Institute of Biophysics, Academy of Sciences of the Czech Republic, v. v. i.

Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno

International Clinical Research Center, St. Anne's University Hospital Brno

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OrganoNET 2014 Conference
BOOK OF ABSTRACTS

Annual conference of project OrganoNET - partnership for education and research in the field of tissue and organ visualization, reg. no.: CZ.1.07/2.4.00/31.0245.
Operation program: Education for Competitiveness

INVESTMENTS IN EDUCATION DEVELOPMENT

Masaryk University
Faculty of Medicine

OrganoNET 2014 Conference
Book of abstracts

June 19–20, 2014
Brno, Czech Republic

Brno 2014

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Dear colleagues,

Stem cell therapy and tissue engineering are promising avenues of modern regenerative medicine that join together experts from basic and applied biomedical research as well as clinically oriented investigators. Close collaboration of internationally recognized scientists and the mutual interactions among students, researches, early career scientists and biomedical companies is supported by European project OrganoNET, in the frame of the Operational Program Education for Competitiveness. After a two year effort, the functional relations have been established among subjects defining, developing, and utilizing imaging approaches, technologies, and instruments, thus contributing to development of various aspects of regenerative medicine and tissue engineering. OrganoNET culminates by organizing its final conference in June 19-20, 2014.

Participation on the OrganoNET 2014 conference is a great opportunity to meet and share experience in various fields of advanced imaging research, ranging from tissue development to stem cell biology and cancer research in four conference sections, *Stem cell biology & Applications, Cancer biology & Therapy, Inflammation and vascular biology and Hypoxia.*

I believe you will enjoy the opportunity to meet and share experiences in the rapidly developing and dynamic field of cell and tissue visualization. On behalf of the conference organizers I wish you the enjoyable meeting.

Aleš Hampl

Head of Department of Histology and Embryology
Vice-dean for research
Faculty of Medicine, Masaryk University

The conference is organized by:

Faculty of Medicine, Masaryk University
Department of Histology and Embryology
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ORGANONET 2014 CONFERENCE
June 19–20, 2014

PROGRAM

Day 1 June 19, 2014

7:30 - 8:40 Registration
8:45 - 9:00 Welcome address

I. Section Stem cell biology & Applications
9:00 - 9:45 Martin Anger (VRI, CEITEC) - *Oocyte spindle assembly checkpoint in space and time*
9:50 - 10:10 Daniela Mináriková (DHE FM MU) - *Phenotypic plasticity and multicentrosomal mitoses in human embryonic stem cells*
10:15 - 10:35 Petr Vaňhara (DHE FM MU) - *Whole-cell mass spectrometry clusters morphologically uniform, yet differentiating hESCs*
10:40 - 11:00 Milan Ešner (DHE FM MU) - *Mitotic divisions with supernumerary centrosomes in human embryonic stem cells*

11:00 - 11:30 Coffee break

II. Section Stem cell biology & Applications
11:30 - 12:15 Ladislav Anděra (IMG ASCR) - *Life and death of stem cells*
12:20 - 12:40 Zuzana Garlíková (DHE FM MU) - *Differentiation of human pluripotent stem cells to lung epithelial cells in air-liquid and 3D organotypic culture*
12:45 - 13:05 Zuzana Koledová (DHE FM MU) - *Regulation of mammary stem cells by receptor tyrosine kinase signalling*
13:10 - 13:30 Pavel Ostašov (FM UK Pilsen) - *Neural stem cells and neuroregeneration*

13:30 - 15:00 Lunch

III. Section Cancer biology & Therapy
15:00 - 15:45 Jan Bouchal (UP Olomouc) - *Recent advances in research and clinico-pathological managment of prostate cancer*
15:50 - 16:10 Tereza Suchánková (DC IBP) - *Characterizing the mechanism of the synthetic lethal interaction between CHK1 inhibition* and DNA damage in normal and cancer cell lines
16:15 - 16:35 Eva Slabáková (DC IBP, CBCE-ICRC FNUSA) - *Regulation of MDM2 and MDMX expression in epithelial-mesenchymal transition*
16:40 - 17:00 Šárka Šimečková (DC IBP, CBCE-ICRC FNUSA) - *Pharmacological inhibition of SCFSkp2 complex activity affects characteristics of cancer stem-like cells*

17:15 - 17:45 Transfer to social evening. Transportation is provided.

18:00 - 23:30 Social evening

Day 2 June 20, 2014

IV. Section Inflammation and vascular biology

9:00 - 9:25 Katarina Szilagyi (Sanquin Research, Amsterdam, Netherlands) - *Decreased atherosclerosis in mice with defective SIRPalpha signaling - role of macrophages*

9:30 - 9:55 Hanke L. Matlung (Sanquin Research, Amsterdam, The Netherlands) - *Antibody-dependent cellular cytotoxicity of neutrophils towards cancer cells involves trogocytosis that can be further potentiated by targeting CD47-SIRPα interactions*

10:00 - 10:25 Simona Hankeová (MU & Karolinska) - *Notch signaling, Vasculature and Alagille syndrome*

10:30 - 10:55 Janne Atosuo (Univeristy of Turku) - *Myeloperoxidase in cell killing*

11:00 - 11:30 Coffee break

V. Section Hypoxia

11:30 - 12:15 Ben Wielockx (Inst. of Pathology, TU Dresden) - *The role of oxygen sensors during pathological and physiological conditions in mice*

12:20 - 12:40 Josef Večeřa (FS MU) - *Development of neural stem cells in HIF1alpha-knockout model*

12:45 - 13:05 Jan Kučera (FS MU) - *Intracellular signaling under hypoxic condition in stem cells*

12:10 - 13:30 Markéta Hanáčková, Kateřina Štefková (FS MU) - *Role of HIF1-alpha in hematopoiesis - in vitro study*

13:30 - 14:30 Lunch

VI. Section Cancer biology & Therapy

14:30 - 15:10 Jiří Kohoutek (VRI, FNUSA) - *Cyclin-dependent kinase 12, a new tumor suppressor?*

15:15 - 15:35 Kateřina Kratochvílová (DHE FM MU) - *TUSC3 alters UPR response and prevents EMT in ovarian cancer*

15:40 - 16:00 Pavel Pitule (FM UK Pilsen) - *Isolation and characterization of circulating tumor cells in colorectal cancer*

16:05 - 16:35 Zuzana Pernicová (DC IBP, CBCE-ICRC FNUSA) - *Modulation of cell cycle leads to phenotypical changes of prostate cancer cell lines*

16:35 - 17:00 Final remarks

List of abstracts

Oral presentation
Poster presentation

1. MALDI-TOF fingerprinting of the whole mammalian cell-lines

Amato, F.[1]; Kučera, L.[2]; Vaňhara, P.[2]; Hampl, A.[2,3]; Havel, J.[1,4,5]

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Robust cell-lines identification requires reliable databases of cell-lines fingerprints. Mass spectrometric fingerprinting of mammalian cell-lines is a promising way to achieve the goal of fast, cheap, simple and reliable cell-lines authentication. However, general and standardized method for mass spectrometric fingerprinting of the whole mammalian cell-lines is not available to date.

We developed, optimized and validated MALDI-TOF mass spectrometric method for reliable, specific and reproducible whole-cell fingerprinting of mammalian cell-lines. Quality and robustness of mass spectrometric fingerprints is demonstrated by statistical analysis of experimental data.

Developed method for the whole mammalian cell-lines fingerprinting, in contrast to actual procedures, is inexpensive, fast and it opens the door towards routine cell authentication.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Projects MSM0021622411, MSM0021627501, MSM0021622430, the Czech Science Foundation (Projects No. 104/08/0229, 202/07/1669), the Grant Agency of Masaryk University (MUNI/M/0041/2013), CEPLANT "R&D Centre for Low-Cost Plasma and Nanotechnology Surface Modifications" project CZ.1.05/2.1.00/03.0086 funding by the European Regional Development Fund and MUNI/A/1014/2013.

