INVITED LECTURES

9

ISOLATION OF ORGANELLES BY FLOW CYTOMETRY: STATE OF THE ART

Gomez-Pacheco M., Gillet C., Le Bars R., Satiat-Jeunemaître B., Bourge M.

Institute of Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198, Gif-sur-Yvette, France

Organelles separation is mostly realised by protocols of centrifugation, or electrophoresis methods. Outputs of these methods are usually efficient, however the purity of the obtained fraction is often questioned. Alternative methods may rely on flow cytometry approaches.
Nowadays, cell sorting by flow cytometry is a powerful tool to design "pure"cell fraction, which may then be used in many downstream applications: cell cultures, but also genomic, proteomic or lipidomic analyses, etc. To go further in such analyses, development of protocols to isolate and concentrate smaller objects (0.5 to 50 µm) such as subcellular organelles would be of high interest. Today the potential resolution of sorting methods is evolving with the complexity of the flow cytometers, increasing both the detection level of fluorescent signals, the quality of the collection process of objects with different size, and the increasing speed of the sorting process. In our laboratory, we have currently sorted nuclei populations on which FISH (Fluorescent in situ Hybridation) analyses were succesfuly applied. Immunolabelling and RNAseq along endoploidy process were also performed on such fractions revealing important correlations between morphogenesis processes and nuclei activity (Pirello et al., 2013; Brown et al., 2017).
This talk will focus on how to identify and sort, by cytometry, the different organelles (chloroplasts, mitochondria, etc.) from plant tissues. The advantages and the limits for such a method in term of purity and structure conservation will be discussed along with the chosen potential downstream complementary studies which have to be performed on such sorted fraction.

10

GENOME EVOLUTION REVEALED THROUGH CHROMOSOME FLOW SORTING IN THE KOMODO DRAGON (*VARANUS KOMODOENSIS*)

Johnson Pokorná M.[1,2], Iannucci A.[3], Altmanová M.[1,2], Kratochvíl L.[1], Rehák I.[4], Pereira J.[5], Ferguson-Smith M.[5], Ciofi C.[3]

[1]*Faculty of Science, Charles University, Prague, Czech Republic*

[2]*Institute of Animal Physiology and Genetics, The Czech Academy of Sciences, Liběchov, Czech Republic*
[3]*Dipartimento di Biologia, Università degli studi di Firenze, Sesto Fiorentino (FI), Italy*
[4]*Prague Zoological Garden, Prague, Czech Republic*
[5]*Cambridge Resource Centre for Comparative Genomics, Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom*

Since the sequencing of the first reptile genome, the green anole lizard (*Anolis carolinensis*), an additional twelve have been published so far. Even though rough drafts are often sufficient to answer important biological questions, a higher level of assembly resolution is required for detailed investigation of evolutionary processes. This level can be reached by building high-quality chromosomal maps and anchoring genomes to chromosomes. Here we present our preliminary work to anchor the genome of *Varanus komodoensis* to chromosomes. We decided to use an approach based on a combination of flow sorting and Next Generation Sequencing. We isolated *V. komodoensis* chromosomes by flow sorting, assigned each flow karyotype peak to its respective pair of chromosomes by FISH, and finally amplified chromosome-specific DNA pools for sequencing by NGS. The resultant data will provide accurate information on the level of genomic organization of this species, and will be a powerful tool for investigating several areas of the evolution of reptiles and amniotes in general. Moreover, we performed cross-species chromosome painting to compare genome organization among varanids and to help to reconstruct the presumptive ancestral karyotype, providing information on the role that genome organization plays in the evolution of species. Last but not least, the production of a high-quality set of genetic information for Komodo dragon will improve our understanding of the life history of this species, and support future in situ and ex situ conservation and management plans.

CELL BIOLOGY AND SIGNALLING

CB-2

NOVEL SURFACE ANTIGENS ASSOCIATED WITH PLASTICITY OF BREAST CANCER CELLS AND DISEASE PROGRESSION

Remšík J.[1,2,3], Fedr R.[1,2], Navrátil J.[4], Souček K.[1,2,3]

[1]Department of Cytokinetics, Institute of Biophysics of the CAS, v.v.i., Brno, Czech Republic
[2]Center of Biomolecular and Cellular Engineering, ICRC-FNUSA, Brno, Czech Republic
[3]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[4]Masaryk Memorial Cancer Institute, Brno, Czech Republic

Both intratumoral heterogeneity and heterogeneity in microenvironmental constituents account for patient prognosis and response and resistance to therapies. Intratumoral heterogeneity, characterized by presence of multiple distinct clones of cancer cells within one tumor, is one of the main obstacles in effective cancer treatment. To uncover the

changes on cell surface during such dramatic phenotypic switches, we introduced flow cytometry-based high-throughput platform for analysis of expression profile of 332 surface antigens with validated antibodies in up to six cell lines, in parallel. We compared surface profiles of three benign epithelial cell lines with their tumorigenic counterparts with complete or partial mesenchymal phenotype and identified a 10-molecule surface signature, associated with cancer plasticity. We further introduced multicolor flow cytometry protocol for analysis of intratumoral heterogeneity in breast cancer patient samples. Using this approach, we proved that all of these surface molecules are expressed within both epithelial and stromal tumor compartments. We believe that deeper mechanistic insight into action of the proposed molecules might help to identify novel biomarkers, associated with cancer invasiveness and progression and potential targets for therapy.

This work was supported by funds from Ministry of Health of the Czech Republic, grant nr. 15-33999A, 15-28628A, all rights reserved (KS), projects no. LQ1605 from the National Program of Sustainability II (MEYS CR) and by the project FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123 (OP VaVpI) by European Union - project ICRC-ERA-HumanBridge (No. 316345) (to LB, RF), and by project HistoPARK (CZ.1.07/2.3.00/20.0185; KS). We would like to thank Iva Lišková, Martina Urbánková and Katka Svobodová for technical assistance.

