supplemented with B27, hrEGF and hrFGF, and cultured for 6 days. At this stage, cells in the EBs were defined as passage 1 (P1). The next several passages (4-8) were also performed using 20 G surgical blades.



For final differentiation into a neuronal phenotype, aggregates were dissociated and plated on coverslips coated with poly-D-lysine/laminin. Plated cells were cultured in serum-free medium supplemented with B27, hrFGF, NT-3, BDNF, IGF-I and AA for 4 weeks. The day after plating the cells and then every 7 days, several coverslips were chosen for immunocytochemical analysis.

Antibodies and immunocytochemistry
Cells plated on cover slips were fixed with 4% paraformaldehyde and 0.25% glutaraldehyde in phosphate buffered saline (PBS) for 30 min. To identify undifferentiated hESC, Human Embryonic Stem Cell Marker Antibody Panel (R&D system) was used. To identify neural precursors and differentiated neurons, antibodies directed against NCAM, PSA-NCAM, nestin, synaptophysin, NG2 (all from Chemicon) and βIII-tubulin, NF-160, GABA, glutamate (all from Sigma-Aldrich) were used. For visualization, the following secondary antibodies were used: goat anti-mouse IgG conjugated with Alexa-Fluor 488 or 594 and goat anti-rabbit IgG conjugated with Alexa-Fluor 594.

Fluorescence-Activated Cell Sorting Analysis
EBs were dissociated by accutase (Sigma-Aldrich) for10 min. Suspensions of cells (10 mln/ml) were used for analysis. Flow cytometric analysis was performed using a FACS sorter (Becton Dickinson FACSAria flow cytometer). Data analysis was performed using BD FACSDiVa software.

Electrophysiology
Cell membrane currents were recorded using the patch-clamp technique in the whole-cell configuration. Tetrodotoxin (TTX)-sensitive Na currents were isolated by subtracting the current traces measured in 1 μM TTX-containing solution from those measured under control conditions. For immunohistochemical identification after patch-clamp measurements, the recorded cells were filled with Lucifer Yellow (LY, Sigma-Aldrich).

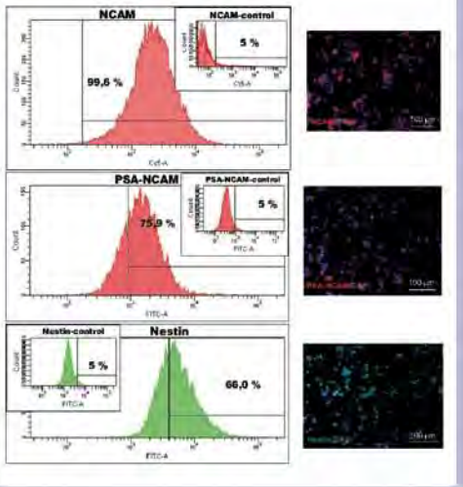
Results

1

The undifferentiated state of the cells was characterized by the expression of the transcription factor Oct-3/4, Nanog and the surface antigen SSEA-4.

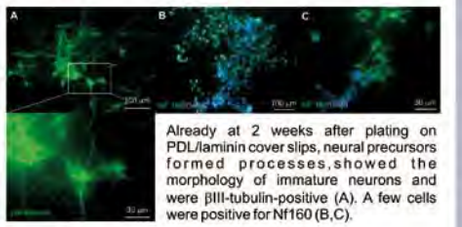
2

FACS characterization and quantification of hESC-derived neural precursors from dissociated EBs revealed 99.6% NCAM-positive, 77.5% PSA-NCAM-positive and 66.0% Nestin-positive cells in the population.

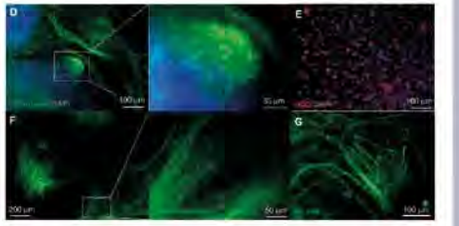


3

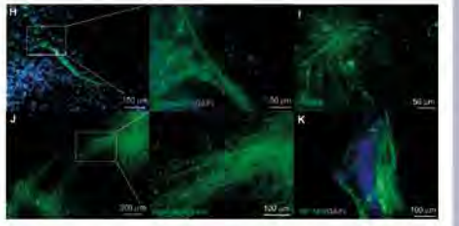
Characterization of hESC-derived neural precursors and neurons by immunocytochemistry.



Already at 2 weeks after plating on PDL/laminin cover slips, neural precursors formed processes, showed the morphology of immature neurons and were βIII-tubulin-positive (A). A few cells were positive for Nf160 (B,C).



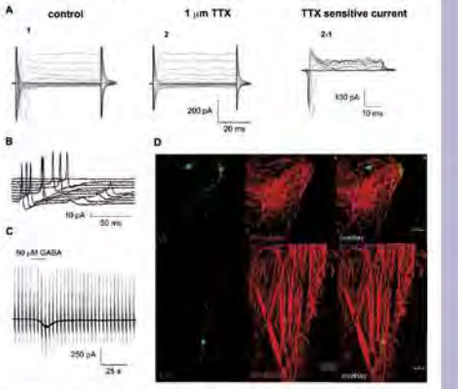
Immunostaining 3 weeks after plating indicated that the majority of the cells were positive for βIII-tubulin (D) as well as NG2 (E). Cell processes formed bundles, which were positive for synaptophysin (F) and NF160 (G).



After 4 weeks of differentiation, hESC-derived neurons expressed neurotransmitters such as glutamate (H) and GABA (I). The main volume of the culture was populated by neurons positive for synaptophysin (J) and NF160 (K).

4

At 3 weeks after plating on PDL/laminin coated cover slips, ESC-derived neural precursors showed neuronal morphology and expressed typical neuronal currents.



A. The membrane current pattern of hESC-derived neurons prior to the application of tetrodotoxin (TTX, A1) and in the presence of 1 μM TTX (A2). The TTX-sensitive current was obtained by subtracting the current after TTX application from the control current (A1-A2).
B. Action potential generation in hESC-derived neurons in response to current steps from a holding potential of -70 mV.
C. GABA-evoked currents recorded at a holding potential of -70 mV.
D. Immunohistochemical identification of recorded cells showing positive staining for βIII-tubulin.

Conclusions

- We developed a protocol for the controlled, directed differentiation of hESCs into a neuronal phenotype

- The protocol yields a highly enriched culture of NP that displays good proliferative potential, that is easy to expand and that can be widely used in cell therapy

- This work serves as a platform for further manipulation of growth and differentiation factors and is an important step towards the potential utilization of hESC in transplantation therapy

The use of bone marrow stem cells in the treatment of spinal cord injury: Preclinical and clinical studies

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INTRODUCTION

Emerging clinical studies of treating brain and spinal cord injury (SCI) led us to examine the effect of autologous adult stem cell transplantation on spinal cord regeneration.

In our experiments we studied the effect of intravenously injected mesenchymal stem cells (MSCs) labeled with superparamagnetic nanoparticles (Endorem), an intravenous injection of the mononuclear fraction of bone marrow cells (BMCs) and the pharmacologically-induced endogenous mobilization of bone marrow by granulocyte colony-stimulating factor (G-CSF) in rats with an acute or chronic balloon-induced spinal cord compression injury (SCI). Functional improvement of the rats was assessed using the Basso-Beattie-Bresnahan (BBB) locomotor rating score and the plantar test. Lesions were evaluated histologically and by magnetic resonance imaging.

Based on our experimental studies, autologous BMC implantation has been used in a Phase I/II clinical trial in patients (n=20) with a transversal spinal cord lesion.

METHODS

Balloon-induced spinal cord compression lesion

- Simple and reproducible
- A 2-French Fogarty catheter is inserted into the dorsal epidural space through a hole in the Th10 vertebral arch
- A spinal cord lesion is made by balloon inflation (volume 15 µl) at the Th8-Th9 spinal level. Inflation for 5 minutes produces paraplegia

Isolation of MSCs

BMCs were isolated from the bone marrow of Wistar rats. MSCs were cultured for 3-5 passages in DMEM/10%FBS with P/S.

Mobilization of BMCs

For the mobilization of bone marrow we used granulocyte colony stimulating factor - Neupogen (Roche) - at a dose of 5mg per 100g body weight, administered once per day intravenously from 7-11 days post-injury.

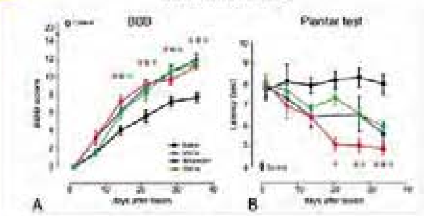
Cell implantation

MSCs or BMCs ($2x10^6$ cells in 0.2 ml PBS) were injected into the femoral vein one week or 4 months after lesioning.

For immunosuppression, a corticoid (Depo-Medrol®, UpJohn) was administered weekly to prevent possible rejection.

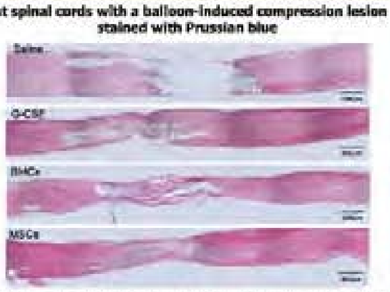
Preclinical study - Acute SCI

1 Behavioral testing



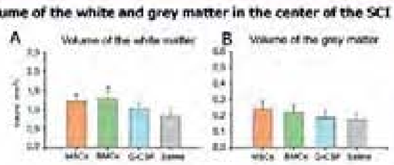
A: BBB scores were significantly higher in rats with implanted cells as well as in G-CSF-treated rats.
B: Sensitivity of the hind limbs as expressed by the withdrawal latency time was significantly greater in rats treated with MSCs, BMCs or G-CSF.

2 Rat spinal cords with a balloon-induced compression lesion stained with Prussian blue



Comparison of longitudinal sections of spinal cords with a balloon-induced compression lesion five weeks after i.v. injection of saline, G-CSF, BMCs or MSCs.

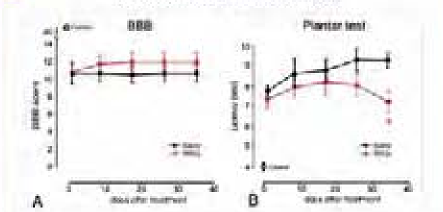
3 Volume of the white and grey matter in the center of the SCI



A: The total volume of the spared white matter in an 11 mm long segment of the spinal lesion was larger in the rats treated with MSCs or BMCs compared to the control group. Mobilization of the BM with G-CSF had no statistically significant effect.
B: No statistically significant differences were observed in the volume of the spared grey matter between treatment groups.
Data are represented as group means ± SEM. *p<0.05 compared to control group

Preclinical study - Chronic SCI

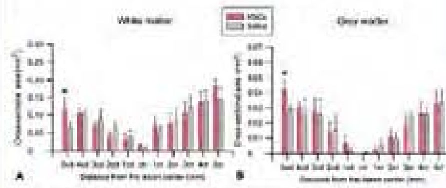
4 Behavioral testing-chronic injury



A: BBB scores did not significantly differ between chronically injured rats injected with either MSCs or saline.
B: The sensitivity of the hind limbs as expressed by the withdrawal latency time was slightly greater in rats treated with MSCs than in saline-injected rats.

Rats were lesioned 4 months before MSC injection.

5 Distribution of the spared white and gray matter in the center of the SCI



A: The area of spared white matter was significantly larger at the caudal end of the lesion in the MSC-treated rats.
B: Differences in the cross-sectional area of the gray matter reached statistical significance at the caudal end of the lesion between the MSC and control groups.
The data represent consecutive cross-sectional areas of the spared white or gray matter at defined distances from the lesion center.
Data are represented as group means ± SEM. cr, cranial; ca, caudal; ctr, center. *p<0.05 compared to control group

Clinical Study

1 Preparation and injection of autologous bone marrow grafts

BMCs were harvested from the iliac bones. Mononuclear cells were separated via sedimentation and adjusted to a final volume of 20-30ml or 500ml (i.v.). Cells were administered using angiography via a. vertebralis (6 cases), intravenously (14 cases), within 5 hours after BMC harvest

- Single application at 11-30 days after injury (8 cases)
- Single application at 2-17.5 months after injury (11 cases)

2 Characteristics of patients with intraarterial BMC implantation

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3 Characteristics of patients with intravenous BMC implantation

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Supported by grant number: AVOZ 50390512, 1M0021620803, NR 8339-3

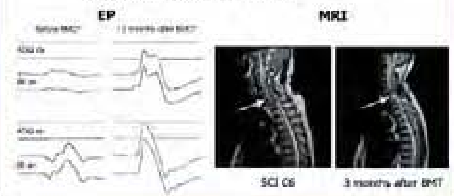
4 Evaluation of cell therapy

The results were evaluated at 3, 6 and 12 months after therapy

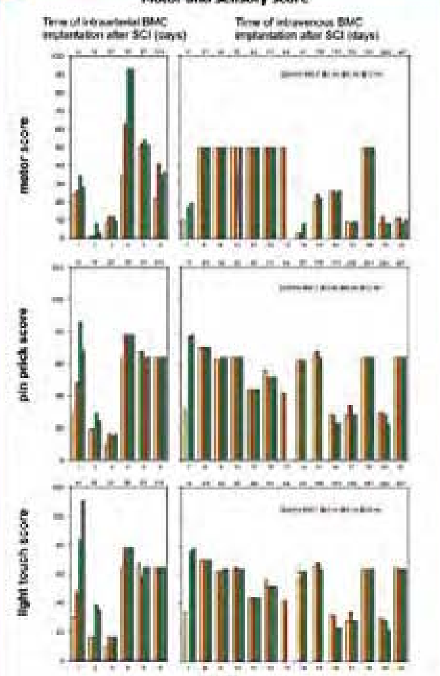
Functional improvement:
neurological improvement (ASIA score)
motor and somatosensory evoked potentials (MEP/SEP)

Lesion size - MRI

C.F., 41y, Incomplete SCI at C6



5 Motor and sensory score



Motor and sensory scores of 20 patients before and 3, 6 and 12 months after BMSC implantation. Motor score was improved in all acute patients and one chronic patient who received BMC via a. vertebralis (cases 1, 2, 3, 4 and 6). Motor score was improved in one acute patient who received BMC intravenously (case 7).

CONCLUSIONS

- Treatment with bone marrow cell populations - nonhematopoietic (MSCs), hematopoietic (mobilized by G-CSF) and all bone marrow cells (BMCs) - had a positive effect on behavioral outcome and on the histopathological assessment of a spinal cord injury. The effect was most apparent after MSC injection.
- Morphometric measurements showed an increase in the volume of spared white matter in animals treated by MSCs, BMCs or G-CSF.
- In chronically injured rats, an i.v. injection of MSCs improved the sensitivity of the hind limbs and increased the spared white and grey matter at the caudal end of the spinal cord segment.
- Autologous BMC implantation has been used in our clinical study in 20 patients with a transversal spinal cord lesion.
- Partial improvement have been observed in all subacute patient and 1 chronic patient who received cells via a. verte-bralis (n=4) and in 1 out of 4 subacute patients who received cells intravenously. A much larger population of patients is needed before any conclusions can be drawn.

Nonwoven nanofiber materials as three-dimensional tissue constructs in spinal cord injury

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[1] *Institute of Experimental Medicine AS CR, Prague*
[2] *Department of Neuroscience, 2nd Medical Faculty of Charles University, Prague*
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INTRODUCTION

Spinal cord injury is accompanied by cellular death and glial scar formation that affects regeneration in the injured region. Extensive research is being done to prevent scarring, to bridge the lesion with polymer implants and to create an environment permissive for cellular ingrowth and the diffusion of neuroactive substances, including growth factors. Some of the limitations of tissue regeneration within polymer implants could be overcome by supplementing the implants with cells that can facilitate the ingrowth of axons; suitable candidates are bone marrow stromal cells (MSCs) and olfactory ensheathing glial cells (OEGs). We have tested implants based on an electrospun nanofiber scaffolds, which are a novel class of biomaterials with high biocompatibility and a large surface area; they support the growth of both MSCs and OEGs. Three-dimensional implants were mechanically rolled from nanofiber layers, seeded with cells and implanted; we have also tested the difference between axially and perpendicularly oriented scaffolds.

MATERIALS AND METHODS

Nanofibers

The nanofiber layer was prepared by electrospinning in a Nanospider® device (International patent WO 2005/024101), developed and patented by the Faculty of Nonwovens, Technical University Liberec, Czech Republic. The nanofiber layer was seeded with cells (1×10^4 cells/implant) and mechanically rolled in order to create the cell-polymer construct.

Cell culture

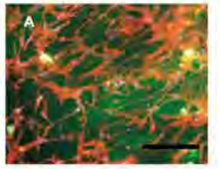
Rat MSCs were isolated from bone marrow by their adherence to plastic from femur bones of 4-week-old Wistar rats in accordance to standardized protocols.
Rat OEGs were obtained from the lamina propria of adult rats. The olfactory mucosa rostral to the olfactory bulb was dissected from the nasal septum, and the lamina propria was mechanically separated from the olfactory epithelium after incubation in Dispase II. The tissue was dissociated with collagenase type I. After 24 h incubation in DMEM/F12, the cells were treated with cytosine-b-D-arabinofuranoside, and incubated in DMEM/F12 with neurotrophin 3.

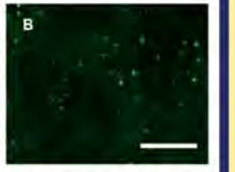
Spinal cord surgery

A rolled nanofiber scaffold was implanted into a hemisection of a rat spinal cord. The dura mater, muscles and subcuta-neous tissue were sutured in anatomical layers. Following surgery, the animals were administered antibiotics and analgesics and left to recover on a heating pad. Animals were sacrificed three weeks after surgery.

RESULTS

Cellular growth on nanofibers

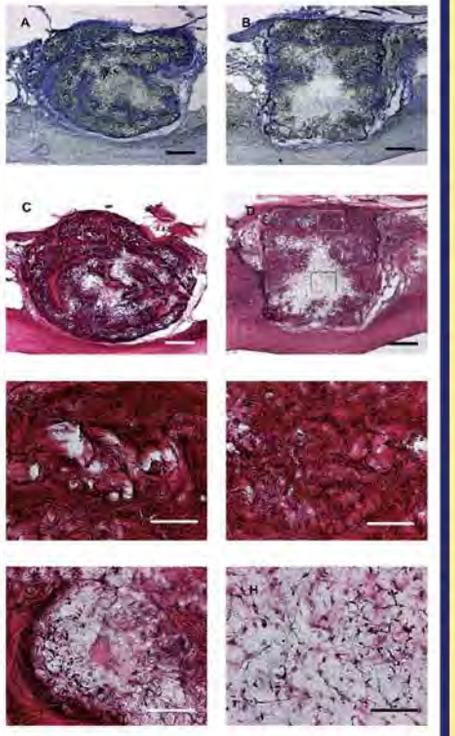




A. The growth of MSCs on a nanofiber scaffold after two days of cultivation (stained with phalloidin - red and DAPI - blue). Scalebar = 100μm.
B. The growth of OEGs on a nanofiber scaffold after 24h of cultivation (cells are stained with CFDA-green). Scalebar = 200μm.
C. Three-dimensional reconstruction of MSCs confluently growing on a nanofiber scaffold (stained with phalloidin) after one week of cultivation.

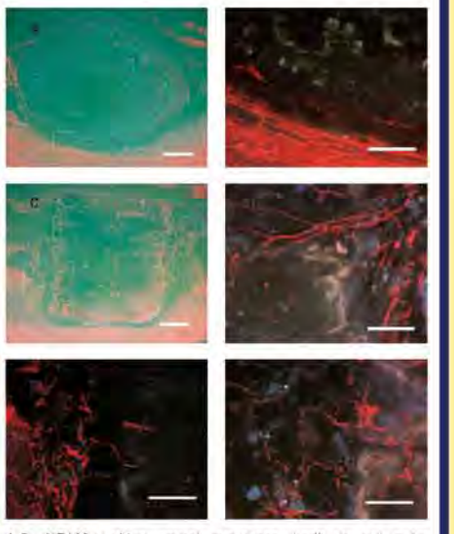
Histology of a nanofiber implant

Three weeks after implantation, the scaffold was filled mostly with the connective tissue, organized into dense and sparse zones, following the structure of the nanofiber layers.



A,B: The ingrowth of tissue into the scaffolds (stained with cresyl violet). Scalebar = 500μm.
C,E,G: The ingrowth of tissue into a perpendicularly oriented implant (stained with hematoxylin-eosin). Scalebar = 500μm (C) and 100μm (E, G).
D,F,H: The ingrowth of tissue into an axially oriented implant (stained with hematoxylin-eosin). Scalebar = 500μm (B) and 100μm (D, F).

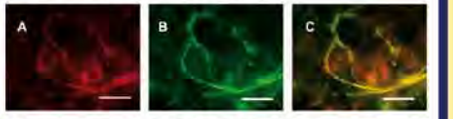
Neurofilaments growing into the nanofiber implants



A,B: NF160-positive neural processes (red) do not enter perpendicularly oriented implants. Scalebar = 500um (A) and 100um (B).
C,D,E,F: We observed the ingrowth of NF160-positive neural processes (red) into axially oriented implants. Scalebar = 500um (C) and 100um (D,E,F).

Growth of OEGs in the nanofiber implant

OEGs were found only in axially oriented implants.



A. Cells stained for anti-GFAP (red). Scalebar = 50μm.
B. The same cells stained for anti-p75-NTR (green). Scalebar = 50μm.
C. Double stained cells (OEGs). Scalebar = 50μm.