2. Nitro-fatty acids serve as regulators of classically and alternatively activated macrophages

Ambrožová, G.[1]; Kubala, L.[1,2]; Martišková, H.[1,3]; Rudolph, T. K.[4]; Klinke, A.[4]; Pekarová, M.[1]

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Nitro-fatty acids are currently suggested as highly promising compounds for treatment of diseases associated with immune homeostasis deregulation. The goal of our experiments was to characterize the role of nitro-oleic acid (OANO2) in classical and alternative activation of macrophages induced by bacterial endotoxin or IL-4. Special attention was focused on determination of specific signalling mechanisms, potentially responsible for OANO2 action in activated cells.

OANO2 modulated production of reactive oxygen species, nitric oxide and cytokines, as well as STATs expression and possible cytotoxic properties in lipopolysaccharide or IL-4 stimulated RAW 264.7 macrophages were detected.

$OANO_2$ is able to decrease the production of TNF-α, RANTES, IL-6, reactive oxygen and nitrogen species in classically activated macrophages, as well as IL-10, IL-4 and TGF in alternatively activated macrophages. Moreover, $OANO_2$ modulates iNOS expression and activation of STAT1, STAT3 and STAT6.

OANO2 was shown to inhibit endotoxin-stimulated expression of iNOS, with subsequent reduction of nitric oxide, as well as superoxide and pro-inflammatory cytokine production. We reported for the first time, that OANO2 effectively down regulates the production of IL-10, IL-4 and TGF in IL-4-activated macrophages. These effects were mediated via down regulation of STATs activation.

This work was supported by the grant of GAČR 13-40824P.

3. Use of microfluidics to achieve native phenotype of endothelial cells

Ambrůzová, B.[1]; Kolářová, H.[1,2,3]; Kubeš, M.; Binó, L.[3]; Víteček, J.[1,2]; Kubala, L.[1,2]

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Vasculature is a complex system composed of many cell types organized in a specific manner to fulfill its physiological function. Our main focus is the inner layer of endothelial cells. They are indispensable for maintenance of vascular tone and immune response. The native phenotype of these cells is characterized by elongated shape, organization along with the blood flow, tight contacts among them and remarkable glycocalyx layer at the luminal surface. Such phenotype is however suppressed under standard static in vitro cultivation. In order to get native phenotype endothelial cells (HUVEC) were cultivated under shear stress in a flow through system operated by IBIDI pump. Flow conditions resulted in elongation of cells and longitudinal orientation with flow. Further, elevated expression of glycocalyx-related genes was detected using RT PCR.

This work was supported by projects HistoPARK (CZ.1.07/2.3.00/20.0185), OganoNET (CZ.1.07/2.4.00/31.0245), FNUSA-ICRC (CZ.1.05/1.1.00/02.0123) and ICRC-ERA-Human Bridge (no. 316 345)

4. Oocyte spindle assembly checkpoint in space and time

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Mammalian oocytes and embryos frequently suffer from aneuploidy, caused by chromosome segregation errors. Most of the time, the aneuploidy originated in meiosis or in first mitotic cycles in developing embryos would prevent further development. However, in several cases, the extra chromosomes are tolerated, which leads into severe mental and developmental disorders, such as Down syndrome. The reason, why chromosome segregation errors are so frequent in germ cells and embryos, is unknown. It seems that the problem lies in less stringent control mechanisms operating in these cells.

In our study we have focused on a surveillance checkpoint mechanism called Spindle Assembly Checkpoint (SAC) during female meiosis I. Using live cell imaging multichannel microscopy we have tested, whether mouse oocytes are capable of detecting univalent chromosomes and single chromatids in meiosis I. We have also monitored the activity of SAC on every single kinetochore within individual cells throughout meiosis I. These events were detected together with chromosome movements, spindle formation, Anaphase Promoting Complex (APC) activation and polar body extrusion (PBE) simultaneously in individual oocytes at various time points during first meiotic division.

Our results showed that SAC in mammalian oocytes works differently, compared to the somatic cells. In contrast to the somatic cells, single chromatids, univalents and unaligned chromosomes are unable to prolong anaphase onset. Moreover, in oocytes from aged individuals, SAC proteins are displaced from individual kinetochores with different dynamics then in young oocytes. This indicates that checkpoint mechanisms operating in oocytes, which are involved in monitoring chromosome segregation, are insufficient in prevention of propagating the aneuploidy to the embryo.

5. Myeloperoxidase in phagolysosomes is not needed for killing the ingested bacteria

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There is an ongoing debate about the specific mechanisms of the killing of ingested microbes in phagolysosomes. Numerous reports demonstrate the involvement of the myeloperoxidase (MPO)-H_2O_2-halide system in the killing of microbes. The current view is that in normal neutrophils, HOCl is primarily responsible for oxidative killing. It has been shown that HOCl is a much more potent killer than H_2O_2 and therefore MPO- H_2O_2-halide system has generally been considered essential in killing. This is, however, disapproved by the fact that the persons suffering from subtotal or total MPO-deficiency with an estimated prevalence of 1 of 2000-4000 individuals have usually a normal health status. Surprisingly, reviews dealing with the role of MPO in killing ignore macrophages known to contain no MPO but capable of killing microbes.

The findings of Segal's group challenge the established view about the role of oxidants in direct killing of bacteria. According to their impression, the main role of NADPH oxidase is not superoxide generation but to polarize the phagosomal membrane via electrogenic transmembrane electron transfer thereby driving the influx of potassium ions to increase the ionic strength of the phagosome. Increased osmolarity then causes the solubilization of elastase and cathepsin G from proteoglycan complexes. Presumably these proteases then kill the ingested bacteria.

In this study, we experimentally show that the phagolysosomal concentration of H_2O_2 is high enough for killing one bacterial cell without the participation of MPO.

6. Hypoxia dependent control of myosine heavy chain expression in heart

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In mammalian heart two major isoforms of myosin heavy chain (MHC) are expressed. In mouse fetal heart MHC beta is the prevalent form. After birth, the expression of MHC alpha is induced. However the adult heart retains the ability to return to the fetal gene program in failing heart. We propose a hypothesis, that hypoxia, a condition common for the fetal and failing heart, particularly hypoxia inducible factor (HIF), plays a role in the postnatal MHC switch and potentially also in the return of the fetal gene program.

Here we present a unique model of the postnatal MHC switch in explanted mouse fetal hearts. Our data show MHC ratio changes in oxygen dependent manner, with hypoxia promoting fetal MHC beta expression and normoxia switching it in behalf of MHC alpha. Normoxic environment induces gradual decrease of MHC beta mRNA. Interestingly, if the heart is after extraction transferred immediately to hypoxic conditions, this MHC beta decrease is slowed down. In silico analysis revealed potential HIF biding sites in MHC coding region. ChIP analysis using one of the predicted sites reveals HIF binding in MHC beta coding sequence. Overall our results suggest that MHC isoform gene expression is regulated by oxygen level.

7. Recent advances in research and clinico-pathological managment of prostate cancer

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Recent studies have shown inherent problems with PSA screening for prostate cancer (CaP) and there are doubts that screening provides appreciable long-term benefit. The semi-random nature of the transrectal ultrasound-guided prostate biopsy process leads to an appreciable false-negative rate, as well as the diagnosis of increasing numbers of insignificant cancers that will not progress in a patient's lifetime. Current proposal for changes for future TNM staging will be presented. The basic and most fundamental challenge in CaP is the prospective separation of the indolent tumours that require little treatment from those that will progress, metastasize, and lead to early death. Markers for CaP monitoring, such as PCA3, AMACR, MSMB, Ki-67 and TMPRSS2-ERG will be described in detail. The second major problem is treatment of metastatic castration resistant prostate cancer. As our knowledge of the molecular circuitry of tumor progression and treatment resistance has become more refined, the number of new targets has grown exponentially. Abiraterone acetate, enzalutamide and immunotherapy will be commented in detail. Research of tumor microenvironment will be illustrated on periostin, asporin and phenomenon of solid stress. Last but not least, importance of interdisciplinary cooperation in both prostate cancer research and clinical managment will be highlighted.

