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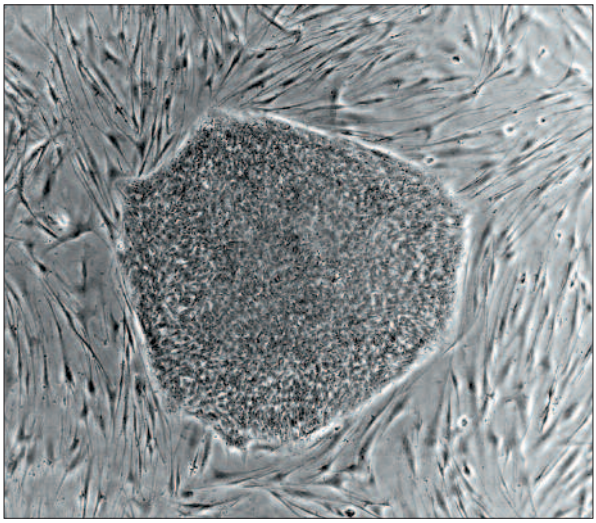

XX. BIOLOGICKÉ DNY

KMENOVÉ BUŇKY – OD REGENERATIVNÍ MEDICÍNY K NÁDOROVÉ BIOLOGII

Plzeň

25.–27. října 2011

Program a sborník abstrakt

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pořádají

XX. BIOLOGICKÉ DNY

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OBSAH

ISBN 978-80-260-0849-1

Vážení přátelé, milí kolegové vážení účastníci XX. Biologických dnů,

Biologické dny, tedy vědecká konference Československé biologické společnosti, mají skutečně dlouhou tradici – první Biologické dny zorganizoval v Praze Prof. Nečas již v roce 1964. Je nám velkou ctí, že organizací jubilejních XX. Biologických dnů jsme se mohli ujmout právě my, tedy ústav biologie Lékařské fakulty University Karlovy v Plzni. Náš ústav není v organizování podobných vědeckých setkání, byť skromnějšího rozsahu, žádným nováčkem – dlouholetý vedoucí našeho ústavu, Doc. RNDr. Josef Reischig, CSc., v Plzni po léta ve spolupráci se společností Olympus organizoval semináře optické mikroskopie, a někteří z vás, účastníků XX. Biologických dnů, už v minulosti Plzeň navštívili právě při této příležitosti. Jakožto vědomý symbol kontinuity jsme se proto rozhodli i XX. Biologické dny zahájit sekcí věnovanou optické mikroskopii v biologii a pojmenovat ji právě po Doc. RNDr. Josefu Reischigovi, CSc. Vedle toho se chceme v rámci letošních biologických dnů věnovat dvěma tématům, které spolu váže mnoho styčných bodů – problematice molekulární biologie nádorů a samozřejmě zejména ústřednímu tématu XX. Biologických dnů – problematice kmenových buněk. Jsme opravdu rádi, že v rámci těchto všech tématických sekcí můžeme v Plzni přivítat osobnosti, které jsou po dlouhá léta v samotném centru vědeckého dění a mohly a mohou tak spoluurčovat směr jeho vývoje. Do programu konference je také zařazeno Mimořádné valné shromáždění Čs. biologické společnosti s prestižní Babákovou přednáškou, které společnost využívá i k předání ocenění významným vědeckým osobnostem.

Kromě této čistě vědecké náplně bychom v rámci XX. Biologických dnů rádi poskytli účastníkům informace a rady přinejmenším ve dvou dalších oblastech. Cenným zdrojem prostředků pro rozvíjení jak vědeckých, tak i edukativních aktivit se v posledních letech stávají strukturální fondy Evropské unie. Jsme proto velmi rádi, že se XX. Biologických dnů účastní i zástupci Ministerstva školství, mládeže a tělovýchovy České republiky, v jejichž kompetenci je konkrétní aplikace těchto evropských prostředků, a seznámí nás s konkrétními a aktuálními možnostmi jejich využití. Tou druhou oblastí, která se dostane do popředí večer 26. října, bude tradiční chlouba našeho kraje – Plzeňský prazdroj, se svou unikátní expozicí tradičního postupu vaření piva. Vzhledem k tomu, že výrobu piva lze dozajista zařadit mezi jednu z prvních široce používaných biotechnologií, tedy praktické aplikace biologických poznatků, návštěvu plzeňského pivovaru pokládáme za integrální součást XX. Biologických dnů a všechny účastníky k ní srdečně zveme.

Podobnou konferenci dnes již není možné zorganizovat jen na bázi registračních poplatků účastníků. Neobešli bychom se tedy bez finanční podpory a pomoci různých šlechetných dárců. Největším dílem k pořádání XX. Biologických dnů přispěla svou dotací Československá biologická společnost. Významnou pomocí byly rovněž sponzorské příspěvky řady komerčních subjektů, jejichž upoutávky najdete na samém konci tohoto sborníku. Na prvním místě je třeba poděkovat zejména firmě OLYMPUS CZECH

GROUP, s.r.o., náš dík rovněž patří všem dalším sponzorů – BARIA s.r.o., Beckman Coulter Česká republika, s.r.o., EAST PORT Praha s.r.o., Ing. Milena Španělová – SpinChem, Maneko, s.r.o., MEGABOOKS CZ, spol. s r.o., M.G.P. spol. s r.o., Scintila, s.r.o. a Sigma-Aldrich spol. s r.o.

Všechny vás srdečně vítám v Plzni a přeji vám zajímavé, přínosné a příjemné XX. Biologické dny!

Doc. Ing. Jiří Hatina, CSc.
předseda organizačního výboru

ORGANIZAČNÍ VÝBOR

Předseda:	Doc.Ing. Jiří Hatina , CSc.
Členové:	MUDr. Jaroslav Voller
	Ing. Ivana Ratajová
	Zdenka Křížková

PROGRAMOVÝ VÝBOR

Předseda:	Doc. Ing. Jiří Hatina, CSc.
Členové (předsedové sekcí):	Prof. MUDr. RNDr. Svatopluk Čech, DrSc.
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VŠEOBECNÉ A TECHNICKÉ INFORMACE

Místo konání	Veškerá jednání budou probíhat v prostorách Lékařské fakulty v Plzni, Šafránkův pavilon, Alej Svobody 31 Slavnostní večeře bude uspořádána dne 26. 10. 2011 v prostorách pivovaru Plzeňský prazdroj, doprava na místo bude zajištěna.
Registrace účastníků	Místo pro registraci účastníků je ve vestibulu Šafránkova pavilonu: 25. 10. 9:00–18:00 26. 10. 9:00–18:00 27. 10. 9:00–12:00
Jednací jazyk	Jednacím jazykem je čeština, slovenština a angličtina. Viz program.
Ústní sdělení	Prezentace ve formátu PowerPoint 2010 nebo Pdf je třeba odevzdat před začátkem příslušné sekce technikovi (p. Ťupa) v přednáškové místnosti.
Plakátová sdělení	Panely pro postery budou připraveny ve foyer budovy Šafránkova pavilonu. Postery budou vyvěšeny po celou dobu konání kongresu.
Nouzová linka	V případě jakýchkoli problémů kontaktujte paní Křížkovou (mobil 608 777 649).
Webová stránka	http://www.lfp.cuni.cz/patofyziologie/bd.html

PROGRAM

Úterý, 25. října 2011

12.15–12.30	**Slavnostní zahájení**
12.30–16.00	**Sekce 1 – Optická mikroskopie v biologii – seminář Josefa Reischiga** **Předsedající: M. Červinka**
12.30–13.00	J. Černý: Life cell imaging jako vhodný nástroj buněčného biologa
13.05–13.35	L. Kubínová: Analýza trojrozměrných obrazových dat získaných konfokální mikroskopií
13.40–14.10	J. Lazar: Analýza struktury a funkce proteinů dvoufotonovou polarizační mikroskopií
14.15–14.45	P. Jůda: Umlčování genů: Hledání „PcG tělísek“ v pralese chromatinu
14.50–15.20	J. Peychl: Spinning disk konfokální mikroskop jakožto nástroj pro světelnou mikroskopii vysokého rozlišení
15.25–15.55	T. Pop: Novinky v mikroskopické technice fy Olympus
16.00–16.30	**Přestávka – postery**
16.30–19.30	**Sekce 2 – Molecular Biology of Cancer** **Předsedající: E. Rudolf**
16.30–17.00	J. Svoboda: Molecular genetics of cancer
17.05–17.35	K. Michalová: Progress in cytogenetic cancer diagnostics
17.40–18.10	O. Slabý: MicroRNAs: biomarkers and potential therapeutic targets in oncology
18.15–18.45	J. Hatina: Teaching oncology in the molecular era
18.50–19.20	A. Zeimet: Ovarian carcinoma – clinical and experimental approaches

Středa, 26. října 2011

7.30–8.30	**Postery**
8.30–12.10	**Sekce 3 – Stem Cells – from Regenerative Medicine to Cancer Biology** **Předsedající: J. Hatina**
8.30–9.10	A. Jung: Colorectal cancer stem cells
9.15–9.45	M. Alison: Current perspectives on normal and malignant stem cells in the liver and intestinal tract
9.50–10.20	C Strell: Tumour-stroma interactions and cancer stem cells
10.25–10.55	M Dahlke: Multipotent adult progenitor cell therapy in transplant medicine
11.00–11.30	C Altaner: Genetically modified human mesenchymal stem cells for cancer treatment
11.35–12.05	E. Syková: Stem cells in regenerative medicine: Preclinical and clinical studies
12.15–13.15	**oběd – postery**
13.15–14.00	**Speciální přednáška – 2. prioritní osa OPVK a aktuální výzvy v oblasti 2.3 – Lidské zdroje ve V a V**
14.00–17.30	**Sekce 4 – Kmenové buňky – od regenerativní medicíny k nádorové biologii** **Předsedající: E. Syková**
14.00–14.30	A. Hampl: Mohou nás embryonální kmenové buňky poučit o kancerogenezi?
14.35–15.05	V. Kořínek: Úloha signální dráhy Wnt při obnově a nádorové transformaci buněk střevního epitelu
15.10–15.40	J. Hatina: Nádorové kmenové buňky urotheliálního karcinomu
15.45–16.15	K. Smetana: Epitelově-mezenchymalní interakce v nádorech a nádorové kmenové buňky
16.20–16.50	J. Mokrý: Biologické vlastnosti kmenových buněk zubní dřeně člověka
16.55–17.25	L. Kafková: Myší embryonální kmenové buňky: regulace buněčného cyklu v podmínkách buněčného stresu
17.30–18.00	**Mimořádné valné shromáždění Čs. biologické společnosti; postery**
18.00–19.00	**Babákova přednáška** J. Motlík: Indukované pluripotentní buňky: od základního porozumění buněčné plasticity k naději pro neurodegenerativní onemocnění a míšní poškození

Společenský večer spojený s prohlídkou pivovaru Plzeňský prazdroj

Čtvrtek, 27. října 2011

9.30–12.30	**Sekce 5 – Přednášky odborných sekcí Čs. biologické společnosti a vybraných přihlášených příspěvků** **Předsedající: R. Janisch**
9.30–10.00	I. Krajčovič: Ukradnuté chloroplasty – kde, kým, ako, prečo?
10.05–10.35	J. Topinka: Nanotoxikologie – nová oblast genetické toxikologie
10.40–12.00	Přednáškové prezentace vybraných původních přihlášených příspěvků
12.00–14.00	**postery**
14.00	Zakončení XX. Biologických dnů

SOUHRNY ÚSTNÍCH SDĚLENÍ

OPTICAL MICROSCOPY IN BIOLOGY – SEMINAR IN HONOUR OF JOSEF REISCHIG

Life cell imaging as ultimate tool to study cell biology

Jan Černý

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Advances in imaging techniques, detection systems and fluorescent probes established a new field in cell biology – life cell imaging. Lack of fixation-based artefacts revolutionized several fields of cell biology, led to rewriting of whole text book chapters. Cell-R imaging system, one of the life cell imaging wide field setups, will be introduced and discussed in the field of vesicular trafficking.

Analysis of 3D image data acquired by confocal microscopy

Lucie Kubínová and Jiří Janáček

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Images of stacks of optical sections acquired by a confocal microscope represent useful data for 3D reconstruction and measurements of biological structures, if suitable image analysis pre-processing and segmentation algorithms as well as methods for estimation of geometrical characteristics are applied. Methods for 3D reconstruction of biological structures using confocal microscopy in combination with image analysis and virtual reality will be presented and demonstrated on capillary bed of placental villi, capillaries in different regions of rat brain, and capillary bed of rat skeletal muscle fibers. Further, stereological and digital methods for measuring geometrical parameters of biological structures, such as components of organs, tissues, cells or cell compartments, will be introduced. The presented methods will be compared from the point of view of their practical applicability, efficiency and precision, with respect to the shapes and arrangements of microscopic structures under study, as well as sources of possible errors due to technical processing of specimens, noise in acquired images, aberrations in microscope system applied, and biased sampling during the relevant biological

experiment. We evaluated the usability, time efficiency and precision of above methods for measurement of specific microscopic structures, such as muscle fibres and capillaries, brain capillaries, or plant cells. Based on our comparison of interactive and automatic methods, we conclude that there is no absolutely universal method which would be optimal for all types of structures. Automatic methods are faster than interactive methods but require automatic segmentation of analyzed objects and they are sensitive to resolution and processing of microscopic image data, e.g. to smoothing which is used for reduction of noise in acquired images. Therefore, they require careful testing and adjusting to the given type of microscopic structure.

This study was supported by the Academy of Sciences of the Czech Republic (grant AV0Z 50110509), by the Ministry of Education, Youth, and Sports of the Czech Republic (grant LC06063, and KONTAKT grant ME09010), and by the Grant Agency of the Czech Republic (grants 304/09/0733 and P501/10/0340).

OBSERVATIONS OF PROTEIN STRUCTURE AND FUNCTION BY TWO-PHOTON POLARIZATION MICROSCOPY

Josef Lazar

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Optical properties of fluorescent labels, including fluorescent proteins, are generally anisotropic: both the rate of light absorption and the apparent rate of light emission depend on spatial orientation of the observed molecules. These anisotropic properties are sometimes, most often in vitro, used for studies of various molecular processes.

Our results show that under a suitable experimental arrangement (two-photon polarization microscopy), anisotropic optical properties of fluorescent proteins can be used for sensitive monitoring of many important molecular processes, directly in living cells. Processes that can be observed by our technique include activation of G-proteins, changes in intracellular calcium concentration, and others. Due to the sensitivity and speed of the method, it should soon allow optical observations of individual electrical pulses (action potentials) in living neurons. An important advantage of our method is that a large number of existing constructs containing fluorescent proteins can now serve as sensitive optical reporters of protein function.

Silencing: Depicting the nature of "Polycomb group proteins bodies" in the chromatin forest

Jana Šmigová, Pavel Jůda, Dušan Cmarko and Ivan Raška

nstitute of Cellular Biology and Pathology, Charles University in Prague, First Faculty of Medicine, Prague, Czech Republic; Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
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Polycomb group (PcG) proteins of the Polycomb repressive complex 1 (PRC1) are found to be diffusely distributed in nuclei of cells from various species. However they can also be localized in intensely fluorescent foci, whether imaged using GFP fusions to proteins of PRC1 complex, or by conventional immunofluorescence microscopy. Such foci are termed PcG bodies, and are believed to be situated in the nuclear intechromatin compartment. However, an ultrastructural description of the PcG body has not been reported to date. To establish the ultrastructure of PcG bodies in human U-2 OS cells stably expressing recombinant polycomb BMI1-GFP protein, we used correlative light-electron microscopy (CLEM) implemented with high-pressure freezing, cryosubstitution and on-section labeling of BMI1 protein with immunogold. This approach allowed us to clearly identify fluorescent PcG bodies, not as distinct nuclear bodies, but as nuclear domains enriched in separated heterochromatin fascicles. Importantly, high-pressure freezing and cryosubstitution allowed for a high and clear-cut imunogold BMI1 labeling of heterochromatin structures throughout the nucleus. The density of immunogold labeled BMI1 in the heterochromatin fascicles corresponding to fluorescent "PcG bodies" did not differ from the density of labeling of heterochromatin fascicles outside of the "PcG bodies". Accordingly, an appearance of the fluorescent "PcG bodies" seems to reflect a local accumulation of the labeled heterochromatin structures in the investigated cells. The results of this study should allow expansion of the knowledge about the biological relevance of the "PcG bodies" in human cells.

Supported by the Czech grants MSM0021620806, LC535 and AV0Z50110509.

Spinning disk confocal microscope as a tool for "super-resolution" light microscopy imaging

Jan Peychl, Thierry Galvez, Yannis Kalaidzidis, Mark Browne, Marino Zerial

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Super resolution light microscopy is becoming increasingly popular in the field of cell biology. There are now several light microscopy methods achieving lateral resolution

below 200 nm: STED – Stimulated Emission Depletion (1), structured illumination (2), PALM – Photo-Activation Localization Microscopy (3), STORM – Stochastic Optical Reconstruction Microscopy (4) and GSD – Ground State Depletion microscopy (5).

The last three methods – PALM, STORM and GSD are based on TIRF – Total Internal Reflection Fluorescence microscopy because this mode of illumination offers the high signal-to-noise ratio required for single molecule registration. Raw data is usually acquired in a TIRF mode and then final "super-resolution" image is calculated. With this approach lateral resolution down to 20 nm can be achieved. Since all three methods are based on TIRF illumination, imaging is usually limited to the objects located within 100 nm of the cover glass or to ultrathin tissue sections. In case that oblique illumination is used then imaging is usually limited to the objects located within 1000 nm of the cover glass.