CONCLUSIONS

- Polymer nanofibers support the growth of MSCs and OEGs; they can be mechanically modified (rolled) in order to create a three-dimensional scaffold.
- Both axially and perpendicularly oriented implants were biocompatible, adhered to the spinal cord tissue and induced tissue formation within the nanofiber scaffolds.
- The ingrowth of neurofilaments is more pronounced in axially oriented implants.
- The implantation of nonwoven polymeric nanofiber scaffolds containing cells may serve as an alternative to conventional grafting technologies.

904.5

Biocompatible hydrogels in spinal cord injury repair

Hejčl A.[1,2,5], Šedý J.[1,2], Lesný P.[1,2], Glogarová K.[1,2], Amemori T.[1,2], Burian M.[2,4], Hájek M.[2,4], Krumbholcová E.[2,3], Přádný M.[2,3], Michálek J.[2,3], Jendelová P[1,2]., Syková E.[1,2]

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INTRODUCTION

Spinal cord injury leads to axonal damage, necrosis, demyelinization and extracellular matrix deposition, which ultimately leads to the formation of a glial scar and post-traumatic pseudocystic cavities. In spinal cord injury biocompatible three-dimensional polymer scaffolds can serve as a bridge between the cranial and the caudal stumps and as a scaffold for the ingrowth of nervous tissue elements. We studied whether we can successfully bridge a chronic spinal cord lesion (5 weeks after injury) by the implantation of a novel hydrogel prepared in our Center and whether this treatment leads to functional improvement.

MATERIALS AND METHODS

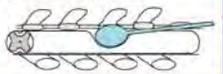
Preparation of HPMA-RGD hydrogels

The hydrogels were prepared by cross-linking radical polymerization of the monomer in a mixture of acetone - dimethyl sulfoxide. As a result of utilizing the surplus dimethyl sulfoxide, the resulting hydrogel was macroporous but not homogenous. The ratio between acetone and dimethyl sulfoxide was chosen in order to create interconnected pores. The surface of the hydrogels was modified by an adhesion-promoting peptide arginin-glycin-asparagin sequence (RGD), bound to the diglycin spacer.

Balloon-induced spinal cord compression lesion

This model is simple and reproducible. It is a reliable model used for behavioral evaluation in experimental spinal cord therapy.

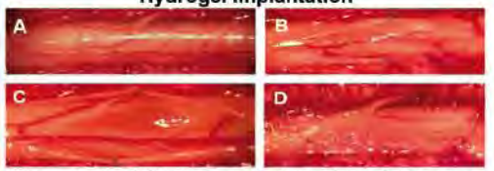
- A 2-French Fogarty catheter was inserted into the dorsal epidural space through a hole in the Th10 verteoral arch.
- A spinal cord lesion was induced by balloon inflation by saline (volume 15 ml) at the Th8-Th9 spinal level.
- Inflation for 5 minutes produced paraplegia.



Seeding rat mesenchymal stem cells on hydrogels

Rat mesenchymal stem cells (MSCs) were isolated from bone marrow by their adherence to plastic (femur bones of 4-week-old Wistar rats). Whole aspirates were plated in DMEM/10% FBS with 100U/ml penicillin and 100U/ml streptomycin. After 24 hours, the nonadherent cells were removed by replacing the medium. On the day of hydrogel implantation, 2 million cells per milliliter in a test tube together with a 2x2x2mm cube of hydrogel were were put ona shaker (500 rpm) for 15 minutes in order to seed the 3D scaffold

Hydrogel implantation



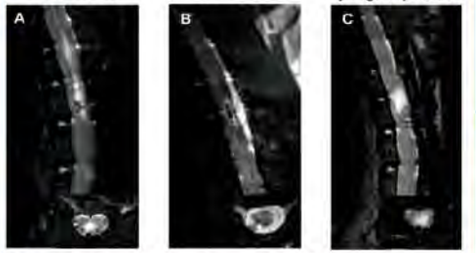
A. Five weeks after the SCI a Th8-9 laminectomy was performed and the SCI site was identified based on spinal cord atrophy and hemorrhage on the dorsal aspect of the spinal cord.
B. The dura was opened. A longitudinal myelotomy was performed and the pseudocystic cavity was devoid of tissue debris.
C. An HPMA-RGD hydrogel was carefully adjusted to the shape of the cavity and implanted inside the lesion.
D. The dura was then closed using 10/0 Ethilon suture to maintain the integrity of the subdural compartment. Muscles and skin were sutured in anatomical layers.

Magnetic resonance imaging

MR images were obtained on a Bruker Biospec 4.7 T equipped with 200 mT/m gradient system. A surface spinal coil together with T2-weighted RARE sequences were used. Sequence parameters were as follows: TE/TR = 70/2500 ms for sagital and TE/TR = 70/2800 ms for axial slices. The slices were 0.5 mm thick with interslice distances 0.5 mm for sagital orientation and 1 mm for axial.

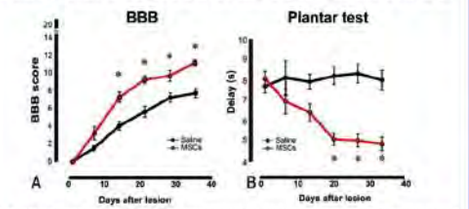
RESULTS

Spinal cord lesion in MRI



A. A cystic cavity (hyperintense signal) developed in the center of the lesion.
B. The center of the lesion further developed and wass dominated by atrophy (increased subarachnoid space) and tissue necrosis (hypointense signal).
C. Three weeks after hydrogel implantation a hyperintense signal was observed indicating that the cavity was bridged by the hydrogel. No atrophy was observed.

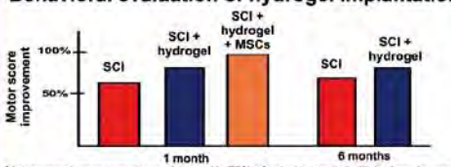
Behavioral evaluation of MSCs treatment in SCI



A,B. MSCs treatment leads to significant motor (A) and sensory (B) score improvement.*

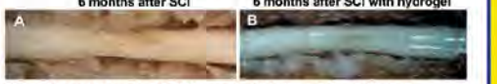
*Sykova E and Jendelova P (2005). Ann N Y Acad Sci. 1049:146-160.

Behavioral evaluation of hydrogel implantation



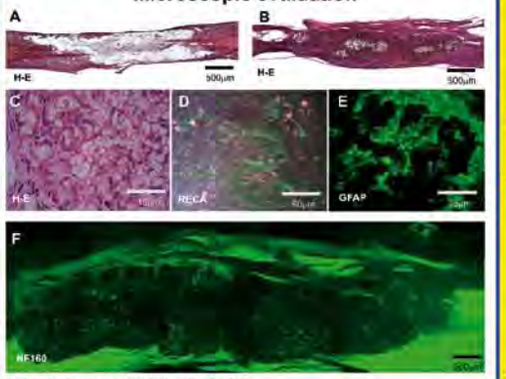
Motor score improvement was observed in 72% of animals treated with hydrogel compared to 56% of controls. Seeding a hydrogel with bone marrow stem cells further improved the motor score after 1 month. The data after six months are under evaluation.

Macroscopic evaluation



A. The spinal cords 6 months after SCI were atrophic in the central part of the lesion.
B. The hydrogel implanation in most cases prevented spinal cord atrophy 6 months after SCI.

Microscopic evaluation

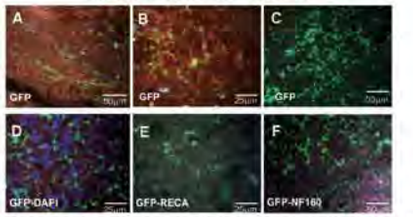


A. A cavity developed at the lesion site after SCI.
B. The implanted hydrogel adhered well to the surrounding tissue and completely bridged the post-traumatic cavity.
C. The porous structure of the hydrogel was completely infiltrated with cellular ingrowth.
D. Blood vessels grew from the spinal cord tissue and infiltrated particularly the border zones of the hydrogel. Fewer blood vessels were found in the central part of the scaffold.
E. Astrocytes were mostly found in the border zone, not in the center of the implant.
F. The hydrogel was infiltrated by neurofilaments both in the peripheral part and in the center, thus serving as a bridge for axonal regeneration.

Microscopic evaluation

G. A detail of the central part of a hydrogel infiltrated with neurofilaments.
H. Schwann cells grow from the spinal cord root into the hydrogel scaffold.
I. Schwann cells grow along the regenerating neurofilaments.

Implantation of HPMA-RGD seeded with GFP-MSCs



A,B,C. MSCs were apparent at the hydrogel border as well as in the center of the hydrogel 1 month after the implantation of a hydrogel seeded with MSCs.
D. The porous structure of the hydrogel was densely infiltrated with cells.
E. Blood vessels infiltrated a hydrogel seeded with MSCs.
F. Neurofilaments infiltrated a hydrogel seeded with MSCs.

CONCLUSIONS

1. Our HPMA-RGD hydrogels successfully bridged a chronic SCI in rats.

2. The hydrogels provided a scaffold for the ingrowth of neurofilaments, Schwann cells and blood vessels. Astrocytes infiltrated only the border zones.

3. HPMA-RGD hydrogels implanted in chronic spinal cord lesions improved the motor function in rats.

4. HPMA-RGD hydrogels can also serve as a 3D carrier for stem cells. Results show that this approach improves motor function in rats compared to hydrogel implantation only.

ACKNOWLEDGEMENTS

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Modified iron oxide nanoparticles for in vivo cell imaging.

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INTRODUCTION

Stem cells and progenitor cells are being investigated for their potential use in regenerative medicine. For the future success of cell therapy in clinical applications, it is important to follow the fate of implanted cells in the host organism. Magnetic resonance imaging (MRI) can be used for dynamic in vivo cell tracking. For MRI detection, cells are magnetically labeled with MR contrast agents [1, 2, 3].

In the current study, we used the WST-1 colorimetric assay to quantify cell proliferation and viability after labeling cells with superparamagnetic iron oxide (SPIO) nanoparticles, which can be used as a cell label for the long-term noninvasive MR tracking of implanted cells in vivo. We assessed the influence of the nanoparticles on several cell types that have been studied as potential tools for cell therapy: a) bone marrow stromal cells (MSCs), b) olfactory ensheathing cells (OECs), c) adipose-derived mesenchymal stem cells (ADMSCs) and d) chondrocytes (CHONs). We tested three types of nanoparticles: first, commercially available dextran-coated superparamagnetic iron oxide nanoparticles (Endorem®), used in several studies [1, 2, 3]; second, polycation-bound iron oxide nanoparticles (PCIO) developed by us (IMC+IEM ASCR, Prague, Czech Republic, patent application no: PV 1006-120); and third, D-mannose modified surface SPIO nanoparticles prepared by IMC, ASCR, Prague, Czech Republic. We also tested the labeling efficiency and the MR detection resolution limit of these new nanoparticles.

METHODS

Cell cultures and labeling with iron oxide nanoparticles

Rat MSCs were obtained from the tibias and femurs of Wistar or Rowett Nude rats. The ends of the bones were cut and the marrow was extruded with DMEM. Marrow cells were plated in DMEM medium containing 10% FBS, 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

Rat OECs were obtained from the lamina propria of Wistar rats. The olfactory mucosa was dissected and the lamina propria mechanically separated from the olfactory epithelium after incubation in dispase II. The tissue was dissociated with collagenase type I in DMEM/F12. Cells were plated in DMEM/F12 medium containing bovine pituitary extract, 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

Human MSCs were obtained from the bone marrow of healthy donors. Bone marrow aspirates were centrifuged through a density gradient. Nucleated cells from the interface were plated in α-MEM medium containing 10% FBS, 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

Human ADMSCs were obtained from orthopedic patients. Adipose tissue from hip joint replacements was digested with collagenase type I. Cells from the stromal vascular fraction were plated in DMEM/F12 medium containing 10% FBS, 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

Human CHONs were obtained from meniscal biopsies of the knee joint. Meniscal fragments were minced and digested in trypsin and subsequently in collagenase type I in DMEM/F12. Cells were plated in DMEM/F12 medium containing 10% FBS, 100 units/ml penicillin, and 0.1 mg/ml streptomycin.

For cell labeling we used either the commercial contrast agent Endorem®, based on superparamagnetic dextran-coated iron oxide nanoparticles (DCSPIO; Guerbet, Roissy, France; 11.24 mg Fe/ml), or polycation-bound iron oxide nanoparticles (PCIO; IMC+IEM ASCR, Prague, Czech Republic, patent application no: PV 1006-120; 1.54 mg Fe/ml). Also D-mannose-coated iron oxide nanoparticles were developed by the precipitation of Fe(II) and Fe(III) salts with ammonium hydroxide followed by the oxidation of the precipitated magnetite with sodium hypochlorite with the subsequent addition of D-mannose solution [4]. After 72 hours the nanoparticles were washed out by replacing the culture medium.

Cells were stained for iron to produce ferric ferrocyanide (Prussian blue); nuclei were counterstained with hematoxylin.

Determination of cell viability

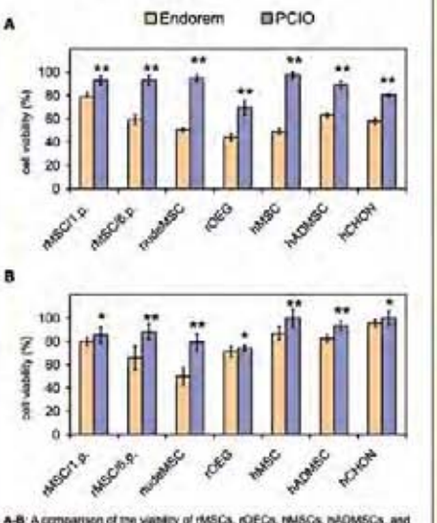
The viability of cells was determined using a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) to a highly water-soluble formazan dye by mitochondrial dehydrogenases in viable cells [5]. rMSCs, rOECs, hMSCs, hADMSCs and hCHONs were plated on 96-well plates at a density of 5×10^3 cells/well. The cells were cultured and labeled with iron oxide nanoparticles as described above. Twelve wells of each cell type were labeled with Endorem®, twelve with PCIO, and twelve wells were not labeled and served as control samples. On the day that nanoparticles were withdrawn (day 3), and 1 week after adding the nanoparticles (day 7), 10 ml of the WST-1 solution was added to 100 ml of culture medium per well, and the cells were kept in the incubator for an additional 2 hours. The absorbance was measured using an ELISA plate reader at a wavelength of 450 nm.

MR measurements

MR images of 1.7% gelatin phantoms containing iron-labeled rMSCs and hMSCs were obtained using a 4.7 T Bruker spectrometer equipped with a standard resonator coil. T2- and T2*-weighted images were aquired, the former using a standard turbo spin-echo sequence (Turbo factor=4, effective echo time TE=42.5 ms, repetition time TR=2000 ms, number of acquisitions AC=16, field of view FOV=6 cm, matrix=256x256, slice thickness=0.75 mm), the latter using a gradient echo sequence (TE=12 ms, TR=80 ms, AC=48, same geometry parameters).

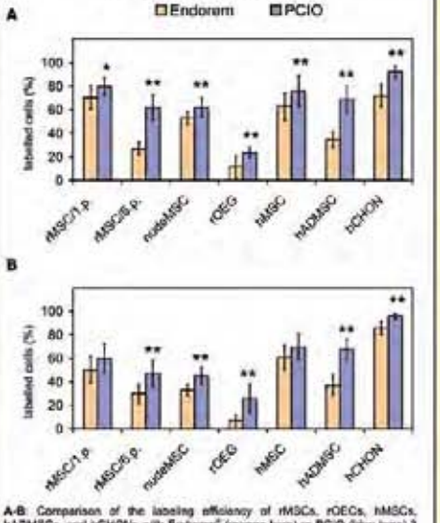
RESULTS

1 Viability of cells labeled with iron oxide nanoparticles



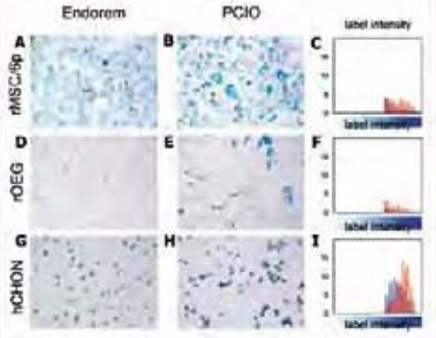
A-B: A comparison of the viability of rMSCs, rOECs, hMSCs, hADMSCs, and hCHONs labeled with Endorem® (orange bars) or PCIO (blue bars) 3 days after the addition of nanoparticles into the culture media, i.e. on the day that nanoparticles were withdrawn (**A**), and 4 days later (**B**). Values for cell viability are expressed as the percentage of the average value in the corresponding control cells.
*significant difference (P<0.05)
**significant difference (P<0.0001)

2 Efficiency of cell labeling with iron oxide nanoparticles



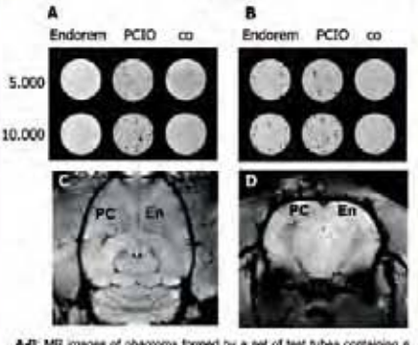
A-B: Comparison of the labeling efficiency of rMSCs, rOECs, hMSCs, hADMSCs, and hCHONs with Endorem® (orange bars) or PCIO (blue bars) 3 days after the addition of nanoparticles to the culture media, i.e. on the day that nanoparticles were withdrawn (**A**), and 4 days later (**B**).
*significant difference (P<0.05)
**significant difference (P<0.0001)

3 Prussian blue staining intensity of cells labeled with iron oxide nanoparticles



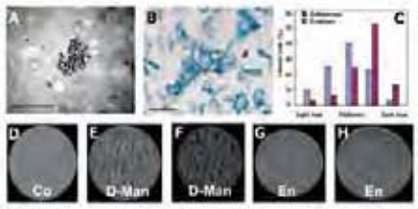
Examples of rMSCs (**A,B**), rOECs (**D,E**) and hCHONs (**G, H**) labeled with Endorem® or PCIO nanoparticles, stained for Prussian blue (iron). **C, F, I**: Histograms showing the intensity of Prussian blue staining for each cell type tested.

4 T2-weighted images of phantoms and rat brains



A-B: MR images of phantoms formed by a set of test tubes containing a suspension of hMSCs (**A**) or rMSCs (**B**) in 0.5 ml gelatin. The cells were labeled with Endorem®, PCIO, or unlabeled controls. The numbers of cells in the phantoms were 5000 or 10000, which correspond to 0.2 or 0.4 cells per image voxel, respectively. The average content of iron per cell, determined by spectrophotometry after mineralization, was 14.6 pg for Endorem® and 35.9 pg for PCIO nanoparticles.
C-D: Axial and coronal (**D**) MR images of a rat brain with 1000 cells labeled with PC-SPIO nanoparticles implanted in the left hemisphere (PC) and 1000 Endorem-labeled cells implanted in the right hemisphere (En). MR images were taken 3 days after implantation.

5 Labeling of cells with D-mannose coated SPIO nanoparticles



A: TEM image shows clusters of D-mannose nanoparticles as black dots scattered in the cell cytoplasm. Cell membrane-bound nanoparticle clusters as a result of endocytosis were not detected. **B**: rMSCs labeled with D-mannose coated SPIO nanoparticles, stained for Prussian blue. **C**: Histograms showing the distribution of the intensity of Prussian blue staining. **D-H**: T2-weighted MR images of gelatin phantoms containing 62,000 unlabeled cells (**D**), 31,100 (**E**) or 62,200 (**F**) cells labeled with D-mannose-coated SPIO nanoparticles and for comparison 31,100 (**G**) or 62,200 (**H**) cells labeled with Endorem. The detectable changes in MR signal correspond to 1, 17 (**E,G**) or 2.34 (**F,H**) cells per image voxel.

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ACKNOWLEDGEMENT

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CONCLUSIONS

1) Polycation-bound iron oxide nanoparticles (PCIO) or D-mannose SPIO nanoparticles used for in vivo cell visualization have better properties than the commercially available dextran-coated contrast agent Endorem®.

2) The efficiency of cell labeling with PCIO and D-mannose SPIO is higher than with Endorem®.

3) Depending on cell type, viability was decreased to 50-75% in the case of Endorem® but only to 80-99% in the case of PCIO nanoparticles after their addition to the culture media.

4) Using a gradient echo sequence, which is more sensitive to superparamagnetic particles, we are able to detect few or single cells. MR contrast provided by cells labeled with PCIO or D-mannose SPIO is visibly greater compared to Endorem® and thus enables the easier detection of cells in vivo.

DECREASED DIFFUSIVITY IN THE BRAINS OF AGED RATS WITH LEARNING DEFICITS

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Introduction

Aging, as well as many neurodegenerative diseases, is accompanied by serious cognitive deficits, particularly impaired learning and memory loss. Cognitive decline in old age has been linked to changes in brain anatomy, morphology, volume, and functional deficits (1). Nervous tissue, particularly in the hippocampus and cortex, is subject to various degenerative processes, including a decrease in the number and efficacy of synapses, neuronal loss, astrogliosis, and changes in extracellular matrix proteins. These and other changes not only affect the efficacy of signal transmission at synapses, but could also affect extrasynaptic (volume) transmission, mediated by the diffusion of transmitters as well as other substances through the volume of the extracellular space (2). Therefore, we studied the diffusion of water in the brain tissue of aged rats with and without a learning deficit.