8. Small molecules promotes *in vitro* cellular reprogramming of human dental pulp stem cells into synaptic neurons

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Macro-molecules like bio-proteins, enzymes and genes having special consideration and imagination of most researchers of different background. During the last five years "Small molecules" i.e. Chir99021, Rock inhibitor, Janus Kinase (JAK)-1 inhibitor, Retinoic Acid (RA) and Dickkopf (Dkk)-1 etc., have captured an exceptional attention of molecular biologists or biophysicist because of their significant role pathogenesis. Neurons and glial cells are the basic information processing structures in the Central Nervous System. Function of a neuron is to receive information (presynaptic) and process that information to the other or neighbouring neurons (post synaptic). Synapses acts as a communication bridge between neurons through information flows. In this study we have explained the role of small molecules (JAK1, Dkk1 and RA) that promotes the cellular reprogramming or differentiation of dental pulp stem cells into synaptic neurons.

After the optimization of all the concentration and time intervals, we used 100nM concentration of RA for 9 days, 10ng/mL of Dkk-1 for 12 days and 20nM concentration of JAK-1 inhibitor for 14 days. Based on correlation among seeded and differentiated cells, we found RA (78.12%) is more efficient in synaptic neurons differentiation followed by Dkk-1 (54.99%) and JAK-1 inhibitor (34.95%). Our data suggested that small molecules are more effective and efficient for synaptic and functional neurons differentiation; that can be helpful against neurodegenerative diseases i.e. Alzheimer, Parkinson's disease and amyotrophic later sclerosis.

This project work was supported by "Education for Competitiveness Operational Programme (ECOP) and co-financed by the European Social Fund and the state budget of the Czech Republic" with Project number CZ.1.07/2.3.00/30.0022.

9. Mitotic divisions with supernumerary centrosomes in human embryonic stem cells

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Centrosome is a key intracellular organelle serving as the organizer of microtubules in animal cells. Number of centrosomes is tightly regulated during the cell cycle. For proper mitotic division it is crucial that cell in M phase contains exactly two centrosomes, which form bipolar mitotic spindle. It was described earlier by our laboratory that cultured pristine human embryonic stem cells (hESCs), and to some extent also human induced pluripotent stem cells (hiPSCs), typically contain a large subpopulation of cells with supernumerary centrosomes in mitotic division. However, it remains unclear how this situation develops and dividing hESCs react to such unfavorable condition.

Here we tried to decipher possible mechanism(s) of acquisition of supernumerary centrosomes and the fate of the affected cells after mitotic division. For direct *in vitro* observation of hESC divisions we created several transgenic hESC lines carrying Histone-2A fused with green fluorescent protein (H2A-GFP) and performed multiple time lapse microscopy analyses followed by detection of multipolar mitotic divisions and by tracking the descending cells.

We detected and analyzed more than 12000 mitotic divisions and found 3% of multipolar mitotic divisions. Approximately 90% of them were tri-polar mitotic spindles. Cells from selected divisions were individually tracked over time. Surprisingly, cells originating from multipolar mitoses were viable and some of them divided further by normal bipolar mitoses. In some cases we also detected fusion of two nuclei before the formation of tri-polar mitotic spindle. We hypothesize that this situation may represent one possible mechanism by which hESCs acquire extra centrosomes.

This work was supported by funds from the Ministry of Education, Youth and Sports of the Czech Republic (CZ.1.05/1.1.00/02.0123, HistoPARK CZ.1.07/2.3.00/20.0185, and MUNI/A/1014/2013).

10. Differentiation of human pluripotent stem cells to lung epithelial cells in air-liquid and 3D organotypic culture

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Human embryonic stem cells and human induced pluripotent stem cells have the capacity to generate derivatives with phenotype of lung epithelial cell types. During differentiation, their phenotype may be affected by spatial organization of the cells within the *in vitro* culture system. We aimed to investigate how morphology of differentiating cells is influenced by air-liquid interface culture and by 3D organotypic culture. We used sequential treatment with soluble factors to drive the differentiation of human embryonic stem cells into the lung epithelial lineage. According to steps which occur during the development of the lung, we induced endodermal differentiation and further specification to anterior foregut endoderm – the origin of lung tissue. Using combination of growth factors, we than directed the differentiation towards distal lung epithelial cell types.

During first few days of differentiation we detected elevated expression of marker of endodermal lineage Sox17 and marker of anterior foregut endoderm Nkx 2.1. After 20 days of differentiation the cells expressed lung surfactant proteins SP-A and proSP-B. When grown in air-liquid interface culture, the cells formed histological structures with epithelial features, such as the basal lamina and tight junctions. Electron microscopy revealed presence of lamellar bodies in the cell cytoplasm.

Using serum-free differentiation and 3D organotypic culture we further tested the morphogenetic capacity of differentiating cells. Such conditions allowed aggregation of the cells and formation of branched structures.

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11. Role of HIF1-alpha in hematopoiesis - in vitro study

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Hematopoietic stem cell (HSC) is root of the longest studied process of differentiation – hematopoiesis. HSCs reside in adult mouse bone marrow or fetal liver but another source of these cells can be found in mouse embryonic stem cells (mESC).
Hematopoietic differentiation from embryonic stem cells can be performed in two basic ways, differentiation in embryonic bodies or co-cultivation with OP9 cell line.

Estimation of hematopoiesis is provided by e.g. qRT-PCR, flow cytometric analysis or functional colony forming assay in semisolid medium.
Induction and progression of hematopoiesis is under both direct and indirect control of hypoxia through modulation of hypoxia inducible factors (HIF). Here we focus on the role of HIF family member HIF-1α in regulation of hematopoiesis.

We have observed that depletion of HIF-1α accelerates hematopoiesis in embryonic stem cells in vitro. In accordance with the effect of HIF-1α depletion, addition of HIF stabilizing drugs ($CoCl_2$ and DMOG) to the culture reduced erytropoiesis in both adult (bone marrow-derived) and fetal (fetal liver-derived) hematopoietic progenitors.

Even role of HIF-1α in erytropoiesis is established, less is known about its influence on myeloid differentiation (monopoiesis and granulopoiesis). Recently we have observed different M-CFU/G-CFU/GM-CFU ratio in adult and fetal hematopoiesis after treatment by HIF stabilizing drugs.

12. Notch signaling, Vasculature and Alagille syndrome

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Alagille syndrome (AGS) is an inherited multisystem pediatric disorder caused by mutations of JAGGED1 (Jag1) or NOTCH2. We previously described a Jagged1 point mutant, "Nodder", which traffics normally but fails to activate Notch signaling. In this project, we characterize Jag1 Ndr/Ndr mice as a model for AGS.

Jag1 is one of the ligands that bind to Notch receptors on adjacent cell. Notch is a cell-to-cell signaling pathway that is crucial for development and homeostasis of organisms and is highly conserved among metazoan species. Notch signaling participates in multiple aspects of vascular development, specifically is important for the development of both mural and endothelial cells.

The most deaths of Alagille patients are due to spontaneous or provoked bleeds, our results point out that the organ defects seen in Nodder mice are due to altered vasculature. For the future experiments we would like to investigate the cross talk between ECs and vascular smooth muscle cells.

13. Higher antitumor effectivity of LA-12 is associated with its ability to bypass the G2 phase arrest induced by oxaliplatin in human colon cancer cells

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Platinum-based chemotherapeutic drugs such as oxaliplatin are used in the therapy of various solid cancers including colon, but their application is limited due to serious side effects and intrinsic or acquired cancer cell resistance. Recently, platinum (IV) adamantylamine ligand-containing complex LA-12 has been introduced and shown as highly effective in many cancer cells including those resistant to conventional platinum-based therapy. Platinum drugs can express their cytotoxicity by creating adducts on DNA and triggering DNA damage response (DDR). Activation of DNA damage pathways can be responsible for halting the cell cycle progression providing the cell with time for DNA repair or induction of cell death. Within these pathways, p53 protein as well as various DDR-related kinases are important players affecting the final cell fate. In our study, we compared the effects of oxaliplatin and LA-12 on modulation of the cell cycle and induction of apoptosis in several colon carcinoma cell lines with different p53/p21 status. We observed that LA-12 exerted effective cancer cell killing independently on p53 and p21, and in significantly lower doses compared to oxaliplatin. Moreover, LA-12-induced cytotoxicity was associated with much weaker p53- and p21-dependent G2-phase arrest and block in M phase entry compared to oxaliplatin-treated cells.