Our goal was to image confocal sections of high lateral resolution which are deeper than 1000 nm into the sample. Therefore we ported dSTORM (6) protocol to standard spinning disk confocal system equipped with "bleaching unit". In addition, we have developed a software tool that determines the coordinates of single molecules in stacks of images and constructs the corresponding dSTORM image. This allows users smoothly zoom from the original to the "sub-diffraction limit" pixel size. Using the dSTORM method on a spinning disk confocal system we have obtained images of endocytic cargos with a lateral resolution of 80 nm. Imaged structures were deeper of the cover glass than 1000 nm.

We conclude that spinning disk confocal system equipped with "bleaching unit" might considered as a system of choice when super-resolution imaging of structures deeper than 1000 nm of the cover glass is needed.

References:
(1) Hell SW. et al., Opt Lett. 1996.
(2) Gustafsson MG. Proc Matl Acad Sci. USA. 2005.
(3) Betzig E. et al., Science. 2006.
(4) Zhuang X. et al., Nat Methods. 2006.
(5) Fölling J. et al., Nat Methods. 2008.
(6) Heilemann M. et al. Angew Chem Int Ed Engl. 2008.

A MODERN LABORATORY NEEDS MORE THAN A MICROSCOPE

Tomáš Pop

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For more information see http://www.olympus.cz/microscopy/

MOLECULAR BIOLOGY OF CANCER

Molecular genetics of cancer

Jan Svoboda

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The grounds of molecular genetics of cancer were laid at the beginning of the last century by T. Boveri this led to the concept of somatic cell mutation as a cause of cancer process. Independently, cancer virus was discovered by Peyton Rous and this discovery led to the proposal of the virus theory of cancer. Viruses, namely retroviruses, were then used for the proof that oncogenic activity of these agents resulted from the acquisition of modified cellular genes, which we now call oncogenes. More than hundred oncogenes have been defined so far and the quest for their further identification is going on. Functions of these oncogenes as well as functions of their ancestors in the normal genome will be discussed on selected examples. Cell hybridization opened the way to the discovery of tumor suppressor genes, the number of which is increasing. Their functions are being investigated. Results obtained so far indicate clearly that there are important quantitative differences in their expression and interactions, which will be again documented on selected examples. In addition to the genetic causes of cancer, there are also involved epigenetic changes, which exert important influence on the process of tumorigenesis. This process is related to a series of other changes, including vascularization, metastatic potential, metabolism, clonal selection and others, which will be summarized and evaluated.

Progress in cytogenetic cancer diagnostics

Kyra Michalová

Center of Oncocytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, General University Hospital and 1st Medical Faculty Prague, Czech Republic
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Malignant cell transformation can be defined as a series of progressive genetic events which take place in one cell clone in limited number of specific genes. These genes could be oncogenes and/or tumor suppressor genes (antioncogenes, recessive

oncogenes). Each change, whether or not it is associated with the initiation or progression of cancer, can be related to a chromosomal rearrangement. Progress in understanding the cytogenetic and recently molecular basis of neoplastic transformation has strengthened the conception of the cancer as genetic disease. If the aberration is above the limit of the light microscope sensitivity, it should be detected by classical cytogenetic techniques. The cryptic rearrangements which are not discernible can be now easily studied with special fluorescence in situ hybridization (FISH) methods as are multicolor FISH, multicolor banding of chromosomes, comparative genomic hybridization and microarray techniques. The molecular characteristics of chromosomal rearrangements leads to identification of genes with a pivotal role in cancerogenesis. Until now many genes important for the origin of tumors were ascertained in recurrent chromosomal breakpoints and more than two thousands breakpoints have been identified. Since the introduction of molecular methods with higher sensitivity (50 to 100kb) oncocytogenetics has developed at a remarkable pace. We present a short review of molecular cytogenetic methods with a survey of specific recurrent rearrangements as are translocations and deletions of chromosomes in leukemia and solid tumors and give their prognostic value.

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MicroRNAs: biomarkers and potential therapeutic targets in oncology

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MicroRNAs (miRNAs) are small non-coding RNAs 18–25 nucleotides in length that downregulate gene expression during various crucial cell processes such as apoptosis, differentiation and development. Changes in the expression profiles of miRNAs have been observed in a variety of human solid tumors. Functional studies indicate that miRNAs act as tumor suppressors and oncogenes. These findings significantly extend concept of molecular pathogenesis of cancer and have shown great potential for miRNA as a novel class of therapeutic targets. Several investigations have also described the ability of miRNA expression profiles to predict prognosis and response to selected treatments in cancer patients, and support diagnosis of origin among cancer of unknown primary site. miRNAs' occurrence has been repeatedly observed also in serum and plasma, and miRNAs as novel minimally invasive biomarkers have indicated reasonable sensitivity for cancer detection. This lecture covers introduction to miRNA biology, miRNA involvement in the hallmarks of cancer, the knowledge regarding miRNAs functioning in pathogenetic signaling

pathways and their potential to serve as disease biomarkers and novel therapeutic targets in the colorectal cancer, breast cancer, renal cell carcinoma and glioblastoma in order to our recent observations.

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TEACHING ONCOLOGY IN THE MOLECULAR ERA

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According to the current models, we can expect a noticeable increase in the total amount of money spent for oncologic care. On the one hand, demographic models predict the population to become increasingly older, and there is a convincing correlation between tumour incidence and age. On the other hand, the advent of molecularly targeted therapies renders many current treatment options very expensive, and this trend toward expensive tailored therapies is likely to progress in next years. Effectiveness of molecularly targeted therapies depends heavily on genetic and epigenetic changes in each individual tumour, nevertheless. From the point of view of a practitioning oncologist, to apply a proper therapy, a thorough insight into molecular biology of cancer is crucial. Therefore, a deep understanding of molecular tumour biology must become an essential part of standard medical education. In addition, for future generations of clinical oncologists, the ability of interdisciplinary communication in a case-oriented manner is indispensable and should be trained as well. With these aspects in mind, we have launched the new teaching initiative in teaching molecular oncology to medical students. Teaching method combines classical lecturing with practical case-oriented teaching, in which small groups of students discuss particular clinical cancer cases with both molecular biologists and clinical oncologists. The educative project is supported by the grant no. CZ.1.07/2.2.00/07.0313 “Molecular Oncology” co-financed by the European Social Fund and the state budget of the Czech Republic. In addition, we are cooperating with similar teaching programme at the Heinrich-Heine University in Duesseldorf.

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Ovarian carcinoma – clinical and experimental approaches

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For decades the various pathways related to oncogenes or tumor suppressor genes have been studied to better understand the tumor biology of ovarian cancer. Results implicated that ovarian cancer is now subdivided into type1, K-Ras or B-RAF mutated cancers and type2, p53 deficient high grade serous cancers. Obviously, type1 originates from ovarian surface epithelium and develops through cystadenomas and borderline tumors, whereas type2 originates from the epithelium of the distal fimbria of the fallopian tube and shows early intraperitoneal dissemination. Moreover, the elucidation of mechanisms related to platinum resistance, which is very common and the most decisive factor for clinical outcome, was focused with priority. In this context we found a non-canonical EGF-Receptor signaling, which via IRF-1/IRF-2 interplay leads to an E2F3a-dependent cell-cycle activation. This short-track pathway appears to be activated particularly in platinum-resistant cancer cells.

Over the last years, the most prominent clinical approaches in ovarian cancer was the implementation of intraperitoneal (IP) chemotherapy and the introduction of targeted mostly anti-angiogenetic therapies in treatment strategies. The targeting of neo-angiogenesis by humanized anti-VEGF antibodies as maintenance therapy, proved to be by far more active in ovarian cancer than in other tumor entities. This was demonstrated by two independent large randomized phase III clinical trials. However, the therapeutic effect was abrogated shortly after treatment termination. These data tempt to speculate that this treatment represent an indirect tumor stem cell therapy in so far that the interaction between the so called vascular niche and the here living precursor- or stem cells is negatively affected. Moreover, the high activity of this strategy especially in ovarian cancer can be explained be its characteristic semi-solid dissemination in form of very small nodules throughout the peritoneal cavity, where in each of these hundreds of spheroid-like clusters neo-angiogenesis plays a crucial role for progression. We hypothesized that in each of these spheroid-like clusters initially one or more tumorigenic precursor/stem cells are the origin of the later metastasis. Considering this ovarian cancer may represent an ideal human in vivo model to study tumor dissemination and stemness. For corroboration, we were able to separate a side population (SP) representing 3 % of all the cells in the green-fluorescence-protein transfected ovarian cancer cell line 2780-V. When SP cells were co-cultured on single layered healthy mesothelium, they formed spheroids, whereas co-cultured non-SP fractions formed small islands which grow as a second layer over the mesothelium monolayer.

STEM CELLS – FROM REGENERATIVE MEDICINE TO CANCER BIOLOGY

CANCER STEM CELLS IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the best understood tumor entities. The driving force for the neoplastic transformation is the accumulation of subsequent mutations in oncogenes (OG, gain of function) and tumor suppressor genes (TSG, loss of function) what is known as the multistep carcinogenesis model. In most if not all CRC the Wnt-signalling pathway is affected either by mutations in the TSG APC (adenomatous polyposis coli) or the OG β-catenin which both lead to the stabilization and accumulation of β-catenin. This protein has an ambivalent function depending on its subcellular localization. In the membrane it is an integral part of the zonula adhaerens (ZA) linking E-cadherin via α-catenin with the β-actin cytoskeleton. In this context it maintains the epithelial phenotype of cells. Eventually, β-catenin translocates into the cell's nucleus either by regular activation of the canonical Wnt-signaling pathway or by the described mutations in APC, β-catenin or more seldom other components of this pathway. In the nucleus β-catenin is an integral part of a transcriptionally active multiprotein complex. The many genes which are activated which confer the hallmarks of cancer like invasion, migration, epithelio-mesenchymal transition (EMT) which is linked to cancer stemness and chemoresistance beside others. Thus, β-catenin induces cancer stem cells (CSC) which is therefore a reversible or regulatable characteristic and helps to explain the histologic heterogeneity found in CRC. In CRC tumor cells with nuclear β-catenin are found especially at the invasive front although all tumor cells harbor mutations that lead to the stabilization of β-catenin what is known as the β-catenin paradox. It seems that factors from the environment, like the HGF (hepatocyte growth factor) are responsible for driving the nuclear translocation of β-catenin. This minority of tumorcells resembles migrating CSC and their amount as well as grade of EMT (budding) significantly correlates with low survival of patients indicating a clinicopathological role of these cells. Additionally, several β-catenin target genes are markers of stemness or CSC like CD44, CD133, CD166, Lgr-5 and others. Though important as markers of CSC their functional contribution to stemness is lacking or only of a minor role. Thus, a mechanistic link between stemness and β-catenin has not been established yet.

Current perspectives on normal and malignant stem cells in the liver and intestinal tract

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The liver and gastrointestinal tract are embryologically related and their stem cells are critically involved in tissue homeostasis and all aspects of cancer development. In the human liver we have suggested that a stem cell niche exists in the periportal area (1) supporting the 'streaming liver' hypothesis. Moreover, cell lineage tracing in mice has suggested that the hepatocyte population in normal liver is replaced once a year from interlobular Sox-9-expressing bile duct cells (2). Sox-9 expression also occurs in other supposed stem cell locations, notably at the base of intestinal crypts. A reserve (potential) liver stem cell population exists in the canals of Hering that gives rise to bipotential liver progenitor cells (LPCs) that are activated and abundant in the setting of chronic liver injury (3).

In the small intestine in particular, a two-tier stem cell system seems to operate. There appear to be slow cycling (so-called label retaining cells, LRCs) stem cells at about 4–5 cell positions from the crypt base; these cells express the likes of Bmi1 and are activated under extreme stress, hence 'potential stem cells'. On the other hand work from the Hans Clevers laboratory over the last 4 years has identified functional stem cells, so-called 'crypt base columnar cells' that are fast cycling, express the Wnt target gene *lgr5*, are multipotential and located amongst the Paneth cells. *In vitro*, single Lgr5-positive cells can create new crypt-like structures in the absence of a stem cell niche.

Experimental studies in mice and observational human studies suggest that most tumours in the gut arise from the stem cell zone ('bottom-up' hypothesis), but in the liver, hepatocellular carcinoma (HCC) can arise from any stage of lineage progression from bile duct cell to fully differentiated hepatocyte. The biliary or bipotential progenitor cell origin of some HCCs can explain the co-existence of hepatocytic and cholangiocytic differentiation in the same tumour.

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Analyses of potential roles of cancer associated fibroblasts in regulation of cancer stem cell niches

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Tumor growth and progression is not just determined by properties of the malignant cancer cells themselves, but also by the tumor stroma. Cancer associated fibroblasts (CAFs) are a major cell type within the tumor stroma. The phenotype of CAFs resembles those of "activated fibroblasts" during wound healing. Recent studies have increased our understanding of the roles of CAFs in tumor growth and confirmed their involvement in control of e.g. metastasis, angiogenesis, drug-sensitivity.

CSCs are defined as cells able to self-renew and to differentiate into heterogeneous progeny that make up the bulk of malignant tumor cells. CSCs are highly resistant to chemotherapy and radiation, and are assumed to be responsible for tumor relapse and metastasis. Targeting of CSCs has therefore been proposed as a novel principle for cancer treatment. CSCs, like normal stem cells, are dependent on a certain niche-environment. In general, a stem cell niche is formed by signalling stroma cells, extracellular matrix and the stem cells. A maintained balance of stem cell quiescence and activity is a hallmark of a functional niche. Following up on already published studies (Vermeulen et al., Nat Cell Biol, 2010) our work explores the potential roles of CAFs the CSC niche.

We have established a set of in vitro co-culture models to investigate the impact of different CAFs on the "stemness" of corresponding tumor cells. Studies are being performed with combinations of primary cultures of CAFs, inflammatory fibroblasts, or growth-factor stimulated fibroblasts, together with pancreatic and breast cancer cells. Endpoints for "stemcellness" include primary and secondary sphere formation, expression of stem cell markers and chemo-resistance. Tumor forming capacity is being established as an additional end-point.

Preliminary results from these studies will be presented, which include evidence that CAFs and other fibroblasts, can enhance the ability of cancer cells to form primary and especially secondary spheres. Spheres formed in the presence of CAF/fibroblast conditioned media showed a greater expression of stemness marker (e.g. embryonic stem cell marker and drug resistance transporters) and an activation of stemness regulating pathways like the Notch or hedgehog pathway. Data on drug sensitivity will also be discussed.

Multipotent adult progenitor cell therapy in transplant medicine

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Multipotent adult progenitor cells (MAPCs) are a unique population of bone-marrow-derived, non-hematopoietic, adherent stem cells with a fascinating range of regenerative abilities. MAPCs and mesenchymal stem cells (MSCs) share a multitude of immunological features holding great immunotherapeutic promise. However, unlike MSCs, MAPCs are more pluripotent, can be cultured indefinitely, and have been shown to function across allogeneic barriers, making them ideal candidates for clinical use. In this work, we demonstrate that third-party MAPCs mediate long-term acceptance of allogeneic, vascularized heart grafts when administered concurrently with low-dose calcineurin-inhibitor (CNI)-free immunosuppression in rats. This allograft acceptance could be transferred via organ upon re-transplantation, or via recipient immune environment following splenocyte infusion, into naēve recipients. We propose a model for MAPC-mediated graft acceptance and transfer of such immune privilege to naïve recipients without additional pharmacotherapy. Our findings substantially add to previous studies in rodent transplant models that have either used (donor-type) MSCs or hematopoietic cell preparations, both of which would pose major obstacles for routine clinical use. Based on these findings, we are currently initiating a phase I clinical trial applying third-party MAPCs to liver allograft recipients.

Inhibition of intracerebral rat glioblastoma C6 by human adipose tissue.derived mesenchymal stem cells expressing yeast cytosinedeaminase::uracil phosphoribosyltransferase

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Prodrug cancer gene therapy driven by mesenchymal stem cells (MSCs) represents an attractive tool to activate the prodrug directly within the tumor mass, thus avoiding systemic toxicity. MSCs lack major histocompatibility complex MHC-II and show only minimal MHC-I expression, therefore, their therapeutic potential can be tested in animal models. We tested the feasibility and efficacy of human adipose tissue-derived mesenchymal stem cells, engineered to express the suicide gene cytosine

deaminase::uracil phosphoribosyltransferase to treat intracranial rat C6 glioblastoma. Experiments were designed to simulate conditions of future clinical application for high-grade glioblastoma therapy, by direct injections of therapeutic stem cells into tumor and by continuous intracerebro-ventricular delivery of 5-fluorocytosine (5-FC) using osmotic pump. Continuous prodrug administration reduced its dose required for the same therapeutic effect and along with repeated administration of therapeutic stem cells increased the survival time. We demonstrated that CDy-AT-MSCs/5-FC system in a therapeutic stem cell dose-dependent manner induced complete tumor regression in a significant number of animals. Results support the arguments to begin clinical studies for treatment of high-grade brain tumors.

Stem cells in regenerative medicine: Preclinical and clinical studies

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Mesenchymal stem cells (MSCs), olfactory ensheathing glia (OEGs) and human neural progenitor cells (PNCs) have the capacity to migrate towards lesions and induce regeneration. In addition to the promising results that we obtained with bone marrow cells in ischemic (diabetic) leg and cartilage repair, degenerative diseases of the brain are also our target. Various cell types labeled with iron-oxide nanoparticles were transplanted into rats with a cortical lesion, a middle cerebral artery occlusion model of stroke, a model of amyotrophic lateral sclerosis (ALS) or a spinal cord compression lesion (SCI). In vivo MRI, used to track their migration and fate, showed that MSCs, OEGs as well as PNCs migrated into the lesion after either intravenous or local administration. PNCs also differentiated into motoneurons and astrocytes. In brain and spinal cord injury, all implanted animals had significantly smaller lesions and better behavioral scores. We found a prolongation of life span in animals with ALS. In a model of chronic SCI, HPMA-RGD hydrogels or nanofiber scaffolds were implanted into the lesion, either with or without seeded MSCs. The animals were tested behaviorally using the BBB and plantar tests for 6 months; we found a significant improvement in rats with SCI after this combined treatment (scaffold and MSCs), compared to the control groups. Combined therapy prevented tissue atrophy, and the scaffolds were infiltrated with myelinated axons, blood vessels and astrocytes. We conclude that scaffolds seeded with MSCs can provide a “bridge” for tissue regeneration in stroke or chronic SCI. Using autologous bone marrow cells, promising results has been found in our clinical studies in patients with diabetic leg and spinal cord injury.