Subjects and methods

Experiments were performed in vivo on 4- (adult) and 22-month-old (aged) Wistar male rats. Diffusion-weighted (DW) MRI was used to determine the mean diffusivity <D> and fractional anisotropy FA (3) in different brain regions of behaviorally characterized rats. Prior to MR measurements (Fig. 1), the ability to cognitively process spatial information was tested in a Morris water maze (Fig. 2) and superior and inferior learners were selected according to their escape latencies (4, 5). DW images were obtained with diffusion weightings (b-factor) of 75, 499, 1235 and 1732 s/mm^2. Diffusion weighting was applied along seven non-collinear directions, the resulting tensors were diagonalised and eigenvalues were used to calculate mean diffusivity and fractional anisotropy. In addition, magnetic resonance spectroscopy (MRS) was used to measure ^{1}H spectra in the hippocampus. Spectra were evaluated using the LCModel (6) to obtain the concentrations of creatine (Cr), glutamate (Glu), inositol (Ins), taurine (Tau) and N-acetyl aspartate (NAA).

Fig. 1: The Morris water maze consists of a round water-filled pool with a diameter of approximately two meters. There is a submerged escape platform made from clear Plexiglas, which is invisible to the swimming animals. A rat is placed into the water and starts to swim. At first the rat will swim around the side of the pool. After a while, it understands that there is no way to escape by doing that. Then, it searches more towards the center of the pool. If it does not find the platform after ninety seconds, it is gently guided to the platform. On the platform, the rat looks around and memorizes the position of the platform in relation to external objects. The next time the animal is usually able to find the platform more quickly.

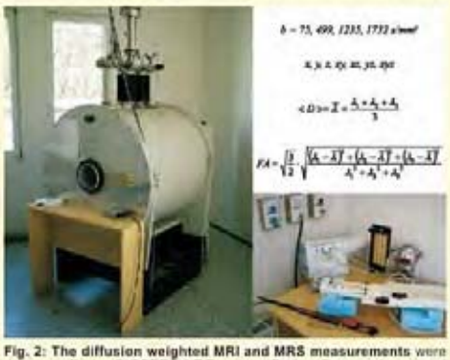

Fig. 2: The diffusion weighted MRI and MRS measurements were done on a Bruker BIOSPEC 4.7 T system (Bruker, Germany) equipped with a 200 mT/m gradient system and a head surface coil.

Results

The aged superior learners did not show significantly longer escape latencies (21 ± 2 s) than adult rats (24 ± 4 s), but the latencies were significantly different in inferior learners (45 ± 9 s). We evaluated mean diffusivity and fractional anisotropy in the primary somatosensory cortex, corpus callosum, CA1 region of the hippocampus, dentate gyrus and striatum (Figure 4). In all these regions, the mean diffusivity was significantly lower in inferior learners when compared either to adult rats or superior learners. There were no such differences between adult rats and aged superior learners except in the corpus callosum. The values are summarized in Table 1. We have not found any change in fractional anisotropy related to learning abilities. However, an age-related decrease in FA in the somatosensory cortex was observed (from 0.179 ± 0.007, N = 8 in adult to 0.153 ± 0.005, N = 16 in aged rats). The concentrations of creatine (Cr), glutamate (Glu), inositol (Ins), taurine (Tau) and N-acetyl aspartate (NAA) in young rats and in both groups of aged rats are summarized in Table 2. The concentrations of Ins and Tau were significantly different in aged rats compared to young rats, but there were no differences between good and bad learners. In contrast, decreases in the concentrations of Glu, which plays a key role in the learning process, of NAA, indicating neuronal loss in the hippocampus, and of Cr were found only in bad learners.

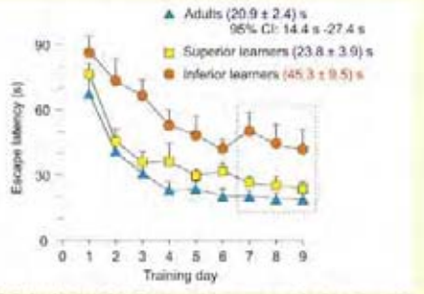

Fig. 3: Behavioral testing. The training program consists of nine days with four trials per day. The average time from the last three days of training is taken as a measure of the animal's ability to solve this spatial learning task. At the end of the training, young animals were able to find the platform, on average, within 21 seconds, aged animals without cognitive deficits in 24 seconds and poorly learning aged rats in 45 seconds. Each group consisted of eight rats.

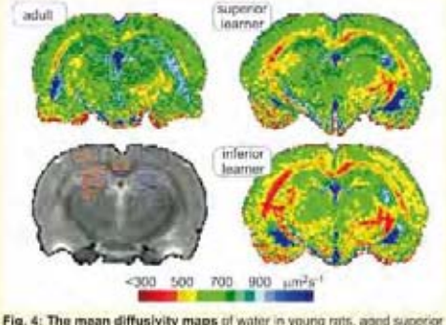

Fig. 4: The mean diffusivity maps of water in young rats, aged superior and inferior learners. Note the mean diffusivity decrease in inferior learners when compared to both young rats and aged superior learners. In the T2-weighted picture, the areas in which the detailed analysis was performed are delineated. These areas are the primary somatosensory cortex, the CA1 area of the hippocampus, the dentate gyrus, the striatum and the corpus callosum.

Acknowledgement

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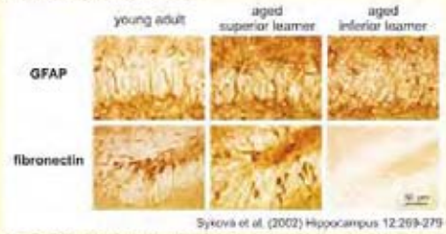
	adult <D> μm²s⁻¹	superior learners <D> μm²s⁻¹	inferior learners <D> μm²s⁻¹
cortex	654 ± 8	637 ± 7	613 ± 4*†
corpus callosum	602 ± 15	546 ± 5*	516 ± 10*†
CA1	705 ± 10	719 ± 4	685 ± 6*†
dentate gyrus	645 ± 10	649 ± 5	605 ± 5*†
striatum	670 ± 13	658 ± 5	611 ± 8*†

Table 1. Mean diffusivity in aged superior learners was not significantly different from that in young rats, except in the corpus callosum. Aged inferior learners were significantly different from both age-matched superior learners and young rats. In contrast, fractional anisotropy was different only in the cortex of aged rats, and there were no differences between aged superior and aged inferior learners in any of the analyzed areas. Significant differences compared to adult rats or superior learners are marked with asterisks or crosses, respectively.



Syková et al. (2002) Hippocampus 12:269-279

Fig. 5: Staining for glial fibrillary acidic protein shows a disruption of the radial organization of astrocytic processes in the inner blade of the dentate gyrus in aged rats, more pronounced in inferior learners. Our previous study (5) found that the loss of radial organization affects extracellular space diffusion anisotropy; however, fractional anisotropy was rather insensitive to these changes. Staining for two components of the extracellular matrix, fibronectin and chondroitin sulphate proteoglycan (not shown), reveals a large reduction in the presence of these macromolecules in aged rat brains, especially in the brains of inferior learners.

	Cr (mM)	Glu (mM)	Ins (mM)	Tau (mM)	NAA (mM)
Young n=6	8.5±0.5	9.6±1.0	5.0±0.1	4.8±0.3	9.3±0.8
Old good learners n=5	8.5±0.4	9.0±0.8	4.5±0.5*	3.5±0.3*	8.5±0.4
Old bad learners n=5	6.1±0.6*†	6.8±0.5*†	4.2±0.4*	3.6±0.4*†	7.1±0.4*†

Table 2. Concentrations of selected metabolites in the hippocampus. Significant differences (two-tailed t-test, $p<0.05$) when compared to young rats are marked by asterisks, differences when compared to good learners are marked with a cross.

Conclusions

1. Our data suggest that learning deficits are not an inevitable result of the aging process.
2. Fractional anisotropy is not a sensitive measure of the structural changes found previously in the hippocampus of aged inferior learners (5).
3. Decreased mean diffusivity was found in aged inferior learners but not in aged superior learners.
4. Decreases in mean diffusivity can significantly influence extrasynaptic transmission and thereby contribute to age-related learning deficits.
5. The decreases in the concentrations of glutamate, creatine and NAA can be considered as a sign of learning disability.

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DIFFUSION AND EXTRACELLULAR SPACE VOLUME FRACTION IN THE BRAIN OF MICE LACKING TENASCIN-R OR HNK-1 SULFOTRANSFERASE

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Introduction

We studied the influence of extracellular matrix (ECM) composition on the diffusion parameters in brain tissue. The diffusion of substances in the extracellular space (ECS) is the foundation of extrasynaptic transmission, which is involved in many brain functions, e.g. vigilance, sleep, alertness, plasticity and memory formation [1], and is altered during many pathological states [2]. Using diffusion-weighted magnetic resonance imaging (DW-MRI) and the real-time iontophoretic tetramethylammonium (TMA) method [3], we investigated the apparent diffusion coefficient (ADC) of water (ADC_w), ECS volume fraction (α = ECS volume/total tissue volume) and the apparent diffusion coefficient of TMA (ADC_{TMA}) in the cerebral cortex of two strains of knockout mice with altered extracellular matrix: TN-R- (tenascin-restrictin) and HNK-1 (human natural killer) sulfotransferase-deficient mice. TN-R represents an important component of the brain ECM, a member of the large extracellular glycoproteins family. HNK-1 is a sulfated carbohydrate epitope that attaches to tenascin molecules and modifies their adhesive or anti-adhesive properties.

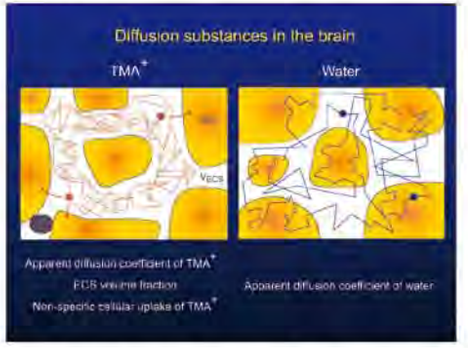

Fig. 1: The ECS diffusion parameters and apparent diffusion coefficient of water (ADC_w)
Three diffusion parameters characterise the diffusion of a substance in the ECS: extracellular space volume fraction (α), the apparent diffusion coefficient of TMA (ADC_{TMA}) and nonspecific uptake (k'). Water diffuses in both the extracellular and intracellular compartments.

Aims of the study

In the present study we investigated:

1. Whether changes in extracellular matrix composition affect the ECS diffusion parameters (α, ADC_{TMA}) and the apparent diffusion coefficient of water (ADC_w) in the brain.

2. The correlation between the ECS diffusion parameters and ADC_w.

Methods

Experiments were performed *in vivo* in the cortex of 8-month-old mice. Two different strains of knockout animals were used along with their respective controls: TN-R-deficient mice (TN-R -/-) and their wild-type littermates (TN-R +/+) [4] and HNK-1 sulfotransferase-deficient mice (ST -/-) and their wild-type littermates (ST +/+) [5]. In TMA experiments, the animals were anesthetized with sodium pentobarbital; during MR measurements, isoflurane was used for inhalation anesthesia.

The diffusion-weighted MRI method measures a weighted average of the apparent diffusion coefficient of water in both the extracellular and the intracellular spaces (ADC_w). Using a stimulated echo sequence, we acquired 4 axial slices with the following parameters: FOV = 2 cm, TR = 1.2 s, TE = 45.7 ms, 0.8 mm slice thickness, 0.4 mm gap, 256 x 128 image matrix, slice diffusion gradient direction and 6 b-values (136 - 1826 s/mm^2). ADC_w maps were evaluated bilaterally in the primary somatosensory cortex.

The TMA method monitors the diffusion of the small TMA cation after its iontophoretic application. The local concentration of TMA^+ can be measured using ion-selective microelectrodes, introduced into the brain. TMA diffuses predominantly in the ECS, and therefore the method can be used to measure the ECS diffusion parameters α and ADC_{TMA}. All TMA measurements were performed in the primary somatosensory cortex (layers II-VI).

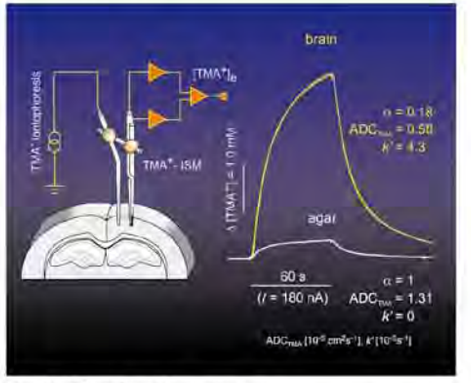

Fig. 2: *Left:* **Experimental arrangement.**
A double-barreled TMA^+-selective microelectrode (TMA^+-ISM) and a micropipette for TMA^+ iontophoresis were glued together with dental cement to stabilize the intertip distance at 100-150 µm.

Right: **Typical diffusion curves evoked by TMA^+ iontophoresis obtained in agar and in the brain.**
Measurement in agar enabled the transport number of the electrode array to be determined. When the electrode array was lowered into the brain, the resulting increase in concentration was much larger than that in agar due to the restricted volume fraction and decreased apparent diffusion coefficient.

Results

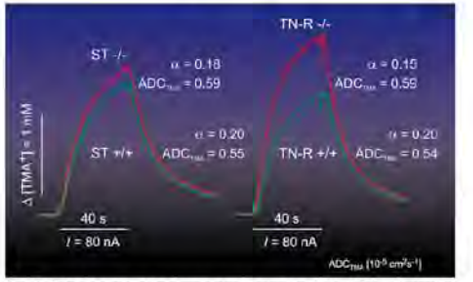

Fig. 3: The extracellular space was lower and the ADC_{TMA} larger in knockouts than in control animals. Typical diffusion curves obtained in the cortex of ST +/+, ST -/- and TN-R +/+, TN-R -/- mice. The diffusion curves in knockout mice were higher, which indicates a smaller extracellular space, when compared to controls. Their more rapid rise/fall in comparison with the control diffusion curves represents an increase in ADC_{TMA}.

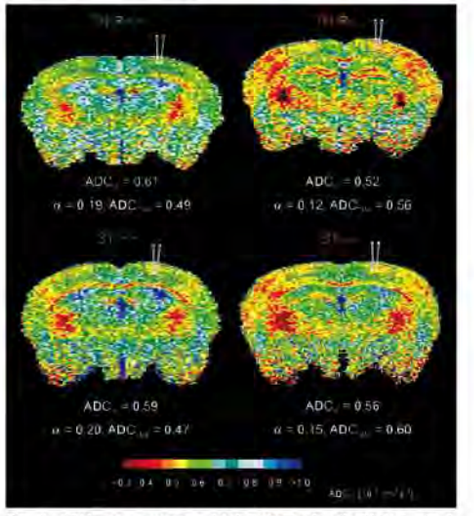

Fig. 4: ADC_w maps were aquired in the brain of TN-R -/-, ST -/- mice and their respective controls. The ADC_w values shown were calculated in the selected areas (see picture). The scale on the bottom of the figure shows the relation between the intervals of ADC_w values and the colors used for visualization. The values of α and ADC_{TMA} below the maps were calculated from TMA measurements in the same animals.

	α	ADC_{TMA} ($\mu m^2 s^{-1}$)	ADC_w ($\mu m^2 s^{-1}$)
TN-R +/+	$0.185 \pm 0.004^{\times}$ n = 8	548 ± 12 n = 8	$576 \pm 8^{\times}$ n = 8
TN-R -/-	$0.118 \pm 0.009^{*}$ n = 10	$612 \pm 19^{*}$ n = 10	$526 \pm 10^{*}$ n = 10
ST +/+	0.196 ± 0.003 n = 7	536 ± 34 n = 7	601 ± 6 n=4
ST -/-	$0.157 \pm 0.008^{*}$ n = 6	594 ± 27 n = 6	$564 \pm 4^{*}$ n = 8

Table 1: Extracellular space volume fraction (α), ADC_{TMA} and ADC_w in TN-R +/+, TN-R -/-, ST +/+ and ST -/- mice. Data expressed as mean ± S.E.M. Significant differences (two-tailed Student's t-test, $p < 0.05$) between knockout animals and their respective controls are marked with asterisks; significant differences between TN-R +/+ and ST +/+ animals are marked with crosses.

Conclusions

1. In the absence of the extracellular glycoproteins TN-R or HNK-1 sulfotransferase, the ECS volume is greatly decreased. The ECS volume fraction (α) was decreased in TN-R -/- mice by 36% and in ST -/- animals by 20%, when compared to the respective controls.

2. There is an increase in ADC_{TMA} which may be the result of the loss of ECM components. In TN-R -/- mice we found a 12% increase in ADC_{TMA}, and a tendency towards an increased ADC_{TMA} was also observed in ST -/- mice.

3. In contrast to ADC_{TMA} values, ADC_w in both knockout animal groups significantly decreased. Therefore, the decrease in ADC_w does not reflect the changes in ECS diffusion barriers. We hypothesize that the values of ADC_w rather depend on the ratio between the intra- and extracellular space volumes.

4. We conclude that the extracellular matrix is important in determining the size of the extracellular space and that it forms diffusion barriers in the ECS affecting the diffusion of neuroactive substances in brain tissue.

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Acknowledgement

This study was supported by grants from the Grant Agency of the Czech Republic 305/02/1528 and 309/04/0753, the Academy of Sciences of the Czech Republic AV0Z50390906, and the Ministry of Education, Youth and Sports of the Czech Republic LN00A065 and J13/98111300004 and the Deutsche Forschungsgemeinschaft. We would like to express our thanks to Achim Dahlmann for mice genotyping.

PSA NCAM and class III beta-tubulin expression in the developing inner ear in mice

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INTRODUCTION

The polysialylated form of neural cell adhesion molecule (PSA-NCAM) and beta III tubulin (TB) are early neuronal markers expressed during inner ear development in vertebrates. Information about their developmental pattern is incomplete. In this study, the expression patterns of PSA NCAM and TB were investigated in C3H mice using immunohistochemistry, from embryonic day 11(E11) to postnatal day 4 (P4).

The aims of the study were to compare the expression patterns of PSA NCAM and TB during inner ear neurogenesis. A comparison was also made between the expression patterns of PSA NCAM and calbindin (CB), as the timing of their expression and their location were similar.

METHODS

In this study mouse embryos and early postnatal mice were used throghout the developmental period from day 11 post-conception up to day 4 after birth. Female C3H mice were checked daily after mating for a vaginal plug; the day when a plug was first observed was defined as E1. Pregnant mice were killed by cervical dislocation on days 11, 12, 14, 17 or 19 of gestation. The embryos were explanted into phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde overnight. Pups (PND 1, 2, 3, 4) were cold anesthetized and beheaded. Their heads were fixed in 4% paraformaldehyde.

Altogether forty-two animals were examined, at least 3 for each age group. Fixed tissue was embedded in paraplim and sectioned. Serial sections in the frontal and sagittal planes were used. Sections mounted on glass slides (TESPA coated) were deparafinized and immunostained. Mouse anti-PSA NCAM (Sigma) and anti-III beta-tubulin (Exbio) were used as primary. Immunohistochemical protocols are described in Buckiova and Syka (2004).

Double immunofluorescent staining was done with mouse anti-PSA-NCAM antibody and rabbit anti-calbindin from Sigma. As secondary antibodies, goat anti-mouse- IgG labeled with Alexa Fluor 488 and goat anti-rabbit IgG labeled with AlexaFluor594 were used.

CONCLUSIONS

PSA NCAM and tubulin are independently expressed during inner ear development in the mouse.

- PSA NCAM immunoreactivity was detected in developing vestibulo-cochlear ganglion neuroblasts since E11.
- Beta-III tubulin started to be expressed in more differentiated spiral ganglion neurons at E13-14.
- PSA NCAM immunoreactivity was present in the precursors of the vestibular sensory cells. TB immunostaining was restricted to vestibular ganglion cells and their fibres at E17.
- PSA NCAM immunostaining was present in the innervation of the hair cells. TB labeling was limited to spiral ganglion nerve fibres at P4.

These data do not support Fanarraga's (1999) concept that PSA NCAM and beta III tubulin expression is linked.

PSA NCAM and calbindin display co-labelling during inner ear development. Immunocytochemistry performed using double labeling revealed that in the developing vestibular sensory epithelium (E17), Kölliker's organ (E17, P4) and the spiral ganglion (P4), PSA NCAM and calbindin immunostaining co-localize.

The joint appearence of PSA NCAM and calbindin was also observed during the development of the cerebral cortex (Jimenez et al., 2002, Varea et al., 2005) and thalamus (Mazetti et al., 2007).

LITERATURE

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Fanarraga et al., 1999, Eur J Neurosci 11:516-527
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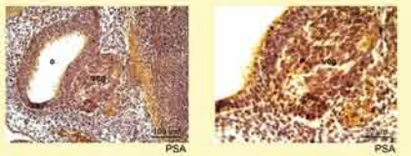
Varea et al., 2005, Neuroscience 136:435-443

ACKNOWLEDGEMENT

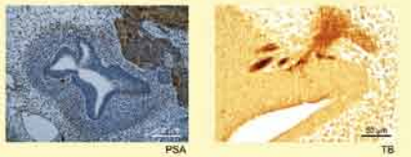
The authors wish to thank Ms. J. Jarosíková and Mr. J. Sztuicka for their technical assistance.

Vestibulo-cochlear ganglion E11-E12



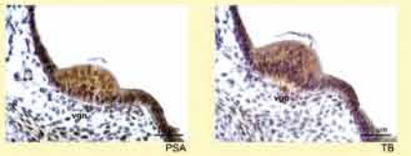
PSA NCAM immunoreactivity is detected in the otocyst (o) and vestibulo-cochlear neurons. TB has no relationship to otocyst differentiation and the formation of the vestibulo-cochlear ganglion (vcg).

Cochlear duct E13-E14



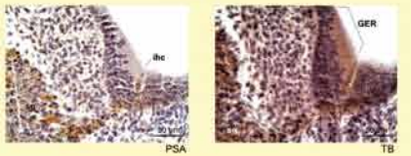
PSA NCAM and TB are expressed in the vestibular and cochlear ganglion.