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14. Inhibition of canonical Wnt signaling deregulates expression of CYP1A1 in colon cancer cells

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Dietary carcinogens, such as benzo[a]pyrene (BaP), a potent carcinogenic polycyclic aromatic hydrocarbon present in various processed food, have been suggested to contribute to the development of sporadic colon carcinomas. The expression of cytochrome P450 1A1 (CYP1A1), one of the principal enzymes contributing to bioactivation of BaP, has been recently shown to be partly under control of canonical Wnt/beta-catenin signaling, which is frequently over-activated in colon cancer. Using cellular models derived from human colon epithelial cells at various stages of carcinogenesis, we inhibited canonical Wnt signaling by using small synthetic inhibitor of beta-catenin or siRNA targeting beta-catenin, and then we analyzed inducibility of CYP1A1 by BaP, and formation of stable BaP-7,8-diol-9,10-epoxide – DNA adducts. We found that inhibition of canonical Wnt signaling by either synthetic inhibitor or by siRNA enhanced formation of DNA adducts. Synthetic inhibitor suppressed induction of CYP1A1 expression at both mRNA and protein level. In contrast, siRNA-mediated beta-catenin knock-down increased the BaP-induced expression of CYP1A1 mRNA, but it reduced levels of CYP1A1 protein. Our data suggest that inhibition of beta-catenin may induce complex changes in CYP1A1 expression and regulation of BaP metabolism.

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15. Cyclin-dependent kinase 12, a new tumor suppressor?

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One of the typical characteristics of cancer development is the acquisition of unlimited cell division potential. Protein complexes driving transition through each phase of cell division consist of the cyclin- dependent kinases (CDKs) and cyclins, their binding partners. Besides already described function of CDKs in the control of cell division, CDKs play an indispensable role in the regulation of transcription. Our work has recently identified CDK12 as a crucial factor regulating transcription of DNA damage response/repair (DDR) genes. Down-regulation of CDK12 not only led to aberrant transcription of DDR genes, such as BRCA1, ATR, FANCI and FANCD2, but it also led to activation of the DDR pathway and increased genomic instability. In summary, we conclude that CDK12 represents, in many aspects, biologically interesting and clinically promising target for further investigation. As an example, CDK12 could be potentially employed as a useful diagnostic marker, since overexpression of CDK12, together with frequent amplification of CDK12 gene, was found in various types of tumors. Similarly, identification of functional impact of CDK12 mutations, identified in broad spectrum of tumors, during the process of cancer initiation and progression is very promising direction in any scientific attempts.

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16. Interaction of Myeloperoxidase with Endothelial Cell Surface

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Myeloperoxidase (MPO), highly cationic enzyme released from activated polymorphonuclear neutrophils, can bind to vascular endothelium and subendothelial extracellular matrix proteins based on electrostatic interaction. However, the importance of the interaction of MPO with endothelial surface glycocalyx composed mainly of glycosaminoglycans and proteoglycans for the glycocalyx integrity and function has not been described. We characterized the extent to which MPO modulates the charge and the three dimensional structure of the glycosaminoglycans, the effect of MPO on the structural integrity of the glycocalyx and the importance for the leukocyte interaction with endothelium. We demonstrate that MPO mediates modulation of glycocalyx function and that MPO provokes the adherence of polymorphonuclear neutrophils through affecting glycocalyx-controlled process of leukocytes adherence to the endothelium. This effect is the most likely based on the change in the electrical charge properties of glycocalyx and glycocalyx structure. These findings extend the knowledge of MPO inflammatory properties in vascular inflammation.

17. Regulation of mammary stem cells by receptor tyrosine kinase signalling

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Mammary gland is a complex tissue that consists of ductal epithelial network, an array of stromal cells and extracellular matrix. Unlike other organs, development of mammary gland is not fully completed by birth. Instead, mammary gland undergoes major developmental changes postnatally, including epithelial growth, branching, differentiation, regression and remodelling. These processes are highly regulated by epithelial-stromal crosstalk and require a concerted function of progenitor cells – mammary stem cells. In this presentation, we will discuss current knowledge on mammary stem cells, methods to assay mammary stem cell activity and our first data on regulation of mammary stem cells by receptor tyrosine kinase signalling.

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18. TUSC3 alters UPR response and prevents EMT in ovarian cancer

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Consequences of deregulated protein N-glycosylation on cancer pathogenesis are poorly understood. *TUSC3* is a gene with a putative function in N-glycosylation, located on the short arm of chromosome 8. This is a chromosomal region of frequent genetic loss in ovarian cancer and in many other epithelial cancers. We established earlier that expression of *TUSC3* is significantly decreased in epithelial ovarian cancer compared to benign controls and even more, hypermethylation of *TUSC3* promoter possesses a strong impact on ovarian cancer patients' survival. Deregulation of *TUSC3* resulted in enhanced proliferation and migration of ovarian cancer cell lines accompanied by phosphorylation of Akt kinase and other components of growth factor signaling pathways. However, precise molecular mechanism of *TUSC3* role in ovarian cancer remains unclear. In this study we demonstrated, that *TUSC3* critically influences stability of endoplasmic reticulum, modulates the epithelial-to-mesenchymal transition of ovarian cancer cell lines *in vitro* and *TUSC3* loss promotes tumorigenicity in mouse model *in vivo*.

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19. Myeloperoxidase modulates neutrophil cell death induced by oxidative burst

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The clearance of inflammatory cells, particularly neutrophil granulocytes, by regulated cell death from the site of inflammation is a key mechanism for the resolution of inflammation. Myeloperoxidase (MPO) is an abundant neutrophil enzyme released upon the neutrophil activation and accumulates at the site of inflammation. Interestingly, in some mouse models MPO deficiency is connected with more severe course of the inflammatory process. This can be due to reduced clearance of MPO deficient neutrophils from the site of inflammation. Thus, the involvement of MPO in neutrophil cell death was studied. Neutrophils isolated from MPO-deficient mice exhibited a significantly lower rate of cell death compared to neutrophils isolated from wild type mice in response to the stimulation of oxidative burst by the various activators. In contrast, the spontaneous cell death rate was independent on MPO-deficiency. The physiological importance of this phenomenon is supported by the observation of a significant accumulation of neutrophils in the lungs of MPO-deficient mice compared to wild type mice in a model of acute pulmonary inflammation. Results suggest that neutrophil-derived MPO can control the course of the inflammation process through the ability of MPO to modulate the life span of neutrophils.

20. Intracellular signaling under hypoxic condition in stem cells

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Hypoxia is known to affect a wide range of biological processes. It plays vital role during development and it is also responsible for stem cell fate commitment. Hypoxic response is mediated mainly *via* family of hypoxia-inducible factors with HIF1 as a master regulator of cell's adaptation to low oxygen environment. Altered reactive oxygen species (ROS) production as well accompanies this condition.

In our work we focus on hypoxia mediated changes in intracellular signaling pathways which are responsible for stem cell maintenance. We used wild type and HIF1alpha deficient mouse embryonic stem cells to investigate activity of MAPK/Erk, PI3K/Akt and p38 pathways upon 24 hours hypoxic (1% O_2) cultivation. To clarify effect of ROS involvement we used ROS scavenger N-acetylcysteine to prevent hypoxia driven redox imbalance. Hypoxia leads to decrease in Erk, p38 and Akt kinase phosphorylation. Interestingly downregulation of Erk and its upstream kinases was HIF1alpha and ROS dependent contrary to Akt and p38. These results demonstrate unknown mechanism of Erk signaling regulation in hypoxia. Our data also indicate that embryonic stem cells are capable of both HIF and ROS dependent and independent sensing of hypoxia.