Can we learn about cancerogenesis from embryonic stem cells?

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Pluripotent human embryonic stem cells (hESC), early upon their derivation from normal human blastocyst, are considered as genetically undamaged. In consonance with this fact, when implanted to immunodeficient host, hESCs give rise to teratomas containing differentiated cells and not malignant tumors. This may change in hESCs propagated in culture for longer periods of time due to the accumulation of a large spectrum of genetic and epigenetic alterations.

Currently, the concept of cancerogenesis is being proposed and evaluated, in which cells with stem cell properties, tumor-initiating cells (TICs), play a key role. The origin of TICs as well as molecular mechanisms underlying their properties are largely ununderstood. One plausible hypothesis is that acquiring stem-like phenotype, not dissimilar to that of hESCs, is the first step in TIC development that makes these cells more prone to further abnormalization.

Normal somatic cells developed a series of mechanisms to prevent accumulation of changes to their genetic complement with the cell cycle checkpoints being of premier importance. Our recent data document that amplifications of chromosomes typically observed in cultured hESCs are at least in part due to the unchecked misbehavior of centrosomes. In contrast, we have also shown that pathways for sensing and reacting to DNA damage caused by UVC light are fully operational in normal hESCs. Still, these pathways are centered around CDC25A and do not involve p53-driven up-regulation of p21 inhibitor of CDKs, which is typical for somatic cells and may represent a slower but more robust mechanism to stop cycling. Here we have further studied this phenomenon and found that in UVC-irradiated hESCs there is 85 microRNAs that undergo up-regulation about 2 fold and higher. Among them there are also microRNAs from family miR-302 that share a seed sequence with p21-regulating microRNAs. Importantly, p21 protein accumulates in hESCs with artificially down-regulated microRNA-processing protein Dicer1 but introduction of miR-302 into such cells reestablishes their low-p21 phenotype. This suggest that undifferentiated hESCs use microRNAs to prevent canonical G1/S checkpoint pathway from being activated. Whether this regulation is operative in only hESCs or also in other types of stem cells including stem cells of cancers should be investigated.

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Wnt signaling in adult stem cells – an insight from the gut

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The epithelia of the gastrointestinal tract represent one of the most rapidly self-renewing tissues in the adult mammalian body. The tract lining is concurrently exposed to chemical stress and mechanical tensions. Consequently, the combination of high proliferation rate and environmental stress results in the accumulation of somatic mutations hitting tumor suppressor genes or proto-oncogenes. More than 50 % of all colorectal tumors harbor inactivating mutations in the APC gene. The majority of these mutations generate premature translation stop signals with resulting production of functionally compromised truncated versions of the APC protein. Intriguingly, some recent discoveries clarified the link between the Wnt signaling pathway, the cellular function of APC and uncontrolled cell growth initiating the path to cancer.

In the healthy intestine the Wnt signaling pathway is active in a small population of the intestinal stem cells. This population is located in the bottom part of the microscopic invagination of the epithelium called the crypts. The stem cell in the crypt divides asymmetrically, generating one stem and one transit amplifying (TA) cells. The TA cells, while migrating from the crypt, differentiate and produce the functional lineages of the epithelium. The differentiated cells are short-lived and after 3 to 5 days undergo apoptosis and are shed into the lumen of the intestine. One exception to this scheme is represented by the Paneth cells. These differentiated post-mitotic cells, mainly found in the small intestine, escape the crypt-villus flow by homing towards the crypt bottom, where they live for several weeks. The loss of the APC gene results in non-physiological activation of the Wnt pathway in the cells outside of the crypt. Therefore, the cells harboring mutant APC preserve typical features of stem cells, i.e. proliferation capacity and “immortality”. Consequently, these cells constantly proliferate and generate polyps. The majority of sporadic human colorectal cancers are initiated by this molecular mechanism.

The talk will be focused on recent discoveries involving the identification of specific markers of the intestinal stem cells and methods how these cells (and their progeny) can be labeled and traced in living experimental animals. The contribution of Wnt signaling to normal stem cell physiology and to tumor initiation and progression will also be presented.

Attempts at initial characterization of urothelial carcinoma stem cells

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There is an accumulating evidence that tumours are organized in a hierarchical fashion, with putative cancer stem cells (CSC) at the top of the differentiation hierarchy. The cancer stem cells are believed to be largely responsible for tumor initiation, metastatic dissemination, relapse and therapy resistance. This hierarchical organization seems to be preserved in some tumour cells lines as well, including cell lines derived from bladder cancer. To characterize these cells in more detail, we have developed several functional in vitro approaches. In a first set of experiments, BFTC905-derived doxorubicin resistant bladder cancer cell line variant with properties suggesting an enrichment of CSC fraction has been stably transfected with a GFP-expression construct under the control of a doxorubicin responsive promoter. This allowed the visualization and tracing of putative stem cells by virtue of their GFP gene expression. Stem cells feature a constitutive expression of multiple efflux pumps of the ABC family, which underlies their ability to eliminate small hydrophobic molecules like drugs and fluorescent dyes. Due to this ability to expel doxorubicin, these cells are unable to activate the doxorubicin responsive promoter and consequently mount GFP expression after doxorubicin treatment. After doxorubicin treatment, we indeed observed a striking heterogeneity in GFP induction; some individual cells or small cell clusters failed to activate it at all (putative stem cells), whereas others activated it only slowly (putative precursor cells preserving certain level of efflux pump expression) or quickly (differentiated cells). Importantly, the GFPlow/- cells were uniquely clonogenic and able to differentiate into cells with full doxorubicin responsiveness. In another set of experiments, we analyzed expression of stem cell enriched cytokeratin-17 in bladder cancer cell lines BC44 and SW780 in pure culture or in coculture with a carcinoma fibroblast cell line. We observed a dramatic enrichment of CK-17 expressing cells in both bladder cancer cell lines in the coculture setting. Moreover, the putative stem cells localized to contact zone with cocultured fibroblasts. This suggests that stromal tumour cells provide a sort of stem cell niche to directly support cancer stem cells.

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Epithelial-mesenchymal interaction in cancer and cancer stem cell

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Adult tissue stem cells represent reserve for tissue self-renewal / regeneration. Their existence elicits outlook for the development of new technologies for regenerative medicine, when some of them were moved from laboratory to clinical practice. However, it is highly probable that similarly to normal tissues, the stem cells are also present in tumors. Their presence seems to be connected with so important clinical complications of cancer such as the multiple drug resistance and/or the minimal residual disease. Unfortunately, the therapeutic targeting of cancer stem cells is complicated and practically not available at clinical scale. The high sensitivity to changes of microenvironment (niche) represents the typical feature of normal stem cells that can. Many studies demonstrated that biological properties of the tumor including metastasation and local aggressiveness are also strongly related to the tumor microenvironment. This compartment is formed by fibroblasts (including their product-extracellular matrix), inflammatory cells and vessels. So called cancer-associated fibroblasts producing panel of growth factors / cytokines / chemokines influence biological properties of cancer cells and they are in the center of interest of many laboratories. These stromal fibroblasts frequently express the smooth muscle actin (but it is not obligatory). This is important that these cells are also active after their separation from cancer cells where they are able to influence the phenotype of normal epithelial cells to be phenotypically similar to cancer epithelium. Unfortunately, their origin has not been fully understood. It seems to be that they are originated from normal local fibroblasts by the paracrine effect of cancer cells. However, they can be also originated by epithelial-mesenchymal transition from cancer cells as well as from endothelial cells, pericytes and macrophages. Participation of mesenchymal stem cell emigrated to cancer site is also probable. Based on these data, the therapeutic manipulation of tumor stroma can represents a new horizon of anticancer therapy. Fructification of these data about epithelial-mesenchymal interaction can be also employed in the improvement of wound healing.

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Biological properties of human dental pupl stem cells

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The dental pulp like other tissues contains tissue-specific stem cells. To expand an initially small number of dental pulp stem cells in vitro the cells grown in vitro were regularly passaged. The cell viability and karyotype were checked regularly. Telomere dynamics was examined with quantitative PCR and results were verified by measurement of terminal restriction fragment length. Dental pulp stem cells expressed mesenchymal markers CD29, CD44, CD73, CD90, CD166, vimentin and STRO-1 at high levels while hemopoietic markers were negative. The cells expressed stem cell markers nanog, SOX-2, nestin, musashi-1, nucleostemin, VEGFR2 and CXCR4. Dental pulp stem cells are multipotent as shown by their osteogenic and chondrogenic potential. Although these cells have a large proliferative capacity they show a decline in their doubling time with prolonged cultivation. All dental pulp stem cell lines were able to grow beyond Hayflick's limit. Measurement of telomere length revealed telomere attrition when samples of the same patients from different passages were compared. Comparison of telomere length between dental pulp stem cells and lymphocytes from the same patient showed that the latter had shorter telomeres than adult tissue-specific stem cells. Our results document that ex vivo expansion contributes to telomere shortening which explains a retarded growth of dental pulp stem cells at later passages. Although telomere loss in tissue-specific stem cells contributes to a tissue ageing, telomere length in stem cells still remained longer when compared with other somatic cells which likely contributes to extended lifespan of these unique cells.

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Mouse embryonic stem cells: regulation of cell cycle under stress conditions

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Mouse embryonic stem cells (mESCs) are cells with high proliferation rate and rapid cell cycle progression. In spite of the lack of G1 checkpoint, mESCs are able to effectively maintain pristine genetic information. In the long term, our team has been focused on description of G1 phase progression of mESCs. Our previous studies explained very short G1 phase and absence of G1 arrest after DNA damage in these cells by high and precocious activity of Cdk2 kinase. In particular, we characterized the response of mESCs to the DNA double strand breaks evoked by ionizing radiation (IR).

Nowadays we analyze cell cycle progression under stress conditions evoked by iron depletion. Iron is essential for cellular metabolism, and regulates activity of ribonucleotide reductase (RR), which supplies cells with deoxyribonucleotides. Iron depletion typically results in a G1/S arrest, rarely in G2/M arrest, and consequently in apoptosis in somatic cells. In our study we are focused on description of checkpoint response, and clarification of the role of RR in mESCs. Our preliminary data question the importance of RR under iron depletion, and accent the role of checkpoint kinases. We show that iron depletion does not result in cell cycle arrest, on the contrary it accelerates G1/S transition due to increase of Cdk2 activity in mESCs. We found decrease of ATR kinase activity in iron depleted cells, while ATM kinase activity remained unchanged. It is known that iron enables formation of reactive oxygen species (ROS), which mediate cellular damage sensed by ATR kinase. We propose that reduction of ROS leads to decrease of ATR activity in iron-deprived mESCs. In somatic cells, Cdk2 is upregulated by Cdc25A phosphatase, which is Chk1, Chk2, ATM and ATR dependent. In mESCs the link between ATR and Cdk2 remains obscure. In mESCs we could not confirm the link between Cdc25A phosphatase and Cdk2 kinase. We assume that other factors than those identified in somatic cells, including aberrant localization of cell cycle regulatory proteins, and possible involvement additional proteins, could play important roles in the regulation of cell cycle progression in mESCs under stress conditions.

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BABÁK'S LECTURE

INDUCED PLURIPOTENT STEM (iPS) CELLS: FROM BASIC UDERSTANDING OF CELLULAR PLASTICITY TO A HOPE FOR NEURODEGENERATIVE DISEASES AND SPINAL CORD INJURY

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Somatic cells reprogramming, originally induced by transcription factors c-Myc, Klf4, Oct4 and Sox2 included in lentiviral vectors, developed during few years in a broad palette of methods increasing efficiency and safety of iPS cells technology. A major barrier to research on neurodegenerative diseases was inaccessibility of diseased tissue for study. A potential solution is to utilize reprogramming technology and to derive iPS cells from patients and differentiate them into neurons affected by a specific disease. This model represents a new experimental system to identify compounds that reduce levels of α-synuclein or mutated huntingtin, and to investigate the molecular mechanism of neurodegeneration caused by mutated protein dysfunction. Miniature pigs are steadily gaining importance as large animal models in the field of regenerative medicine and in stem cell research. Several somatic stem cell populations, including mesenchymal stem cells or neural stem cells were successfully isolated in pigs, but establishment of proper embryonic stem (ES) cell line, fulfilling all classical ES cells landmarks, including germ-line transmission remains so far elusive. Discovery of induced pluripotency makes preparation of pig induced iPS cells an attractive alternative to ES cells. First, we have taken advantage of our experience with the isolation and culture of fetal porcine neural progenitors (NPC) and used them as a starting population for preparation of minipig iPS cells. The several porcine NPC lines were transduced with either the combination of all four "Yamanaca factors" (Oct4, Sox2, Klf4 and c-Myc), 3 factor combination without Sox2 or with Oct4 only. Second, we were able to derive cell clones displaying morphology similar to human ES cells, but proliferating on feeders in LIF containing medium, similar to mouse ES cells. The B8 clone transduced with 3 factor displayed alkaline phosphatase positivity, expression of Nanog, endogenous Sox2 and Oct4. B8 minipig iPS cells formed embryoid bodies when placed in a suspension culture, and further differentiated when placed onto gelatin coated dishes. Third, we have recently isolated NPC lines from F2 generation of transgenic minipig for N-terminal part of the human mutated huntingtin as a unique model for preparation of disease specific iPS cells.

LECTURES OF SPECIALIZED SECTIONS OF THE CZECHOSLOVAC BIOLOGICAL SOCIETY

NANOTOXICOLOGY – NEW AREA IN GENETIC TOXICOLOGY

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Increasing importance of nanotechnologies leads to increased human exposure to nanoparticles by various routes: inhalation, ingestion or injection. New area of toxicology – nanotoxicology – was established to study adverse effects of nanoparticles. Current knowledge of possible toxic effects of nanoparticles is very limited. Some studies demonstrate specific risk connected with the exposure to nanomaterials. According to some experts, it is impossible to predict adverse effects of nanoparticles from the known effects of larger particles. Basic difference is related to particle size: 1) Higher specific surface of nanoparticles per unit of mass; 2) The ability of nanoparticle to penetrate biological membranes. The size of particles and their surface are from the point of view of toxicology highly important parameters, since they determine number of reactive sites on the surface of particles. These reactive sites might be responsible for generation of reactive oxygen species. Nanomaterials may penetrate the nuclear membrane physically. Once in contact with DNA, the chemical and physical characteristics of the nanomaterial determine, whether it can induce DNA damage, gene mutations, or chromosome alterations. Nanomaterials may also affect the mitotic or meiotic apparatus, producing numerical chromosome abnormalities. In addition, genotoxic effects may be produced indirectly. There are indications that some nanoparticles can provoke inflammation and generation of reactive oxygen species (ROS) and lipid peroxidation. Various ROS and lipid peroxidation products are known to be genotoxic, but in general the possible association between inflammatory and genotoxic effects of nanomaterials is insufficiently understood. In case the elimination of nanoscale particles is ineffective, nanomaterial will accumulate in cells, which may contribute to genotoxic effects in target tissues. It is unclear whether or not nanomaterials can have systemic genotoxic effects. Besides systemic effects, the present OECD in vivo genotoxicity tests only cover the bone marrow and the liver as target organs. This may not be adequate, if effects are primarily expected in the route of entry such as the lungs, gastrointestinal tract, and skin.

Stolen plastids – perspectives on plastid acquisitions

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The spread of plastids/chloroplasts appears to have had the most significant impact on the diversification of major eukaryotic lineages. Chloroplasts were once free-living cyanobacteria that underwent an evolutionary transformation into metabolic compartments of oxygenic photosynthesis within eukaryotic cells. A number of eukaryotic lineages have acquired photosynthesis directly from cyanobacteria (that is primary endosymbiosis – the Archaeplastida – green, red, and glaucophyte algae) or indirectly via secondary or even tertiary endosymbiotic events involving eukaryotes in the role of both host and endosymbiont (e.g., chromalveolates, euglenoids, chlorarachniophytes). Most hypothetical examples of this process evoke a predator-prey relationship such as a phagotrophic eukaryote continually feeding on algae. Most plastids are long-established organelles, resulting from ancient events and are drastically different from their free-living ancestors, having lost or transferred most genes to the host nucleus. The discovery of several organisms that have undergone more recent endosymbioses may provide insights into the first crucial steps of this process. Plastid retention from prey, also known as kleptoplastidy, is an example of a specific relationship between two organisms that could represent an early stage of plastid acquisition. Plastid retention is a form of mixotrophy whereby a feeding cell temporarily sequesters the plastids of prey in order to benefit from the photosynthesis occurring in the stolen organelle. These transient plastids, called kleptoplasts, are found in many eukaryotic lineages including dinoflagellates, ciliates, other unicellular eukaryotes, and even several invertebrate animals such as sea slugs (e.g. genus Elysia), sponges, reef corals, and clams. However, despite the ability of pathogenic organisms to enter vertebrate cells, there is an apparent absence of mutualist endosymbionts in vertebrates. The inability of symbionts to enter vertebrate host cells may be attributable to the adaptive immune system that recognizes and destroys foreign cells. Associations between microbes (green algae Oophila amblystomatis) and vertebrate embryos (the spotted salamander Ambystoma maculatum) are possible candidates for intracellular symbioses because such early associations may precede an adaptive immune response. The invasion of algae into salamander host tissues and cells represents a unique association between a vertebrate and a eukaryotic alga, with implications for research into cell-cell recognition. The enormous array of biological and biochemical characteristics presented by algae offers great opportunities for exploitation across a wide range of technologies, e.g. in the production of biodiesel, industrial chemicals, and even nanotechnologies.