Macula utriculi E17



PSA NCAM immunostaining is present in the developing sensory cells, vestibular ganglion neurons (vgn) and their nerve fibres. TB immunostaining is present in the vestibular ganglion neurons and their nerve fibres.

Cochlea E17

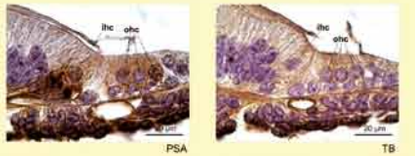


PSA NCAM and TB are expressed in the spiral ganglion (sg).
PSA NCAM immunostaining is present in precursors of inner hair cells (ihc).
TB marks great epithelial ridge (GER)

Macula utriculi E19

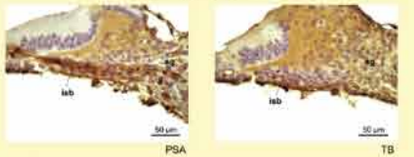
PSA NCAM immunostaining is present in the developing sensory cells, vestibular ganglion neurons and their nerve fibres. TB immunostaining is present in the vestibular ganglion neurons (vgn) and their nerve fibres.

Afferent and efferent innervation P4



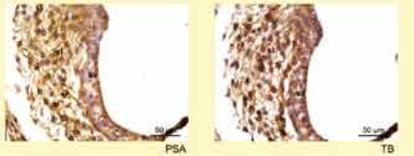
PSA NCAM immunoreactivity surrounds the inner hair cells (ihc). White arrows mark efferent inervating fibres. TB immunostaining is not involved in the synaptic connections of the hair cells. In addition, TB immunoreactivity is present in outer hair cells (ohc).

Spiral ganglion P4



PSA NCAM is expressed in some spiral ganglion (sg) neurons and their dendrites. Also the inner spiral bundle (isb) is labeled. TB immunostaining is present only in the dendrites of the spiral ganglion.

Cochlear lateral wall P4



PSA NCAM and TB are expressed in the spiral ligament (sl) but not in the stria vascularis (sv).

Double immunofluorescence of PSA NCAM and CB

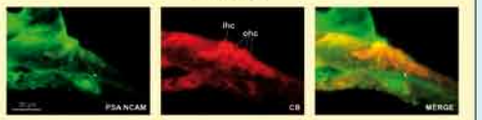
Crista ampularis (CA) E17

PSA NCAM-positive vestibular neurons and the positive fibers inervating the presumptive developing sensory domain appear. Co-labeling of vestibular sensory and neuronal cells. In addition, both PSA NCAM and CB mark the stereocilia of sensory cells.

Cochlea E17

Co-labeling in Kölliker's organ (k) and spiral ganglion (sg).

Cochlea P4



PSA NCAM immunoreactivity is seen at the base of the outer hair cells (arrow). CB labeling is strong in the outer and inner hair cells.

Kölliker's organ P4

Co-labeling in Kölliker's organ (k).

Spiral ganglion P4

Co-labeling of spiral ganglion (sg) neurons.

Modulation of excitatory synaptic transmission by $GABA_B$ receptors and SK channels in the MNTB

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Introduction

Calcium-dependent potassium channels (SK) are activated by low concentrations of Ca^{2+} and hyperpolarize a wide range of neuronal cells. $GABA_B$ receptors are G-protein coupled receptors that are inhibitory to voltage-gated Ca^{2+} channels (VGCC). The tight coupling between intracellular Ca^{2+} and the activity of SK suggests that the inhibition of VGCC by $GABA_B$ receptors could subsequently result in a reduction of SK currents and SK-induced hyperpolarizations. Principal cells of the medial nucleus of the trapezoid body (MNTB) accurately convert excitatory signals to inhibitory signals and the precise action of the MNTB synapses is important for sound source localization in mammals. The firing pattern of principal cells is profoundly affected by the afterhyperpolarization phase (AHP) following a postsynaptic action potential. The aim of this study was to investigate the indirect modulatory effects of $GABA_B$ receptors on SK and SK-mediated AHP in MNTB principal cells of mouse.

Methods

Brainstem slices (250-300 µm) were prepared from 8 - 30 day-old mice. Action potentials were evoked by current step injections into principal neurons and recorded at a room temperature by the patch clamp technique in the current clamp mode (Axopatch 200B). For the conductance clamp experiments, SM-1 amplifier was used to simulate excitatory postsynaptic conductances (EPSGs). Trains of excitatory postsynaptic currents (100-500 Hz) previously recorded in voltage-clamp mode were used as the conductance waveforms. EPSGs were normalized such that the peak conductance in the waveform corresponded to 250-300 pS. Recordings were performed at physiological temperature. Errors reported as ± 1 SD and statistical significance was tested by both paired and nonpaired t-tests (p=0.05).

For immunohistochemistry experiments slices were fixed with paraformaldehyde (4%) and incubated with primary antibodies raised against SK channel subunits. Secondary antibodies were conjugated with Alexa fluor 488. The immunofluorescence was analyzed by the confocal microscopy.

Results

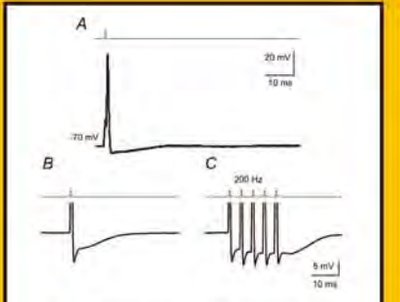

Fig. 1. Two components of the action potential afterhyperpolarization.

A, An example of an action potential (AP) evoked by a current step (0.5 nA/ 0.5 ms) injected to a MNTB principal cell.
B, The AP was followed by the afterhyperpolarization (AHP) consisting of a fast and a slow component.
C, The slow component was potentiated after a train of APs (5@200Hz).

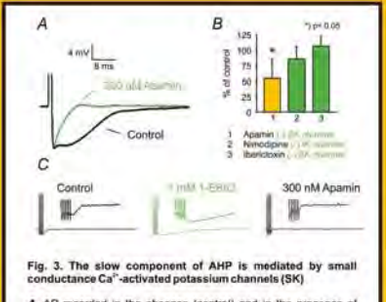

Fig. 3. The slow component of AHP is mediated by small conductance Ca^{2+}-activated potassium channels (SK)

A, AP recorded in the absence (control) and in the presence of apamin, a specific inhibitor of SK. Only the slow component of AHP was sensitive to apamin indicating that it is mediated by SK channels. ***B,*** Bar graph summarizes the effects of specific inhibitors of 3 types of Ca^{2+}-activated potassium channels on the amplitude of slow AHP. A significant inhibition was observed only with apamin. Data for each treatment were collected from 7-9 cells. ***C,*** Examples of slow AHP recorded after a train of APs (5@100Hz) in control conditions, in the presence of 1-EBIO, a specific positive modulator of SK, or in the presence of apamin. Note a strong potentiation of the AHP by 1-EBIO.

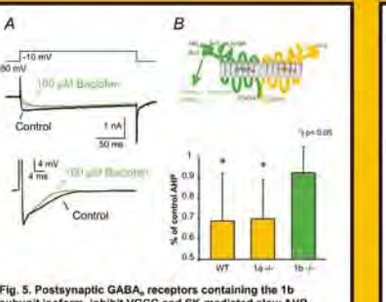

Fig. 5. Postsynaptic $GABA_B$ receptors containing the 1b subunit isoform inhibit VGCC and SK-mediated slow AHP.

A, upper, Postsynaptic Ca^{2+} current evoked by a voltage step to –10 mV and recorded in the absence (Control) or in the presence of baclofen, a specific $GABA_B$ receptor agonist. ***Lower,*** postsynaptic AP recorded from wild-type (WT) MNTB principal cell in the absence (Control) or in the presence of baclofen. Only the slow component of AHP was sensitive to baclofen. ***B,*** Summary of the effects of baclofen on the amplitude of slow AHP in the MNTB principal cells isolated from WT mice and from mice with deleted $GABA_{B1a}$ or $GABA_{B1b}$ subunits.

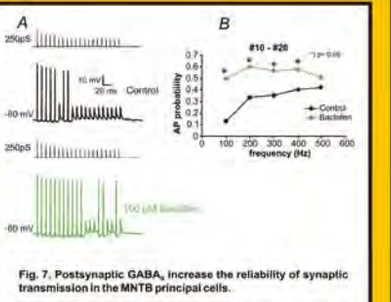

Fig. 7. Postsynaptic $GABA_B$ increase the reliability of synaptic transmission in the MNTB principal cells.

A, Examples of voltage responses to a train of 20 current injections at 100 Hz recorded with the dynamic-clamp technique at 37°C from the same principal cell in the absence ***(upper)*** or in the presence ***(lower)*** of baclofen. The amount of action potential failures was strongly suppressed by baclofen.
B, Summary graph showing the effect of baclofen on the probability of action potential generation by trains of stimuli delivered at fast frequencies. Note that baclofen significantly increased the probability over the range 100 to 400 Hz indicating the frequency-specific excitatory effects of postsynaptic $GABA_B$ receptors.

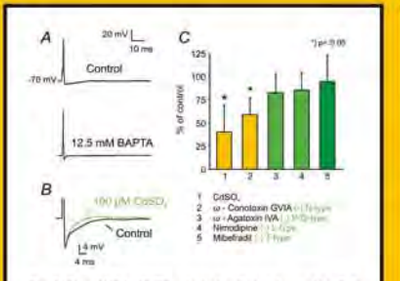

Fig. 2. Slow AHP in MNTB principal cells is triggered by Ca^{2+} influx mediated by N-type voltage-gated calcium channels.

A, upper, Single AP evoked by a current step under control conditions; ***lower,*** single AP recorded from a cell filled with a fast intracellular Ca^{2+} chelator, BAPTA. Note the monophasic AHP. ***B,*** Slow AHP was sensitive to extracellular Cd^{2+}, a nonspecific inhibitor of voltage-gated calcium channels (VGCC). ***C,*** Summary of the effects of subtype-specific inhibitors of VGCC on amplitude of slow AHP in MNTB principal neurons (6 - 9 for each treatment). A significant inhibition was produced only by Cd^{2+} and by ω-conotoxin GVIA, a selective blocker of N-type VGCC.

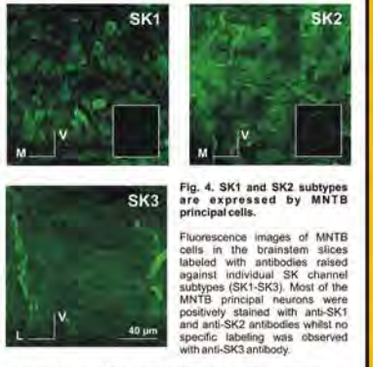

Fig. 4. SK1 and SK2 subtypes are expressed by MNTB principal cells.

Fluorescence images of MNTB cells in the brainstem slices labeled with antibodies raised against individual SK channel subtypes (SK1-SK3). Most of the MNTB principal neurons were positively stained with anti-SK1 and anti-SK2 antibodies whilst no specific labeling was observed with anti-SK3 antibody.

Insets: Membrane localized clusters of SK channels expressed in a principal cell.

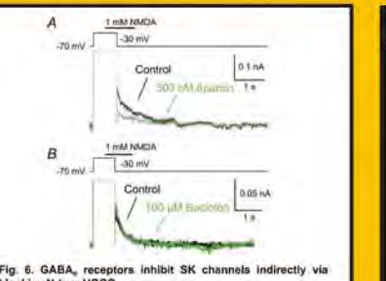

Fig. 6. $GABA_B$ receptors inhibit SK channels indirectly via blocking N-type VGCC.

A, Current response of MNTB principal cell evoked by an application of N-methyl-D-aspartate (NMDA) during a depolarizing voltage step and recorded in the absence (Control) or in the presence of apamin. Activity of VGCC was blocked by Cd^{2+} whilst the SK channels were continuously stimulated by 1-EBIO. Note the relaxation current to be sensitive to apamin indicating the activation of SK channels by NMDA-induced Ca^{2+} influx. ***B,*** The NMDA-induced SK-mediated current was not blocked by baclofen. This suggests that the mechanism underlying the modulation of SK channels and the slow AHP by baclofen is indirect, involving $GABA_A$-mediated inhibition of N-type VGCC.

Conclusions

MNTB principal cells fire action potentials terminated by a biphasic afterhyperpolarization. The slow component is dependent on N-type voltage-gated Ca^{2+} channels and it is mediated by Ca^{2+}-activated potassium channels.

Postsynaptic $GABA_B$ receptors inhibit the slow afterhyperpolarization and the SK channels indirectly, due to modulation of calcium influx through voltage-gated calcium channels. The $GABA_B$ coupled to the afterhyperpolarization contain the $GABA_{B1b}$ subunit.

Postsynaptic $GABA_B$ receptors increase the reliability of repetitive synaptic transmission at MNTB excitatory synapses.

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IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF D6/GFP-NEURAL STEM/PROGENITOR CELLS DURING IN VTRO DIFFERENTIATION AND AFTER TRANSPLANTATION INTO THE INJURED RAT BRAIN

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INTRODUCTION

D6 is a promotor of the *mDach1* gene, which is involved in the development of the neocortex, including the ventricular zone and hippocampus, and is expressed in the proliferating neural stem/progenitor cells of the brain cortex (Machon *et al.*, 2002). Neural stem/progenitor cells were isolated from E12 embryos of D6/GFP mice, and their differentiation potential was studied *in vitro*, 6-8 days after the onset of differentiation. To study their survival and differentiation *in vivo*, the cells were transplanted into the intact or injured cortex of 6-week-old rats. The properties of D6/GFP cells were then studied 1-4 weeks after transplantation using the whole-cell patch-clamp technique, and immunohistochemical analyses were carried out.

Machon *et al.*, 2002. Forebrain-specific promoter/enhancer D6 derived from the mouse Dach1 gene controls expression in neural stem cells. Neuroscience 112(4): 951-966.

METHODS AND MATERIALS

Cell culture

Cells were isolated from E12 embryos of D6/GFP mice (Machon *et al.*, 2002) and cultured as neurospheres, maintained in Neurobasal-A medium supplemented with B27, 2 mM glutamine, 1% penicilin/streptomycin and growth factors bFGF (10 ng/ml) and EGF (20 ng/ml) at 37 °C and 5% Co_2.

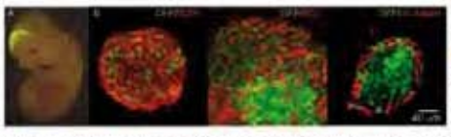


Fig.1 A: Sagital view of E12 embryo, **B:** D6-GFP neurospheres predominantly expressed BLBP and RC2; on the neurosphere surface βIII-tubulin-positive cells were detected.

In vitro differentiation

For *in vitro* analysis the cells were trypsinised and plated on PLL-coated coverslips at a cell density of $6x10^4$ /cm^2 and treated with Neurobasal-A medium supplemented with B27, 2 mM glutamine,1% penicilin/streptomycin and 20 ng/ml bFGF. Immunohistochemistry and patch-clamp analyses were carried out 6-8 days after the onset of differentiation.

Fig.2 6-8 days after the onset of *in vitro* differentiation D6/GFP cells gave rise predominantly to neurons (βIII-tubulin-positive cells).

Photochemical lesion

Wistar rats (150-200g) were anesthetized by isoflurane. Rose bengal (a potent photosensitizing dye) was injected intravenously into the femoral vein (8 mg/100 g). The scalp was incised to expose the skull surface. A 1x1 mm area of the skull above the right somatosensory cortex (bregma -2 mm, lateral 2.4 mm) was exposed to a beam of a cold light for 10 min. The infarct area extended approximately 1-1.5 mm in diameter.

Transplantation of D6-GFP cells

To follow the differentiation potential of D6-GFP cells *in vivo*, the spheres were trypsinised, counted and diluted to a final concentration of $2x10^5$/µl. 3µl of this suspension were aseptically transplanted into the intact/injured brain of adult rats, using a Hamilton syringe.1-4 weeks after transplantation brain slices were used for immunohistochemistry or for a patch-clamp analysis.

Patch-clamp technique

The coverslips with cells or the tissue slices were placed into a chamber mounted on the stage of a Zeiss microscope and perfused with oxygenated ACSF. Membrane currents were recorded with the patch-clamp technique in the whole-cell configuration. Current signals were amplified, filtered at 3 kHz and sampled at 5 kHz by an interface connected to anAT-compatible computer system.

Current patterns of the cells were obtained by clamping the cell membrane from the holding potential of -70 mV to values ranging from -140 mV to +40 mV or -160 mV to +20 mV, respectively, at intervals of 10 mV. Pulse duration was 50 ms.

The A-type K^+ (K_A) current component was isolated by subtracting the current traces clamped at -110 mV from those clamped at -50 mV.

The delayed outwardly rectifying K^+ current (K_{DR}) component was obtained by clamping the cell membrane from a holding potential of -50 mV and isolated by passive current subtraction.

TTX-sensitive Na^+-currents were isolated by subtracting the current traces measured in 1 µM TTX-containing solution from those measured under control conditions.

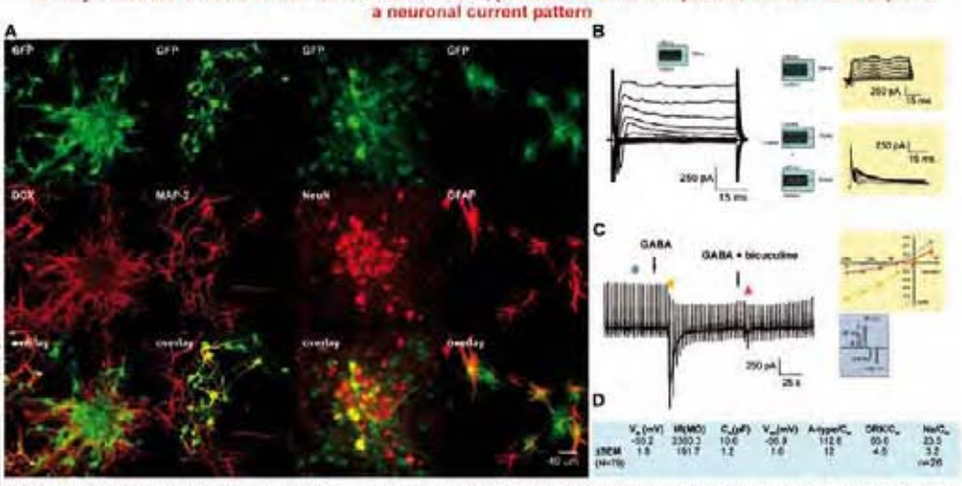
Currents evoked by GABA were obtained by clamping the cell membrane potential to different values, by rectangular voltage steps, from the holding potential of -70 mV to potentials of -140, -105, -35, 0 and +35 mV.

Immunohistochemical analysis

A LSM5 DUO confocal microscope equipped with an Arg/HeNe laser was used for immunohistochemical analysis. Cells were identified using antibodies against glial fibrilary acidic protein (GFAP), brain lipid binding protein (BLBP), Rc2, doublecortin (DCX), NeuN, neurofilaments 68 kD (NF68), microtubule associated protein 2 (MAP-2), and βIII-tubulin.

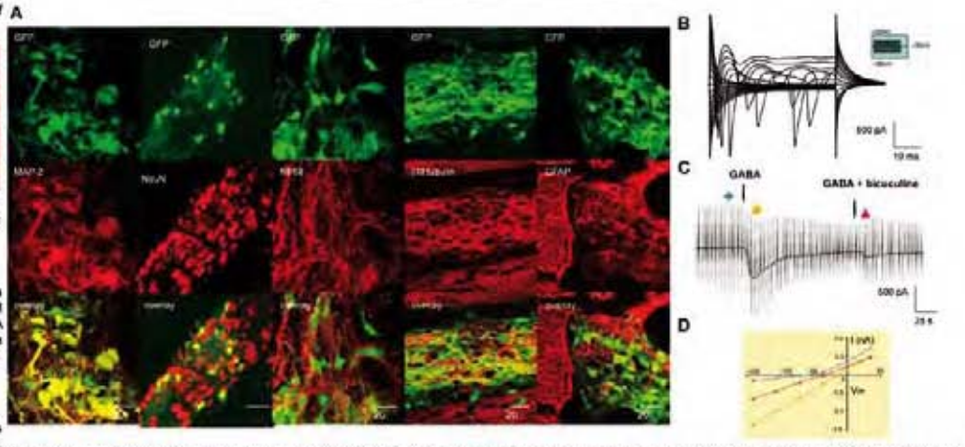
RESULTS

6-8 days after the onset of *in vitro* differentiation DCX-, βIII-tubulin- or MAP-2-positive D6/GFP cells express a neuronal current pattern



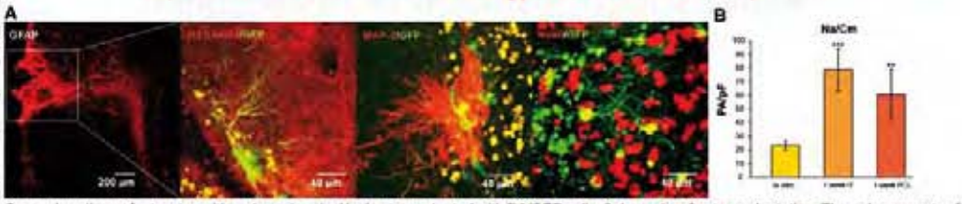
A: 6-8 days after the onset of *in vitro* differentiation, a large number of D6/GFP cells expressed neuronal markers such as DCX or MAP-2 and also markers of mature neurons such as βIII-tubulin or NeuN. However, cells expressing GFAP were also detected. **B:** 6-8 days after the onset of *in vitro* differentiation, DCX/βIII-tubulin/MAP-2 positive D6/GFP cells expressed a neuronal current pattern: large amplitudes of K_{DR} and K_A currents only, or together with TTX-sensitive I_{Na} currents (33% of cells) The subtracted K_{DR} and K_A currents are in the yellow insets. **C:** In 68% of cells, 100 µM GABA evoked an inward current sensitive to bicuculine. For the resulting current/voltage (I-V) relationship of the GABA response, see the yellow inset. **D:** Membrane properties of D6/GFP cells expressing neuronal markers after 6-8 days after the onset of differentiation *in vitro*. V_m membrane potential, V_{rev} reversal potential, C_m membrane capacitance, IR input resistance, K_{DR}/C_m, K_A/C_m and I_{Na}/C_m - K_{DR}, K_A and I_{Na} current densities (pA/pF).