21. The role of ATP-binding transporters associated with multi-drug resistance in stem cells

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ATP-binding transporters (ABC-t) play various roles in regulation organism function and homeostasis from prokaryota to mammals. ABC-t mediate transport of mainly lipophilic substances through cellular membranes. Some ABC-t are important in cell protection against endogenous and importantly also exogenous toxins. These transporters are called ABC-t associated with multi-drug resistance (ABC-t/MDR), according to their role in resistance of tumor cells to pharmacotherapy. ABC-t/MDR are also over-expressed in stem cells, where their protective role is expected, too. Particularly, ABCB1, ABCC1, and ABCG2 are common ABC-t/MDR expressed in stem cells. However, substrates of ABC-t/MDR are not only toxins, but also important signaling molecules as well leukotriens and/or glutathione conjugates and porphyrins, which mediated balance in intracellular oxidation-reduction processing. Thus we hypothesize the role of ABC-t/MDR also in regulation of stem cells fate. To test this hypothesis we analyzed effect of modulation of ABC-t/MDR activity in embryonic and neural stem cells. We observed that ABCC1 and ABCG2 are the most expressed ABC-t/MDR in our tested stem cells. Importantly, inhibition of these ABC-t/MDR leads to decreasing of stemness and induction of differentiation in both embryonic and neural stem cells. Analysis of mechanism of observed effect and identification of studying ABC-t/MDR substrates, which may be responsible for this effect, are in progression.

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22. Antibody-dependent cellular cytotoxicity of neutrophils towards cancer cells involves trogocytosis that can be further potentiated by targeting CD47-SIRPα interactions

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Cancer therapeutic antibodies are used in the clinic for the treatment of certain types of cancer. In the present study we have investigated the mechanism(s) underlying antibody-mediated cytotoxicity of neutrophils against cancer cells, and how this process is regulated by CD47-SIRPα interactions.

CD47-SIRPα interactions promote close cell-cell contact by selective regulation of integrin function. Unlike NK cells, which use granzyme B and perforin for target cell apoptosis induction, antibody-dependent lysis of cancer cells by neutrophils involves a fundamentally different process. This is not depending on either of the two classical anti-microbial effector mechansisms of neutrophils, including ROS generated by NADPH oxidase and proteases released from intracellular granules, but rather seems to be mediated by 'trogocytosis' (greek: trogo=gnaw) in which neutrophils actively tear membrane-parts off cancer cells, thereby triggering a form of necrotic cell death that is termed trogoptosis. Finally, neutrophil-mediated trogoptosis of antibody-opsonized target cells could be strongly potentiated by targeting CD47-SIRPα interactions. Collectively, these findings provide insight into the mechanism of antibody-dependent destruction of cancer cells by neutrophils and its regulation by CD47-SIRPα interactions.

23. Phenotypic plasticity and multicentrosomal mitoses in human embryonic stem cells

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Human embryonic stem cells (hESCs) are prone to genetic instability during their in vitro maintenance. In some cancer types, deregulation of centrosome number was shown to induce genetic instability at the earliest stages of cancer development. In hESCs cultured on mouse embryonic fibroblasts (MEF), the presence of supernumerary centrosomes is associated with early passage hESCs, whereas in late passage hESCs the frequency of multicentrosomal mitoses declines. We show that the culture medium from early passage cells can increase this frequency in late passage cells. Cripto-1 (CR-1), an embryonic gene that promotes tumorogenesis, may play a role in inducing this phenomenon. We show, that high expression of CR-1 correlates with high frequency of multicentrosomal mitoses observed in early passage cells and late passage cells upon medium change. Addition of neutralizing antibody or recombinant CR-1 protein significantly affects the presence of supernumerary centrosomes. Signaling pathways MAPK p44/42 and SAPK/JNK are activated in early passage hESCs, whereas in late passage they are activated upon medium change or addition of recombinant CR-1 protein. Despite the evidence of CR-1 involvement in centrosomal abnormalities, we didn't confirm the hypothesis, that the culture medium from early passage cells contained higher level of CR-1 protein. In addition to these findings, we also investigated the frequency of multicentrosomal mitoses in hESC cultivated on Matrigel. Early and late passage cells were transferred from MEF to Matrigel and surprisingly, low frequency of multicentrosomal mitoses was associated only with early passage, whereas the late passage frequency was high. Thus, different culture conditions may prevent the occurrence of supernumerary centrosomes that represent a major risk to hESC in terms of their genetic stability.

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24. The role of HIF-1alpha in the regulation of cardiomyogenesis *in vitro*

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A cardiac cell formation (cardiomyogenesis) is critically dependent on cell microenvironment. Oxygen availability is one of the most important factors which play an essential role in cardiomyocytes differentiation. It is known that hypoxia modulates the differentiation of murine embryonic stem cells to cardiomyocytes *in vitro*. Hypoxic conditions lead to the stabilization of hypoxia-inducible factor-1 alpha (HIF-1alpha). However, the role of HIF-1alpha in the regulation of cardiomyocyte differentiation is not well understood. With the aim of better understanding the role of HIF-1alpha within cardiomyocyte development and progenitor cell reprogramming, cardiomyogenesis in wild type and HIF-1alpha depleted murine embryonic stem cells was analyzed *in vitro*.

The level of expression of individual cardiospecific genes was higher in the HIF-1alpha deficient cell line compared to wild type cells in the early phase of differentiation (on Day 5). In contrast, in the late phase of cardiac cell development (on Day 15 and later), these markers were lower in HIF-1alpha deficient cells. This suggests a higher state of maturity in HIF-1alpha deficient cells at the beginning of cardiomyogenesis differentiation. At the same time, the profile of cardiospecific markers suggests that a lower number of progenitor cells enter the differentiation process. However, the final cardiomyocyte maturity status of cells which enter the cardiomyogenic process is similar in both wild type and HIF-1alpha deficient cell lines. This is suggested on the basis of their similar beating characteristics and morphology.

In conclusion, HIF-1alpha is important for induction of the development of cardiomyogenic progenitors and has a significant effect on the maturation of early cardiomyocytes in *in vitro* culture.

25. CDK12 - new tumor suppressor candidate

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Eukaryotic transcription is mediated by action of RNA-Polymerase II (RNAPII). Its proper function is orchestrated at the level of posttranslational modifications of its C-terminal domain (CTD). Family of transcription-associated cyclin dependen kinases (CDK) phosphoryate the CTD of RNAPII and CDK12 is one of the key players in transcriptional regulation process. Phosphorylation of CTD on Serine 2 by CDK12 is associated with the elongation phase of transcription. CDK12 forms active complex with its regulatory partner Cyclin K. Depletion of CDK12 results in lowered expression of key DNA-damage response associated genes (e.g. BRCA1, ATR, FANCI, FANCD2) and it leads to genome instability as well as increased sensitivity to DNA damaging agents. Several studies have shown that CDK12 is mutated in various types of cancer. We used mutations found in High-grade serous ovarian cancer screen as a model for studying CDK12 function. Mutated CDK12 in most of these cases abrogates its binding to Cyclin K, along with loss of its kinase activity towards CTD of RNAPII. Mutations in CDK12 lead to defects in the DNA damage response, to genome instability and to cancer development. We suggest that CDK12 functions as a tumor suppressor.

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26. Modulation of cell cycle leads to phenotypical changes of prostate cancer cell lines

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Tumor heterogeneity and cancer cells plasticity present challenges for effective clinical diagnosis and therapy. Such challenges are epitomized by neuroendocrine transdifferentiation (NED) and the emergence of neuroendocrine-like cancer cells in prostate tumors. NED frequently arises from androgen-depleted prostate adenocarcinoma and is associated with the development of castration-resistant prostate cancer and poor prognosis.

Here we showed that NED was evoked in prostate epithelial cancer cell lines by long term androgen depletion and by high cell density cultivation. Both conditions were associated with cell cycle arrest and deregulated expression of several cell cycle regulators. Therefore, we targeted these regulators (cyclin D1, cyclin D3, Cdk1, Cdk2) and investigated the effect on cell cycle and consequent NED induction. Finally, we focused on uncovering the signaling pathway involved in high cell density-promoted NED. We demonstrated that the cyclic adenosine 3′, 5′-monophosphate (cAMP)-mediated pathway is activated in these conditions. Our results imply a new relationship between cell cycle attenuation and promotion of NED.