Operační program Vzdělávání pro konkurenceschopnost (OP VK) je víceletým tematickým programem v gesci Ministerstva školství, mládeže a tělovýchovy ČR (MŠMT), v jehož rámci je možné v programovacím období 2007-2013 čerpat finanční prostředky z Evropského sociálního fondu (ESF), jednoho ze strukturálních fondů Evropské unie (EU). OP VK se zaměřuje na oblast rozvoje lidských zdrojů prostřednictvím vzdělávání ve všech jeho rozmanitých formách s důrazem na komplexní systém celoživotního učení, utváření vhodného prostředí pro výzkumné, vývojové a inovační aktivity a stimulace spolupráce participujících subjektů. Informace o OP VK je možné nalézt na webových stránkách MŠMT www.msmt.cz.

SOUHRNY
PLAKÁTOVÝCH SDĚLENÍ

① Microorganisms studied under polarized light microscope

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Several groups of microorganisms were studied under polarized light microscope to find any anisotropic structures in their cells. The biological material was collected in the ponds of the villages Sýkořice and Zbečno located in the Křivoklátsko Natural Reserve (Czech Republic). Prokaryotes (Cyanobacteria) and eukaryotes (algae and Protozoa) were observed under research light polarized microscope LOMO Sankt Petersburg Company eqipped with polarizer, analyzer, compenzator plates, rotating stage and photomicrography device Carl Zeiss Jena Company with digital SLR camera Nikon D 70. In Cyanobacteria, e.g. genus *Oscillatoria* were not found any anisotropic structures. Some filamentous green algae, e.g. genus *Mougeotia* had strong anisotropic cell walls, the other, e.g. in the genus *Gloetila* only several anisotropic granules in cytoplasm were observed. Colonial algae, e.g. *Scenedesmus* sp., *Pediastrum* sp. etc. contained large amount of anisotropic granules in their individual cells. Also several species of diatomes from the genus *Navicula* had two agregates of minute anisotropic granules next to the nucleus. Flagellates, e.g. *Euglena* sp. contained two large anisotropic bodies close to the nucleus. On the other hand, amoebae, e.g. genus *Amoeba* had only several small anisotropic granules in the cytoplasm. In conclusion we can tell, that the presence of these anisotropic granules in the cells of microorganisms shows on the great importance of those storage materials for the metabolism of studied organisms.

② CCHM – a novel technique of digital holographic microscopy for in vitro study of cell motility reactions

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Coherence Controlled Holographic Microscopy **(CCHM)** is a novel technique of wide-field light microscopy. The difference from classic Digital Holographic Micro-

scopy **(DHM)** lies in a possibility to illuminate with arbitrarily low coherence of light. This means that holographic observation is possible without laser light using an illumination system of standard wide-field microscopes. Owing to the ordinary illumination source the **CCHM** images are of low noise, deprived of coherence noise (speckles) and the lateral resolution is improved by a factor of 2 compared to classic **DHM**. These achievements further improved **Quantitative Phase Contrast (QPC). QPC** from cell biology point of view represents the main methodical contribution of **DHM.** It ascribes numerical value of object beam phase shift in nm compared to reference beam for every pixel. For phase shift is proportional to equivalent of dry mass the **QPC** allows detection of both cell translocation as well as intracellular motion. Based on **QPC** we elaborated a method of **Dynamic Phase Differences (DPD).** Typical results of **QPC** and **DPD** will be shown on **videos** of raw and processed data together with static images in only 2D *(x, y)* format that thus enable **printing/publishing** the dynamic video 3D information (*x, y* images in time).

The work is supported by the Ministry of Education of CR (grant MSM0021630508).

③ Selected digital techniques applied to biological research

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Various recent types of digital instruments and techniques is possible to use in biology. Some of them were tested under laboratory and/or field conditions. They involve following selected techniques and equipments: **web camcorders and mini-camcorders-** small or miniature devices for catching of movies (dynamic processes); **infrared digital photography-** additional function of some digital cameras and camcorders with ability to shot still photographs or movies in complete dark conditions; **laser digital photography-** potentially useful technique for study of some internal parts of dense samples; **high speed digital photography-** ability of some digital cameras to make movies at frequency till 1200 frames per second and present it in slow mode; **simple negative and slide scanner-** simple devices for scanning of flat and transparent samples; **accessories for digital photography-** group of devices and even small adaptations often substantially increase power of basic devices (cameras and camcorders); **digital microscopes-** recently well growing area of biological devices for daily use; **digital endoscopes, fiberscopes and boroscopes-** several types of devices for documentation of life under water, in cavities in soil or trees or under stones; **high dynamic range photography and high dynamic range-art photography-** photographic technique based on ability of particular cameras to manage high dynamic range of complicated photographic situations; **mobile device**

photography and movies making- cameras in mobil phones, in notebooks, netbooks and tablet etc.

Most of the above mentioned devices and techniques are usable in various biological projects including microscopy. More details will be given at the presentation.

④ "Click & Seed" System – biomimetic modification of 2D and 3D surfaces to control the cell behaviour

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The surface modification of various materials is an important technique for tissue engineering and regenerative medicine. Cell migration, proliferation and differentiation is controlled via signalling pathways of various cell receptors (e.g. integrins, cadherins), hence the key role is played by specific interactions of ligands and cell receptors. However the additional adsorption of proteins from cultivation medium in vitro or tissue fluid in vivo can non-specifically influence cell behaviour.

Recent methods do not provide the immobilization of ligands in precise concentration and the suppression of non-specific protein adsorption, therefore we designed a simple, versatile method for the surface modification with peptides and protein fragments applicable to any type of material.

Murine fibroblasts and embryonal carcinoma cells were seeded on protein-repulsive substrates with several peptide concentrations, which were precisely evaluated by direct measurement of activity of radiolabeled ligands. This work shows the influence of the adhesion site of fibronectin, RGDS, and its scrambled analogue RDGS on adherent cells. Specific and non-specific interactions were observed on cells with diverse expression profile of integrins. RGDS supports cell adhesion and spreading on protein-repulsive layer of murine fibroblasts, but it has selective properties for heterogeneous population of human embryonal carcinoma cells. Results of experiments demonstrate functional dependence of cell adhesion, cytoskeletal organization and proliferation on nature of ligands, its concentration and types of cells.

Unique “Click&Seed” system enables the spatial control and patterning of cells on planar surfaces and 3D biomaterials which is essential for tissue engineering of scaffolds and studies of cell-material interactions.

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⑤ THE NUCLEAR PORE COMPLEXES CHANGED AFTER ULTRASOUND EXPOSURE

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Changes in the size of diameter of nuclear pore complexes fractured along a protoplasmic and an exoplasmic face of the inner and outer membrane of a nuclear envelope (freeze-etching method) in HL-60 cells after ultrasound exposure were studied. A cell suspension (10^7 per ml, in cultivation medium) exposed at 1 MHz frequency and 1 W/cm^2 intensity in a continuous mode for 10 min was placed in a 37 °C water bath; samples were collected at four different intervals (0 min, 10 min, 20 min, 30 min) and fixed in 2 % paraformaldehyde + 2.5 % glutaraldehyde in 0.1 M cacodylate buffer. The assessment showed significant differences in the size of diameter of nuclear pore complexes on the protoplasmic face between ultrasound-exposed and unexposed cells ($P_0 = 0.001$, $P_{10} = 0.000$, $P_{20} = 0.000$). On the exoplasmic face there was a significant difference between ultrasound-exposed and unexposed cells only in the sample fixed immediately after sonication ($P_0 = 0.004$). In the other samples the differences were not statistically significant.

The study shows that therapeutic ultrasound has an effect on cellular structures formed from subunits and separate proteins whose bonds can be disintegrated by mechanical waves. The visual manifestation of it is an increase in the size of diameter of nuclear pore complexes on fractured membrane faces.

⑥ Light and fluorescent microscopy as principal method of cytogenetic diagnostics

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In spite of technical progress the optical microscopy remains a principal research as well as routine diagnostic method in lots of disciplines of biological and medical science including clinical cytogenetics.

Cytogenetics is a borderline discipline of genetics dealing with the study of chromosomes; chromosomal pathology and its influence on patientęs phenotype is studied by clinical cytogenetics. The development of cytogenetics was influenced by progress of microscopic technique through a lot of important cytologic findings. Clinical cytogenetics is one of the branches of clinical medicine, which utilize directly the findings obtained by optical microscopy for establishing diagnose and choice of appropriate medical care for both postnatal and prenatal cases.

We present the examples of light and fluorescent microscopy application including digital image analysis and computer karyotyping of working out interesting clinical cases in our institute. Light microscopy analysis of metaphase chromosomes by G-banding method and fluorescent microscopy focused on both interphase nuclei and metaphase chromosomes (fluorescence in situ hybridization using whole chromosome painting, centromeric, subtelomeric and locus-specific probes) were used for our examinations. The presented cases illustrate the necessity of suitable method choice according to the aim of analysis as well as the fact that relevant results can often be obtained only through the combination of several methods.

For recognizing unbalanced aberrations undetectable by optical microscopy basic cytogenetic analysis can be extended by molecular cytogenetic methods, e.g. array CGH. However, in vast majority of clinical cases the methods of optical microscopy remain irreplaceable.

⑦ Life time microscopy study of cells A375 after sonodynamic therapy

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Presented research was aimed to evaluate viability of human melanoma cells line A375, after combined cisplatin and therapeutical ultrasound treatment in vitro. The

study included life time microscopic observation focused on the detection of presence of apoptosis and evaluation of cell's adhesion.

There were compared three modes of cell's treatment: exposure to ultrasonic far field (of intensity 1 $W \cdot cm^2$), application of cisplatin (concentration of 2.3 μM), and application of cisplatin followed by exposure to application ultrasound.

Obtained data showed that a maximal suppression of cells viability came for cells which were exposed by the ultrasound in presence of cisplatin. It can be concluded that, at the experimental set-up, ultrasound, as a physico-mechanical factor, influences the cells so that a higher effect of co-acting anticancer agents is possible. The mechanism of ultrasound action could be explained by changes in cell's membrane porosity or their reorganization.

Accrding to a subjective monitoring of manifestations of apoptosis by the life time microscopy, it was found that the most apoptotic cells (after 24 hours of incubation) were present in the experimental groups affected by cisplatin and affected by combination of ultrasound field and the cisplatin; the number of apoptotic cells in the experimental groups affected by ultrasonic field alone was lower.

By subjective comparison of experimental groups which were affected by cisplatin, by combination of cisplatin and ultrasound and by ultrasound alone, the difference of cell adhesion for 24 hours time interval was not observed, i.e. none of the experimental groups showed a significant delay in adhesion. There was detected difference of the cells adhesion for time 3 hours after treatment, which shows that the cells which were affected by ultrasound showed a similar adhesion as the control group and the number of adhered cells was about 50 %. The cells affected by cisplatin and by cisplatin combined with ultrasound were adhered in a very small extent for this time interval.

The author thanks Ing. Josef Jaroš, Masaryk University, Faculty of Medicine, Department of Histology and embryology, for his help with the microscopic part of the study.

(8) Comparison of cancer of unknown primary diagnosis with diagnostic outcome after pathological-anatomical autopsy

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Oncogenic disease can occur in every tissue of human body. Diagnosing cancer of unknown primary site (CUP, C80,…) is very problematic field of histopathology, though it ranks as the fourth most common cause of cancer deaths. Since CUP is identified as

histological confirmed metastases in the absence of identifiable primary tumor, its diagnostic is very time-consuming and financially heavy . It follows type 2 progression, it is malignant to begin with. Only 27 % of CUP cases are diagnosed by bio-optical methods in live patients. The aim of this study was to statistically compare clinical diagnosis of CUP with diagnostic outcome after pathological-anatomic autopsy. Study considers data from years 2008-2010, patients with cancer treated and autopsied in Slovak republic. Over 50 % of cases were clinically categorized as tumors of unknown primary origin. Autopsy and histopathological evaluation has revealed the origin of the malignant disease to be 20.1 % in the lungs and 34 % in the GIT. In 13 cases autopsy confirmed CUP diagnosis. In 15.5 % autopsy did not found any malignant disease despite clinical suspicion. Most cases of female patients were histological evaluated as adenocarcinoma while male patients suffered mostly with squamous cell carcinoma.

(9) DNA PLOIDY AS INDEPENDENT PROGNOSTIC FACTOR IN SOME OF THE GYNAECOLOGICAL MALIGNANCIES

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DNA aneuploid solid tumours have significantly worse prognosis than the diploid tumours of the same grade and stage. DNA ploidy has been found to have a prognostic impact in endometrial carcinoma in several studies. We established the method of DNA ploidy assessement in endometrial and ovarian carcinoma using flow cytometry in our laboratory. The first results in the sample of endometrial carcinoma patients showed that patients could profit from the preoperative evaluation of DNA ploidy – DNA aneuploid tumours should be intended for more extensive and complete surgery even if other prognostic parameters do not show high risk. The study of DNA ploidy prognostic value in the second sample, the patients with borderline ovarian tumours, was inconclusive for the present. Further increase of the patients number, close and long follow-up could help to clarify the relation between DNA ploidy and other prognostic factors and clinical outcome.

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(10) Reciprocal translocation as recurrent form of 13q14 deletion in chronic lymphocytic leukemia

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13q14 deletion is the most frequent chromosomal aberration in chronic lymphocytic leukemia (CLL). It is associated with good prognosis on condition that it is not a part of complex karyotype and that it encompasses only a minimal deleted region. The minimal deleted region size is approximately 900 kbp and it contains several genes including DLEU7 and miR15a, miR16-1 recognized as tumor suppressor genes. 13q14 deletion is found predominantly as an interstitial deletion, but it was previously described in a form of reciprocal translocation with deletion at the breakpoint at 13q14. In contrast with 13q14 deletion, the presence of translocation affecting any chromosome in CLL is associated with impaired prognosis.

13q14 deletion was found by interphase FISH in 62 % of our cohort of patients (146/235). Detailed metaphase analysis (G band, FISH) could be accomplished in 135 patients and it revealed the translocation form of 13q14 deletion in 10 % of them (13/135). Surprisingly, coexistence of a clone with the translocation form of deletion and other clone with the interstitial deletion was often found (5/13). In one suitable case, subsequent clonal evolution analysis by appropriate FISH probes proved that the clone with reciprocal translocation with deletion arose independently of the other clone with interstitial deletion in this patient.

We confirmed the reciprocal translocation form of 13q14 deletion as a recurrent form of 13q14 deletion in CLL and we established its frequency at 10 %. Based on our results we assume that formation of reciprocal translocation with deletion is independent of more common interstitial deletion. A possible mechanism of its formation in one step would be microhomology mediated break-induced replication. Whether the translocation form of 13q14 deletion could modify the disease prognosis has not been known yet, therefore the research of a larger cohort of patients is needed.

(11) Conventional cytogenetic analysis of human lymphocytes and health safety of workers exposed to mutagens and carcinogens

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The number (percentage) of chromosomal aberrations is the endpoint of the method of cytogenetic analysis in peripheral lymphocytes (CAPL). Chromosomal aberrations (CHA) detect resulting biological effect of so-called internal biological active dose of genotoxic factors (for example mutagens, carcinogens) of environment (reversible biomarker of early biological effect, not biomarker of disease). Because epidemiological studies confirmed evidence on the existence of significant associations between the frequency of CHA and the incidence of cancer CAPL has been using in select cases in connection with professional exposure (at least eight hours a day, five days during a week, for many years). In addition CHA detection in peripheral blood of employees professionally exposed to mutagens, carcinogens and its mixtures makes the possibility to detect real biological (genotoxic) effect in vivo of a complex mixture from the whole environment (cumulative effect).

Because the Czech population level of CHA for not professionally exposed adults is known (between 0–2 % of aberrant cells AB.B.) it is possible to characterize groups of workers with the same exposure scenario as a group with not effective genotoxic exposure (0–2 % AB.B.), group with elevated exposure and with the high exposure to genotoxicants.

This paper presents results of observed groups of workers (select by hygienist) during 2009–2010 periods carried out in laboratories of institute of public health in Ostrava.

(12) Occupational exposure to genotoxic agents and chromosomal aberrations

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The workers of numerous workplaces are periodically, excessively and long-time exposed to low doses of genotoxic agents. Their mutagenic and carcinogenic effect is

permanently discussed. In oncological units are workers exposed to antineoplastic agents, in hospital theatres to volatile anaesthetics, in rubber industry to butadiene (BD) and in some chemical factories to vinylchloride (VC) and ethylene oxide (EO).

Aim of the study was the evaluation of genotoxic risk of these agents by means of quantification the total chromosomal aberrations (CAs), and specific types – chromatid-type of aberrations (CTA-type) and chromosome-type of aberrations (CSA-type) of using method of cytogenetic analysis in peripheral blood lymphocytes.

The group exposed to antineoplastic agents (E1) was consisted of 249 medical workers of 9 selected hospitals; group exposed to anaesthetics (E2) of 247 persons from 6 hospitals of middle region of Slovakia; 257 workers exposed to BD (E3) and 258 workers (E4) of given chemical foundry in Middle Slovakia. The control group presented 250 unexposed persons.

Statistically higher frequency of total CAs (presented in per cent) was detected in all four exposed groups with comparison to control (E1 – 1.97, E2 – 2.34, E3 – 1.73, E4 – 1.72 vs. control 1.19, respectively, $P = 0.001$). In groups E2 and E4 was detected statistically higher frequency of CSA-type aberrations in comparison to CTA-type (E2 – 1.43 vs. 0.91, $P = 0.001$, E4 – 1.13 vs. 0.59, respectively $P < 0.05$), however in group E2 and E3 waseţ detected any difference in frequency CSA-type and CTA-type of aberrations (E2 – 0.92 vs 1.05 and E3 – 0.91 vs. 0.82).