1 week after transplantation into the intact rat brain, D6/GFP cells express neuronal markers and a typical neuronal current pattern



A: Coronal sections of rat cortex transplanted with D6/GFP cells 1 week after transplantation. Photomicrographs of the tissue sections illustrate GFP/MAP-2-, GFP/NeuN-, GFP/NF 68-, and GFP/βIII-tubulin- positive cells. There were no GFP/GFAP- positive cells. Yellow colour indicates positively stained cells **B:** 1 week after the transplantation into the intact rat cortex, D6/GFP cells expressed a neuronal current pattern: large amplitudes of K_{DR} and K_A currents only, or with TTX-sensitive I_{Na} currents (77% of cells) **C:** In100% of cells, 100 µM GABA evoked an inward current which was blocked by bicuculine. **D:** Resulting I/V relationship of the GABA response. **E:** Note that the Na/C_m in transplanted cells was significantly higher than in cells differentiated *in vitro*.

D6-GFP cells transplanted into the site of a photochemical lesion survived up to 4 weeks and expressed neuronal markers and a typical neuronal current pattern



Coronal sections of rat cortex with a photochemical lesion transplanted with D6/GFP cells 2-4 weeks after transplantation. The enlargements of the tissue sections illustrate GFP/βIII-tublin-, GFP/MAP-2- and GFP/NeuN-positive cells. Yellow colour indicates positively stained cells. **B:**Note that the Na/C_m in transplanted cells was significantly higher than in cells differentiated *in vitro*.

SUMMARY

•D6/GFP neural stem/progenitor cells give rise predominantly to cells expressing neuronal markers and express a typical neuronal current pattern.

•After transplantation into the non-injured cortex or a site of a photochemical lesion, D6/GFP neural stem/progenitor cells survive and give rise to neurons responding to GABA and are able to generate action potentials.

Oxygen-glucose deprivation reveals two astrocytic populations in the cortex of GFAP/EGFP mice: in situ quantification of cell volume changes

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Introduction

Various pathological states, such as ischemia or traumatic brain injury, lead to significant volume changes in astrocytes, followed by regulatory volume processes. The majority of studies to date have been performed on cell cultures, and little information is available about the effect of pathological states on glial cells in acute brain slices.

In the present study we have used real-time 3D confocal morphometry to quantify volume changes of astrocytes in the cortex of GFAP/EGFP mice after exposure to ischemia modeling solutions. The contribution of chloride channels and K-Cl and Na-K-Cl cotransporters to astrocyte volume regulation during ischemia was studied using inhibitors.

Methods

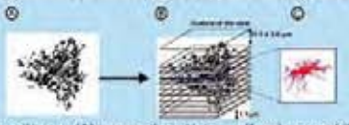
Ischemia:

Transversal 400-µm thick slices of 30-40-day old GFAP/EGFP transgenic mice (Nolte et al., Glia, 2001) were used. The brain slices were perfused for 20 or 40 minutes in modified ACSF without glucose, saturated with 90% N_2 / 5% CO_2 / 5% O_2 (**OGD**). Acidification (**pH shift** from 7.4 to 6.8) was modeled by saturation of ACSF with15% CO_2 / 85% O_2.

All **inhibitors**, 100 µM Cd^{2+}, 100 µM NPPB, 200 µM DIDS, 30 µM tamoxifen (inhibitors of Cl-channels), 100 µM bumetanide (an inhibitor of Na-K-Cl cotransporter) and 100 µM DIOA (an inhibitor of K-Cl cotransporter) were applied for 20 minutes during ischemia or a pH shift, for 40 min during reperfusion.

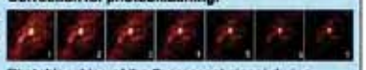
Cell morphometric measurements:

Changes in cell morphology were quantified by analyzing the total cell volume and the volume of the cell soma and cell processes from images of astrocytes expressing EGFP, acquired with a LEICA TCS SP system confocal microscope equipped with an Ar/HeNe laser.

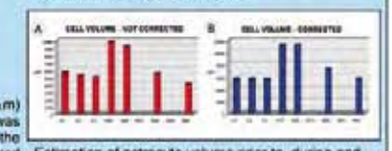


The cell image (**A**) was sectioned into a uniformly spaced (0.12 µm) set of 2-dimensional parallel images (**B**). The cell surface was found in each image using an edge-detecting algorithm (**C**) and the area of the image surrounded by the edge (indicated by the red color) was calculated for each image. The cell surface area and the volume of individual cells were obtained by integrating the values of the edge length and area from all images in a set.

Correction for photobleaching:



Photobleaching of the fluorescent signal during repetitive scanning of astrocytes.

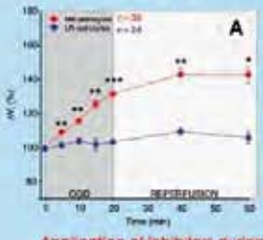


Estimation of astrocyte volume prior to, during and after ischemia not corrected (A) and corrected (B) for photobleaching.

Results A: OXYGEN-GLUCOSE DEPRIVATION

Confocal images of astrocytes prior to, during and after a 20-minute application of oxygen-glucose-deprived ACF.

Cell Volume

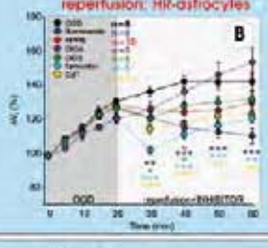


A: Real-time confocal morphometry of GFAP/EGFP astrocytes during OGD revealed the presence of two distinct cell populations, one expressing large cell volume changes (HR-astrocytes) and one expressing small volume changes (LR-astrocytes).

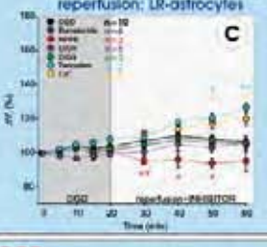
B: Inhibitors of chloride channels and the inhibitor of Na-K-Cl cotransporter significantly reduced cell swelling during 40-min reperfusion after OGD in HR-astrocytes.

C: In LR-astrocytes, the application of NPPB led to a volume decrease during 40-min reperfusion after OGD, the application of tamoxifen and Cd^{2+} led to the volume increase.

Application of inhibitors during reperfusion: HR-astrocytes

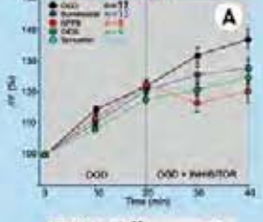


Application of inhibitors during reperfusion: LR-astrocytes

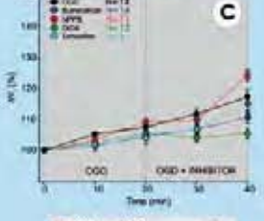


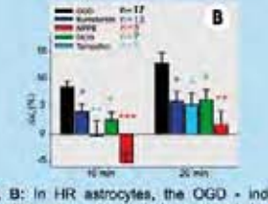
Confocal images of astrocytes prior to and during a 40-minute application of oxygen-glucose-deprived ACF.

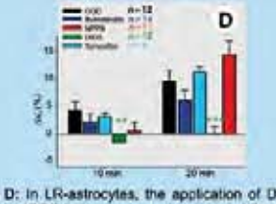
Application of inhibitors during OGD: HR-astrocytes



Application of inhibitors during OGD: LR-astrocytes



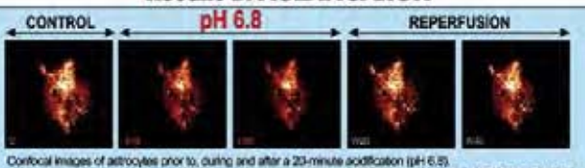




A, B: In HR astrocytes, the OGD - induced swelling was significantly reduced by the application of all used inhibitors.

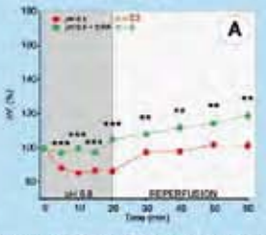
C, D: In LR-astrocytes, the application of DIDS during OGD led to a volume decrease, while other inhibitors had no significant effect.

Results B: ACIDIFICATION

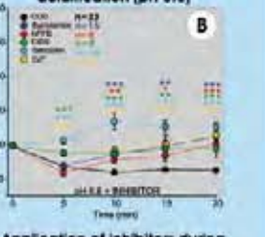


Confocal images of astrocytes prior to, during and after a 20-minute acidification (pH 6.8).

Cell Volume



Application of inhibitors during acidification (pH 6.8)

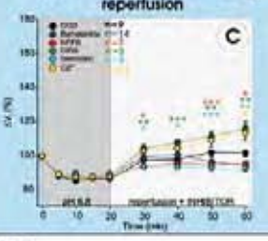


A: A 20-min shift of pH from 7.4 to 6.8 led to astrocyte shrinkage, sensitive to 50 µM EIPA (an inhibitor of Na^+/H^+ exchanger).

B: The application of tamoxifen during acidification led to a marked increase in astrocyte volume, while the application of other inhibitors caused smaller volume changes when compared to pH shift alone.

C: During reperfusion the application of DIDS and Cd^{2+} led to an aditional volume increase when compared to control, while NPPB and tamoxifen had an opposite effect; they partially inhibited the cell volume recovery.

Application of inhibitors during reperfusion



Conclusions

- Two populations of astrocytes differently responding to **OGD** exist in the cortex of GFAP/EGFP mice.
- During **OGD**, the inhibition of chloride channels or Na-K-Cl cotransporters reduces the cell swelling in HR-astrocytes. In LR-astrocytes, only DIDS reduces astrocyte swelling. The chloride channel inhibitors (tamoxifen and NPPB) reduce the cell swelling in HR-astrocytes, while in LR-astrocytes an aditional volume increase is detected.
- The chloride channel inhibitors (tamoxifen and Cd^{2+}) applied during reperfusion reduce the cell swelling in HR-astrocytes, while in LR-astrocytes an aditional volume increase is detected.
- The **shift of pH** from 7.4 to 6.8 leads to astrocyte shrinkage; all measured astrocytes display consistent volume changes.
- During the **pH shift**, the application of chloride channel inhibitor (tamoxifen) completely abolishes the cell shrinkage, while NPPB, DIDS, Cd^{2+} and Na-K-Cl cotransporter inhibitor (bumetanide) reduces it only partially.
- During reperfusion, the application of chloride channel inhibitors (tamoxifen, NPPB) blocks the volume recovery, while DIDS or Cd^{2+} cause a marked volume increase compared to pH shift alone.

Supported by GACR305/06/1316,GACR305/06/1464,AVOZ50390512,LC554,1M0538

Morphogenesis of a supernumerary cheek tooth in *Spry* null and *Tabby/Eda* mice

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INTRODUCTION:

Each quadrant of mouse dentition comprises only one incisor and three molars, separated by a toothless diastema **(Fig. 1A).** In both the upper and lower diastema, ancestral tooth primordia transiently occur in mouse embryos [1]. Two large diastemal buds in front of the molars have been homologized to the premolars lost during mouse evolution [1-3]. In mandible, the second (more posterior) diastemal bud (R2) is incorporated into the anterior part of the lower first molar (M1) [2], (Fig. 1B). Either a mutation in the *Eda* gene or a loss of *Sprouty (Spry)* gene function can stimulate revival of the diastemal buds leading to origin of a supernumerary cheek tooth (S) [4,5]. Such a tooth can be classified as an atavism [6] **(Fig. 1).**

Aim:

Comparison of the supernumerary tooth S morphogenesis and its developmental relationship to the diastemal premolar buds in the mandible of *Spry 2* and *4* null mice and of *Tabby/Eda* homo/hemizygous mice.

Fig. 1. Tooth pattern in a lower jaw.

(A) A scheme of adult mouse mandible.

(B) Comparison of the mandibular tooth pattern in mouse embryos (left) and mouse ancestors (right).

(C) The cheek teeth in *Tabby/Eda* heterozygous mouse. Note the unilateral presence of a supernumerary tooth (S), which is accompanied by a reduction of the anterior part of the first molar (M1). I - incisor; M2, M3 - the second and third molar, respectively; P, the last premolar of primitive rodents (kept in some of their actual descendants). D - diastema. MS - the anterior (first) diastemal bud; R2 - the posterior (second) diastemal bud, which is incorporated into the anterior part of M1 in WT mice. Dashed line - epithelial thickening in the anterior part of the prospective diastema. Dotted line - prospective tooth primordia. Black apoptosis.

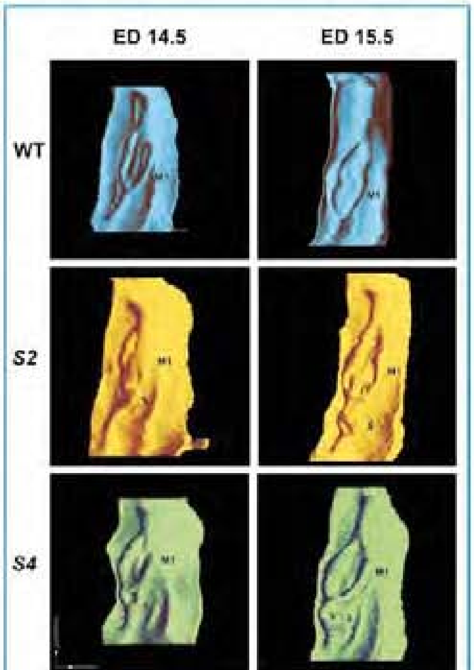

Fig. 2. Dental and adjacent oral epithelium of mouse embryos in 3D reconstructions.

The epithelium is shown from the mesenchymal aspect in WT, *Spry 2-/- (S2)* and *Spry 4-/- (S4)* mice at embryonic days (ED) 14.5 and 15.5. Note the progressive differentiation of the supernumerary tooth (S) in the anterior part of the dental epithelium in the cheek region of mandible. This is accompanied by a more posterior location of the anterior limit of the first molar (M1), (compare to Fig. 3).

METHODS:

Morphogenesis of the lower cheek dentition was analyzed in *Spry 2* and *4* null mice and in *Tabby/Eda* homo/hemizygous mice at embryonic days 12.5-16.5. We used series of 7μm thick histological sections and computer-aided 3D reconstructions **(Figs 2, 3).**

At embryonic day 13.5, the mitotic index was evaluated on 7μm thick frontal histological sections in two regions of the dental epithelium located in the cheek part of mandible: in the R2 diastemal bud and in the molar area. The mean values were calculated from 6 jaws (3 heads) of *Spry 2* null *(Spry 2-/-)* embryos and 10 jaws (5 heads) of wild type (WT) embryos **(Fig. 4).**

ACKNOWLEDGEMENTS:

The technical assistance by Mrs I. Kopperova, Z. Martonova, and Mr. J. Fluek is acknowledged. Financial support: GACR (grants 304/05/2665, 304/07/0223), MSMT CR (COST B23.002), COST B23 (Brussels, EU).

Conclusions:

- The loss of *Spry* function or EDA signaling enhanced autonomous development of the diastemal rudimental primordia, which is normally suppressed in WT mice.
- The diastemal bud R2 was not incorporated into the M1, as it was in WT mice. The R2 was either involved in the formation of a supernumerary tooth (S) primordium (Figs 2, 5) or failed to develop (in some *Tabby/Eda* jaws). Some S primordia regressed during further development.
- The formation of S was accompanied by abnormal segmentation of dental epithelium including the shift of the anterior limit of the M1 in a posterior direction (Fig. 3).
- The autonomous development of the R2 was in line with the high level of mitotic activity in its epithelium in *Spry 2-/-*, which was similar to the molar region in both *Spry 2-/-* and WT embryos. In contrast, the mitotic activity was significantly decreased in the R2 bud in WT jaws (Fig. 4).
- An improved understanding of the genetic and cellular pathways leading to the development of the supernumerary atavistic tooth may help to develop approaches of controlled tooth replacement [4,7], and to explain mechanisms driving tooth suppression during evolution.

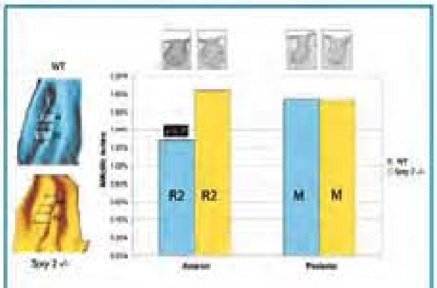

Fig. 4. Mitotic activity in the R2 diastemal bud and molar epithelium in *Spry 2-/-* and WT embryos at ED13.5.

Left: the 3D reconstructions of dental and adjacent oral epithelium are presented from mesenchymal side. The lighter strips on a 3D reconstruction show the two areas, where the mitotic index was analysed: in the R2 bud (on 10 histological sections) and, after a gap of further 10 histological sections, in the molar epithelium (M) (on 10 histological sections).

Top: the dental epithelium of the R2 or in the molar region is documented in *Spry 2-/-* and WT embryo on frontal histological sections.

The graph shows the mean values of the mitotic index in particular areas. The mitotic activity was similar in the molar epithelium of *Spry 2-/-* and WT embryos and, surprisingly, also in the R2 of *Spry 2-/-*. In contrast, the mitotic activity was significantly ($p < 0.01$) lower in the bud R2 of WT embryos.

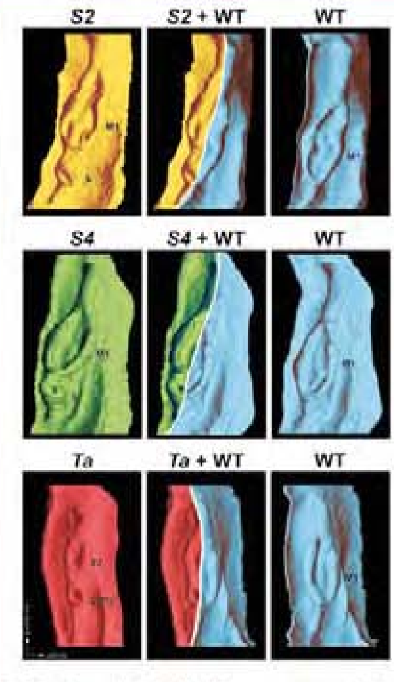

Fig. 3. Developmental relationship of the supernumerary tooth (S) and the first molar (M1) in mouse mandible.

The 3D reconstructions of the dental and adjacent oral epithelium are presented from mesenchymal view in *Spry 2-/- (S2)*, *Spry 4-/- (S4)*, *Tabby/EDA (Ta)* and in the corresponding wild type (WT) mice at embryonic day 13.5. For each genotype, a comparison is made between the genetically altered and wild type dental epithelium using a graphical chimera: *S2*+WT, *S4*+WT, *Ta*+WT. The S forms at the place corresponding to the anterior part of the M1 in WT mice. Consequently, the anterior limit of M1 is located more posteriorly than in WT jaws.

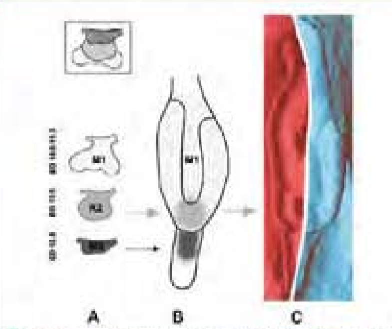

Fig. 5. Developmental relationship between the diastemal rudiments, the M1 in normal mice, and S in mutants (according to ref. 4).

(A) The most conspicuous structures of dental epithelium in WT mice at ED 12.5, 13.5 and 14.5. These shapes do not correspond to the developing central part of the first molar (in the rectangle), but to the individual segments of the dental epithelium that appear sequentially in the posterior direction: the first diastemal bud (MS darkest), the second diastemal bud (R2 middle grey), and the first molar (M1 - light grey). The MS and R2 are vestigial structures (Fig. 1B).

(B) The R2 becomes incorporated mesially into the M1 cap. The MS regresses in front of the M1, but its remnant can be incorporated later into the extending M1. Black dots, concentration of apoptosis.

(C) A chimera made from the 3D reconstruction of *Tabby/Eda* and WT dental epithelium (Fig. 3C). The S originates from the mesial portion of the normal M1, where the R2 bud is incorporated in WT mice.

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The early prenatal development of the dental and vestibular epithelia in humans

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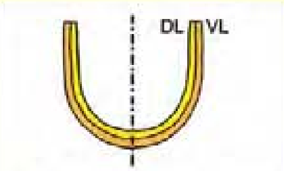

Figure 1: According to the generally accepted concept, the oral vestibule arises from a vestibular lamina (VL), which is a continuous structure running externally and parallel to the dental lamina (DL).

Introduction:

Using 3D imaging method, we have previously documented that the vestibular epithelium does not form a continuous lamina going parallel to the dental mound in the upper human embryonic jaw, as generally present the textbooks on human embryology (Fig. 1). Instead of this, the vestibular epithelium includes a complex system of epithelial bulges and ridges. The ridges reiteratively fuse with the dental epithelium behind the developing upper deciduous canine and the first and second molars. The upper dental and vestibular epithelia are regionalised in parallel along the mesio-distal axis (Fig. 2, Hovorakova et al., 2005).

Aim:

-to investigate the early morphogenesis and developmental relationships of the dental and vestibular epithelia in the human lower jaw

-to compare and contrast the results with the data on the upper jaw (Hovorakova et al., 2005).