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27. The Importance of Experimental Milieu in Regulation of Macrophage Activation

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Background: Macrophages are important source of cytokines and other compounds, which recruit additional cells to sites of infection or tissue injury. Importantly, their functions might be directly controlled by the composition of milieu in their immediate vicinity. Therefore, the analysis of their function under different *in vitro* as well as "simulated" *in vivo* condition is of particular interest.

Materials and methods: Mice peritoneal macrophages were stimulated with different activators (e.g. LPS, IFN-γ, TNF-α and interleukins or their combination) under different experimental conditions (*in vitro* and "simulated" *in vivo* conditions). Consequently, the physiological functions of macrophages were assessed using different luminometric, spectrophotometric, molecular, and immunohistochemical methods.

Results: Our data demonstrate that there exists crucial difference in activation of macrophages under classical *in vitro* and "simulated" *in vivo* conditions. These abnormalities are accompanied by significant changes in inflammatory response of macrophages as well as in activation of intracellular signaling pathways.

Conclusions: The activation of mice peritoneal macrophages is dependent on the composition of milieu in their immediate vicinity and the "classically used" *in vitro* systems seem to be not suitable for correct analysis of their functions.

28. Isolation and characterization of circulating tumor cells in colorectal cancer

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Circulating tumor cells (CTC) are cells released from the tumor into the blood or lymphatic circulation. These cells play important role in metastatic cascade, as they are able to spread all around the body via blood or lymph stream. When in circulation, they can be use for relatively non-invasive detection of disease, its prognosis or prediction of treatment efficacy. Nowadays, CellSearch is the only clinically validated method for CTC identification in metastatic breast, colorectal and prostate cancer, but it has its limitations. We apply a novel approach, called HD-CTC, to describe changes of CTC quantity and quality in time in patients with metastatic colorectal cancer. Advantage of this system is analysis of CTC on the background of all nucleated blood cells without the need of enrichment step typical for most other methods for CTC identification resulting in no loss of potentially interesting cell populations. Furthermore, coupling of HD-CTC with single-cell DNA sequencing to assess copy number variations in individual cells allows description of heterogeneity of tumor cell populations within individual patient. Goal of our study is to use CTCs as a predictive marker, which provide us “on line” view on treatment response and disease evolution.

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29. Neural stem cells and neuroregeneration

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Regeneration capability of the central nervous system is very limited and therefore diseases accompanied with marked loss of neurons lead to mostly irreversible functional deficit. Neural stem cells (NSC) represent one of endogenous regeneration mechanisms. Thus, their proper activation or their transplantation could have the potential to overcome limitations of natural regeneration.

However, for successful NSC driven regeneration it is necessary to fine-tune their proliferative activity and differentiation potential. These properties are strongly modulated by neurogenic properties of local microenvironment, which differs between various structures of the CNS and also by various signalling molecules.

One of the endogenous molecules which affect NSC differentiation is sonic hedgehog (Shh). This molecule is involved in control of neural cells proliferation and differentiation in developing cerebellum. Shh is also capable of induction of neurogenesis in the adult tissue. Shh not only helps to maintain NSC in low differentiation state, but also improves survival of differentiating cells. Nevertheless, the efficiency of neurotransplantation therapy strongly depends on the graft and the host tissue state.

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30. The role of EMT/MET in regulation of phenotype of breast cancer cells

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Unfolding the mechanisms of cancer cell plasticity might contribute to future therapeutical approaches.Breast cancer stem cells (BCSC) are suggested to be the drivers of metastasis, resistance to chemotherapy and radiation. BCSC were previously shown to co-exist in diverse mesenchymal and epithelial states in different
tumor sites. To understand the relationship between EMT (epithelial-to-mesenchymal transition), MET (mesenchymal-to-epithelial transition) and BCSC we used HER2/neu-overexpressing primary mouse mammary carcinoma cell line and its relapsed HER2/neu antigen-negative variants (ANV). Analysis at the single cell level revealed different expression of surface stem cell markers (CD44, CD49f, CD133, Trop-2 and Sca-1), transcription factors responsible for stemness (Oct-3/4, Sox-2, Nanog) and EMT/MET regulators (Snail, Slug, Axl) amongst cell lines. We examined basal expression of epithelial and mesenchymal markers and also EMT regulators in various BCSC populations. These changes in phenotype confer that the stemness and EMT might be related in mouse mammary carcinoma cells and their relapsed variants.

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31. Identification of cancer stem-like cell in prostate cancer cell lines and analysis of epithelial-to-mesenchymal transition

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Cancer stem cells (CSCs) have long been implicated in numerous tumour formations and therapy resistance including prostate cancer disease. Importantly, mechanisms of their origin have not been elucidated in prostate cancer yet. It has been demonstrated that epithelial-to-mesenchymal transition (EMT) is phenomenon which can lead to the acquisition of stem cell properties in various types of cancer. This was first discovered by research on immortalized human mammary epithelial cells, where induction of EMT resulted in the acquisition of mesenchymal properties and in the expression of stem cell markers. Furthermore those cells had properties similar to mammary epithelial stem cells (Mani *et al*., 2008). In prostate cancer, cells with EMT phenotype have been shown to express stemness factors and to have increased clonogenic capacity (Kong *et al*., 2010). However phenotypic heterogeneity and EMT status of routinely cultivated prostate cell lines remains unknown.
In this study we aimed to screen surface expression of selected CSCs markers (Trop2, CD133, CD44, CD24 and CD49f) in a panel of prostate benign and cancer cell lines using multicolour flow cytometry. We found out that each cell line express different set and combinations of these CSCs markers. Next, to elucidate the EMT status in putative CSCs subpopulations, we combined analysis of selected surface stem cell markers with detection of regulators and markers of EMT (Snail, Slug, E-cadherin, N-cadherin) in CD24/CD44 subpopulation of PC-3 and DU-145 cells.
In summary, we described different subpopulations of CSCs in a panel of prostate epithelial cell lines and determined EMT status of those subpopulations.

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1. Kong, D., S. Banerjee, et al. (2010). "Epithelial to Mesenchymal Transition Is Mechanistically Linked with Stem Cell Signatures in Prostate Cancer Cells." PLoS ONE 5(8): e12445.
2. Mani, S. A., W. Guo, et al. (2008). "The Epithelial-Mesenchymal Transition Generates Cells with Properties of Stem Cells." Cell 133(4): 704-715.

32. Teratogenic effects of lithium carbonate on cardiogenesis in *in vitro* systems– Possible protective role of myo-inositol

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The drug lithium carbonate (Li_2CO_3) use during pregnancy increases the possibility of cardiovascular anomalies. The earlier studies confirm its phosphatidylinositol cycle (PI) inhibition and Wnt pathways mimicking properties, which might contribute to its teratogenic effects. In this study the toxic effects of Li_2CO_3 in chick embryonic cardiomyocyte micromass system (MM) and embryonic stem cell derived cardiomyocyte (ESDC) were evaluated, with possible protective role of myo-inositol. In MM system the Li_2CO_3 did not alter the toxicity estimation endpoints, whereas in ESDC system the cardiomyocytes contractile activity stopped at 1500 µM and above with significant increase in total cellular protein content. In ESDC system when myo-inositol was added along with Li_2CO_3 to continue PI cycle, the contractile activity was recovered with decreased protein content. The lithium toxic effects depend on the role of PI cycle at particular stage of cardiogenesis, while relation between myo-inositol and reduced cellular protein contents remains unknown.

33. Docosahexaenoic acid effectively stimulates TRAIL-induced apoptosis of colon cancer cells at the level of mitochondria

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Docosahexaenoic acid (DHA) is an n-3 polyunsaturated fatty acid (PUFA) known for its beneficial effect in a wide variety of diseases including colorectal cancer. In cancer cells, mitochondria-dependent apoptotic pathways are very frequently suppressed. Mitochondria belong to the subcellular sites where n-3 PUFAs are rapidly incorporated. DHA causes oxidative stress and alternation of membrane barrier properties and may also facilitate the toxic effects of certain antitumour agents. Our aim was to elucidate a potential sensitizing effects of DHA on apoptosis induced by a cytokine TRAIL (TNF-related apoptosis inducing ligand) in human colon cancer cells. TRAIL has been shown to selectively induce apoptosis in tumor but not in normal cells. However, many tumor cells may still exhibit a TRAIL-resistant phenotype.