Only in group E2 we detected statistically higher frequency of total CAs in women as compared to men (2.44 vs. 1.88, $P < 0.01$), and in anaestesiologic nurses as compared to physicians (2.47 vs. 2.17, $P < 0.01$); in group E3 higher frequency in smokers as compared to non-smokers (1.92 vs. 1.60, respectivelly $P < 0.05$). In remaining groups we detected not any difference in dependence to gender, smoking and job category.

This study was supported by VEGA grant 1/0576/10, grant MZ SR 2007/48-UK-13 and projects "Carcinogenic and toxic metals in working environment" and "Translation medicine Centre" co-financed from EU sources.

(13) Chromosomal aberrations and polymorphisms of selected genes in relation to occupational exposure to inhaled anaesthetics

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This study was focused on evaluation of the genotoxic risk of anaesthesia personnel, who was regularly professionally exposed to low-dose volatile anaesthetics.

The aim of this study was to assess the frequency of total chromosomal aberrations (CAs), and their specific types ie. chromatid (CTA) and chromosome (CSA) type and also to assess individual susceptibility factors by the evaluation of selected polymorphisms of genes for xenobiotic metabolised enzymes (XME), *CYP1B1, EPHX1, GSTM1, GSTP1* and *GSTT1*.

Evaluated groups consisted of 76 persons exposed to the action of volatile anaesthetics and 76 persons who were not exposed to anaesthetics or other carcinogens.

Assessing the overall CAs in the exposed group, the statistically highly significantly higher incidence compared with the control group (2.53 ± 1.37% vs. 1.47 ± 0.93%, Mann-Whitney U-test, P = 0.0008) was found. Three times higher incidence of type aberrations CSA compared with CTA type (Mann-Whitney U-test, P = 0.0009) was found in the exposed group. Statistically significant differences in the incidence of total CAs or their specific types were not identified based on gender, smoking status and job position. Statistically significant differences depending on the presence of variant alleles (*CYP1B1* Asn453Ser, Leu432Val *CYP1B1, EPHX1, GSTP1* Ile105Val), or in the presence of positive or neutral variant genes *GSTM1* and *GSTT1* were not found at evaluation of selected polymorphisms of genes for xenobiotic metabolised enzymes. To detect individuals with higher susceptibility in the preventive protection of workers is utmost important.

This study was supported by VEGA grant 1/0576/10, grant MZ SR 2007/48-UK-13 and project „Translation medicine Centre" co-financed from EU sources.

(14) CHROMOSOMAL ABERRATIONS AND POLYMORPHISMS OF DNA REPAIR GENE HOGG1 IN MEDICAL WORKERS EXPOSED TO CYTOSTATICS

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In this study we compared the incidence of chromosomal aberrations (CAs) and their chromosome (CSA) and chromatide (CTA) types in medical workers occupationally exposed to cytostatics with respect to DNA polymorphisms of repair gene *hOGG1*. Occupational exposure to cytostatics may cause the increased frequency of chromosomal aberrations. Human organism has developed many protective mechanisms against DNA damage. Such protection may be executed by the xenobiotics biotransformation and by DNA damage repair mechanisms.

Polymorphisms in DNA repair genes may be associated with differences in the capacity to repair DNA damage. 8-oxoG (7,8-dihydro-8-oxoguanine) is arguably the major mutagenic lesion in DNA because during DNA replication is able to form pairs with an adenine and oxidized derivative of guanine as well.

The study was performed on population of 71 medical workers and 67 control individuals that were not exposed to any known carcinogen and mutagens. The cytogenetic analysis was used for determination of chromosomal aberration's frequencies and PCR-RFLP method for determination of gene polymorphisms.

We detected statistically higher frequency of total chromosomal aberrations in exposed group in comparison to control (1.76 ± 1.22 vs.1.28 ± 0.90; $P < 0.01$). In exposed group we found statistically significant differences between CTA-type aberrations in comparison to control group (0.83 ± 0.95 vs. 0.54 ± 0.61; $P < 0.05$). Elevated frequency of chromosomal aberrations was detected in exposed individuals with wild type Ser326Ser *hOGG1* genotype in comparison to control group with the same genotype (1.89 ± 1.10 vs. 1.42 ± 0.84; $P < 0.05$). We did not find statistically higher frequency in individual type (CSA and CTA) of CAs in association with polymorphic alleles of *hOGG1* repair gene.

(15) Lung cancer incidence, tumour histological type and survival in individuals exposed to chromium

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Workers chronically exposed to hexavalent chromium have higher incidence of lung cancer. Our study investigates incidence of lung cancer types, age of onset of the disease and surviving time among chromium exposed workers (smelters, tapers, crane operators) in comparison to non-exposed persons.

77 chromium exposed workers and 104 male controls with diagnosed lung cancer were analysed. The average exposure time among workers was 18.38 years (range 1–41 years).

Chromium exposure decreases the age at the onset of the disease of 4.18 years (65.51 years in control; 61.33 years in exposed group). Significant difference between the age at the onset of the disease was found between smokers (65.1 years) and non-smokers (68.6 years) (P = 0.008) in the control group. Non-exposed non-smokers had explicitly higher age at the onset of the illness in relation to other groups. In exposed group the significant effect of smoking on the age at the diseases onset was not found (P = 0.775). Small cell lung carcinoma (SCLC) forms 25.71 % of all cases in chromium exposed workers and 16.34 % in non exposed individuals. The survival time of patients with NSCLC and SCLC within non-exposed were 12.33 and 17.75 month respectively and in exposed group 14.8 and 4.44 month respectively. No correlation was found between the age at the diseases onset and time of exposure.

Occupational exposure to chromium was identified as an important risk factor of lung cancer even overlaying effect of smoking. Both chromium exposure and smoking decrease the age at the diseases onset. Higher percentage of SCLC was found in chromium exposed individuals.

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(16) Association between DNA polymorphisms of selected repair genes and risk of lung cancer

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Polymorphisms in DNA repair genes may be associated with differences in DNA repair capacity, thereby influencing the individual susceptibility to the development of lung cancer. We investigated the association between polymorphisms in DNA repair genes hOGG1 (Ser326Cys) and XRCC1 (Arg399Gln), which play an important role in the DNA base excision repair pathway. In this case-control study, we recruited 309 lung cancer cases and 339 healthy controls. Genomic DNA was extracted from peripheral blood by phenol/chloroform extraction. The genotypes of hOGG1 and XRCC1 alleles were determined by polymerase chain reaction-restriction endonuclease length polymorphisms (PCR-RFLP) analysis. The differences in the distribution of genotypes and alleles between cases and controls were analysed using x2-test. The odds ratio (OR) and 95 % confidence intervals (95 % CI) were adjusted for the risk of developing

lung cancer, alone polymorphisms or their combinations. The presence of polymorphisms of genes hOGG1 and XRCC1 in women represents elevated risk of lung cancer compared to men. The frequency of the genotype XRCC1 Gln/Gln (OR = 2,32; P = 0,19) represented 2-fold higher risk of lung cancer compared with genotype Arg/Arg in women. Genotypes Arg/Arg + Ser/Cys (OR = 2,45; P = 0,19), Arg/Gln+Ser/Ser (OR = 2,3; P = 0,08), Arg/Gln + Cys/Cys (OR = 2,45; P = 0,76) had a 2-fold increase risk of lung cancer compared with Arg/Arg + Ser/Ser genotype. The frequency of the Gln/Gln + Ser/Ser (OR = 7,36; P = 0,012) genotypes was statistically significantly higher compared with Arg/Arg + Ser/Ser genotype. In summary, variants alleles Cys326 and Gln399 are associated with reduce enzyme activity of the DNA repair genes and lead to increase susceptibility at risk of developing lung cancer.

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(17) EXPRESSION OF SELECTED REGULATORY PROTEINS IN LUNG TISSUE OF PATIENTS WITH DIAGNOSED PULMONARY CARCINOMA

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Apoptosis is the fundamental process necessary for eliminating damaged or mutated cells. Alterations in the apoptotic pathway appear to be key events in cancer development and progression Bcl-2 is the founding member of the Bcl-2 family of apoptosis regulator proteins with anti-apoptotic effect. Survivin acts as an inhibitor of apoptosis as well and has been implicated in both the inhibition of apoptosis and mitosis regulation. P 53 is one of the tumour suppressor proteins; prevents tumour formation through cell cycle blocking and eliminates damaged cells via activation of apoptosis. The Ki-67 protein is a cellular marker for proliferation. To investigate the possible interactions of mentioned proteins we examined their expression in 76 patients with diagnosed lung cancer using immohistochemical visualisation. Ki-67 protein was expressed in the cancer cells of all patients with small cell lung cancer type. We found negative correlation between survivin and p53 expression. Decreased intensity of

expression and less number of positive cells for surviving (66,66 %) in small cell lung cancer in comparison to other lung cancer types (97,98 %) was detected. Reversely, expression of Bcl-2 was found in more than 90 % of cases with small cell lung cancer. We hypothetise that high expressivity and intensity of Bcl-2 protein could probable influence worst biological behaviour of its clinical-pathological entity.

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(18) Prognostic Significance of ERCC1, RRM1, BRCA1 and TS in surgically treated Non-small Cell Lung Cancer patients

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Introduction: Chemotherapy is an important modality of treatment of non-small cell lung cancer (NSCLC). The recent studies show relevance of estimation of response rate by the use of predictive molecular markers. In early stages, the surgical resection is the curative treatment. With the exception of stage I the resection is usually followed by adjuvant chemotherapy.

The goal of our study was to assess the relation of surgically resected tumor tissue mRNA levels of ERCC1 (excision repair cross-complimentary group 1), RRM1 (ribonucleotide reductase subiunit M1), BRCA1 (breast cancer 1 gene) and TS (thymidylate synthase) to DFI and OS in patients who had undergone the adjuvant therapy.

Patients and Methods: The studied group consisted of 90 patients with NSCLC, who had undergone curative lung resection at the Department of Surgery and 59 of them were indicated to adjuvant chemotherapy. Ninety paired (tumor and control) lung tissue samples were taken directly from the tumor tissue and from adjacent, histologically cancer-free lung tissue (normal lung tissue). Quantitative estimation of mRNA of selected genes was performed by RT real-time PCR method with UPL probes (Universal Probe Library, Roche).

Results: We evaluated relation of expression level of ERCC1, RRM1, BRCA1 and TS to DFI and OS in NSCLC and histological subtypes of NSCLC and also in stage groups. We found longer OS in patients with adenocarcinoma with higher expression of RRM1 mRNA ($p = 0.002$).

We found longer OS in patients with squamouse cell carcinoma with higher expression of BRCA1 mRNA (p = 0.041). In NSCLC patients of stage 3, we found longer DFI in patients with higher expression of RRM1 and ERCC1 (p = 0.004, p = 0.038; respectively).

We evaluated also the expression level of ERCC1, RRM1, BRCA1 and TS in normal lung tissue in relation to adverse effects of chemotherapy (cytotoxicity). We found no relationship of expression and cytotoxicity reaction.

Conclusion: We investigated if the possible residual tumor cells after resection reflect the properties of primary tumor and response to chemotherapy according to level of predictive markers with the respect to current knowledge. We found that the level of ERCC1, RRM1, BRCA1 is in relation to prognosis but we did not recorded the relation to response rate.

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19 mRNA EXPRESSION LEVELS OF RESISTANCE-RELATED PROTEINS IN CELLS FROM PATIENTS WITH ACUTE LEUKAEMIA

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Resistance of tumor cells to chemotherapeutic agents is an important factor that limits the successful treatment of a wide range of malignancies. The multidrug resistant (MDR) phenotype is well recognized in clinical samples, and it has been extensively studied, particularly in acute myeloid leukemia. One of the principle mechanisms underlying this MDR phenotype is the active cellular extrusion of chemotherapeutic agents by the multidrug resistance protein MDR1 (p-glycoprotein). More recently, other drug resistance proteins, notably the multidrug resistance-associated protein, MRP, and the breast cancer resistance protein, BCRP, have also been implicated in multidrug resistance. Apoptosis and anti-apoptosis pathways are also deeply related to drug sensitivity and resistance. The aim of the present study was to analyze the expression of three ABC-transporters (P-gp, MRP and BCRP) and four apoptotic proteins (p53, bax, bcl-2 and bcl-x) in leukaemic cells. In addition, our study focuses on determination levels of mRNA apoptotic proteins among leukaemic cells and normal lymphocytes from healthy donors. The expression of mRNA was measured by Reverse Transcription – PCR. We have demonstrated that acute leukaemia, both myeloblastic (AML, n = 16) and lymphoblastic (ALL, n = 10), is associated with significantly elevated levels of p53 and bax mRNA in leukaemic cells. With respect to ALL, significantly elevated levels of

bcl-xl mRNA could explain for relative resistance of ALL cells to p53-dependent apoptosis. P-gp exhibited strong variation in transcription level among different leukaemia patients; however, it was significantly higher in relapsed than in de novo patients. The expression of MRP was more consistent and no significant differences between de novo and relapsed patients were observed. The expression level of BRCP was very low, however, significantly higher in relapsed than in de novo patients. These results suggest the efficacy of this quantitative analysis of resistance-related proteins for the prediction of clinical drug resistance in acute leukaemia.

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(20) CHANGE IN THE EXPRESSION OF SELECTED GENES DURING THE DEVELOPMENT OF COLORECTAL CANCER

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Background: Colorectal cancer is one of the most common malignant disease in the Czech Republic and prediction of disease progression or selection of the best treatment for the particular patient is very complicated. We are trying to find new markers of the disease progression or treatment response to help to select appropriate care for the patients and to improove quality of their lives.

Study Aims: We would like to identify genes with the different expression levels between the tumor and healthy tissue in the well-defined group of patients. Our ultimate goal is to use this expression change during the diagnosis as the prognostic or predictive factor.

Methods and Materials: We performed literature and database search and selected set of 12 genes for our study (ACLS5, VSNL1, DSTN, SAMD3, CTHRC1, MAPK1, CLDN23, SLC26A2, MIER3, CAPN10, VCAN a LGR5). Some of these genes are already connected to the malignant process in other types of cancer. First, we have identified best reference genes and optimalized each quantitative real-time PCR reaction with SYBR Green I stain and then measured the expression of candidate genes. Expression changes were statistically validated.

Results: For the pilot study we have used paired samples from 25 patients. Out of the twelve genes, four had significantly different expression between the tumor and

healthy tissue, two of them were downregulated in tumor (CLDN23, SLC26A2) and two upregulated (CTHRC1, VSNL1), two other genes (MIER3, LGR5) were at the border of the significant expression change.

Conclusions: In the pilot study we have identified four genes with the different expression between the tumor and healthy tissue. In the subsequent study on the larger group of patient we would like to correlate these expression changes with the clinical data (overall survival, disease free survival) and response to the particular treatment.

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(21) TIMP-1 PLASMA LEVELS IN PATIENTS WITH LIVER METASTASES

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Background: TIMP-1 (tissue inhibitor of metalloproteinases 1) regulates extracellular matrix turnover, but the major effect in tumor tissue is a promotion of cell growth and anti-apoptotic activity. The aim of our study was to evaluate the relation of TIMP-1 plasma levels to prognosis in patients with liver metastases with a special respect to possible early prediction of the recurrence of disease. Nowadays we know that repeated liver surgery for metastases significantly prolongs overall survival.

Methods: We studied a group of 87 patients with metastatic liver disease (72 primary tumours being CRC) who underwent the surgery of liver metastases (39 cases of radiofrequency ablation, 44 resections, 4 other type of surgery) and assessed their preoperative protein TIMP-1 plasma levels. These levels were evaluated according to prognosis. Furthermore we measured plasma TIMP-1 in the postoperative period and tried to relate the changes of the levels to the diagnosis of relapse. Quantitative estimation of plasma levels of TIMP-1 protein was performed by ELISA technology (Quantikine Human kits (R&D Systems, USA).

Results: We found statistically significant relation of preoperative TIMP-1 protein plasma levels and overall survival (OS) in the whole group of patients with metastatic liver disease ($p = 0,0042$) and in the group of patients with CRC metastases ($p = 0,0208$), higher level was associated with adverse outcome. Significantly shorter OS was observed in patients with higher level of TIMP-1 in a subgroup treated by RFA ($p = 0,0314$) and also in the subgroup treated by liver resection ($p = 0,0457$). We investigated if there is relationship of plasma TIMP-1 increase in postoperative period

and disease recurrence, but it was not found to be statistically significant. Nevertheless, due to limited number of patients where this parameter could be determined, more data should be collected to confirm this result.

Conclusion: We found there to be a relationship between TIMP-1 preoperative plasma levels and prognosis (OS) of patients with metastatic liver disease. Higher plasma level of TIMP-1 is related to an adverse prognosis of patients.

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(22) Detection of novel genetic changes in the RAS genes in papillary thyroid carcinoma

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Objectives: Activating point mutations in the RAS genes (H-RAS, K-RAS, N-RAS) are reported in follicular thyroid adenoma (FTA), follicular thyroid carcinoma (FTC) and follicular variant of papillary thyroid carcinoma (FVPTC). The aim of this study was to determine the frequency of RAS mutations in thyroid tumors.

Methods: DNA was extracted from 98 samples, including 72 fresh frozen thyroid samples and 26 paraffin-embedded formalin-fixed samples. The cohort contained 83 PTCs (56 FVPTCs, 14 mixed follicular-classical types, 11 classical variants and two other rare variants), one FTA, 7 FTCs, 4 poorly differentiated carcinomas (PDC) and 3 anaplastic carcinomas (ATC). The presence of RAS mutations in exon 1 and exon 2 of the H-RAS, K-RAS, N-RAS genes was determined by direct sequencing and detected missence RAS alterations were evaluated in silico using PolyPhen-2, Align-GVGD and SIFT software.