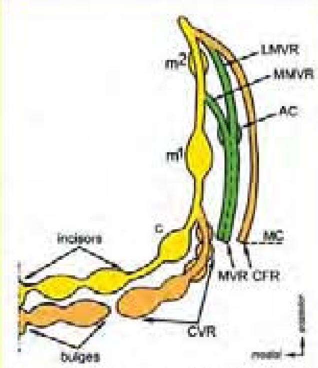

Figure 2: Data on the upper jaw (Hovorakova et al., 2005) have shown epithelial bulges that initially emerge externally to the dental epithelium (yellow) in the lip vestibular region. They fuse together being transformed into the canine vestibular ridge (CVR) in the posterior direction. The CVR fuses with the dental mound behind the deciduous canine primordium (c). In the cheek region, the vestibular epithelium forms the molar vestibular ridge (MVR) and the cheek-furrow ridge (CFR = the epithelium lining the mucosal inflection between cheeks and the alveolus with teeth). The MVR splits posteriorly into the medial (MMVR) and lateral (LMVR) branches. The prospective fornix of the oral vestibule (orange) originates from the bulges and CVR in the lip region and from the CFR in the cheek area (Hovorakova et al., 2005). The residual vestibular epithelium is green. AC - accessory epithelial cap, MC - mouth corner, m^1 and m^2 - the upper first and second molars, respectively.

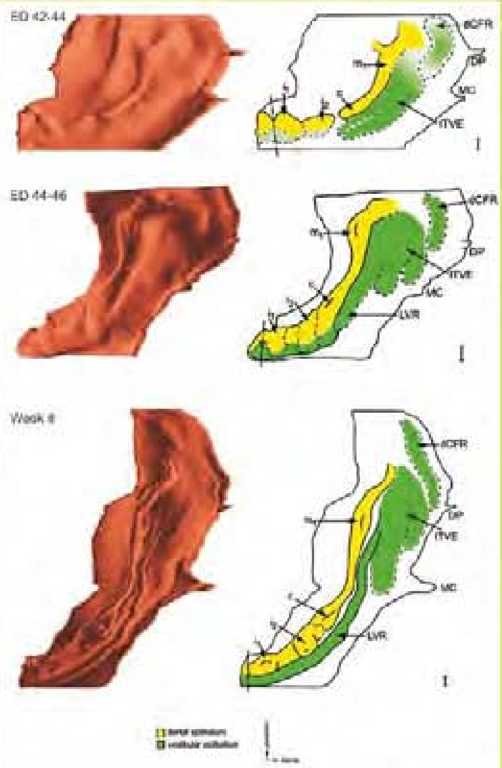

Figure 4: Computer-aided 3D reconstructions of the epithelium on the oral surface of the lower jaw and their schematic interpretation. The deciduous lower tooth primordia are labeled: central incisor (i_1), lateral incisor (i_2), canine (c) and first molar (m_1). Different vestibular structures are shown: the labial vestibular ridge (LVR), the irregularly thickened vestibular epithelium (ITVE) and the mandibular cheek furrow ridge (dCFR). The positions of the mouth corner (MC) and *ductus parotideus* (DP) are indicated. The midline is shaded.

Material:

The development of the dental and vestibular epithelia was examined in 53 series of frontal histological sections and in 7 series of sagittal histological sections of human embryos and fetuses using an old collection of serial histological sections held by the Department of Teratology IEM in Prague. Since homogenous data allowing stage determination were not available for all specimens, the staging was checked and expressed according to Carnegie Collection (O'Rahilly and Müller 1987) and according to Streeter (1951) and correlated to the morphological criteria proposed by Moore and Persaud (1993).

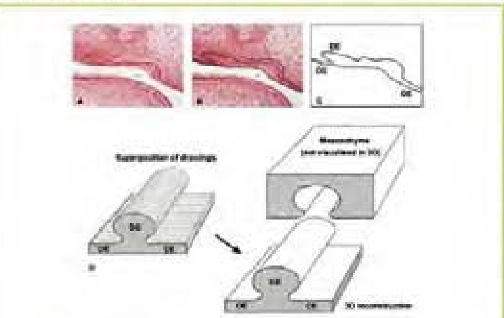

Figure 6: Method of 3D-reconstructions. Drawing of contours of the basement membrane on frontal histological section (A, B, C). Scheme of the superimposed drawings (D) giving a template for the digitalization. Scheme of the 3D reconstruction of the dental (DE) and adjacent oral epithelium (OE) with the underlying mesenchyme, which is not visualised in 3D (E).

Conclusions:

-Similarly to the upper jaw (Fig. 2), a continuous anlage of the oral vestibule did not exist in the mandible (compare Figs 1 and 3).

-In contrast to the upper jaw, where the dental and vestibular epithelia originated separately, they have a common origin in the lower incisor region. Two dento-vestibular epithelial bulges are present there (Figs 2, 3, 4). The lingual parts of the bulges gave rise to the respective deciduous central and lateral incisors. The labial parts of the bulges gave rise to the vestibular epithelium (Fig. 5).

-In the lower canine and molar regions, the dental and vestibular epithelia developed separately, similarly to the upper jaw (Figs. 2, 3, 5).

-In both, the upper and lower jaw, the prospective fornix of the oral vestibule had different origin in the anterior (lip) and posterior (cheek) jaw regions (Fig. 1).

-The vestibular structures could eventually play a role in the development of pathologies located externally to the functional dentition (eg. Ozan et al., 2007).

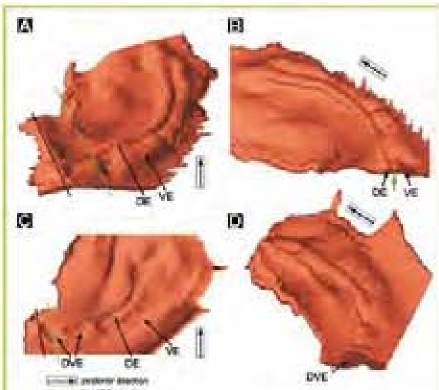

Figure 5: Comparison of the upper (A, B) and lower (C, D) incisor regions of the embryo at ED 42-44 on 3D reconstructions. Dental (DE) and vestibular (VE) epithelia are presented on aerial view (A, C) and on a sagittal section through the incisor region on 3D reconstruction (B, D). In the upper incisor region, DE and VE differentiate apart from each other, being separated by a groove indicated by a green arrow (A, B). However, in the lower jaw, one bulge of the dento-vestibular epithelium (DVE) is present in each the central and lateral incisor region (C, D). At later stages, the lingual parts of the two bulges give rise to i_1 and i_2, respectively. The labial parts differentiate into the vestibular epithelium. The midline is shaded.

Methods:

Histology:

After fixation in Bouin-Hollande fluid or in 10% formol, the heads were embedded in paraffin, cut in a series of 10 µm frontal sections and by haematoxylin-eosin, alcian-blue-haematoxylin-eosin, or by a Periodic Acid Schiff method. The early stages of tooth development (epithelial thickening, dental lamina, tooth bud) were distinguished according to Peterkova et al. (1996).

Three-dimensional (3D) reconstructions (Fig. 6):

3D analysis of the developing dentition and oral vestibule was undertaken in 9 lower jaw quadrants at different stages of development. Contours of the dental and adjacent oral epithelium were at 10 µm intervals (magnification x 120-260, depending on the size of the specimens) using a LEICA DMLB or JENAVAL microscope equipped with a drawing chamber. The superposition of the drawings was performed by the "best-fit" method with respect to the middle line and horizontal level for correct spatial positioning of the reconstructed structures.

The digitalization of serial drawings and the correlation of successive images have previously been described (Lesot et al. 1996). 3D images were generated using a volume rendering program (Sun Voxel Sun Microsystems, Santa Clara, CA, USA).

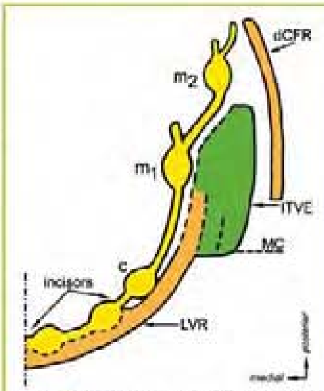

Figure 3: Data on the lower jaw (Hovorakova et al., 2007, in press). The two bulges emerge in the incisor region, comprising both the prospective dental and vestibular epithelia (dento-vestibular bulges, see also Figs 4, 5). The lingual parts of these bulges give rise to the prospective buds of deciduous central and lateral incisors. The labial parts progressively separate from the incisor germs as the vestibular epithelium. In the canine and molar region, the band of thickened vestibular epithelium is present externally to the dental epithelium at earliest stages of the development. In the cheek region, the area of the irregularly thickened vestibular epithelium (ITVE) differentiated. At ED 44-46, the vestibular and dental epithelia transiently fused in the canine region (Fig. 4B). Later, the vestibular epithelium again separated from the canine primordium (c) and fused posteriorly with the medial part of the ITVE and anteriorly with the vestibular epithelium in the incisor region (Fig. 4C) giving rise to the labial vestibular ridge (LVR). The mandibular cheek furrow ridge (dCFR) located laterally to the ITVE lined the mucosal inflection (the prospective lower part of the oral vestibule) between the developing alveolus and the cheeks. MC - mouth corner, m_1 and m_2 - the lower first and second molars, respectively.

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Acknowledgements:

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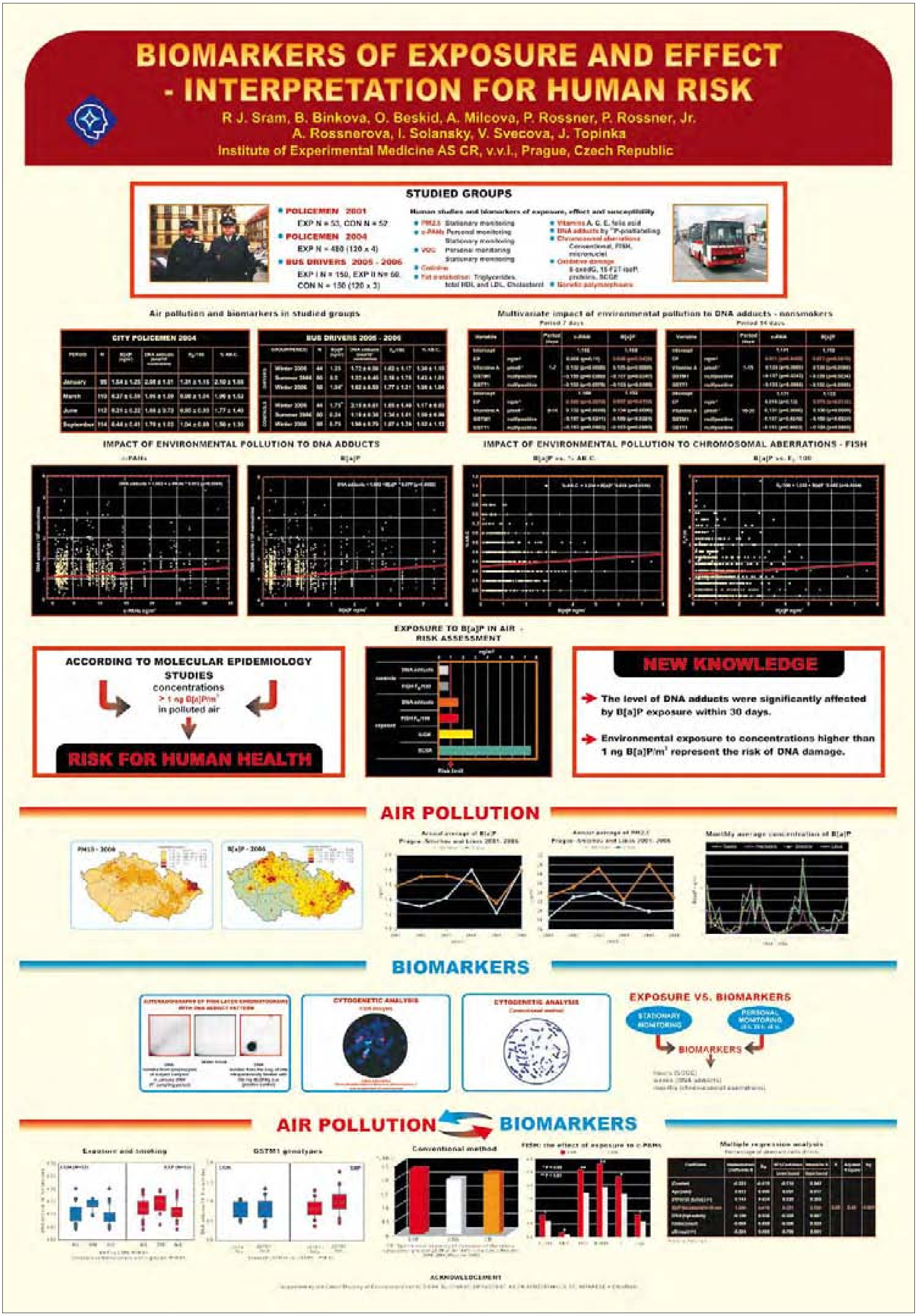
BIOMARKERS OF EXPOSURE AND EFFECT
- INTERPRETATION FOR HUMAN RISK
R J. Sram, B. Binkova, O. Beskid, A. Milcova, P. Rossner, P. Rossner, Jr.
A. Rossnerova, I. Solansky, V. Svecova, J. Topinka
Institute of Experimental Medicine AS CR, v.v.i., Prague, Czech Republic
STUDIED GROUPS
POLICEMEN 2001
EXP N = 53, CON N = 52
POLICEMEN 2004
EXP N = 480 (120 x 4)
BUS DRIVERS 2005 - 2006
CON N = 150 (120 x 3)
Air pollution and biomarkers in studied groups
CITY POLICEMEN 2004
BUS DRIVERS 2005 - 2006
IMPACT OF ENVIRONMENTAL POLLUTION TO DNA ADDUCTS
IMPACT OF ENVIRONMENTAL POLLUTION TO CHROMOSOMAL ABERRATIONS - FISH
EXPOSURE TO B[a]P IN AIR - RISK ASSESSMENT
ACCORDING TO MOLECULAR EPIDEMIOLOGY STUDIES
concentrations
> 1 ng B[a]P/m3
in polluted air
RISK FOR HUMAN HEALTH
NEW KNOWLEDGE
The level of DNA adducts were significantly affected by B[a]P exposure within 30 days.
Environmental exposure to concentrations higher than 1 ng B[a]P/m3 represent the risk of DNA damage.
AIR POLLUTION
BIOMARKERS
CYTOGENETIC ANALYSIS
EXPOSURE VS. BIOMARKERS
STATIONARY MONITORING
PERSONAL MONITORING
BIOMARKERS
AIR POLLUTION
BIOMARKERS
Exposure and smoking
GSTM1 genotypes
Conventional method
Multiple regression analysis
ACKNOWLEDGEMENT

Evaluation of nucleotide excision repair capacity in healthy individuals – a pilot study

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**Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic*

Introduction:

• Potentially important sources of interindividual variability in cancer development are 1) **heritable polymorphisms** in genes encoding biotransformation and DNA repair enzymes and 2) **DNA repair capacity (DRC).**

• Differences in DRC may be responsible for variability in cancer risk (1). Epidemiological studies have shown that deficiency in DRC contributes to the accumulation of DNA damage involved in human carcinogenesis (2, 3).

• **Especially in sporadic cancer**, affected most likely by gene-environment interactions, assays measuring individual DNA repair capacities appear to be useful tool define DNA repair characteristics in malignancies. Moreover, they provide unique information on the relationship between the genotype and phenotype, i.e. functional consequences of the polymorphisms.

• Nucleotide excision repair (NER) is the major pathway in humans for the removal of damage produced by UV light and a variety of bulky lesions formed by chemical agents (4). There are few studies investigating NER repair capacity among patients with various type of cancer (5, 6), but scarce data are available on background DRC levels in healthy individuals. **The assessment of DNA repair capacity in normal population is the crucial step in understanding possible relation between cancer risk and altered DNA repair capacity.**

Aim of the study:

• To develop simple, reliable method for NER capacity measurement based on challenge assays by UV light and chemical agent in peripheral mononuclear cells.

• To evaluate variability in DNA repair capacity among healthy individuals.

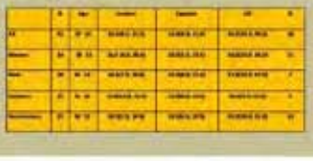
• To compare NER capacities after the employment of different challenge assays.

• To assess the modulation of NER capacity by genetic polymorphisms in relevant genes of this DNA repair pathway.

• To determine how this variation may relate to cancer risk (under investigation).

The obtained data from healthy population may serve as a background for further investigation of DRC among cancer patients with following goals: prediction of cancer risk, efficiency of the anticancer therapy and prognosis.

Study population:



Methodology:

In the present study we have employed a **challenge assay** (based on the comet assay) which measures DNA single-strand breaks appearance/disappearance (incision and ligation steps), following different in vitro treatments generating lesions repaired mainly by NER.

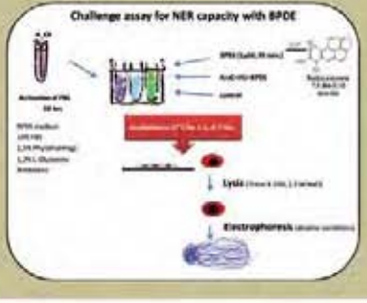
Freshly isolated and stimulated peripheral blood mononuclear cells from healthy individuals were challenged by:

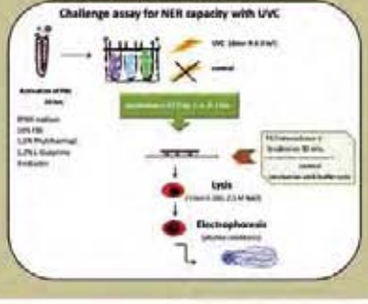
1. **Benzo(a)pyrene diolepoxide (BPDE)**: where 2 types of controls were used, control without any treatment as a background damage, and control with BPDE + 1-(β-D-Arabino-furanosyl)-cytosine hydrochloride (AraC; 10^{-4}M) + Hydroxyurea (HU; 0.01M), inhibits DNA resynthesis and ligation.
2. **UVC**: where 2 steps of treatment were used:

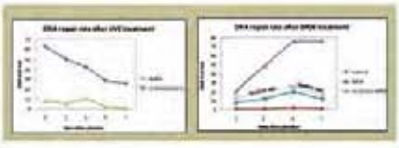
cells are challenged by UV light to induce dimers

cells are incubated with Endonuclease V enzyme to convert dimers into the breaks detectable by the comet assay.

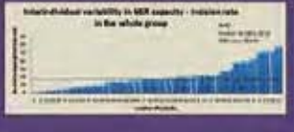
After challenge with BPDE/UV light, cells were incubated in the whole medium to allow DNA repair within intervals ranging from 0 to 7 hours. The balance of SSBs was detected by alkaline version of the comet assay.

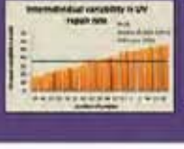




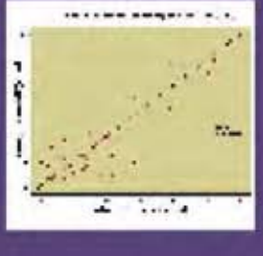


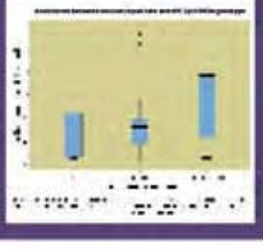
Preliminary results:

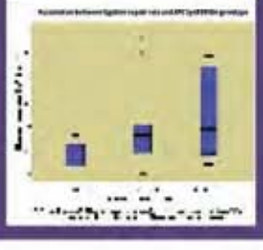


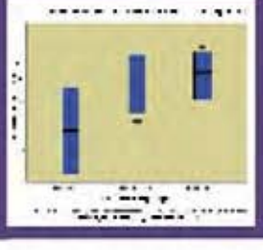


Preliminary results:









Conclusions:

• From preliminary results we have observed a substantial inter individual variability in the repair of lesions induced by both agents (BPDE, UVC).

• No direct correlation was observed between DRC assessed by the two different challenge assay.

• The DRC following the BPDE treatment was investigated separately for incision and ligation steps of NER and both parameters strongly correlated each other (r=0.91, p<0.0001).

• The association between rates of incision and smoking habit was of borderline significance (r=0.29, p=0.059), suggesting possible induction of DRC by tobacco smoke.

• DRC levels were not different between males and females and did not correlate with age.

• Higher DRC in incision, ligation and also UV repair rate among carriers of variant Gln/Gln genotype in XPC lys939Gln was observed, however, this association was not significant (small cohort).

• DRC is currently being assessed in additional volunteers (controls and cancer patients). The DRC levels along with expression analyses of selected relevant genes at transcriptional level will be also investigated.

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APPLICABILITY OF NITRIC OXIDE PLATFORM TO SCREENING THE CYTOKINE-INDUCING ACTIVITY OF DRUGS

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BACKGROUND, HYPOTHESIS AND GOALS

The high-output nitric oxide (NO) production depends largely on the cytokine-mediated activation of inducible NO synthase (iNOS). The direct activator of NO is IFN-γ. A number of other cytokines can either upregulate (*e.g.* IL-1β, TNF-α) or downregulate (*e.g.* IL-13, TGF-β) the immune-stimulated NO biosynthesis.

- **It may be hypothesized** that a drug which directly enhances the NO production is an inducer of IFN-γ. Compounds enhancing NO only when applied together with the priming stimulus provided by the exogenous IFN-γ, might do so via the activation of the secretion of NO-upregulatory cytokines.
- **The aim of our work** was to evaluate the validity of the hypothesis using a novel class of antivirals, acyclic nucleoside phosphonates (ANPs).

CONCLUSIONS

ANPs are upregulators of NO production. Statistically highly significant correlation exists between the extent of NO production and levels of cytokines produced by the cells of both mouse and human origin. It is concluded that the animal NO platform is a rapid and economical pilot screening approach allowing reliable prediction of cytokine-stimulatory potential of drugs in human cell system.