Here we demonstrate that DHA is capable to significantly increase TRAIL-induced apoptosis in SW620 colon cancer cells and highlight the importance of the mitochondrial apoptotic pathway in this process. Combined treatment with TRAIL and DHA effectively triggered the activation of proapoptotic Bcl-2 family proteins, release of mitochondrial cytochrome c, decrease in mitochondrial membrane potential and inhibition of mitochondrial respiration.

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34. Regulation of MDM2 and MDMX expression in epithelial-mesenchymal transition

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Most cancer–related deaths are associated with advanced disease and metastasis. Epithelial-mesenchymal transition (EMT) is viewed as an essential step facilitating dissemination of tumor cells. While the EMT-inducing transcription factors from the Snail, Twist and ZEB families are important mediators of cancer progression and metastasis, reports about the role of MDM2 and MDMX in EMT and cell migration are conflicting.

Analyzing paired benign and tumorigenic cell lines derived from prostate and breast tissue, we observed that cancer transformation is accompanied by EMT, downregulation of MDM2 and upregulation of MDMX. Similar correlation was confirmed in a significant proportion of samples obtained from paired patient prostate cancer tumors and metastases. The effects of modulated MDM2 expression on cellular motility were cell type specific.

To gain insight into the regulation of MDM2 and MDMX expression in benign and tumorigenic clones derived from BPH-1 cell line, we studied regulation of gene and protein expression. We observed a predominant transcriptional regulation of MDM2 and posttranscriptional regulation of MDMX. Interestingly, experimental downregulation of Slug and Twist enhanced MDM2 expression in BPH-1 CAFTD03 cells, suggesting an interplay between the regulation of MDM2 and EMT.

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35. Combined treatment of PPAR ligand with oxaliplatin affect the cell cycle and death in colon cell line both sensitive and resistant to oxaliplatin

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The platinum drugs represent a unique and important class of anti-tumour agents. They are extensively used in the treatment of many solid tumors. Anticancer effects of platinum drugs involve target in nucleus or other cytoplasmic targets, such as mitochondria. Oxaliplatin is the newest platinum drug used in standard chemotherapy of solid tumors. Serious side-effects and resistance to treatment are the main disadvantages of oxaliplatin. Therefore, new combination with for example tumor suppressors, transcription factors etc. are needed. PPAR (Peroxisome Proliferator - Activated Receptors) are transcription factors which belong to large family of nuclear receptors. PPARγ has been detected in colon cancer cells where it has been indirectly linked to colon cancer pathogenesis. We supposed that combination of the PPARγ ligand and oxaliplatin could efficiently affect colon cells. Moreover, we focused on the possibility to overcome cancer cell resistance to oxaliplatin by this combination. Our results bring new knowledge of molecular mechanisms of platinum drug and specific PPARγ ligand effects in colon cancer cells.

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36. Characterizing the mechanism of the synthetic lethal interaction between CHK1 inhibition and DNA damage in normal and cancer cell lines

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The concept of synthetic lethality has been a new direction in rational anticancer drug development. Two genes are synthetic lethal if mutation of either alone is compatible with viability but mutation of both leads to death. (Kaelin WG. et al. Nat Rev Cancer. 2005;5(9):689–98.). During tumorigenesis, cancer cells have developed mutations that distinguish them from their wild-type counterparts. These mutations represent new vulnerabilities which can be exploited as synthetic lethal interaction partners for cancer-specific targeted therapies. One of the promising strategies is using drugs which target cell cycle checkpoints as sensitizers of classic DNA damaging chemotherapy.
Recently, several CHK1(Checkpoint 1) inhibitors have been developed and evaluated in clinical trials as a chemopotentiating strategy. As one of the most potent and selective compound was identified SCH 900776 (Guzi, T.J., et al., Mol Cancer Ther, 2011. 10(4): p. 591-602).

In our work, we used combination treatment by new CHK1 inhibitor SCH900776 and DNA damaging chemotherapeutics such as hydroxyurea and gemcitabine in a large panel of cell lines with different genetic backgrounds. Lung, breast, ovarian, colon, pancreatic, kidney and prostate cancer cell lines were chosen for screening and comparison of their response to synthetic lethal treatment. Our results indicate that CHK1 inhibitors enable reduction of the concentration of chemotherapeutics to obtain the same growth inhibition in the most of cancer cells. In contrast, the concentrations of drugs which are effective in tumors, do not affect cell lines from healthy tissues (e.g. breast cell line MCF10A or dog kidney tissue MDCK), which were chosen as controls. Moreover, the synthetic lethal effect of DNA damage and CHK1 inhibition was also confirmed in cancer cells grown under three-dimensional cultivation condition. Consequently, cell lines with the most considerable effect observed, will be further studied to determine the molecular mechanism of combination therapy.

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37. Defective signal regulatory protein alpha signaling reduces atherosclerosis in mice

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Background: We tested the hypothesis that defective cytoplasmic signaling of signal regulatory protein alpha (SIRPα) in hematopoietic compartment affects development of atherosclerotic lesions.

Materials and methods: Bone marrow of sublethally irradiated LDL receptor deficient mice was reconstituted with bone marrow from WT mice or mice expressing SIRPα lacking cytoplasmic tail, followed by 10 weeks of high fat diet and analysis of atherosclerosis development.

Results: Both the size and severity of cardiac atherosclerotic lesions was considerably decreased in SIRPα mutant chimeric mice. This was associated with a prominently increased amount of newly recruited small macrophages in lesions of SIRPα mutant mice, while the numbers of other inflammatory cells did not differ. RNA sequencing of bone marrow-derived macrophages of mice lacking SIRPα revealed decrease in expression of interferon gamma (IFNγ)-stimulated genes and chemokines which are known to act as atherogenic factors.

Conclusions: SIRPα signaling on myeloid cells has an important role in development of atherosclerosis probably via regulating expression of pro-inflammatory genes related to IFNγ.

38. The role of autophagy in modulation of 5-FU-induced death response in colon cancer cells with different p53 status

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Autophagy is a physiological process that maintains cellular homeostasis by degrading aged or damaged organels and misfolded proteins. In fact, it may serve either to promote cell survival or execute cellular demise. Autophagy also plays an important role in carcinogenesis, and can be induced by various chemotherapeutic agents.
5-Fluorouracil (5-FU) is a drug used in the treatment of colorectal cancer that blocks thymidylate synthase, or incorporates into DNA and RNA, thus inducing cell cycle arrest or apoptosis. Autophagy has also been reported as an important mechanism in the cellular response to 5-FU.

We investigated the ability of 5-FU to trigger autophagy in human colon cancer cells with different p53 status, and the essential molecules involved. Using chemical inhibitors of autophagy (3-methyladenine, bafilomycin A1) or specific siRNAs against crucial autophagy regulators of the Atg family, we studied the functional role of autophagy in modulation of the overall death response of colon cancer cells to 5-FU.

We showed that the ability of 5-FU to trigger autophagic response may significantly affect the cancer cell sensitivity to its cytotoxic effects, in dependence of p53 status. The results obtained, together with the role of selected molecular regulators involved in these effects will be presented.

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39. Pharmacological inhibition of SCF^{Skp2} complex activity affects characteristics of cancer stem-like cells

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Skp2 protein is a crucial component of SCF^{Skp2} complex that functions as an E3 ubiquitin ligase and therefore is involved in protein degradation. High level of Skp2 was described in various types of cancer, including prostate and colorectal cancer. Binding NEDD8 to Cullins is necessary for SCF^{Skp2} formation and its activity (Kawakami et al., 2001). Recently, inhibitor of NEDD8 pathway, MLN-4924, was described (Soucy et al., 2009). Disruption in NEDD8 pathway using this inhibitor leads to accumulation of SCF^{Skp2} substrates and triggers cellular processes such as cell cycle arrest and apoptosis.