Results: Mutations in six patients was found in codon 61 of the activating domain of K-RAS (2 PTC patients) and N-RAS genes (4 PTC patients). The polymorphism 81T-C in H-RAS gene was found in 41% PTC, in one FTA, 3 FTC, 3 PDC and one ATC. We detected other 7 silent, 6 missence and one nonsense genetic changes. The alterations were found only in follicular variant of PTC. The association with phenotype was not apparent.

Conclusion: RAS mutations in our cohort of thyroid cancer were screened. In addition to six mutations in codon 61 (GTPase domain), we revealed other genetic changes. However, their influence on the development of PTC needs to be confirmed.

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(23) Sodium butyrate-induced death of HL60 cells – implication of intrinsic apoptosis pathway

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Sodium butyrate (NaBu), inhibitor of class I histone deacetylases (HDACs), induces differentiation, cell cycle arrest and death of several cancer cell lines. The aim of presented study was to determine effect of NaBu on survival of leukemic cell line HL60.

Incubation of leukemic cell line HL60 with NaBu led to inhibition of proliferation and death of cells in concentration- and time-dependent manner. While concentrations of NaBu less than 5 mM blocked the proliferation of HL60 cells, 5 mM NaBu led to death of HL60 cells observed after 48 hours of incubation. Analysis of cell extracts revealed down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-Xl. In addition, activation of both caspase 9 and caspase 3 were observed, whereas involvement of cathepsin-mediated cell death was not documented. Our data indicate that inhibition of class I HDACs is associated with death of HL60 cells via mechanism typical for intrinsic (mitochondrial) pathway.

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(24) Functional analysis of Mdm2 mutants suggests differences between Mdm2 homodimers and Mdm2/MdmX heterodimers

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Mdm2 can mediate p53 ubiquitylation and degradation either in the form of Mdm2 homodimers or Mdm2/MdmX heterodimers and these interactions are mediated mainly by their respective RING domains plus the adjacent C-terminal tails. Interestingly, studies of the crystal structures as well as NMR studies failed to show any significant differences in the structure of Mdm2/Mdm2 homodimers and Mdm2/MdmX heterodimers.

In order to study the structure and function of Mdm2/Mdm2 and Mdm2/MdmX complexes in more detail, we have created a series of Mdm2 mutants with extended C-terminal domain. All mutants lost their ubiquitin ligase activity towards p53 even though they were still capable of forming Mdm2 homodimers either through RING-RING or RING-acidic domain interactions. All extended mutants also retained the ability to interact with MdmX and this interaction led to reactivation of their ubiquitin ligase activity and p53 degradation by the mdm2/MdmX complex. Surprisingly, in contrast to the interaction with MdmX RING, only a subset of extended Mdm2 mutants were activated by the interaction with Mdm2 RING domain, suggesting that there might be yet unidentified structural and functional differences between Mdm2/Mdm2 homodimers and Mdm2/MdmX heterodimers.

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(25) Polo-like kinase I controls nuclear envelope break down and chromosome dynamics in meiosis I

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Plk1 belongs to the extended family of serine/threonine kinases controlling the cell cycle. It is involved in the control of mitosis and contributes also to the regulation of meiotic division.

In somatic cells, Plk1 plays a role in centrosome maturation, nuclear envelope breakdown, spindle formation or APC activation. It interacts with other extensively studied mitotic molecules like Cdk1 or Aurora A and it is known, such as these kinases, as a potential target of cancer therapy.

There are not many studies dealing with the role of Plk1 in oocytes, still it has been shown that it plays some part in the formation of spindle or in germinal vesicle breakdown (GVBD).

On a basis of live cell imaging of mouse oocytes – control ones or with pharmacologically inhibited Plk1, we can describe the role of Plk1 in these cells as a kind of synchronizer of meiotic events. As expected considering results from somatic cells, oocytes with inhibited Plk1 exhibit delayed GVBD and delayed start of a condensation of chromatin. But also, these two events – GVBD and chromatin condensation are somehow desynchronized in cells with inhibited Plk1: in contrast to control oocytes, Plk1 inhibition leads to almost full condensation of chromatin in oocytes with nuclear envelope still present. Also duration of GVBD and chromatin condensation are prolonged in oocytes with inhibited Plk1. Bipolar spindle is formed after inhibition of Plk1, but only with corresponding delay and oocytes are then arrested in metaphase I.

So in conclusion, Plk1 is an important player in mouse oocytes meiosis with significant role in GVBD, condensation of chromatin, spindle assembly and anaphase onset.

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(26) GALECTIN-1 -ITS POTENTIAL ROLE IN WOUND REPAIR AND IN CANCER

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Adult stem cells as well as the tumor cells need for their survival special microenvironment. The extracellular matrix is known to play the key role in this “niche”. Studying the histological sections of cancer stroma and wounded skin, we observed a high incidence of myofibroblasts as well as presence of an endogenous lectin, i. e. galectin-1. We studied whether human galectins induce the conversion of humen dermal fibroblasts into myofibroblasts and the production of bioactive extracellular matrix scaffold in series of in vitro experiments employing human recombinant galectins (type 1, 2, 3, 4, 7 and 8).

Galectin-1 was found the most potent inducer of myofibroblasts formation and extracellular matrix production. The effect of galectin-1 was found to be independent on TGF-beta1 , (known to activate the fibroblast – myofibroblast transition) but both substances had an additive effect. Examining cell features associated with galectin stimulation, the galectin-exposed fibroblasts/myofibroblasts produced massively an extracellular scaffold rich in fibronectin and also galectin-1. When tested as substratum, it turned out to be beneficial for cultivation of keratinocytes without feeder cells. Interestingly, the keratinocytes cultured on this scaffold changed their phenotype towards low-differentiated cells.

Stimulating the genesis of myofibroblasts and production of specific extracellular matrix, some galectins could on one side enhance wound healing and on the other one play an important role in the cancer stroma creation and metastasizing of a tumour.

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(27) ANTICANCEROGENIC PLANT NUCLEASE TBN1 AND ITS GLYCOSYLATION MUTANTS SHOW LOW TOXICITY ON MOUSE EARLY EMBRYO MODEL

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Anticancerogenic properties of bifunctional apoptotic nuclease/dsRNAse TBN1 from tomato (EC 3.1.30.x) was discovered as the original finding of our group (e.g. Matoušek et al., Oncol. Res. 18: 163–171, 2009). TBN1 have been recently patented (Invention No 302164) as an antitumor therapeutic with low side-effects. Along with other unique ribonucleases and onconase, its potential applicability in medicine was reviewed (Fang and Ng, Biochim. Biophys. Acta. 1815: 65 74, 2011). It is expected that TBN1 that effectively suppress proliferation of various solid tumors acts on stroma, micro- or dsRNA levels or blocks angiogenesis. It is of interest to test TBN1 action on cancer stem cells, methastases and cell differentiation processes. The principal achievement

of successful crystallization of TBN1 (Dohnálek et al., J. Synch. Rad. 18: 29–30, 2011) enabled its first modifications to study biological action. Activity of plant bifunctional nuclease as well as cytotoxicity of onconase depends on protein glycosylation pattern. In the present work hypo and hyperglycosylated mutant forms of TBN1 were prepared and in vitro mouse embryo assay (MEA) was employed to test their embryotoxicity as a one of important parameters of its applicability in medicine. Two-cell mouse embryos were isolated from oviducts of superovulated mice (C57/BL6) ca. 36 hours after mating. To check nuclease toxicity embryos were cultured in CZB medium supplemented with 15 μg/ml of either TBN1 variants or BS-RNAse. Development of embryos was evaluated under a stereomicroscope during culture (Chatot CL, et al., J Reprod Fert. 86: 679–88, 1989) and blastocyst rate was scored after 72 hours (Roussev RG, et al., Am J Reprod Immunol. 33: 171–7, 1995). In all treatments blastocyst rate exceeded 70 % except for BS-RNAse. The results of MEA revealed that the blastocyst development rate was not affected significantly when cultured in medium supplemented with either TBN1 or four different glycosylation TBN1 mutants, while the splitting process was strongly blocked after application of BS-RNAse. These results confirm much lower TBN1 toxicity in comparison to animal ribonucleases.

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(28) Intracellular sequestration of ellipticine: role of vacuolar-ATPase

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Ellipticine exhibit antitumor activities. The main reasons for the interest in ellipticine clinical purposes are its efficiency against several types of cancer, limited toxicity particularly lack of hematotoxicity. Ellipticine arrests cell cycle progression by regulating the expression of cyclinB1 and Cdc2 and by phosphorylation of Cdc2 and to induce apoptosis by the generation of free radicals, the activation of Fas/Fas ligand system, the regulation of Bcl-2 family proteins and, an increase of wild-type p53, and the initiation of the mitochondrial apoptosis pathway. Ellipticine also uncouples mitochondrial oxidative phosphorylation. However, the precise molecular mechanism responsible for these effects has not yet been explained. It was suggested that the prevalent mechanisms of its antitumor activity is intercalation and topoisomerase II inhibition. We have demonstrated that ellipticine covalently binds to DNA after enzymatic activation, suggesting a third possible mechanism of action.

Vacuolar (V)-ATPase is the enzyme complex necessary for the acidification of intracellular compartments (trans-Golgi network, endosomes, lysosomes, secretory granules) and protonated chemicals may be trapped into these vacuoles. The V-ATPase-dependent trapping of the cationic form of drugs in acidic vesicles is followed by their osmotic swelling and dilatation. Sequestration of drugs into subcellular compartments can influence anticancer action of protonated anticancer drugs including ellipticine. Role of V-ATPase and lysosomal compartment in metabolism and toxic action of ellipticine is not known.

Ellipticine treatment is a causes of significant and concentration-dependent cytoplasmic vacuolization of the neurobalstoma, glioblastoma and meduloblastoma cells. The vacuoles were detectable already 30 minutes after the treatment and ellipticine was concentrated to the vesicular structures in cytoplasm. Vacuolization and intravesicular ellipticine-associated fluorescence was abolished by co-treatment with the specific V-ATPase inhibitor bafilomycin A1 and lysomotropic agents chloroquine. Chloro-quine pretreatment increased the efficiency of ellipticine. Ellipticine exposed cells were analysed by immunoblotting for autophagy marker MAP1 LC3. Increased expresion of LC3 II persisted even after 24h. We observed dilatation and multiplication of lysosomes without the of share autophagosomes using electron microscopy.

Conclusion: ellipticine is sequestrated to the vesicular structures probably of lysosomal origin. Specific Vacuolar-ATPase inhibition or using of lysosomotropic drug chloroquine increased ellipticine efficiency.

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(29) EPIGENETIC MODULATIONS OF ANTIGEN PRESENTING MACHINERY GENES IN TUMOUR CELLS

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Epigenetic changes play important roles as genetic alteration in carcinogenesis and in the course of the tumour growth. From immunological point of view, it is noteworthy that downregulation of genes crucial for antigen presentation and costimulation involve epigenetic events, e.g. silencing of genes encoding *MHC* or costimulatory molecules. We have shown, using *MHC* class I deficient tumours that expression of immune response genes can be restored by epigenetic agents (e.g. *DNA*-methyltransferase and histone deacetylase inhibitors). We have used *MHC* class I deficient tumours to know, whether expression of immune response genes can be restored by epigenetic agents. We try to demonstrate, whether *DNA*-methyltransferase, histone deacetylase inhibitors upregulate of *MHC* class I surface expression on tumour cells deficient in

MHC class I expression. It is known, that *IFN*γ-mediated *MHC* class I surface expression is associated with upregulation of antigen presenting machinery genes *(TAP-1, TAP-2, LMP-2, LMP-7).* This work is focused on epigenetic mechanisms (*DNA* methylation, histone acetylation) in *IFN*γ pathways . We have performed a pilot study based on transcriptome analysis of *MHC* class I-deficient cell line *TC-1/A9* in control and *INF*γ and *5AC/TSA*-treated samples. *MHC* class I positive tumour line *TC-1* and *TC-1/A9,* an *MHC* class I-deficient subline was used. We specialize on transcriptome and methylome analyses of tumour and immune cells.

This project is focuse on analysis of the epigenetic regulation of selected genes responsible for activation or suppression of anti-tumour immune response in tumour cells. The effect of epigenetic agents may involve both activation and repression of immunoactive genes in different cell lineages. The results of this study will contribute to elucidation of the immune consequences of the anti-tumour treatments with the epigenetic agents.

(30) CYTOKINE EXPRESSION DURING SPONTANEOUS REGRESSION OF THE SWINE MELIM MELANOMA

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Melanoma is the most serious skin cancer with growing incidence worldwide in Caucasian populations. Melanomas are highly chemo- and radio-resistant. Moreover, various immunotherapeutic procedures show still a low effectiveness in human. Thus, patients with melanoma progression show high mortality. Proinflammatory cytokines produced by the tumour cells and/or tumour-associated leucocytes contribute to malignant progression. The spontaneous regression (SR) of human melanoma appears very exceptionally. Th1 cytokines secreted by activated T helper cells may mediate SR. Precise understanding of biological processes that participate in SR could bring new knowledge utilisable also in clinical oncology.

The MeLiM (Melanoma-bearing Libechov Minipig) strain with hereditary melanoma was developed in the IAPG Libechov as a suitable animal model for human melanoma. Multiple skin nodular melanomas develop in affected animals and roughly two thirds of them show SR. Thus, this model can be used for detailed study of melanoma progression as well as SR. The SR tumours show mass lymphocyte infiltration, melanoma cell damage and tumour rebuilding in fibrous tissue. Variable extent of depigmentation of the skin and bristles is often observed.

Objective of this study was to monitor temporal changes in concentration of cytokines secreted into blood serum of the MeLiM piglets with SR. Blood samples were taken from two control (melanoma-free) piglets and four piglets with SR at 4, 6, 12 and 18

weeks of age. The Quantibody Porcine Cytokine Array 1 (Ray Biotech, USA) was utilized for quantification of ten cytokines. In the control animals, concentrations of IL-4, IL-6, IL-12, GM-CSF, IFNγ and TNFα were low at 4 and 6 weeks, highly increased at 12 weeks and decreased back to low values at 18 weeks. TGFß1 showed a gradual decrease to negligible values at 18 weeks. Expression of IL-1ß, IL-6, IL-8 and IL-10 was usually below the limit of detection (LOD). On the contrary, all detected cytokines were below LOD in SR animals with exception of IL-8 that showed increased levels at 12 and 18 weeks. Higher concentration of IL-8 correlated with higher extent of depigmentation. These results suggest: 1) natural immunosuppression in melanoma-bearing animals and 2) expression of the proinflammatory cytokine IL-8 as the serum marker of SR.

This work was supported by the MEYS CR (grant No. 2B08063).

(31) CHARACTERIZATION OF MYELOID-DERIVED SUPPRESSOR CELLS INDUCED BY CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE

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Myeloid-derived suppressor cells (MDSC) play important role in tumour escape due to their ability to suppress T-cell responses. They accumulate in lymphoid organs and blood under different pathologic conditions, such as during tumour growth. Their mobilization was also reported after cyclophosphamide (CY) administration. In mice, they are characterized by markers of monocytes (CD11b) and neutrophils (Gr-1).

The aim of our experiments was to compare the phenotype and function of spleen Gr-1+CD11b+ MDSC accumulating after CY therapy (CY-MDSC) with those accumulating in mice bearing HPV16-associated murine TC-1 carcinoma (TU-MDSC) and to evaluate whether these cells could be affected by all-trans-retinoic acid (ATRA), agent that is known to stimulate differentiation of immature myeloid cells.

Even though both CY-MDSC and TU-MDSC accelerated growth of TC-1 tumours in vivo, their phenotype and function differed. CY-MDSC consisted of higher percentage of monocyte-like subpopulation and lower percentage of polymorphonuclear-like subpopulation. They had lower relative expression of genes responsible for immunosuppressive function (TGFß, Arg-1, iNOS, gp91, VEGF) and displayed lower suppression of T-cell proliferation. However, when stimulated with IFNγ, the relative expression of iNOS in CY-MDSC was higher and higher was also NO production when compared with TU-MDSC. CY-MDSC were more susceptible to the ATRA treatment, which resulted in their differentiation. This effect was associated with decrease in

relative expression of immunosuppressive genes and lower VEGF production. To confirm the effect of ATRA on MDSC in vivo, mice bearing TC-1 tumours were treated with CY in combination with ATRA. The chemotherapy resulted in accumulation of MDSC in spleens, whereas subsequent ATRA treatment reduced this accumulation and improved the antitumour effect of CY.

Our results indicate that CY-MDSC are less immunosuppressive than TU-MDSC and they consist of higher percentage of monocyte-like subpopulation. This may be the cause of their susceptibility to ATRA treatment. Our findings supported the rationale for utilization of ATRA after CY chemotherapy and provided useful information for elaborating the optimal immunotherapeutic protocols for the treatment of HPV 16-associated tumours.

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(32) THE ROLE OF MOUSE MESENCHYMAL STEM CELLS IN DIFFERENTIATION OF T-CELLS INTO ANTI-INFLAMMATORY REGULATORY T-CELL OR PROINFLAMMATORY HELPER T-CELL 17 POPULATION

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Bone marrow-derived mesenchymal stem cells (MSCs) can produce significant levels of transforming growth factor-ß (TGF-ß) and interleukin-6 (IL-6). These 2 cytokines represent the key factors that reciprocally regulate the development and polarization of naive T-cells into regulatory T-cell (Treg) population or proinflammatory T helper 17 (Th17) cells. We demonstrated that MSCs and their products effectively regulate expression of transcription factors Foxp3 and RORγt and control the development of Tregs and Th17 cells in a population of alloantigen-activated mouse spleen cells. The immunomodulatory effects of MSCs were more pronounced when these cells were stimulated to secrete TGF-ß alone or TGF-ß together with IL-6. Unstimulated MSCs produce TGF-ß, but not IL-6, and the production of TGF-ß can be further enhanced by the anti-inflammatory cytokines IL-10 or TGF-ß. In the presence of proinflammatory cytokines, MSCs secrete significant levels of IL-6, in addition to a spontaneous production of TGF-ß. MSCs producing TGF-ß induced preferentially expression of Foxp3 and activation of Tregs, whereas MSC supernatants containing TGF-ß together with IL-6 supported RORγt expression and development of Th17 cells. The effects of MSC supernatants were blocked by the inclusion of neutralization monoclonal antibody anti-TGF-ß or anti-IL-6 into the culture system. The results showed

that MSCs represent important players that reciprocally regulate the development and differentiation of naive T-cells into anti-inflammatory Foxp3+ Tregs or proinflammatory RORγt+ Th17 cell population and thereby can modulate immunopathological or transplantation reactions.