CB-3

THE NOVEL C/EBPα TARGET GENE EVI2B REGULATES MYELOID DIFFERENTIATION AND HEMATOPOIETIC PROGENITOR CELL FUNCTION

Zjablovskaja P.[1,2], Kardosova M.[1], Danek P.[1], Angelisova P.[1], Benoukraf T.[3], Wurm A.[4], Sian S.[3], Kalina T.[2], Brdicka T.[1], Balastik M.[5], Delwel R.[6], Tenen D. G.[3,7], Malissen B.[8], Fiore F.[9], Behre G.[4], Horejsi V.[1], Alberich-Jorda M.[1]

[1]Institute of Molecular Genetics of the ASCR, Prague, Czech Republic
[2]Childhood Leukaemia Investigation Prague, 2nd Faculty of Medicine, Charles University; University Hospital Motol, Prague, Czech Republic
[3]Cancer Science Institute, National University of Singapore, Singapore
[4]Leipzig University Hospital, Leipzig, Germany
[5]Institute of Physiology of the ASCR, Prague, Czech Republic
[6]Erasmus University Medical Center, Rotterdam, The Netherlands
[7]Harvard Stem Cell Institute, Harvard Medical School, Boston, USA
[8]Centre d'Immunophénomique, Aix Marseille Université, Marseille, France
[9]Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, Marseille, France

C/EBPα and its target genes are known as important regulators of myeloid differentiation and maintenance of hematopoietic stem cells. Here, we identified a novel C/EBPα target gene, *EVI2B*, and demonstrated its abundant expression in the majority of hematopoietic populations, with remarkably high levels in myeloid lineage cells. We demonstrated that *Evi2b* depletion alters myeloid lineage development *in vitro*, both in murine and human models. Further, we observed that *Evi2b*-depleted hematopoietic stem and progenitor cells (HSPC) demonstrate impaired colony forming ability as well as decreased capacity

to reconstitute the hematopoietic system of lethally irradiated mice. In addition, we demonstrated increased apoptosis and decreased proliferation in progenitor cells with reduced levels of EVI2B, which explains the affected functionality of these cells. To deepen our understanding of the mechanistic role of EVI2B in hematopoiesis, we aimed to identify EVI2B interacting proteins. Using immunoprecipitation followed my mass spectrometry analysis we identified a list of potential EVI2B binding partners. In within those, CD97, a G protein-coupled receptor involved in adhesion and migration, was present. The interaction between EVI2B and CD97 was verified by western blot analysis in murine and human cells. Both EVI2B and CD97 localize in the cellular membrane, and we hypothesize that EVI2B interacts with CD97 and possibly regulates its function. In addition, alterations in *Evi2b*-depleted HSPCs might be caused by impaired performance of the EVI2B/CD97 complex leading to changes in cell adhesion and/or migration. Altogether, our work provides an insight into the role of EVI2B in myeloid differentiation and functionality of hematopoietic progenitors, and suggests its involvement in cellular adhesion and/or migration.

CB-4

CRIPTO-1 INDUCES MULTICENTROSOMAL MITOSES IN HUMAN EMBRYONIC STEM CELLS

Porokh V.[1], Vaňhara P.[1,2], Mináriková D.[1], Holubcová Z.[1,2], Bárta T.[1], Jurečková L.[1], Doležalová D.[1], Hampl A.[1,2]

[1]*Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2]*International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic*

Human embryonic stem cells (hESC) are pluripotent cells derived from embryoblast of blastocyst-stage embryos. When propagated in *in vitro* culture, hESC suffer from harboring chromosomal abnormalities. We have reported previously that hESC cultured *in vitro* develop abnormalities in centrosomal numbers. The molecular mechanism driving the centrosomal instability, however, remains unknown. The multicentrosomal phenotype is influenced by culture substratum and becomes eliminated in culture-adapted hESC. In culture-adapted hESC, curiously, the supernumerary centrosomes can be induced by media collected from cultures of early passage hESC. Recent studies have shown that Cripto-1 (CR-1) has multiple binding partners and can modulate a variety of intracellular signaling pathways including those converging at components of mitotic apparatus. Here we have treated high-passage culture-adapted hESC by recombinant CR-1 and found that this treatment resulted in increased frequency of multicentrosomal mitoses. This centrosome-amplifying effect of CR-1 was then abolished by neutralizing anti-CR-1 antibody added into culture media. We hypothesize that non-natural CR-1 signaling occurring in hESC cultures may belong to the factors that are responsible for genetic instability of these pluripotent stem cell.

This study was supported by funds from the Czech Science Foundation (15-11707S), from the Faculty of Medicine of Masaryk University (MUNI/A/1369/2016), and from the National Program of Sustainability II (project no. LQ1605, MEYS CR).

CB-5

OPTIMIZATION OF MASS CYTOMETRY FOR IMMUNOPROFILING OF CANCER PATIENTS

Poláková I.[1], Šmahel M.[1], Pelák O.[2], Pokrývková B.[1], Simonidesová S.[3], Grega M.[4], Tachezy R.[1]

[1]Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, BIOCEV, Czech Republic
[2]CLIP, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic
[3]Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University, Motol University Hospital, Prague, Czech Republic
[4]Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

Immune cells infiltrating the tumor microenvironment play a crucial role in tumor generation and development. From the point of view of cancer therapy, particularly immunotherapy, it is essential to know what types of immune cells are present in tumors and how these cells influence the tumor cells. Immunomonitoring may thus contribute to accurate prognosis and the choice of an appropriate treatment. Mass cytometry represents a convenient way for multi-parametric phenotyping of immune cells as it is capable of detecting over 40 parameters. In this study, we designed a panel of 31 antibodies to reveal various types of immune cells (T, B, NK and dendritic cells, monocytes, macrophages) in the tumor and blood. We tested this panel in samples of patients with head and neck squamous cell carcinoma. First, preparation of samples was optimized with respect to the number of immune cells and the proportion of tumor cells and dead cells. To perform a one-tube analysis of tumor and blood cells, two different methods of barcoding were compared: a commercial barcoding kit based on palladium isotopes and the CD45-based barcoding technique. As sample preparation, barcoding, staining, and measurement is a long and expensive procedure, possibilities of interruption of this procedure and conditions of sample storage were examined to keep the staining and measurement expenses low and measure samples from several patients at one day.