The cancer stem cells (CSCs) represent an attractive target for anticancer therapy. However effects of MLN-4924 on these cells are not described in details. Therefore we aimed to investigate whether inhibition of SCF^{Skp2} will affect properties of the cancer cells characterized by expression of cancer stem cell markers.

Firstly, we investigated effects of MLN-4924 treatment on cell cycle deregulation in prostate cancer cell lines and compared it with another inhibitor, SKPin C1. This inhibitor is specific only for $p27^{Kip1}$ or $p21^{Cip1}$ recognition by SCF^{Skp2} (Wu et al., 2012). Further, we analysed subpopulations of DU-145, PC3 or BPH1 CAFTD 04 cancer cell lines that were determined by expression of cancer stem cell surface markers, such as CD44, CD133 or CD24.

We compared the base Skp2 protein level within these subpopulations. Next, we used MLN-4924 inhibitor to investigate effects of SCF^{Skp2} inhibition on clonogenic capacity of cells using ACCA (Automatic cell cloning assay; Fedr et al., 2013) or abitity of cell to form spheroids in 3D condotions.

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1. Fedr et al. Automatic cell cloning assay for determining the clonogenic capacity of cancer and cancer stem-like cells. Cytometry A., 2013 May;83(5):472-82
2. Kawakami et al. NEDD8 recruits E2-ubiquitin to SCF E3 ligase. EMBO J, 20**,** 4003-12, 2001
3. Soucy et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature, 458**,** 732-6, 2009
4. Wu et al., Specific Small Molecule Inhibitors of Skp2-Mediated p27 Degradation; Chem Biol. 2012 December 21; 19(12): 1515–1524

40. Whole-cell mass spectrometry clusters morphologically uniform, yet differentiating hESCs

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Discrimination of genetically and morphologically uniform population of *in vitro* cultured cells represents a major obstacle in cell authentication. The molecular, genetic and/or light-microscopy analyses are inefficient in case of small and subtle but critical changes arising in cultured cells. Matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF MS) possesses manifold applications in classical analytical and structural chemistry that can be adapted for cell profiling. Processing of mass spectra by sophisticated mathematical analysis, such as artificial neural networks, reduces unwanted inconstancy and can identify hidden patterns in mass spectra. We applied this approach on morphologically uniform, yet innerly different hESCs. The hESCs were stimulated with retinoic acid (RA) or left untreated and then processed with whole-cell MALDI-TOF MS. The correctly trained ANNs were able to reach significantly high level of discrimination while the standard cluster and factor analysis did not identify differences. In summary, we developed and optimized a simple, robust and efficient cell authentication tool aimed for routine use in clinical grade cell cultures.

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41. Role of HIF-1α in formation and maintenance of neural stem/progenitor cells in vitro

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Hypoxia inducible factor 1α (HIF-1α) is a master regulator in adaptation of cells of many tissues to hypoxic conditions, including neural stem/progenitor cells (NSPC). Studies of last decade have also revealed that HIF-1α plays a pivotal role in stemness maintenance of NSPC through crosstalk with signaling pathways like Notch or Wnt. However, information about role of HIF-1α in early neural development is still uncomplete or missing. In our experiments, we use mainly NSPC derived from murine embryonic stem cells (ES) for neurospheres cultivation, an *in vitro* counterpart of *in vivo* developing embryonic neural tissue. We try to find out how absence of HIF-1α affects the formation, selfrenewal capacity and expression of specific protein markers of NSPC using qRT-PCR, WB and immunocytochemistry approach. Preliminary results indicate that ES with HIF-1α-knockout have increased number of neurospheres with neurogenic potential and decreased selfrenewal capacity compared to *wt*. Further, we would like to investigate HIF-dependent regulatory mechanisms of NSPC formation and selfrenewal with respect to other signaling pathways involved in regulation of neural stem cells.

42. Role of HIF-1α in selfrenew and differentiation of neurospheres derived from embryonic stem cells

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Hypoxia inducible factor 1α (HIF-1α) is a main factor which responds to hypoxia and regulates adaptation to hypoxic conditions. HIF-1α is important for stemness maintenance by stabilizing activated Notch-1 and for maintenance of neurogenic niche by upregulating vascular endothelial growth factor.

These informations are important for our experiments with neural stem cells (NSC). We use NSCs derived from murine *wt* and HIF-1α deficient embryonic stem cells and NSCs dissected from embryonic brain. We monitor influence of HIF-1α knock-out on formation, selfrenewal capacity and transition ability of NSCs by neurosphere cultivation and by screening of neural markers using qRT-PCR, WB techniques and flow cytometry.

According to our preliminary results from qRT-PCR and colony forming assay, HIF-1α supports selfrenewal of NSCs and knockout of HIF-1α enables neurospheres to express more neural markers. Moreover, gene expression of Hes1 and Hes5 transcription factors is affected by HIF-1α knockout which points to interaction of HIF-1α and Notch pathways in selfrenewal abilities of NSC.

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43. Cell death signaling in human pluripotent stem cells

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Pluripotent stem cells (PSCs) are similarly as somatic stem cells and progenitors susceptible to by stress- or other stimuli-induced cell death. They appear to be especially sensitive to mitochondria-dependent, DNA damage-induced apoptotic cell death, largely mediated by p53 transcriptionally-independent signaling and shifted balance between pro- and anti-apoptotic proteins from the Bcl-2 family. Their increased sensitivity to DNA damage- or stress-induced apoptosis is likely linked to their immortality - protection of the accuracy of their genetic information and prevention of possibly harmful mutations or even transformation. In addition to the stress-induced apoptosis, the second major natural apoptotic signaling in mammalian cells originates at death receptors from the TNFR family.

We analyzed whether also they are expressed and could play a role in cell death signaling in human PSCs. We found that from major death receptors are in PCSs expressed only TRAIL receptors DR4 and DR5 but nor TNFR1 or Fas. Unstressed cells are however resistant to TRAIL-induced apoptosis likely due to the increased expression of caspase-8 inhibitor cFLIP. However, FLIP downregulation or mild stress efficiently sensitized them also to TRAIL-induced apoptosis, which could represent another layer protection against transformation.

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44. The role of oxygen sensors during pathological and physiological conditions in mice

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Low oxygen tension (hypoxia) is a characteristic feature during physiological and pathological processes in vivo. The transcriptional response to deprived pO_2 is to a large extent regulated by the hypoxia inducible factors (HIF), mainly HIF1α and 2α. Moreover, both factors can in general actively promote oxygen delivery and adaptive processes to hypoxia such as erythropoiesis, angiogenesis, anaerobic glycolysis and hematopoiesis. 'Oxygen-sensing' is therefore indispensable as it enables the cells to instantaneously adapt to this type of stress situation. The machinery behind this relies on the HIF-prolyl hydroxylases (PHD1-3), enzymes that hydroxylate and, consequently, lead to the inactivation of HIFα in the presence of oxygen. Research in our group has been focused on the role of these oxygen sensors during many different settings in vivo, including tumor development, hematopoiesis/erythropoiesis, skin wound healing and bone homeostasis in different genetically modified mouse strains.

45. PPARγ ligand sensitizes prostate cancer cells to the oxaliplatin treatment

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Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily. These transcription factors regulate metabolic processes, inflammation, proliferation and differentiation of cells. Thiazolidinediones are synthetic ligands of PPARγ. Platinum-based drugs are broadly used for the cancer treatment, but they are often inefficient and have serious side effects. Therefore, the new combined chemotherapeutic strategies are considered with the aim to suppress these unfavorable effects of platinum-based drugs.

We analyzed the interactive effects of PPARγ ligand, rosiglitazone (RGZ), and platinum-based cytostatic drug, oxaliplatin (L-OHP) on prostate cancer cell lines. Our results indicated that pretreatment of cancer cells with RGZ sensitizes BPH-1 CAFTD 03 cell line to the effects of L-OHP. We detected increased inhibition of proliferation and a block in the G2/M phase of the cell cycle, together with changes in expression of cyclins. Moreover combined treatment increased percentage of the dead cells analyzed by flow cytometry after Annexin V staining. The combined treatment decreased PPARγ and MDMX protein expression. These data suggest a possible link between PPARγ and MDMX.

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