(33) INFILTRATION OF COLORECTAL CARCINOMA BY S100+ DENDRITIC CELLS AND CD57+ LYMPHOCYTES AS PROGNOSTIC FACTOR AFTER RADICAL SURGICAL TREATMENT

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Introduction: S100+ dendritic cells (DC) and CD57+ lymphocytes are factors reflecting the immune system ability to suppress the progress of tumor growth. Group of CD 57+ cells includes natural killers (NK) and late stages of T effectors lymphocytes.

Aim: The authors evaluated the relationship between the known clinical and histological factors and the presence of S100+ and CD57+ cells in the tissue of colorectal carcinoma with the aim to detect patients with high risk of short overall survival (OS) or short disease free interval (DFI) after radical surgical treatment.

Methods: There were data of 150 patients (97 males and 53 females) in analyzed patientsę cohort that underwent elective radical surgical procedure for colorectal cancer. The influence on DFI and OS of following parameters was evaluated: grading, staging, positivity of S100 and CD57 by immunohistochemical staining.

Results: OS 1, 3 and 5 years was 92,2 %, 76,5 % and 70,2 %, DFI 1,3 and 5 years 85,3 %, 64,3 % and 49,4 %. CD57 positivity in the tumor mass was proved as a positive prognostic factor for OS. Risk of short OS is 2,5-fold higher in patients with the low tumor infiltration by CD57+ lymphocytes. The combination of N2 stage of lymph nodes and absence of CD57+ cells was proved as the strongest negative prognostic factor for OS. No significant influence of CD57 positivity on DFI appeared. Authors did not prove any significant influence of S100 positivity on OS or DFI.

Conclusion: The presented study proved infiltration of colorectal cancer tissue by CD57+ cells as an important prognostic factor for OS.

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(34) Authentication of cell lines of colorectal cacinoma

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Background: Cell lines are routinely used in basic and applied research including drug development as models of normal and tumor tissues. A cross-contamination and misidentification seem to be a serious problem in validity of data obtained from the experiments. Recent efforts to correct this situation have led to establishment of standards for authentication of human cell lines. Examination of STR (short tandem repeats) polymorphisms, the method widely used in forensic genetics, was found to be the most appropriate solution. The aim of our work was to introduce cell line authentication in our laboratory to study genome integrity (STR instability) of cell lines during long-time cultivation and transfection experiments.

Methods: We have studied cell lines of colorectal carcinoma Caco 2, Colo 320, HT-29, SW 480 and SW 620. Cell lines were grown in standard DMEM medium supplemented with penicillin/streptomycin and mercaptoethanol. DNA was isolated from the harvested cells (DNeasy Blood & Tissue kit) and polymerase chain reaction (PCR) was performed to amplify regions of interest containing selected STR loci (CSF1PO, D13S317, D16S539, D5S818, D7S820, TH01, TPOX, vWA). Subsequently, fragment analysis by capillary electrophoresis (ABI 3700) was performed to assess the length (bp, number of repeats) of selected STR alleles.

Results: We have approved correct identities of 4 cell lines out of 5 examined, nominally Caco 2, HT-29, SW480 and SW620. On the other hand, cell line Colo 320 was different in 3 of 8 STR loci (vWA, D13S317, CSF1PO), this means that our cell line Colo 320 fails to satisfy condition of identity of this cell line. Surprisingly all lines used in our laboratory have been incorrect in marker CSF1PO in relation to declared.

Conclusion: We have introduced a new method in our laboratory. Cell lines of colorectal carcinoma Caco 2, HT-29, SW480 and SW620 with confirmed authenticity will be used in following research. However, cell line Colo 320 used in our laboratory is disqualified for further use. The cause could be long term cultivation which caused acumulation of mutations and resulted in phenotype deviation. Based on our results we conclude that marker CSF1PO is more sensitive to change in sequence than other markers.

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(35) BIOLOGICAL EFFECT OF ORGANOSELENIUM COMPOUND ON HUMAN TUMOR COLORECTAL CELLS IN VITRO

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Colorectal cancer is among the most common cancers. Diet, its specific component, is one of the risk factor in the development of the disease.

Selenium as an essential trace element play role in enzymatic functions in organism, it is also important antioxidant. Selenium is involved in many biological processes as selenocysteine incorporated to enzymes.

In present, there are many studies, which are engaged in chemoprevention effect of selenium, which encompass stimulation of protective cellular mechanisms in control cells and inhibition of proliferation in malignant cells. Cellular and molecular effects of selenium compound depends on chemical form, dose and type of in vitro model.

The aim of our study is to examine the effect of organoselenium compound 1,4-phenylenebis(methylene)selenocyanate (p-XSC) on two colorectal carcinoma cell lines HT 29, which has mutation in gene p53 and APC gene and HCT 116 +/+ with wild type p53. Our results show first information about cytotoxic and growth-suppressing effect of p-XSC. Cytotoxic and growth-suppressing effect of p-XSC was measured by WST-1 assay, which indicate the activity of mitochondrial dehydrogenase. Cell proliferation was measured by EdU assay. The method is based on incorporation of thymidine analogue to DNA during it's synthesis.. Cell cycle progression and apoptosis was evaluated by flow cytometry,using propidium iodide,which binds to DNA, one dye per 5 base pairs of DNA. In all cases, cells were incubated 24 h with tested compound.

In future, we will focus on western blott analysis of selected apoptotic and proliferating markers.

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(36) CHEMOSENSITIVITY TESTING OF HUMAN OVARIAN CANCER CELLS AND ITS IN VITRO MODEL

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Ovarian cancer is the most common cause of death from gynecological mallignities in Europe and North America, being the third most frequent and the first as to the mortality. Standard first-line chemotherapeutic treatment involves the administration of carboplatin, in advanced stages combined with paclitaxel. Introducing chemoresistance testing of human ovarian cancer cells may help to choose optimal drug and customize the individual chemotherapeutical regimens in patients. One of approaches of individualization of chemotherapy is in vitro chemosensitivity testing. In our study, we evaluated the cytotoxic effects of six selected cytostatics – cisplatin, paclitaxel, carboplatin, gemcitabine, topotecan and etoposide – in cells isolated from ovarian tumours and ascites of individual patients. Effects of selected cytostatics on cell viability were determined by MTT assay. In our group of clinical samples the highest sensitivity showed cells to topotecan, sensitivity to cisplatin was higer than to carboplatin and paclitaxel used in clinical practice showed the most often only the marginal reactivity. Resistance to carboplatin and most of the time to gemcitabine and etoposide was commonly present. We realized the same in vitro chemosensitivity testing in a model system, human ovarian cancer cell line A2780. In these cells the highest sensitivity was repeatedly detected to topotecan, gemcitabin, paclitaxel and cisplatin. The lowest sensitivity showed A2780 cells to carboplatin.

(37) *IN VITRO* AND *IN VIVO* CHARACTERISTICS OF THREE RAT SARCOMA CELL CLONES

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The C4, C7 and D6 clones were isolated from the R5-28-2 rat sarcoma cell line that was derived from spontaneous tumour developed in the Lewis rat female. Clones differed in morphology observed in vitro. Both C7 and C4 clones showed fibroblast-like

shape with a smaller size of the C4 cells. On the contrary, D6 cells were almost round with short processes. Slightly higher proliferation was found in this clone (50 %) in comparison with the C4 and C7 clones (both 40 %) as revealed by the BrdU technique. Immunocytochemistry also detected similar expression of vimentin, fibronectin, laminin, collagen IV, PCNA, MMP-2 and MMP-9 in all cells of the three clones.

We found high expression of MCP-1, TIMP-1 and VEGF in all three clones secreted into culture medium by protein chip assay. They secreted also lower and variable amount of CINC-2 with the highest level in the D6 clone. Moreover, the C7 clone was characteristic by high expression of LIX.

Karyotype analysis classified the clones to the group of sarcoma cells with chaotic karyotype. Differences in chromosome number were detected among three clones and among cells of individual clones. The highest chromosome number was found in the C4 clone (2n = 130 ± 8). Lower chromosome number and higher intercellular variability was observed in both the C7 clone (2n = 94 ± 20) and the D6 clone (2n = 55 ± 20).

To test tumorigenic potential the tumour cells of each clone were injected subcutaneously to eight Lewis rats. It produced tumours in all animals. The most rapid tumour growth was in the D6 clone. Tumours appeared 2 weeks after cell injection and rats had to be killed due to large tumour size after next 2 weeks. Tumours arising from the C4 and C7 clone started to develop 4–5 weeks after cell inoculation and grew for 2–4 weeks. Spontaneous tumour regression (62.5 % of animals) was observed in the C4 clone only. No metastases in inner organs were detected in any of the clones.

This work was supported by the MEYS CR (grant No.2B08063).

(38) Influence of pulsed electromagnetic field and field of pulse vector magnetic potential on sarcoma cells and skin fibroblasts of the Lewis rat

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The vector magnetic potential was artificially created as a mathematical help for cross-translation of equations describing magnetic and electric field without any force demonstration. One of possible sources of vector magnetic potential is the toroidal coil. A pulse magnetic field of magnetic induction is generated in the toroid core when a pulse

signal produced by pulse power generator supply the toroid winding. The pulse magnetic field is accompanied by the field of pulse vector magnetic potential (PVMP) outside of the core. Effects of various electromagnetic fields are well documented in studies of normal and cancer cells. However, data about influence of PVMP are missing.

Cytokines are biologically active small proteins produced by various cells. They are involved in normal developmental and pathological processes. Aims of these in vitro experiments were to analyse cytokines expression in normal and cancer cells of rat and to detect if the pulsed electromagnetic field (PEMF) and field of PVMP is capable to change expression of secreted cytokines.

The Lewis rat sarcoma cell line R5-28-7 derived from spontaneous subcutaneous tumour and skin fibroblasts isolated from explanted ear culture were used for this study. Petri dishes with the cells were placed on the applicator with electromagnetic and/or toroidal coils in thermoregulation box (37 °C) and irradiated with PEMF and/or field of PVMP of the same characteristics (125Hz, 2ms pulses, duty cycle 25 %) for 20 min. Control cells were placed in thermobox outside the applicator. Cultivation medium was collected 24 hours after the treatment and screened for expression of 19 cytokines by protein chip antibody assay. Both cell types secreted similar levels of MCP-1, TIMP-1, VEGF and b-NGF into culture medium. In addition, sarcoma cells expressed CINC-2 and IL-10 whereas fibroblasts produced MIP-3α. Neither PEMF nor field of PVMP caused any changes in the cytokine expression between irradiated and control cells.

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(39) TOXICITY AND ORGAN DAMAGE ASSOCIATED WITH OVERPRODUCTION OF GM-CSF BY GENE-MODIFIED MOUSE CELLS TRANSFORMED BY FUSION GENE (BCR-ABL) – KINETICS DAMAGE

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Background: Granulocyte-macrophage colony-stimulating factor (GM-CSF) is strong stimulator of the immune system.

Methods: Mouse bcr-abl-transformed 12B1 cells which induce leukemia and also subcutaneous tumors in syngeneic animals were transfected with mouse GM-CSF gene. Groups of mice were inoculated subcutaneously with either 12B1-GM-CSF cells or parental 12B1 cells. At intervals two mice from each group were sacrificed. Blood sample was taken for cytological investigation and for determining the GM-CSF level

in sera. At autopsy several organs were taken for histopathological, immunohistological, cytogenetic investigation and FACS.

Results: After administration of 12B1 GM-CSF cells the first clinical symptoms were observed on day 6, i.e. about one week prior to appearance of subcutaneous tumors. Simultaneously a raise of GM-CSF level in serum was detected, which gradually increased until day 16. On day 10 the first signs of damage to lungs (haemorrhagy), On day 16 the signs of damage – heart (hypertrophy), spleen (megakaryocytes were detected, extramedullary haematopoiesis), liver (extramedullary haematopoiesis, apoptosis, activated of endothelium) and kidneys (tubular cell damage) were detected. These progressed in the subsequent days. Except of hepatosplenomegaly similar changes were not seen in mice inoculated with parental 12B1 cells. Starting on day 8 a marked increase of myeloid- derived suppressor cells was seen in 12B1-GM-CSF inoculated mice. Their numbers steadily increased and on day 18 they surpassed more than 10 times their counts in 12B1-inoculated mice and control animals On the other hand , similar counts of T-reg cells were detected in both 12B1- and 12B1-GM-CSF inoculated animals.

Conclusion: The data strongly suggest that the extensive organ damage seen was caused by the overproduction of GM-CSF by the gene-modified cells.

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(40) Stem Cells in the Process of Metastatic Breast Cancer

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Background: Metastasis cascade is a series of biological process which includes cancer cells intravasation, their circulation in blood and lymphatic veins, extravasation and subsequent growth in the distant organs forming metastatic locus. Circulating tumor cells (CTCs) play a crucial role in this process. These cells present very heterogeneous population on a single cell level.

As explanation of this phenomena might be a process of epithelial-mesenchymal transition (EMT). Cancer cells obtain within this process similarly to mesenchymal cells

a high mobility and invasiveness. Further, there are data about existence of specific CTCs subpopulation, which behavior reminds stem cells – "cancer stem cells" (CSCs). They can be developed by transformation from "normal" stem cells as well as induction of EMT in more differentiated cancer cells. Some studies showed that 60–70 % of CTCs has shown expression of stem cell associated genes.

The research goal of our team was to characterize CTCs of breast cancer patients, within chemotherapy treatment with a focus to monitor their gene expression profiles. Within the gene expression profiling, several EMT- process associated genes (e.g. TWIST1, ALDH, PI3KA) and stem cell associated genes (CD24L, CD44, FOXO-3, CXCR1) have been included. The first comparison of clinical characteristics and CTC- gene expression profiles will be presented with a clear target to detect the risk of breast cancer disease recurrence in chemotherapy treated patients.

Methods: In primary BC five ml blood was collected before and after chemotherapy (n = 87 patients). In metastatic BC 5 ml blood of 72 patients was studied either at the time of relapse of BC or at a documented progressive BC before receiving new therapy. All samples were analyzed for CTCs using the AdnaTest BreastCancer (AdnaGen). CTC-cDNA was gene-specifically pre-amplified for multimarker qPCR analysis on the Biomark® (Fludigm, USA) microfluidic chip for 48 genes in each of 48 samples (2034 rxn in total). qPCR data were analyzed with GenEx ver. 5.0 (MultiD, SE) and correlated to available clinical data.

Conclusion: Gene expression profiling of CTCs is a potential discriminator of patients with therapy sensitive and therapy resistant tumor cell populations and therefore with good and inferior prognosis.

(41) Immunization against markers of cancer stem cells

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In recent years, cancer stem cells (CSC) have been identified in numerous human cancers. As these cells are able to initiate and propagate tumor growth they need to be eradicated to cure patients with malignant diseases. Unfortunately, CSCs are resistant to chemotherapy, radiotherapy, and targeted molecular therapy. Thus, immunization against CSC markers could be an important method of CSC elimination.

To identify CSCs, cell surface markers are usually utilized, but unique CSC-specific markers are still missing. Gene expression profiling have shown a shared transcriptional program of embryonic stem cells (ESC) and CSCs which suggests that the regulatory network controlling the function of ESC may also be active in CSCs. The key regulators

of ESC identity including self-renewal are transcription factors Oct-4, Sox2, and Nanog. The production of these proteins has also been shown in various human tumors. For development of DNA vaccines against CSCs, we chose Sox2 and Nanog as target antigens. Their potential oncogenicity was reduced by the mutagenesis of nuclear localization signals. To break tolerance and increase immunity induced against these self-antigens, the corresponding genes will be modified by the addition of cell-localization signals and sequences from the tetanus toxin (TT) gene encoding strong helper epitopes activating Th cells.

To study the effect of helper-epitope localization in a protein molecule and the effect of chimerical-protein cellular localization, we fused the TT helper epitope p30 (aa 947 to 967) with the N- or C-terminus of the mutated E7 oncoprotein (E7GGG) of human papillomavirus type 16 (HPV16) and altered cellular localization of the fusion constructs with signal sequences targeting proteins into endoplasmic reticulum and endosomal/lysosomal compartment. After DNA immunization with a gene gun, we found induction of CD4+ T cells recognizing p30 and enhanced response of E7-specific CD8+ T cells. The impact on these immune responses was affected by cellular localization of the proteins.

As embryonal carcinoma cell lines produce Sox2 and Nanog they can be used for evaluation of the efficacy of developed vaccines. To find other models for examination of these anti-tumor DNA vaccines, we searched for the expression of Sox2 and Nanog in various mouse tumor cell lines and proved Sox2 production in TC-1 cell line derived from lung cells.

This project was supported by grant NT/11541-4 from the Czech Ministry of Health.

(42) Effect of histone deacetylase inhibitor valproate on expression of CD133 in neuroblastoma cell line

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Recently, it is suggested that cancer stem cells (CSCs) play an important role in carcinogenesis and tumor progression. CD133, marker of the stem and progenitor cells in various tissues, is sign of worse prognosis in tumors. Neuroblastoma (NBL), a tumor of the peripheral sympathetic nervous system, is biologically heterogeneous, prognosis of high-risk (HR-NBL) tumors is poor, because chemo-resistance arises in those patients.