The project was supported by grant # 17-28055A from the Agency for Medical Research of the Ministry of Health of the Czech Republic and by the Ministry of Education, Youth and Sports of CR within the National Sustainability Program II (project BIOCEV-FAR) LQ1604 and by the project ªBIOCEVº (CZ.1.05/1.1.00/02.0109).

CB-6

LIPIDOME IN EPITHELIAL COLON CANCER CELLS: EXPRESSION OF GENES INVOLVED IN REGULATION OF LIPID METABOLISM – TUMOR VS. NON-TUMOR TISSUE DEFFERENCES

Karasová M.[1,2], Straková N.[1], Zapletal O.[1,2], Tylichová Z.[1,2], Levková M.[3], Hušková Z.[3], Slavík J.[1,4], Ciganek M.[1,4], Ovesná P.[1,5], Skalický P.[6], Bouchal J.[3], Ehrmann J.[3], Kolář Z.[3], Hofmanová J.[1,2], Vondráček J.[1,2], Machala M.[4], Kozubík A.[1,2]

[1]*Biofyzikální ústav AV ČR, v.v.i., Brno, Czech Republic*
[2]Ústav experimentální biologie, Přírodovědecká *fakulta MU, Brno, Czech Republic*
[3]Ústav klinické a molekulární patologie, LF UP a FN Olomouc, Olomouc, Czech Republic
[4]*Výzkumný ústav veterinárního lékařství, v.v.i., Brno, Czech Republic*
[5]*Institut biostatistiky a analýz MU, Brno, Czech Republic*
[6]*I. Chirurgická klinika, Fakultní nemocnice Olomouc, Olomouc, Czech Republic*

Cell transformation is associated with a radical modification of cellular metabolism, including activation of biosynthetic pathways providing the rapidly dividing cells with necessary structural, energetic and signaling molecules. Activation of lipid biosynthesis, in particular *de novo* synthesis of fatty acids, is a major step in this metabolic transition. Colorectal cancer (CRC) is the second leading cause of cancer-related deaths and early diagnosis is a key factor in successful therapy. Several pilot studies suggested differences in specific fatty acid (FA) composition between colon cancer and the surrounding non-tumor tissue, as well as abnormalities in plasma and erythrocyte lipid and FA profiles in CRC patients. However, as many aspects of lipid alterations in cancer remain unclear, it is difficult to derive drugs specifically targeting enzymes or regulatory molecules controlling cancer-specific lipid metabolism. In our work, we aim to identify lipid-related biomarkers suitable for better diagnosis or as alternative therapeutic targets in CRC patients. Our major goal is to evaluate lipid composition changes and modulation of molecules and pathways regulating lipid metabolism in CRC epithelial cells, as compared with non-tumor colon epithelial cells. PCR analyses of lipid metabolism-related genes (including e.g. SCD1, FASN, ACLY, caveolin-1, CD36, etc.) were performed using custom-derived qPCR arrays and we identified several molecules differentially expressed in tumor epithelial cells, in particular SCD1 (stearoyl-CoA-desaturase 1) as promising candidates for further detailed studies. SCD1 is a transmembrane protein catalyzing conversion of C16:0 and C18:0 saturated fatty acids to the corresponding monounsaturated fatty acids. It plays a major role in formation of cell membranes and cancer cell survival/proliferation. We are currently evaluating the functional role of SCD1 in alterations of CRC cellular lipidome. [Supported by projects No. 15-30585A of the Agency of Health Research of the Czech Republic.]

CB-7

INHIBITION OF CHK1 STIMULATES THE COOPERATIVE CYTOTOXIC ACTION OF PLATINUM-BASED DRUGS AND TRAIL IN HUMAN PROSTATE CANCER CELLS

Krkoška M.[1,2], Herůdková J.[1,2], Vondálová Blanářová O.[1], Paruch K.[3,4], Sova P.[5], Hyršlová Vaculová A.[1*]

[1]Department of Cytokinetics, Institute of Biophysics, Czech Academy of Sciences, v.v.i., Brno, Czech Republic
[2]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Czech Republic
[4]Department of Chemistry, CZ Openscreen, Faculty of Science, Masaryk University, Brno, Czech Republic
[5]Platinum Pharmaceuticals, a.s., Brno, Czech Republic
**correspondence to: vaculova@ibp.cz*

Checkpoint kinase 1 (Chk1) is involved in regulation of the cell cycle, DNA damage response and cell death. Chk1 has been recognized as an attractive target for pharmacological inhibition in modern oncology. Small-molecule inhibitors of Chk1 kinase have been identified and intensively investigated as single agents or in combination with various chemotherapeutic drugs, and showed to significantly enhance the chemosensitivity of numerous tumor types.
Herein, we provide the first demonstration that pharmacological inhibition of Chk1 using the potent and selective inhibitor SCH900776, currently profiled in phase II clinical trials, significantly potentiates the cooperative cytotoxic effects of platinum(II) (cisplatin) or platinum(IV) (LA-12) complexes and TRAIL cytokine in human prostate cancer cells. SCH900776 mediated enhancement of cisplatin/LA-12 and TRAIL combination-induced DNA damage and cell death, associated with stimulation of mitochondrial apoptotic pathway. Compared to SCH900776, the specific siRNA-mediated silencing of Chk1 induced similar sensitizing effects on cisplatin/LA-12 and TRAIL combination-induced apoptosis in prostate cancer cells, further supporting the important role of this kinase in modulation of cytotoxic potential of the drugs. The molecular mechanisms involved in Chk1 inhibition-mediated potentiation of anticancer effects of platinum drugs and TRAIL will be further presented and discussed within our contribution.