COMPOUNDS (upper right table), METHODS

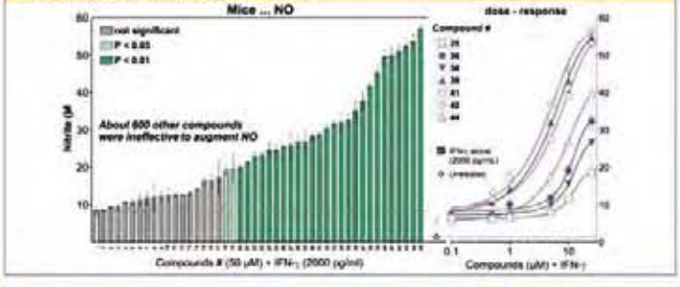
Acyclic nucleoside phosphonates (ANPs) are synthetic analogues of natural nucleoside monophosphates. They inhibit replication of both DNA-viruses and retroviruses (*tenofovir* and *adefovir* are broadly used for the therapy of AIDS and hepatitis B, resp.). Several hundreds of ANPs of distinct chemical structures were screened for the potential to

- enhance **production of NO** in mouse resident peritoneal cells;
- activate **secretion of cytokines** in mouse and human peripheral blood mononuclear cells.

All compounds were prepared in-house (AH). Possible LPS contamination was excluded using chromogenic Limulus amoebocyte test. NO was determined (Griess reagent) after the 24-h of culture. Cytokines were assayed after the 6-h of culture (ELISA).

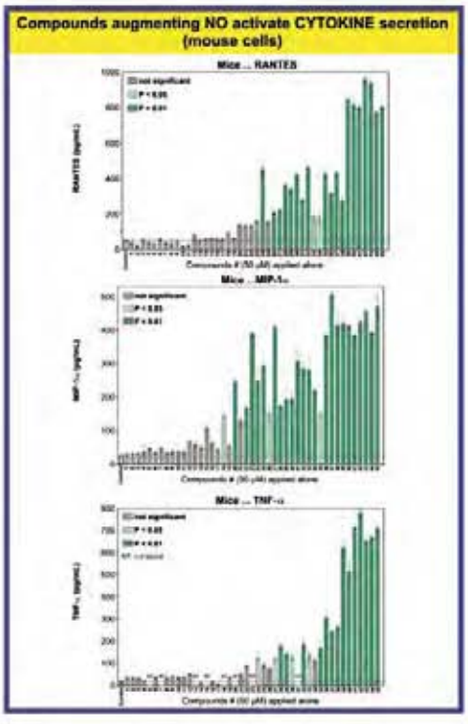
RESULTS

The compounds do not stimulate NO production on their own (not shown). However, they enhance production of NO triggered by IFN-γ.

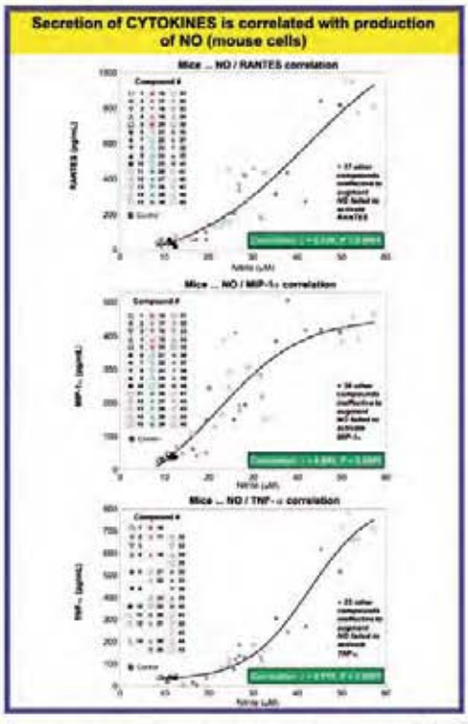


IDENTIFICATION of the compounds
Symbols / Codes / Chemical names

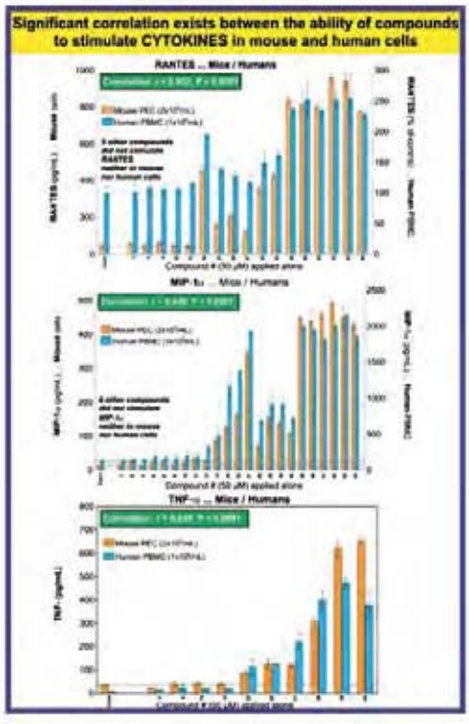
Compounds augmenting NO activate CYTOKINE secretion (mouse cells)



Secretion of CYTOKINES is correlated with production of NO (mouse cells)



Significant correlation exists between the ability of compounds to stimulate CYTOKINES in mouse and human cells



Acknowledgements. The work was supported by the grant 1M0508.

DEVELOPMENT OF DEFINED ANIMAL PROTEIN-FREE CULTURE SYSTEM FOR HUMAN EMBRYONIC STEM CELLS

estools
Advances with Human Embryonic Stem Cells

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INTRODUCTION

Previously we demonstrated that the high passage hESCs (p>100) can be cultured in the chemically defined medium based on the plant hydrolysate VegetaCell and other defined supplements in the combination with human foreskin fibroblasts.

Here we investigated the effect of completely humanized VegetaCell medium in the culture of low passage hESCs, either with human feeders or in feeder-free conditions using Matrigel.

Material and methods

hESCs culture

hESCs: CCTL14 (derived at Department of Molecular Embryology in Brno, 2003); p23 – p44; 46, XX
CCTL12 (used in some additional experiments; data not shown)

feeder cells: mouse embryonic fibroblasts (MEF, CF-1) or human foreskin fibroblasts (hFF, ECACC 10401)

culture medium:
standard medium: DMEM/F12, 15% KnockOut Serum Replacement, β-mercaptoethanol, non-essential amino acids, L-glutamine, penicillin / streptomycin and 10 ng/ml FGF-2

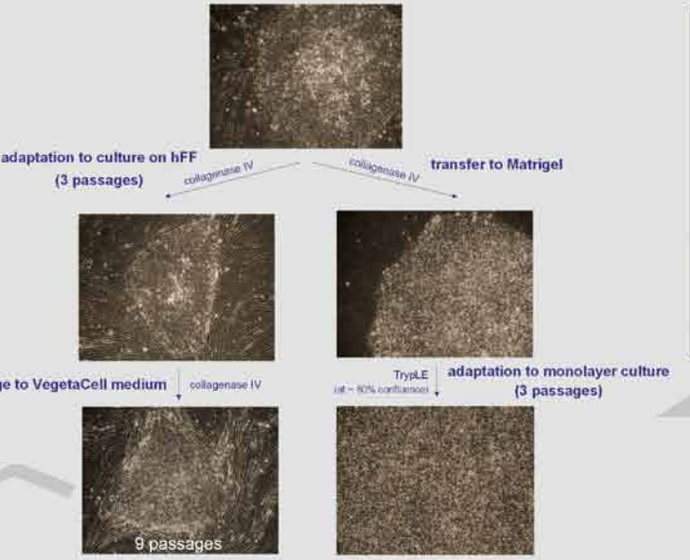
VegetaCell medium: without KnockOut Serum Replacement, supplemented by: 5% VegetaCell, 3 mg/ml human serum albumin, 0.4% cholesterol, 5.5 µg/ml human holo-transferrin, 1 µg/ml human insulin, 0.6 µg/ml sodium selenite and 50 µg/ml ascorbic acid

feeder-free conditions:
Matrigel combined with MEF-conditioned medium (10 ng/ml FGF-2) or VegetaCell medium

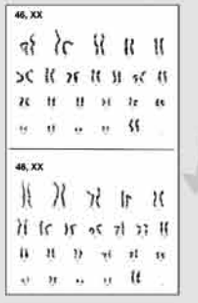
karyotype analysis: performed by Duncan Baker (University of Sheffield)

duration of the cell cycle: measured by EdU incorporation assay

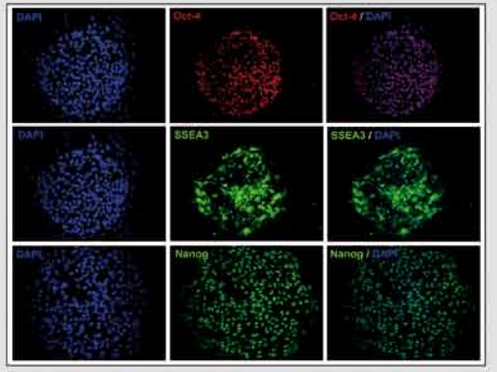
1 Adaptation of hESCs to culture conditions using VegetaCell medium and hFF or Matrigel



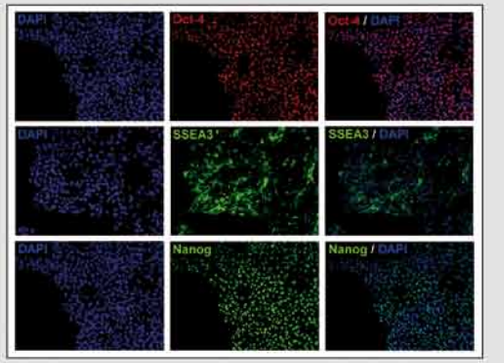
2 hESCs in the VegetaCell medium preserve normal karyotype



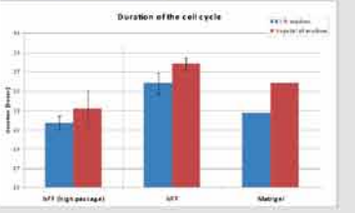
3 Colonies of hESCs in VegetaCell medium on human feeders express Oct-4, SSEA3 and Nanog



4 hESCs in ~70% confluence on Matrigel in VegetaCell medium express Oct-4, SSEA3 and Nanog



5 hESCs in VegetaCell medium have a prolonged duration of the cell cycle



CONCLUSIONS

Culture adapted as well as normal low passage hESCs grow in the culture system based on humanized VegetaCell medium

- in colonies on human feeders or
- in monolayer on Matrigel.

hESCs cultured in these conditions

- express SSEA3, Oct-4 and Nanog and
- maintain the normal karyotype.

Acknowledgements

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Human embryonic stem cells can activate checkpoint control pathways

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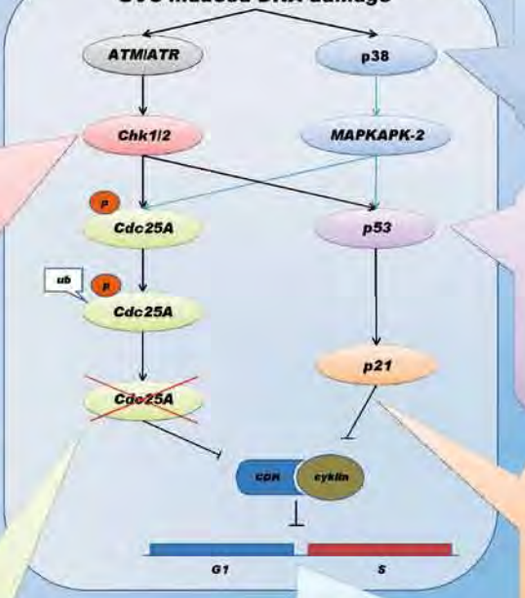
Introduction

Alterations to the genome of hESC → MECHANISM? → Failure of checkpoint control?

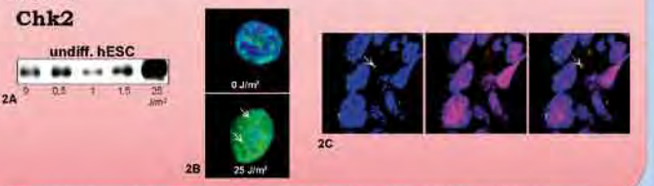
UVC-induced DNA damage



Chk1

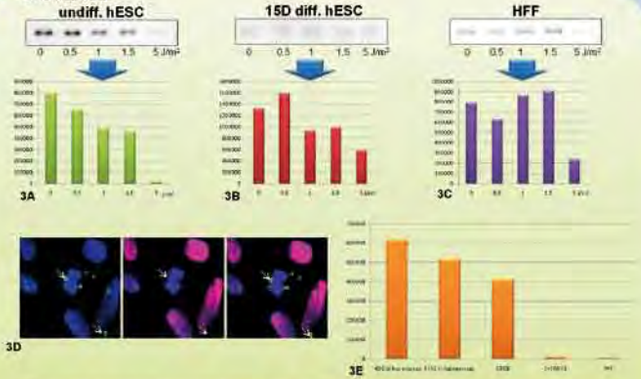
1A) Phosphorylation of Chk1 (ser345); **1B)** Localization of Chk1 in hESC – arrows show centrosomes

Chk2



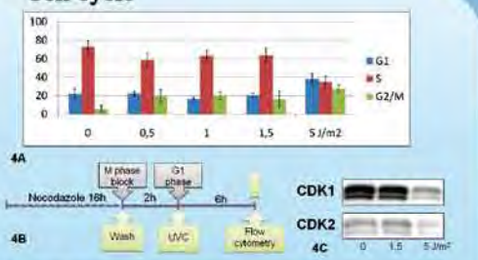
2A) Phosphorylation of Chk2 (thr68); **2B)** Localization of phosphorylated Chk2 – arrows show domains of nuclear accumulation; **2C)** Localization of Chk2 in hESC – arrows show centrosomes

Cdc25A



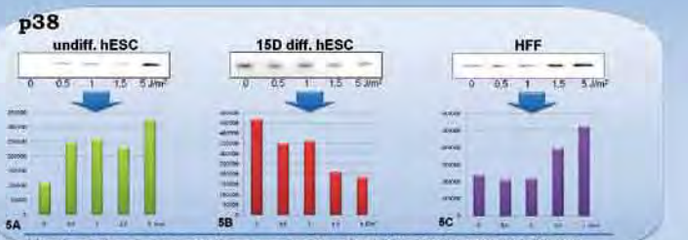
Quantity of Cdc25A 3h after UVC treatment in: **3A)** undiff. hESC, **3B)** 15D diff. hESC, **3C)** HFF; **3D)** Localization of Cdc25A phosphatase in undiff. hESC – arrows show centrosomes; **3E)** Quantity of Cdc25A in hESC at low passage, hESC at high passage, 5D diff. hESC, 15D diff. hESC and HFF

Cell cycle



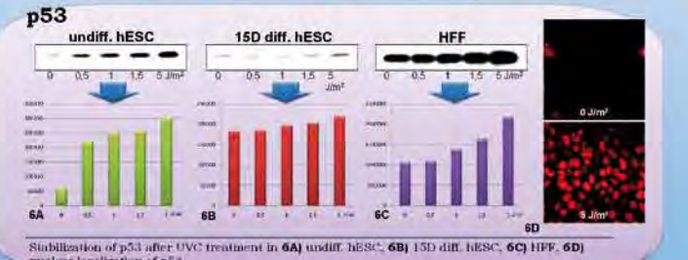
4A) Changes to cell cycle distribution in response to UVC treatment; **4B)** Design of experiment; **4C)** Activities of CDK1 and CDK2 in undiff. hESC after UVC treatment

p38



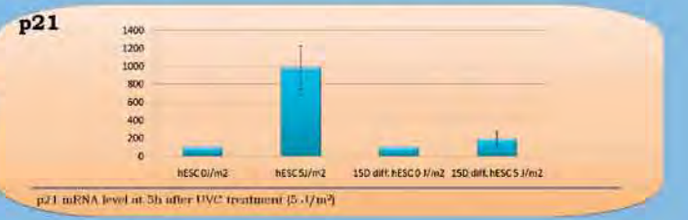
Phosphorylation of p38 after UVC treatment in: **5A)** undiff. hESC, **5B)** 15D diff. hESC, **5C)** HFF

p53



Stabilization of p53 after UVC treatment in **6A)** undiff. hESC, **6B)** 15D diff. hESC, **6C)** HFF, **6D)** nuclear localization of p53

p21



p21 mRNA level at 3h after UVC treatment (5 J/m²)

Summary

In undifferentiated hESC UVC irradiation causes:

- G1/S arrest
- activation of p38 and degradation of Cdc25A phosphatase
- stabilization of p53 and transactivation of its target gene p21
- rapid decrease of activities of CDK1 and CDK2

Some components of checkpoint control pathways are activable in undifferentiated hESC

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Successful Repair of Osteochondral Defects with Composite Artificial Scaffold in Six-month Study in Miniature Pigs

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Introduction

Cartilage defects have a poor reparative capacity. Their repair is improved by autologous chondrocytes either in suspension or mainly in biodegradable scaffolds, such as hyaluronic acid, fibrin, collagen (1,2,3). We prepared new composite hyaluronate/collagen/fibrin scaffold and studied its ability to repair osteochondral defects in miniature pigs in a six-month study.

Methods

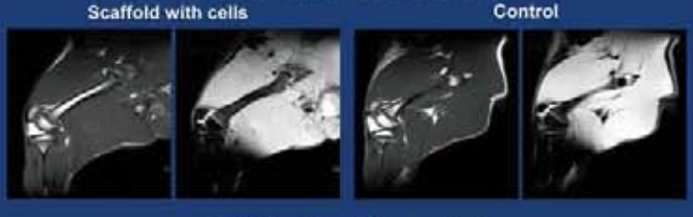
1. Preparation of composite scaffold from hyaluronate sodium, type I collagen, and fibrin (HA - Mr 1,5 x 10^6)
   Group with cells – 3 animals, 9x10^6 autologous chondrocytes/mL
   Group without cells – 3 animals, implanted scaffold without any cell
   Control group – 3 animals, the same defects were left empty
2. Implantation of scaffolds into 6mm wide and 2 mm deep osteochondral defect in the middle of condylus medialis
3. Analysis of the repaired defects
   Histology – 24 weeks after the implantation (alcian blue and PAS at pH 2.5, haematoxylin-eosin, immunohistochemistry - type II collagen)
   MRI – 12 and 24 weeks after the implantation - sagittal orientations using T1 weighted spin echo (TR/TE=500/12 ms) and FLASH (TR/TE=999/11 ms) sequence, FOV=280 mm, slice thickness 3 cm. The same T1 weighted sequence was repeatedly applied after contrast agent (Gd-DTPA) application

Conclusion

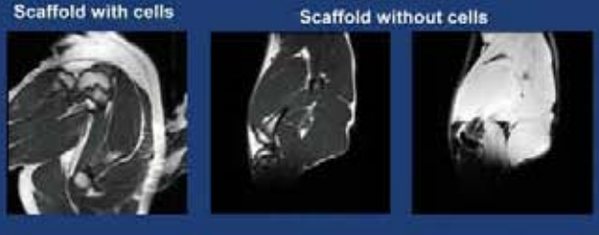
Scaffolds with autologous chondrocytes repaired the osteochondral defects in miniature pigs after 24 weeks; the defects were filled with hyaline and partially fibrous cartilage. Scaffolds without cells repaired defect predominantly with fibrous cartilage.

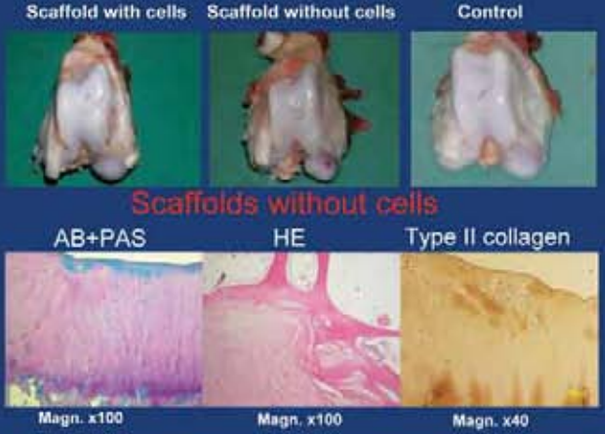
Results

MRI after 12 weeks



MRI after 24 weeks





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Lateral Plasma Membrane Compartmentation: Composition, Structure and Function of Stable Patchy Domains in Yeast

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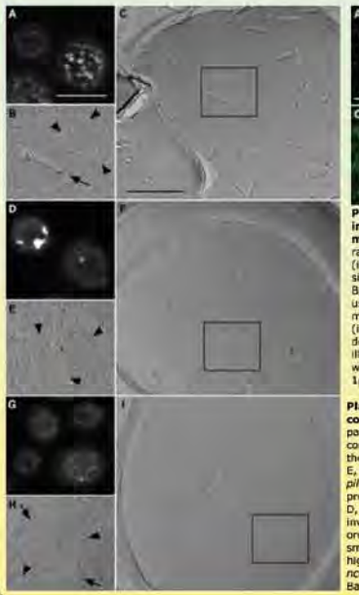
ABSTRACT

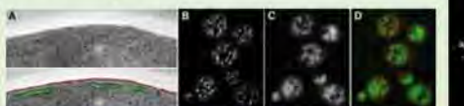
It becomes generally accepted that the plasma membrane is laterally compartmented into domains of different lipid and protein composition. So far, we identified nine proteins accumulated in ergosterol-rich 300 nm stable patchy areas (the MCC, membrane compartment of arginine permease Can1) in the plasma membrane of *S. cerevisiae*. Among them were three transporters with twelve transmembrane domains and six proteins of unknown function with predicted tetraspan structure. In addition, the distribution of cortical patches formed by at least twelve cytosolic proteins was matched with the MCC pattern. Using various electron microscopy approaches, we were able to show that MCC patches are fluorescence microscopy correlates of furrow-like plasma membrane invaginations observed on freeze-fractured cells. The presence of stably curved membranes in these structures is in agreement with the proposed lipid composition of MCC.