Nowadays, epigenetic therapy is considered as a promising approach to target CSC. Histon deacetylase inhibitors (HDACi) are tested as anticancer agents which can induce reexpression of epigenetically silenced genes in tumors. Valproic acid (VPA) that is

used for epilepsy treatment, is a member of HDACi possesses anticancer properties such as inhibition of the cell cycle and induction of apoptosis. The aim of our study was to identify the effect of the VPA on the expression of CD133 in UKF-NB3 cell line derived HR NBL.

UKF-NB3 was cultured in both IMDM medium with 10% fetal serum, and serum free medium (SFM) consisted of: Advanced DMEM/F12 medium with EGF, BFGF, Heparin and B27. Cells were harvested in three consequent days incubation in 1mM of VPA. Expression of CD133 was detected by flow cytometry and quantitative RT-PCR. The percentage of live, apoptotic, and dead cells after drug treatment was estimated by PI/Annexin-binding assays. Cell cycle analysis of CD133+ and CD133– ratio was done using flow cytometer.

The flowcytometric analysis of CD133 showed significant increase of CD133+ cells in the samples treated with VPA in both media compared with controls. The values of RT-PCR were correlating with the cytometric results. There was not difference in viability and apoptosis between the control and sample treated with VPA in first two days, which can exclude hypothesis that the increase in CD13t3+ cells is just relative to death of CD133- cells.

We have suggested some reasons which can contribute to this enlargement; either directly by VPA action or indirectly. One is that CD133+ cells are resting more in S-G2-M phase where it may escape the cell cycle shift toward G0/G1 induced by valproic acid and SFM. Moreover, the increase in CD133 ratio could be a sign of acquiring chemo-resistance.

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(43) Spatial correlation of B-Myb/c-Myb expression and proliferation in stem cell niche of mouse incisors

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Mouse incisor development starts in the second half of embryogenesis but the tooth keeps growing continuously during postnatal life. This suggests the presence of stem cells in the incisor. As a stem cell niche was described an area in the apical part of the tooth epithelium (cervical loop) with a very high proliferation activity (1).

The Myb family of transcription factors is known to be involved in proliferation, differentiation and apoptosis. B-Myb has been proposed to represent one of the several

characteristics of the stem cell state (2), and c-Myb is commonly produced in undifferentiated cells (3).

Therefore, we aimed to investigate whether Myb molecules are present in the proposed stem cell niche of mouse incisors. For this purpose, immunohistochemistry was applied in saggital sections of the mouse lower jaw at 17.5 days of embryonic development. Proliferating cell nuclear antigen (PCNA) was colocalized with B-Myb and c-Myb in serial sections.

The B-Myb and c-Myb proteins were found in both parts of the incisor, the epithelium and the mesenchyme, however, neither of them was restricted to the area of extensively proliferating stem cells of the cervical loop. Nevertheless, the posterior part of the superior cervical loop displayed high expression of all molecules under study. Notably, PCNA negative region of the mesenchyme facing the epithelial tip of the cervical loop was also negative for B-Myb and c-Myb. Moreover, B-Myb expression spatially copied the pattern of Notch 1 expressed in the cervical loop cells and stratum intermedium (4) and implicated in early tooth morphogenesis and tooth differentiation. The c-Myb as well as B-Myb and PCNA proteins were predominantly present in the apical half of the tooth germ, whereas the basal mesenchyme between the cervical loop tips was almost negative. As in the molars (5), incisor odontoblasts in general were c-Myb positive but B-Myb negative.

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(44) Scalable High-density monolayer culture of human pluripotent stem cells increases rate of teratoma formation

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The high-density monolayer culture on Matrigel matrix is being used for many applications including differentiation and transfection. In this work, we show that this culture system is an efficient and robust method to maintain high-quality pluripotent stem cells, which are more viable for any detrimental manipulations than colony-based growth. After more then 70 passages, these cells retain the key characteristics of pluripotent cells including expression of marker genes, differentiation potential, and stable karyotype. Interestingly, cells from high-density culturing markedly increase the efficiency of teratoma formation when injected into the immunodeficient mouse (95.5 % for monolayer cells vs. 45 % for colony growth). After series of dilution experiments and viability test, we found that this phenomenon is most likely caused by superior viability of high-density cultured human embryonic stem cells. This improves their survival shortly after injection into the mouse and subsequently increases the efficiency of teratoma formation. Importantly, these cells are not transformed as they are sensitive to suboptimal culture conditions, resulting in differentiation and/or cell death. Moreover, this monolayer culture system is fully reversible, as these cells can be reverted to colonies possessing normal features of colony growth. Taken together, the high-density monolayer culture enhances the viability of human pluripotent stem cells, which provides a more robust and effective culture system.

(45) The two faces of FGF2 in metabolic pathway regulation in hESCs

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Human embryonic stem cells (hESCs) are distinguished by pluripotency and self-renewal and thanks to these features are the potential source of cells for therapy.

It has been known, that FGF2 maintains pluripotency in hESC. While the effect of FGF2 on pluripotency signaling has been under careful investigation, not much is known about its effect on energy metabolism. The idea that hESCs rely preferentially on a “clean” glycolysis rather than on “dirty” oxidative phosphorylation matches the need of hESCs to maintain intact genome. It is also manifested by rather undeveloped, circular mitochondria with absent cristae in hESCs compared to their differentiated counterparts. In this study we have focused on defining the role of FGF2 in the switch between glycolysis based metabolism in hESCs and oxidative phosphorylation based metabolism in differentiated cells.

By the use of chemical inhibitor dichloroacetate (DCA) we inhibited pyruvate dehydrogenase kinase (PDHK), leading to increased pyruvate flux into the mitochondria and thus shifting the metabolism of the cells from glycolysis toward the oxidative

phosphorylation. Eight days of DCA treatment resulted in rapid decrease of the proliferation capacity of cells cultivated in absence of FGF2, while the presence of FGF2 partially rescued the phenotype, enabling the cells to proliferate even under the conditions of limited glycolysis. Viability of the cells remained constant under all tested conditions making the difference only in the function of proliferative capacity. Although the DCA treatment significantly affected proliferation rate, these changes were not accompanied by a decrease of expression of undifferentiated stem cell markers NANOG and SSEA3, suggesting that it is not differentiation which enables the cells to survive in the presence of DCA, despite the fact that mitochondria undergo structural changes during the DCA treatment that are similar to changes observed during differentiation.

These results shed new light on the FGF2 function in metabolism. While it maintains the pluripotency status, including non mature mitochondria and glycolysis based metabolism, it can also support survival of pluripotent cells under conditions favoring the oxidative phosphorylation.

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(46) Complex CDK2/cyclin B1 and its role in human embryonic stem cells

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Human embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of human blastocyst. When human ES cells are propagated in culture, they must ensure their pluripotent state. The capability of having undifferentiated status in vitro is maintained by unique cell cycle regulation. However, little is still known about cell cycle regulation and sustaining pluripotency in embryonic stem cells.

It has been previously shown that cyclin-dependent kinases (CDK), key molecules involved in cell cycle regulation, provide a link between pluripotency and self-renewal

in human ES cells (Hoof et al., 2009). Especially, CDK2 (the key molecular player in G1/S transition) emerged as central molecule in controlling self-renewal and cell-fate decision (Neganova et al., 2009).

Here we indentified novel interaction between CDK2 and cyclinB1. CDK2 in human ES cells physically associates with cyclin B1. This complex is able to phosphorylate histone H1 and RB in vitro with maximal activity in mitosis of human ES cells. Double knockdown of CDK2 and cyclin B1 cause accumulation of hESC in G2/M, suggesting that this complex might play a crucial role in mitosis of human ES cells.

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(47) Intracerebellar transplantation of P19 carcinoma stem cells and P19-derived neuroprogenitors in Lurcher mutant and wild type mice

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Degenerative diseases including those of CNS and cerebellum could potentially be treated using stem cells (SC), including the embryonic carcinoma cells which could disrupt degenerative vicious cycle. Lurcher mutant (Lc) mice represent a model of olivocerebellar degeneration. They suffer from postnatal loss of Purkinje cells. The aim of the work was to assess survival of P19 embryonic carcinoma cells (EC) and neuroprogenitors derived from these cells grafted into the cerebellum of adult B6CBA Lc and wild type (WT) mice.

The EC cells were cultured without differentiation or differentiated into neuroprogenitors using the retinoic acid. In 25 WT and 25 Lc mice naive P19 cells were

injected. Neuroprogenitors were transplanted in 26 WT and 25 Lc mice. 3 weeks later the mice were sacrificed and grafts were identified according to GFP.

The graft was found in 53.8 % and 32 % WT mice that received neuroprogenitors and naive EC cells respectively. Both naive EC cells and neuroprogenitors survived only in 16 % in Lc mice. The differences are not significant. In WT mice treated with naive P19 cells, expansive appearance of the graft was more frequent ($p = 0.0017$) than in mice treated with neuroprogenitors. In mice that received naive P19 cells no signs of destruction were observed, while the destruction appeared in 42.9 % and in 25 % of neuroprogenitor treated WT and Lc mice respectively. In Lc mice both types of grafts were localised usually in the mesencephalon. If the grafts were in contact with the cerebellum they did not invade it while the demarcation against the mesencephalon was not strict. On the contrary, in WT mice the grafts were found mostly in the cerebellum. These differences are significant (for NPG $p = 0.0049$; for P19 $p = 0.0101$). This would indicate that neurodegenerative changes of the cerebellar tissue could have a negative impact on the fate of EC cells grafted into the cerebellum of Lc mice.

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(48) IMMUNOHISTOLOGICAL CHARACTERIZATION OF GRAFTED TISSUE AFTER NEUROTRANSPLANTATION IN THE MOUSE MODEL OF CEREBELLAR DEGENERATION. A PILOT STUDY

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Regeneration in the damaged central nervous system (CNS) is limited and therapy of CNS diseases accompanied with neuronal loss is problematic. Neurotransplantation based therapies have great potential for future treatments of various diseases not only by replacing missing or malfunctioning cells but mainly by supporting the inner regenerative processes.

Lurcher (Lc) mutant mice represent a model of olivocerebellar degeneration – heterozygotes suffer from a complete loss of cerebellar Purkinje cells and a secondary reduction of granule cells and inferior olive neuron number.

The aim of our project was to characterize the phenotype of cellular elements that develop from the GFP positive stem cells (partially differentiated into neuroprogenitors) and cerebellar embryonic tissue (solid graft and cell suspension) transplanted into the cerebellum of Lurcher mutant and healthy control wild type mice.

The cells were characterized by immunohistochemistry for presence of specific markers for neuron-restricted progenitors (MAP 2), Purkinje cells (calbindin) and glia – e.g. astrocytes (GFAP). The first results show that grafts of all types are composed of a lot of glia cells situated especially on the graft periphery and that Purkinje cells are present in solid embryonic grafts as well as in embryonic cerebellar grafts injected as a cell suspension. However, Lurcher mutant cerebellum seems to provide less permissive milieu for the cell suspension grafts than normal cerebellum.

In this pilot study, we focused on the basic examination of graft survival and we determined the cell types in which grafted cells and embryonic tissue differentiated in the host cerebellum.

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(49) Proliferation and death of cells in the brain of young-adult and old mice in restrospection and perspective

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Proliferation of cels in mature brain is extremely low except the forebrain periventricular/ subependymal zone (PVZ), the cell progenitor source. Here we report frequency of dividing cells in different regions of the mouse brain, their changes with age and turnover detected by 3HTdR labelled DNA (3H- DNA). As shown earlier by autoradiography (1, 2), the DNA synthesizing cells in mature parenchyma of all brain regions represents only several promiles of total population while in the PVZ it ranges around 20 %. Except the PVZ, the microgranular populations of the olfactory bulbs and the hippocampus, dividing cells were of glial or vascular type or of an immature and unspecified dark phenotype. With age, number of these cells decreased so that they almost disappeared in the hippocampus at age of 9 month while they were still present in other brain regions in 16 month old i.e. senescent mice. The half-time life of the newly

synthesized bulk isolated 3H-DNA was, however, only 72–96 h in all brain regions (3). As shown by autoradiography, labelled cells (expecially in the PVZ) occurred often in small groups/nests of irregular size, frequency and intensity of labelling suggesting different proliferation rate/pool or disappearence of postmitotic daughter cells or apoptosis (4). Migration of cells from the PVZ in our study is indicated by translocation of some 3H-DNA to the olfactory bulbs. Its peak value, reached on post-injection day 7, was followed by the drop similar to other brain regions (4). The cell death is suggested by the short half-life time of the 3H-DNA and occasional pyknoses. We assume that cell division randomly bursts in solitary or small groups of precursor/progenitory cells scattered throughout the whole brain and that under physiological conditions many of daughter cells die e.g due to absence of survival signals. Alterations in cell division/cell loss equilibrium may result in local or diffuse hypo- or hyperplasias. The cell escaping this control may also represent a germ for some brain tumors.

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(50) THE CELL DAMAGE AND REGENERATION IN THE IMMATURE RAT BRAIN AND GLIOMA CULTURES IRRADIATED BY EPITHERMAL NEUTRON BEAM OF THE LVR-15 NUCLEAR REACTOR IN ŘEŽ

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Interaction of epithermal/thermal neutrons with boron-10 (^{10}B) provides a possibility of higly circumscribed targeting of lethal damage of tumors even to solitary cells interspaced in normal tissue. The nuclear reactors used for the Boron-Neutron-Capture-Therapy require, however, individual tuning and monitoring by physical and biological methods. Here, were report effects of epithermal beam of the LVR-15 in Řež (7.8×10^8 n/cm^2, 9 MW, 16.1 cGy/min) on the immature rat brain and the rat C6 glioma culture, the models developed for this purpose (Mares et al. 1997, Burian et al. 2006).

Irradiation of animals for 4 to 7 min induced radiation dose- and cell differentiation degree-dependent apoptosis-like death of cells most expressed in the immature/germinative zones of the cerebellum and around the forebrain ventricles, exceeding 2.1 times gamma radiation equivalent. Within the next 3 days, the cellular debris disappeared and process of regeneration has started. The latter was inversely proportional to the radiation dose and was highly incomplete in the animals treated with higher radiation doses In cultures (irradiated for 30 to 45 min), cells slowed-down cycling and the cell death reached peak values at 72 to 96 h. The regeneration process, measured by re-growth of cell populations 3 to 21 days after irradiation, was also inversely proportional to radiation dose. In addition, surviving cells hypertrophied and formed thick and long processes resembling reactive astrocytes. Catalytic histochemistry showed that these cells up-regulated γ-glutamyltranspeptidase and dipeptidylpeptidase-IV activity suggesting new enzyme defense mechamisms of tumor cells against the radiation induced metabolic/oxidative stress. The acute and late/regeneration radiation changes were significantly amplified in presence of ^{10}B delivered by sodium borocaptate and ^{10}B-phenylalanine-fructose complex, Katchem, Řež) in both models used and the damage extented to more mature early postmitotic cells.

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Lonza

Média - séra - transfekce - agarózy - detekce endotoxinu

Lonza je specialistou v primárních a kmenových buňkách, klasických i bezsérových kultivačních médiích a reagenciích. Je hlavním a největším světovým producentem agarózových produktů pro klasickou elektroforézu (Gambrex). Dále nabízí kity pro detekci mykoplazmy a především kity pro detekci endotoxinu (LAL kity). Nabízí také Amaxa® Nucleofector® technologii pro transfekci primarních buněk a těžko transfekovatelných buněčných linií a inovační StellARray™ systém pro výzkum genové exprese.

Molecular Devices MDS

ELISA readery - luminiscence - fluorescence

Absorbanční (ELISA), fluorescenční, luminiscenční i multimode mikrodestičkové readery, SOFTmax® PRO software a validace, promývačky, dispenzery a rozplňovací soupravy Aquamax, automatizace - StakMax Microplate Handling System, buněčné sběrače, IonWorks™, Treshold, reagenční kity; CellKey Systems, NEW CellKey384 System, Cellular Imaging, Electrophysiology, Fluorometric Imaging Plate Readers, Liquid Handling.

Biohit

Manuální pipety - elektronické pipety - multipipety

Manuální jedno- i multikanálové pipety Proline, Proline PLUS, mLINE s fixním i nastavitelným objemem, jedno-, čtyř- a osmikanálové elektronické pipety s nastavitelným objemem eLINE, Proline a ePET, elektronické dispenzery, mechanické stěrky a lahvové dávkovače. Široká nabídka příslušenství: špičky s filtrem i bez, sypané i v krabičkách, sterilní i nesterilní, stojánky otočné i lineární.

Origio

IVF - PICSI - média - mikropipety

Origio - nové obchodní jméno sdružující firmy Medicult, (média pro humánní In Vitro fertilizaci, IVF), Humagen Fertility Diagnostic (mikropipety pro IVF) a MidAtlantic Diagnostic (instrumentace pro IVF)

Cell Biosciences

Dokumentační systémy - záznam a digitální zpracování obrazu

Dokumentační systémy pro záznam, archivaci a analýzu gelů; CCD kamerové systémy pro detekci signálu-chemiluminiscence, fluorescence, microarrays.Digitální zpracování obrazu dle požadované aplikace od funkční genomiky po analýzu genové exprese.

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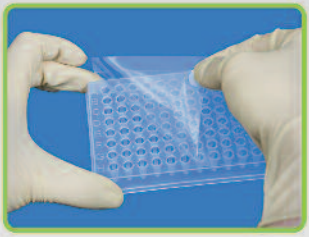

ISOHELIX
integrovaná strategie práce s DNA

- pouze ISOHELIX DNA bukální tampóny, systém Spin + Collect a DNA Quality Check kit umožní efektivní a ekonomickou izolaci DNA ze stěru slizničních buněk
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MGP

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