This work was supported by Czech Science Foundation (15-06650S), HistoPARK (CZ.1.07/2.3.00/ 20.0185), and The Brno City Municipality (Brno Ph.D. Talent).

CB-8

DECODING BLADDER CARCINOMA THROUGH HIGH DIMENSIONAL ANALYSIS

Koladiya A.[1], Otavová K.[2,3], Drbal K.[1]

[1]Laboratory of Molecular dynamics of immune response, Department of Cell Biology, Faculty of Science, Charles, University, Prague 2, Czech Republic
[2]Third Faculty of Medicine, Charles University, Prague 10, Czech Republic
[3]Thomayer Hospital, Department of Urology, Prague 4, Czech Republic

Bladder tumors show a heterogeneous biological behavior depending upon tumor subtype, growth potential, and several other characteristics. Their clinical management is complicated by the unpredictability of the malignant dyscrasia in individual patients. Relapse and treatment failure in most patients have often been attributed to chemoresistance in tumor cells and their metastasis. Emerging evidence indicates that tumor heterogeneity may play an equally important role and extends to virtually any measurable property of cancer cells. The aim of this study is to perform a cluster analysis of individual tumor cell populations and correlate it with the standard histopathology assessment (tumor stage and grade). It reveals cell surface molecules as potential biomarkers associated with the pathology and used for solid tumor profiling in general.
In the present study, we have analyzed advanced bladder carcinoma (BCa) patient samples after radical cystectomy with the main focus on the prognostic importance of cell surface marker profiles. The viSNE analysis was done to study 14-color monoclonal antibody panel data plus a cell viability probe to identify subpopulations of epithelia, endothelia and leukocytes in the multidimensional space including progenitor, inflammatory, angiogenesis and EMT signatures.
Our results show highly heterogeneous expression of all *in silico* predicted phenotypic markers on the populations of BCa cells. The association between individual molecular signatures and the patient clinical status is linked to the relapse/progression prediction using EORTC risk tables and finally the prognostic value of flow cytometry data in solid tumors will be discussed.

CB-9

MOJOSORT ™NANOBEAD CELL ISOLATION USING A COMMERCIAL MAGNETIC COLUMN PROVIDES HIGH PURITY, YIELD AND PRESERVED CELL FUNCTION

Zemlickova E.

BioLegend UK, Oxford, United Kingdom

Magnetic cell sorting has been widely used to isolate different cell types as it is a fast and reliable method to obtain discrete populations with high purity, and yields. Several platforms have been developed to isolate the cells. The two more widely used are based on columns and stand alone magnets. As researchers balance the equivalence between

these two systems, we evaluate important parameters when using the two methods, and the use of Biolegend reagents in magnetic separation columns.

CB-10

POLYCHROMATIC FLOW CYTOMETRY USING THE NEW BD FACSYMPHONY ANALYSER: PANEL DESIGN AND CHALLENGES IN DATA INTERPRETATION

Fleischer J.

BECTON DICKINSON GmbH , Heidelberg, Germany

Flow cytometry has evolved fast during the last years with more and more dyes being developed for multiparameter research. Until now, most instruments were not able to record more than 18 colours, thus limiting researchers in panel design. We now present the BD FACSymphony, a high-parameter flow cytometer, allowing researchers to run 28-colour panels and go even beyond with new dyes in development. The instrument can handle up to 50 channels, resulting in 48 colours plus FSC and SSC. We will present some of the new dyes and provide an outlook into the future for new dye developments. Example data from the instrument will be shown, and data interpretation needs to be addressed when we enter the realm of high-dimensional data. This new hardware together with new dyes will enable deeper insights into cell biology than ever before.

CB-11

ER STRESS ALTERS SENESCENT PHENOTYPE OF OVARIAN SURFACE EPITHELIAL CELLS

Vašíčková K.[1,2], Vaňhara P.[1,2], Hampl A.[1,2]

[1]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2]International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic

Ovarian surface epithelium (OSE) is a simple layer of squamous-to-cubic cells that covers the ovary. *In vivo*, OSE is a highly dynamic structure undergoing regular cycles of wounding and repair, dealing constantly with a variety of stressful signals. Clinically, OSE contributes to more than 70 % cases of epithelial ovarian cancer[1]. Therefore, understanding the molecular mechanisms of OSE stress response, regeneration and aging is necessary to elucidate various pathologies, including ovarian dysfunction, infertility and cancer. In our work, we examined the link between endoplasmic reticulum (ER) stress and senescence in murine OSE (mOSE).
ER is a principal cell organelle primarily responsible for transmembrane and secreted protein synthesis and folding. Disruption of ER function triggers a protective machinery called the unfolded protein response (UPR). UPR then arrests the RNA translation and

increases production of ER chaperones to alleviate ER stress, or, if the stress cannot be resolved, UPR induces phenotypic change or apoptosis.
Cellular senescence is a complex cell phenotype characterized by permanent cell cycle arrest and can be induced telomere shortening, DNA damage, various endo- or exogenous stressors and also by activation of some oncogenes[2]. Senescent cells accumulate with age in tissues and are assumed to play a role in age-associated diseases including cancer.
Our results indicate that mOSE activates UPR signaling upon entering senescence while ER stress induction by tunicamycin halts cell proliferation and causes increased expression of senescent marker p21. Senescent or ER-stressed cells shared a common expression pattern of UPR hallmarks. ER stress in mOSE also upregulates mesenchymal markers suggesting a link between ER stress, senescence and cellular plasticity.

Supported by the project no. LQ1605 from the National Program of Sustainability II (MEYS CR) and by Masaryk University (MUNI/A/1369/2016). We acknowledge the core facility Cellular Imaging (CELLIM), institution CEITEC MU supported by the Czech-BioImaging large RI project (LM2015062 funded by MEYS CR) for their support with obtaining scientific data presented in this paper.