Among the integral MCC components, Nce102 seems to play a major role in the membrane domain composition. Deletion of Nce102 resulted in the homogenous patterns of MCC-specific transporters. Accordant changes in the membrane ultrastructure were detected in the deletion strain. We document that the lack of Nce102 as well as that of Pil1, a primary component of eisosomes, cytosolic structures co-localizing with MCC, accelerate substrate-induced endocytosis and/or turnover of MCC-specific transporters. Proteins essential for classical endocytosis as for example Ede1, Sla2 and Rvs161 do not co-localize with MCC areas. This suggests that proteins within the MCC are protected from degradation, and that in general, lateral segregation of plasma membrane constituents controls protein turnover.

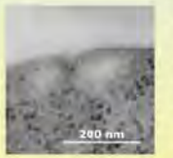
Membrane Compartment of Can1 (MCC) corresponds to furrow-like invaginations of the plasma membrane



Plasma membrane invaginations in the optics of a confocal microscope. A field of 1000 randomly distributed model objects (invaginations; A, red in C), and its simulated confocal image (patches; B, green in C) are presented. PSF used for the simulation of the microscope imaging is also shown (insert in A). D and E represent details of B and C, resp. A cartoon illustrating the size of a yeast cell was added into C-E. Bars: 5µm (A), 1µm (insert).

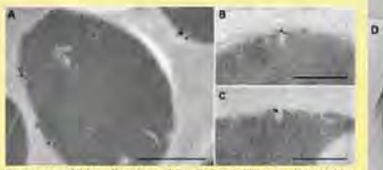


MCC patches do not co-localize with cortical ER. Thin section of a high pressure-frozen wild type cell (A), and a tangential confocal section of the cells co-expressing an MCC marker Sur7-mRFP (B, red in D) and ss-GFP-HDEL (C, green in D) are presented. Plasma membrane (red) and cisternae of cortical ER (green) are highlighted in A. Bars: 200nm (A), 5µm (B-D).



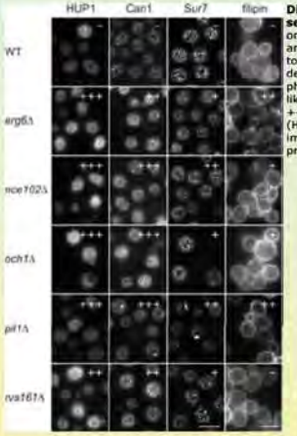
Elongated MCC patches and furrow-like invaginations in *ypr050cΔ* cells. Cell surface distributions of MCC patches (marked by Sur7-GFP; A, B) and the furrow-like invaginations of the plasma membrane (C, D) in *ypr050cΔ* mutant are compared. Superposition of four consecutive confocal sections (A), depth-coded 3D stack covering whole confocally sectioned cells (B), surface ultrathin resin section (C) and a freeze fracture surface view (D) are presented. Bars: 5µm (A, B), 500nm (C, D).

Plasma membrane in *nce102Δ* and *pil1Δ* as compared to wild type cells. Fluorescence patterns of Can1-GFP (A, D, G) on tangential confocal sections and freeze fracture views of the plasma membrane (outer layer; details in B, E, H, overviews in C, F, I) in BY4741 (A-C), *pil1Δ* (D-F) and *nce102Δ* (G-I) cells are presented. Note the dispersed Can1 pattern in D, G, and the absence of membrane invaginations in E, H. In B, E, H, hexagonally ordered grain clusters (arrowheads) and smooth elongated areas (arrows) are highlighted. In contrast to wildtype (B), in *nce102Δ*, these areas are not invaginated (H). Bars: 5µm (A, D, G), 500nm (C, F, I).

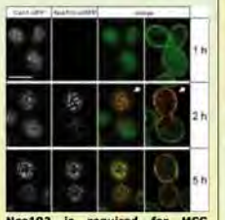


Immunogold localization of Sur7. Sur7-GFP was detected by anti-GFP (A) and anti-Sur7 (B, C) using pre-embedding labeling approach. The protein was clustered in areas of invaginated plasma membrane. Bars: 1µm (A), 500nm (B,C).

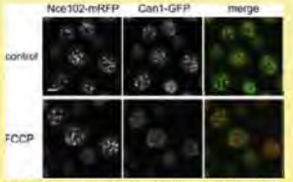
Nce102 anchors transporters within MCC



Distribution of MCC markers in selected knockout strains. Distributions of HUP1-GFP, Can1-GFP, Sur7-GFP, and filipin-stained sterols were monitored in the library of single gene deletion strains. Examples of detected phenotypes (classification: wild type-like: -; weak: +; medium: ++; strong: +++) on tangential confocal sections (HUP1, Can1, Sur7), or wide field images (filipin; transversal sections) are presented. Bars: 5 µm.

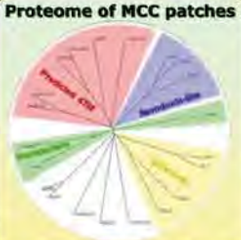


Nce102 is required for MCC localization of Can1. Plasma membrane distribution of Can1-GFP was observed in *nce102Δ* cells expressing Nce102-mRFP under the control of a galactose-inducible promoter. After the induction, gradual restoration of the wild type-like patchy distribution of Can1-GFP was followed on tangential confocal sections. As notable on transversal sections of the same cells, the pattern of bud membrane was restored earlier (arrows). Bar: 5 µm.



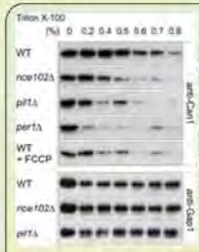
Can1-GFP dissociates from Nce102-mRFP upon membrane depolarization. Surface views of cells expressing Can1-GFP and Nce102-mRFP before (upper) and 2 minutes after addition of 50µM FCCP to the medium (lower panels) are shown. Bar: 2 µm.

Proteome of MCC patches



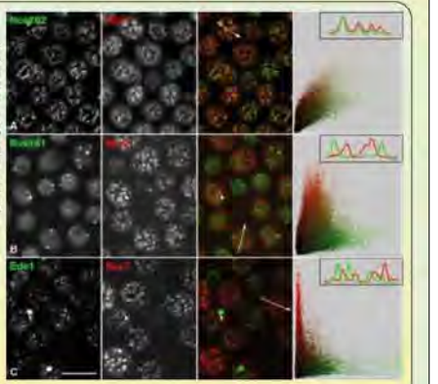
Twenty-one proteins were co-localized with MCC patches. Nine of them are integral MCC components, twelve others are soluble.

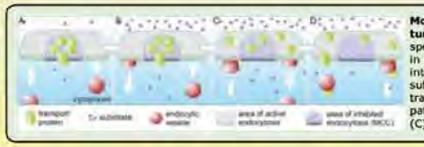
MCC regulates turnover of transporters



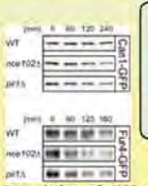
Extractability of the transport proteins Can1 and Gap1 by Triton X-100. Membranes were isolated from exponentially growing cells. Aliquots corresponding to 50 µg of membrane protein were treated with increasing concentrations of Triton TX-100. The non-solubilized proteins were resolved by SDS-PAGE, and detected by specific antibodies on western blots.

Sites of classical endocytosis do not co-localize with MCC. The plasma membrane distributions of Ede1 and Rvs161, markers of early and late endocytic steps, respectively, were tested for co-localization with MCC marker Sur7. For comparison, localization of MCC resident Nce102 was analyzed. Tangential confocal sections showing the cell surface are presented. Because of a high mobility of Rvs161 patches, a maximum intensity projection of 36 frames (5 s/frame) instead of a single frame is shown in (B). In this arrangement, a higher number of Rvs161 patches could be localized towards the stable Sur7 pattern at the same time. The rate of co-localization was quantified by fluorescence intensity profiles (upper diagrams) and 2D scatter plots. Note the diagonal orientation of Nce102-derived scatter plot demonstrating the co-localization of red and green fluorescence signals and a clear separation of red and green pixels in the two other cases. Bar: 5 µm.

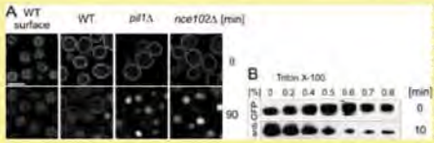




Model of spatially confined protein turnover. In the absence of substrate, specific transporters are concentrated in MCC and protected against internalization (A). After the excess of substrate is supplied (B), the transporters are released from the MCC patches to the surrounding membrane (C) and subjected to endocytosis (D).



Degradation of MCC transporters is accelerated in mutants affected in the domain formation. Exponentially growing cultures of wild type, *nce102Δ* and *pil1Δ* cells expressing Can1-GFP or Fur4-GFP were treated with cycloheximide. At given time points, total membranes were isolated from the culture aliquots. The membrane proteins were resolved by SDS-PAGE and Can1-GFP and Fur4-GFP were detected by anti-GFP antibody on western blots. Into each lane, 2.5 µg of the total protein were loaded.



Can1 is released from MCC patches prior to endocytosis. (A) Can1-GFP was localized in the wild type, *nce102Δ* and *pil1Δ* cells before (upper) and 90 minutes after the addition of 5 mM arginine (lower panel). Arginine-induced loss of patchy Can1-GFP pattern on the surface confocal sections (left), and the amount of the internalized protein on transversal sections could be easily followed. Note significantly more intensive vacuolar staining in the mutants lacking the MCC patches as compared to wild type. Bar: 5 µm. (B) Extractability of Can1-GFP in Triton X-100 was detected using anti-GFP antibody in the membranes of wild type cells, before and 10 minutes after the addition of 5 mM arginine.

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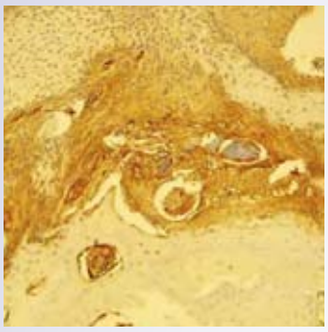

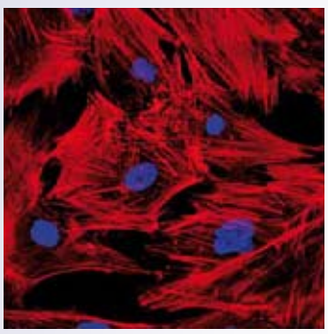

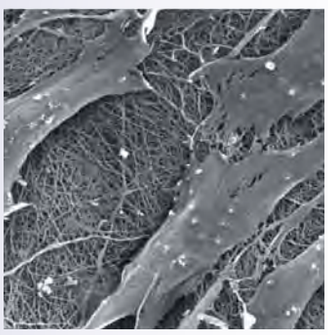

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LOCATION

TRAVEL DIRECTIONS TO THE INSTITUTE OF EXPERIMENTAL MEDICINE ASCR

You can, of course, take a taxi to the Institute. It is best to phone for a taxi from, for example, AAA taxi service (phone: 9614014 or 222 333 222); do NOT hail a taxi on the street, as the cost of such a taxi could be unpleasantly high. The Institute's street address is Vídeňská 1083, in the district of Krč in Prague 4. Please note that our Institute is a few hundred meters away from a well-known hospital and transplant center with a very similar name, the Institute of Clinical and Experimental Medicine, known by its Czech acronym "IKEM". Unfortunately, most taxi drivers are more familiar with IKEM than with our Institute, so visitors are often mistakenly transported to IKEM.

Alternatively, you can travel by public transportation. Enter the metro at the nearest station, consult the maps located in all metro stations, and then make your way to the metro line C (the red line) and take a train in the direction "Háje". Travel on metro line C until the station "Budějovická". Exit the station "Budějovická" in the same direction that the train was traveling, following signs for "Poliklinika". After leaving the statiton, continue walking through a small below-ground shopping area in the same direction until the last stairs on the right, again following signs for "Poliklinika". At the top of the stairs is a bus stop. Take bus 193 in the direction "Ústavy Akademie věd" until the final stop. The entrance to the Academy of Sciences campus is directly opposite the bus stop. Enter the campus, turn to the left, and follow the road for approximately 150 meters until the first large building on the left, building LA. This is the Institute of Experimental Medicine AS CR, v. v. i.

General public transportation information: Prague has an excellent public transportation system of buses, trams, and three metro lines, identified as A, B and C. Transfers between the three lines are possible at certain metro stations, and each metro station displays a detailed map of the entire metro system, showing all the stations and transfer points. In order to travel on any form of public transportation in Prague, you must purchase a valid ticket before entering the metro or boarding a tram or bus, then have the ticket validated by inserting the ticket (with the arrow face up and pointing frontwards) into one of the small yellow validation machines located at all metro entrances and also on all trams and buses.

Ticket Sales/Advance Sales in the Metro Sales locations in the metro have the entire assortment of tickets available, especially passes for fixed calendar periods (monthly, quarterly, yearly) and passes with an arbitrary validity period (30-day, 90-day, 365-day).

The sales location in the building of the Central Dispatch at Na Bojišti 5, Prague 2 is open Mon–Fri, 6. 30 a. m.–6. 30 p. m.

Saturday sales hours are the same in all advance sales locations: 7: 30 a. m.–12: 00 p. m. (except for the sales loca tion at the Hlavní nádraží/Main Train Station (C), which is closed Saturdays). Transferable tickets without the holder's personal information are only sold at the Central Dispatch at Na Bojišti 5, Prague 2, and at the Roztyly metro station.

Single-trip tickets are sold at all sales locations.

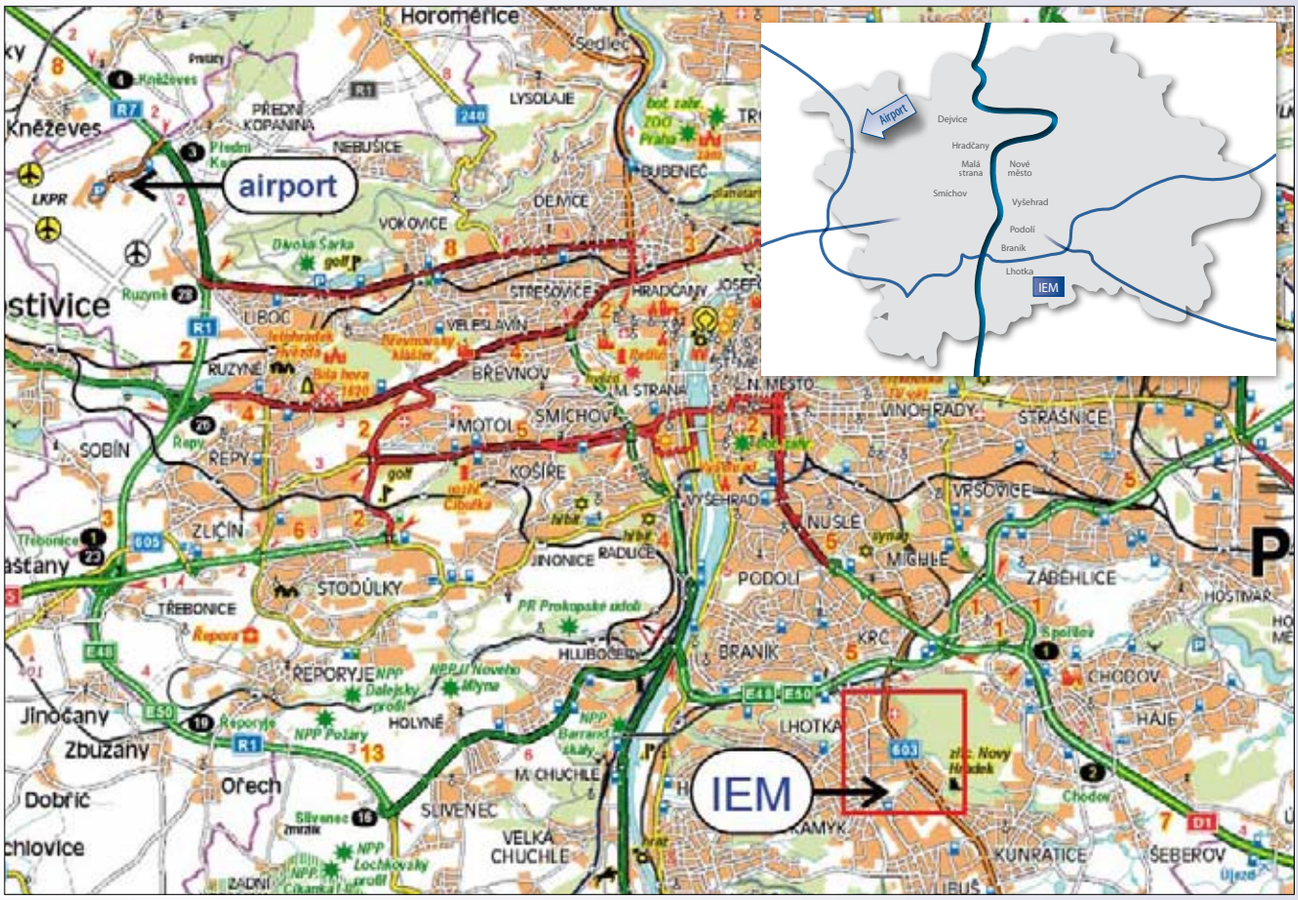
Credit and debit cards can be used at the following sales locations: Roztyly (C), Hůrka (B), Karlovo náměstí (B), Hloubětín (B), Nádraží Holešovice (C), Želivského (A) and at the Central Dispatch at Na Bojišti 5, Prague 2.

Ticket/Pass type	Adult	Child	Student	Senior
Limited * 20/30 min.	18 CZK	9 CZK	18 CZK	9 CZK
Basic 75 min.	26 CZK	13 CZK	26 CZK	13 CZK
1 day 24 hrs	100 CZK	50 CZK	100 CZK	50 CZK
3 days** 72 hrs	330 CZK	•	330 CZK	•
5 days ** 120 hrs	500 CZK	•	500 CZK	•

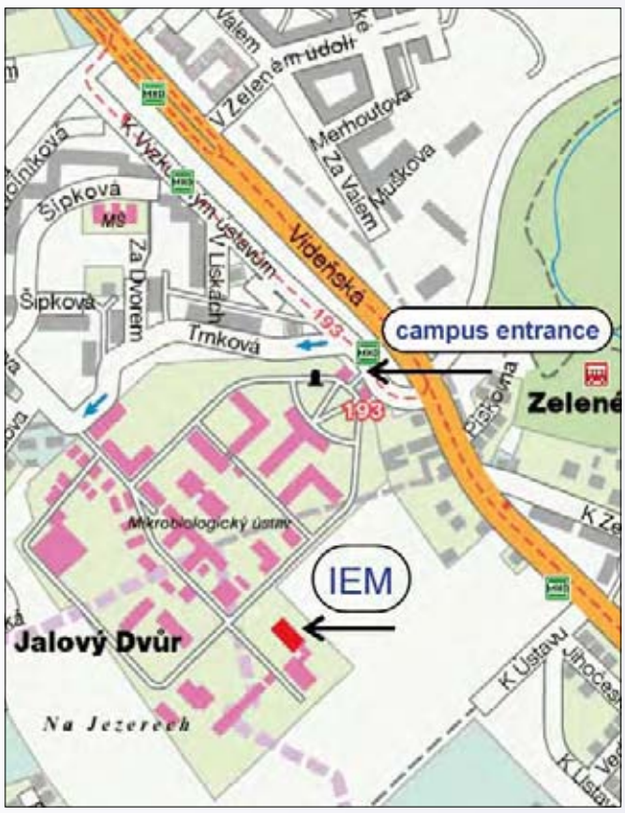
If you are caught riding any form of public transportation without a valid ticket, you will have to pay a large fine on the spot. Therefore, please do not attempt to travel on any part of the public transportation system without a valid ticket.

Basic ticket can also be purchased by sending an SMS with the text "DPT" to the numter 9020626 (only with SIM of one of the Czech operators).

TRANSPORT IN PRAGUE

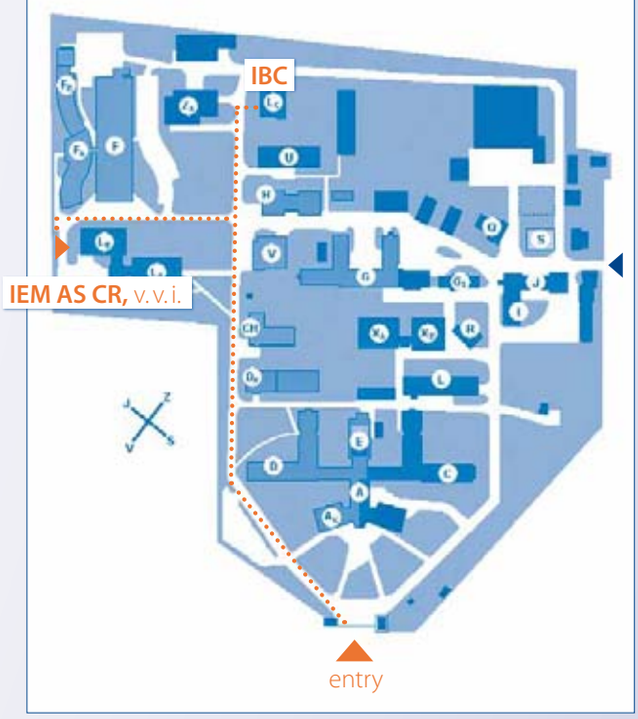


IEM ASCR LOCATION IN PRAGUE 4



THE CAMPUS OF RESEARCH INSTITUTES AS CR IN PRAGUE 4-KRČ

GPS Loc: 50°1'3. 964"N, 14°27'59. 812"E



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We thank Cell Therapy for generously supporting the publication of this brochure.