1. McCluggage, W. G.: Pathology 43, 420-432, (2011).
2. Campisi, J.; d´Adda di Fagagna F.: Nat. Rev. Mol. Cell 8 (2007).

CB-12

THE TRANSCRIPTION FACTOR C/EBPγ REGULATES MAST CELL DEVELOPMENT AND FUNCTION

Kardošová M.[1*], Potůčková L.[2*], Halová I.[2], Zjablovskaja P.[1], Dráberová L.[2], Dráber P.[2], Alberich-Jorda M.[1]

[1]Laboratory of Haematooncology, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]Laboratory of Signal Transduction, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic
**Equal contribution*

Although mast cells are important part of immune system having protective role in bacterial resistance or toxin degradation, their uncontrolled activation is related to the pathogenesis of many inflammatory and allergic diseases. Consequently, it is critical to fully understand the transcriptional network that controls mast cell development and function. Differentiation of progenitors to mature mast cells is driven by several transcription factors, such as GATA1, GATA2, STAT5 and MITF, and requires downregulation of C/EBPα. Recently, we identified *Cebpg* as a direct C/EBPα target gene and observed that *Cebpg* is abundantly expressed in mast cells. In order to investigate its role in murine mast cells, we employed *Cebpg* conditional knockout mice, which allow excision of *Cebpg* in the hematopoietic system. Flow cytometry analysis of *Cebpg*$^{f/f}$ Vav-1Cre^{-} and *Cebpg*$^{f/f}$ Vav-1Cre^{+} mice, referred

here as WT and *Cebpg* KO respectively, revealed similar numbers of peritoneal mast cells in steady state conditions. Interestingly, mice lacking *Cebpg* presented defective peritoneal mast cell repopulation upon ablation with distilled water injection. To further explore the role of C/EBPγ in mast cells, we generated bone marrow-derived mast cells (BMMCs). We observed that bone marrow from *Cebpg* KO mice produced reduced number of mature mast cells in comparison to WT controls. Further analysis of BMMCs showed that deletion of *Cebpg* reduced mast cell migration towards antigen, SCF or PGE, and degranulation upon FcεRI-mediated activation. Next, we revealed that BMMCs exhibit increased expression of C/EBPα transcription factor in the absence of C/EBPγ. In summary, we demonstrated that C/EBPγ is important transcription factor which suppresses C/EBPα expression, thereby favoring mast cell development and function. Our data identifies a new component of the mast cell transcriptional network and provides a better understanding of mast cells in normal physiological conditions and disease.

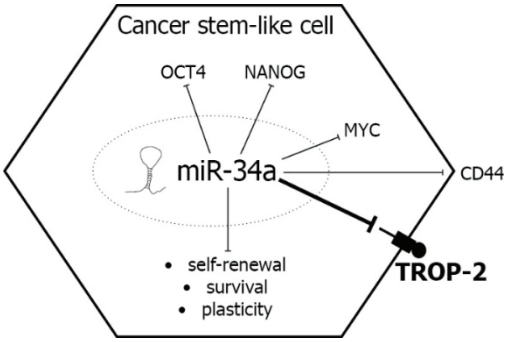
CB-13

STEM CELL MARKER TROP-2 IS A TARGET OF TUMOR SUPPRESIVE MIR-34A

Slabáková E.[1], Remšík J.[1,2,3], Kubík M.[1,3], Culig Z.[2,4], Souček K.[1,2,3]

[1]Department of Cytokinetics, Institute of Biophysics of the CAS, v.v.i., Brno, Czech Republic
[2]Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic
[3]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[4]Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Austria

MiR-34a is an important regulator of cell cycle control, apoptosis, motility and stem-like properties which is frequently downregulated in cancer. The strategy of miR-34a re-expression in advanced tumors has been investigated in clinical trials. Trop-2 is a transmembrane glycoprotein encoded by the TACSTD2 gene. It manifests stem-cell like qualities, abundant expression in distinct cancer cell populations and has been proposed as a target for antibody-based therapy.
We demonstrated that miR-34a modulation by miRNA mimics or inhibitors affects Trop-2 protein and TACSTD2 transcript levels in multiple epithelial cell lines of breast and prostate origin. A reporter assay confirmed an interaction of miR-34a with a predicted binding site in TACSTD2 3'UTR, which was disrupted by targeted mutation or deletion. Altogether, we propose Trop-2 as a new target of tumor suppressive miR-34a.



This work was supported by funds from The Czech Science Foundation, grant nr. 15-11707S, Ministry of Health of the Czech Republic, grant nr. 15-28628A, all rights reserved (KS), projects no. LQ1605 from the National Program of Sustainability II (MEYS CR), by the project FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123 (OP VaVpI), and by project HistoPARK (CZ.1.07/2.3.00/20.0185; KS). We would like to thank Iva Lišková, Martina Urbánková and Kateřina Svobodová for technical assistance.

CLINICAL IMMUNOLOGY

CI-1
MONITORING CHANGES IMMUNITY DURING ALLERGEN-SPECIFIC IMMUNOTHERAPY

Vlas T., Vachová M., Panzner P.

Department of Immunology and Allergology, Faculty Hospital, Pilsen, Czech Republic

Allergic diseases are characterized by complex changes in the immune system.
Specific allergen immunotherapy (SIT) is a challenging process that induces changes in the immune system. Previously, this process was referred to as desensitization or hyposensitization. Treatment with low concentrations of allergens leads to the induction of a physiological immune response. Drug therapy is now being implemented with antihistamines. These drugs have no effect on the mechanism of allergic reaction. Here is the advantage of SAIT, which affects the processes of allergic reaction.
In our study we observed in patients with allergen-specific immunotherapy for wasp and bee venom. We monitor patients with severe anaphylactic shock (type IV.). The tracking time is 5 years. Every year we monitor the parameters of humoral and cellular immunity.
SAIT induces tolerance to allergens. We were observing clinical response by reductions in reactivity in skin tests, by decreasing the intensity of symptoms, and by lower consumption of the anti-allergy drugs. We found an increase in specific IgG4 and a decrease in specific IgE. Changes in levels of specific IgE were significant, ranging from 2 % to 10% reduction. Furthermore we monitored changes in the quantitative expression of the FCeRI receptor on

the surface of basophilic granulocytes. We also discovered changes in the representation of T regulatory lymphocytes. This subpopulation of CD4-positive lymphocytes increased during specific allergen immunotherapy. SAIT effected the Th1 / Th2 system balance as well. In our case there was a decrease in IL-4 production in T helper lymphocytes. These changes had an impact on the synthesis of immunoglobulin's and led to the production of specific IgG4. SAIT affects also B-lymphocytes, which are converted to B regulated lymphocytes (Br1). These cells with a specific CD19 positive / CD71 negative / CD73 positive phenotype produce high levels of IL-10. This subpopulation of B lymphocytes was monitored *in vitro* after stimulation with the specific antigen of Ap1 m1. We observed an increase in the population of Br1 lymphocytes during the SAIT. We also recognized a change in the representation of peripheral circulating dendritic cells (Dc) measured as CD123 +, HLA-Dr ++ cells. Their level in the peripheral blood increased during treatment and later, after completing the SAIT, the circulating DC count went to normalize.
With SAIT, there is a decrease in specific IgE levels. Levels of specific antibodies in IgG4 increase. The FCeRI receptor expression on basophilic granulocytes was reduced. The presence of T regulatory and B regulatory lymphocytes increased at the same time. There was a reduction in T helper cell production and a transient increase of dendritic cells in peripheral blood during the SAIT period. The SAIT may affect the development of an allergic disease. By understanding the exact effect of SAIT we can improve the effectiveness of treatment, find a suitable biomarkers of the efficacy of specific allergen immunotherapy.

CI-2

IMMUNOMODULATORY EFFECT OF PROBIOTIC STRAIN E. coli O83:K24:H31 AND ITS ROLE IN ALLERGY PREVENTION

Hrdý J.[1], Súkeníková L.[1], Novotná O.[1], Petrásková P.[1], Boráková K.[2], Prokešová L.[1]

[1]*Institution of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic*
[2]*Institute for the Mother and Child, Prague, Czech Republic*

Allergy belongs to one of the most common diseases with steadily increasing incidence. Early administration of selected probiotic strains could prevent allergy development. We have shown the decreased allergy incidence in children after postnatal application of the probiotic strain *Escherichia coli* O83:K24:H31 (EcO83). The effect of EcO83 on newborn´s immune system and the capacity of EcO83 to promote dendritic cell (DC) maturation and polarisation of immune responses were followed. Increased presence of activation marker CD83 was observed on DC stimulated *in vitro* by EcO83, DC of newborns of allergic mothers having significantly higher increase of CD83 surface expression than DC of children of healthy mothers. Increased gene expression and secretion of IL-10 was detected in DC stimulated with EcO83, the increase being higher in DC of newborns of healthy mothers in comparison with that of allergic ones. Coculture of EcO83 stimulated DC with CD4+T cells generally increased the presence of intracellular cytokines tested. Intracellular presence of Th1 and Th2 cytokine was more pronounced in T cells of newborns of allergic

mothers whereas IL-10 was increased CD4+T cells of newborns of healthy mothers. We can conclude that newborns of allergic mothers have generally increased reactivity of both DC and CD4+T cells which together with decreased capacity of DC of newborns of allergic mothers to produce IL-10 could support inappropriate immune responses development after allergen encounter. EcO83 induces dendritic cell maturation and increases their production of IL-10. This modulation could help to suppress allergy development. This work was supported by AZVCR15-26877A, Charles University program Progres Q25/LF1, UNCE 204017 and SVV 260 369.

CI-3

CD MAPS – REVISITING CD1 TO CD100 EXPRESSION ON HUMAN IMMUNE CELL SUBSETS

Kalina T.[1], Kužílková D.[1], Fišer K.[1], Cuenca M.[2], Álvarez E. B.[3], Bartol S.J.W.[4], Andres-Perez M.[3], van Zelm M.C.[4,5], Engel P.[2]

[1]Charles University in Prague, Prague 5, Czech Republic,
[2]Univ of Barcelona, Barcelona, Spain,
[3]Departamento de Medicina y Servicio de Citometria, Universidad de Salamanca, Salamanca, Spain,
[4]Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands
[5]Department of Immunology and Pathology, Monash University, Melbourne, VIC, Australia

Cluster of differentiation (CD) is a nomenclature dividing mAbs into groups of same antigen specificity. Defined by Human Leukocyte Differentiation Antigens (HLDA) Workshops it has been the cornerstone of immunology and flow cytometry for more than three decades.
However the mapping of CD antigens to haematopoietic cell populations is influenced by the weaknesses of individual studies and by flow cytometry technical limitations dating up to over 30 years back. To overcome such limitations HCDM started a CDMaps project. CDMaps aim to systematically map CD markers to haematopoietic cell populations in a standardised environment using modern flow cytometry.
Here we present the results of first phase of CDMaps, mapping first hundred (n = 118) CD mAbs to 47 immune cell subsets from three tissues (blood, thymus and tonsil). Using he standardized approach developed by the EuroFlow consortium we measured PE conjugated CD antibodies as part of 8-color panels allowing cell subset definition. For each CD mAb at least $2x10^6$ cells were acquired. The level of CD mAb bound was measured as an antibody binding capacity (ABC) using QuantiBRITE beads. Resulting dataset consists of Median ABCs (MedQB), absolute and relative cell count and other derived statistics of over 5500 CD - cellular subset combinations.
To facilitate analysis of this large dataset we developed a web based application with several data interrogation scenarios: 1) CD expression over cell population sets (e.g. CD10 and CD38 expression over B cell subsets in blood), 2) Comparison of CDs inside cell population subsets (e.g. CD20 to CD30 on naïve CD8 T cells), 3) Differential CD expression

between cell subsets (e.g. which CDs are significantly different between all CD4+ and all CD8+ cell subsets), 4) Principal component analysis and 5) Hierarchical clustering analysis both providing means to find cell subset groupings.
In conclusion CDMaps systematically map over a hundred of CD mAbs to a spectrum of haematopoietic populations and provides web based platform facilitating user friendly analysis of the whole dataset. This application will serve as a useful resource to widen and advance studies into basic, translational and clinical immunology.

CD Maps project is supported with reagents by BD Biosciences, BioLegend and Exbio. TK is supported by Ministry of Health Czech Republic grant 15-26588A and LO1604.

CI-4

ASSOCIATION OF CIRCULATING $CD4^+CD8^+$ T-CELLS WITH SOLID CANCER IN HUMANS

Šinkorová Z.[1], Vaňásek J.[2], Kolářová I.[2], Vilasová Z.[2], Zuzulová M.[3], Jarčušková J.[3], Zárybnická L.[1], Šinkora J.[4]

[1]*Radiobiology, Faculty of Health Sciences, Hradec Kralove, Czech Republic*
[2]*Oncology, Multiscan s.r.o., Pardubice, Czech Republic*
[3]*Clinical Immunology and Alergology, Cancer Institute St Elisabeth, Bratislava, Slovakia*
[4]*Life Sciences, Becton Dickinson Czechia, s.r.o., Prague, Czech Republic*

Introduction: $CD4^+CD8^+$ double positive (DP) T-cells represent a prominent population of peripheral lymphocytes in some species. While DP T-cells can easily be detected in circulation in e.g. pigs and chicken where their numbers significantly increase with age, they are rather rare in most intensively studies mammals – mice and humans – where they are frequently overlooked or even considered doublets of single positive (SP) T-cells. We have recently proven that clonally expanded DP Tcells are associated with spontaneous as well as induced tumor regression in the melanoma pig model. In some patients with solid cancer, we have observed a clearly visible population of DP T-cells strongly resembling DP T-cells in melanoma rejecting swine.
Materials and Methods: Multicolor immunophenotyping and flow cytometry have been used to characterize peripheral DP T-cells in circulation of patients suffering from different types of solid cancer (ovarian, melanoma, cervix). Surface marker expression and the presence of granzyme B and perforin were studied in the attempt to bring some evidence that, similar to pigs, such cells can be involved in specific anti-tumor immunity.
Results and Discussion: Both in patients and control samples, two major subpopulations with DP phenotype have been defined based on the level of CD4 and CD8 expression: $CD8^{hi}CD4^+$ and $CD8^{lo}CD4^+$ T-cells the frequencies of which had an increasing trend with age. Within both of subpopulations, several subsets differing in surface marker expression and intracellular presence of granzyme B and/or perforin were found. Interestingly, in some patients with solid cancers, a phenotypically homogeneous subset bearing lower level of CD4 dominated in the DP compartment strongly resembling DP T-cells associated with melanoma regression in pigs. Such T-cells were negative for CD27, CD28, CD25, CCR7, HLA-

DR and CD127 possessed CD56 and CD57 NKT-cell markers on the surface, exerted Th1 cytokine profile and had granzyme B and perforin in cytoplasma. Taken together, such a subset represents activated/memory T-cells with cytotoxic potential phenotypically different from the majority of SP $CD4^+$ lymphocytes, both helper and regulatory T-cells. Another step in its classification will be testing its clonal character upon sorting and TCR characterization.

CI-5

MONITORING OF INTRAHEPATIC AND CIRCULATING IMMUNE SYSTEM FEATURES IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

Macek Jilkova Z.[1,2], Kurma K.[1,2], Granon A.[1,2], Marche P.[1,2], Decaens T.[1,2,3]

[1]Université Grenoble Alpes, France
[2]Institute for Advanced Biosciences, Research Center UGA / Inserm U 1209 / CNRS 5309, Grenoble, France
[3]Clinique Universitaire d'Hépato-gastroentérologie, Pôle Digidune, CHU Grenoble, France

During development of hepatocellular carcinoma (HCC), an effective antitumor immune surveillance in liver microenvironment is impaired. Thus, enhancement of antitumor immune responses by immune checkpoint modifications is a promising treatment strategy. However, the network of inhibitory and stimulatory checkpoint molecules is very complex and extensive studies need to be carried out to clarify the co-expression of various inhibitory and stimulatory checkpoint molecules such as PD-1, CTLA-4, TIM-3, LAG-3, OX40 or 4-1BB and their compensatory changes induced by different treatments.
The aim of this study was to investigate intrahepatic and circulating immune system in advanced-stage HCC patients i) before treatment by performing direct immunomonitoring on fresh liver biopsies and fresh whole blood, ii) to follow the immunological changes induced during treatment by blood analyses 1 and 3 months after start of treatment and iii) finally to evaluate again intrahepatic and circulating immune system in case of tumor progression. To date, 20 HCC patients were included. Fresh liver biopsies from tumor and from non-tumor tissue were immediately mechanically homogenized and stained for multi-parametric FACS analyses. Similarly, whole fresh blood was stained by following markers: Tube 1: CD45, CD3, CD56, CD16, CD15, CD19, CD8, CD69, CTLA-4, CD274 (PD-L1) and CD279 (PD-1); Tube 2: CD45, CD3, CD56, CD16, CD15, CD19, CD8, CD137 (4-1 BB), LAG-3, CD134 (OX40), TIM-3. Samples were measured using BD-LSRII flow cytometer (BD Biosciences), data were collected with BD FACS Diva 6.3.1 software and analyzed using FCS Express V6 software.
We constantly observed differences in checkpoint molecule expressions on intrahepatic immune cells compared to peripheral immune cells. The frequency of PD-1+ intrahepatic T lymphocytes was much higher compared to peripheral T cells and PD-1 was mainly expressed by CD8+ T lymphocytes in the tumor tissue. Similarly, around 50% of CD8+ T cells in tumor tissue expressed LAG3. The frequency of intra tumoral T cells expressing inhibitory checkpoint molecules Tim3 and CTLA4 was around 15%, while expression of

stimulatory checkpoint molecules OX40 or CD137 was negligible (less than 5%). The compensatory upregulation of alternative inhibitory immune checkpoints after therapeutic PD-1 blockade in non-responding HCC patient was observed. In conclusion, our study helps to understand the complexity of the inhibitory receptor network that regulates immune system to generate an immunosuppressive microenvironment of HCC and represents an opportunity to derive predictive factors for response to treatment.

CLINICAL HEMATOLOGY & CELL THERAPY

CT-1

ADVANCED PROFILING OF SWITCHING B CELL PRECURSOR LEUKEMIA TOWARDS MONOCYTIC LINEAGE

Mejstříková E., Žaliová M., Musilová A., Fišer K., Nováková M., Slámová L., Vakrmanová B., Starková J., Rejlová K., Vášková M., Trka J., Hrušák O.

CLIP – Childhood Leukaemia Investigation Prague, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Transdifferentiation of B cell acute lymphoblastic leukemia (BCP ALL) into monocytic lineage is more frequent than expected and it is typically a stepwise process during which cells express monocytic and B-lymphoid markers simultaneously. So far we did not find a single unifying (epi)genetic background, however, in comparison to other BCP ALLs, swALL is enriched for deletions, alterations and rearrangements of the ERG, IKZF1 and DUX4 genes, respectively. We observed signs of the transdifferentiation in 66 patients out of 1155 centrally analyzed BCP ALL cases. *Aims:* 1) What are the typical features of individual categories of swALLs? 2) Is it possible to define markers, ideally suitable for flow and mass cytometry, differentially expressed at diagnosis of swALLs? 3) What is the expression pattern of transdifferentiating cells? How similar it is to that of normal monocytic cells? Results: 1) Largest subgroup (55%) of swALLs represents patients with expression profile consistent with DUX4 gene rearrangement. They typically have no clear signs of transdifferentiation at diagnosis and their blasts start to transdifferentiate during the prednisone prophase. Cases with CD19posCD2pos ALL and low DUX4 expression (n=6) typically present with signs of transdifferentiation already at diagnosis. 2) Among the 23 most significantly differently expressed genes between swALLs and control BCP ALLs, two encode proteins measurable by flow cytometry: CLEC12 (CLL-1, CD371) and MS4A1 (CD20) with higher and lower expression in swALLs, respectively. 3) Although we frequently observe absence of B cell antigens (namely CD19) by flow cytometry on transdifferentiatied blasts, the expression of B cell differentiation genes) is present at higher levels in comparison to healthy mature monocytes. Following markers are being tested for discrimination of switched and healthy monocytes: CD45RA, CD52, CD22, Ki-67, CD70 and CD21.
Conclusion: The newly described subgroup of BCP ALL with DUX4 gene rearrangement

largely overlaps with previously described swALL. CLL-1 seems to be a sensitive marker for identification of swALL. Transcriptomic profile of transdifferentiated monocytoid cells differ from that of healthy monocytes, and residual expression of B cell genes speaks for true transdifferentiation rather than for clone independence.
Supported by Ministry of Health of the Czech Republic, grant nr. 15-28525A, by Ministry of Education, Youth and Sports NPU I nr.LO1604 and by UNCE 204012.

CT-2

MESENCHYMAL STROMAL CELLS FOR STEROID-REFRACTORY GvHD – LABORATORY RESPONSE DURING A CLINICAL TRIAL

Holubova M., Karas M., Hrabetova M., Vlas T., Jindra P., Lysak D.

Hemato-oncology dept., Faculty Hospital in Pilsen, [2]Czech National Marrow Donor Registry

Mesenchymal stromal cells (MSC) are tested in many studies dealing with their regenerative or immunomodulatory potential. In our clinical trial we use their ability to reduce the symptoms of a steroid-refractory graft versus host disease (GVHD) which negatively affects quality of life after allogeneic stem cells transplant.
MSC were applied for the treatment of patients with GVHD nonresponding to standard therapy (14 acute and 14 chronic GVHD). A basic lymphocyte´s subsets and dendritic cells (key players in GVHD pathology) were measured before application and 14-30-60-100-180 days after treatment. The peripheral blood samples were stained with the panel of antibodies for detection of B-cells, NK cells, T-cells (Tregs; Th1/Th2/Th17; naïve/memory subsets), dendritic cells and measured on BD FACSCanto II flow cytometer. The percentage representation was evaluated with FlowJo software and the absolute counts of the individual subsets were calculated using flow cytometry and haematology analyser.
The day+100 response evaluation showed the reduction of corticosteroids dose in all aGVHD pts. (to 17% of the starting dose) and in 83% chGVHD pts. (to 56%). The majority of lymphocytes subsets did not show any typical trend correlating with GVHD severity. Surprisingly, we detected Tregs decrease in both groups without changes in naïve vs memory portion. B cells involved mainly in cGVHD decreased in this group. In contrast, NK cells playing a key role in aGVHD decreased in patients with this form of GVHD. Plasmacytoid dendritic cells increased in both group whereas myeloid cells grew only in aGVHD patients. Our results indicate the influence of MSC on activated NK cells in acute GVHD and B-cells in chronic GVHD. The increase of dendritic cells (used as a marker of GvHD severity in many studies) correlated with good response to MSC and can be used as a biomarker for evaluation of this experimental treatment.

The work was supported by grants Ministry of Health FNPL-00669806.

CT-3

AMBIGUOUS LEUKEMIAS IN CHILDREN: HOW TO GET FROM THE UNCERTAINTY

Hrusak O.[1,4], De Haas V.[2,3], Luks A.[4], Janotova I.[4], Mejstrikova E.[1,4], Bleckmann K.[6], Moricke A.[7], Irving J.[8], Konatkowska B.[9], Alexander T.[10], Inaba H.[10], Schmiegelow K.[12], Stokley S.[13], Rossi J.[14], Felice M. S.[15], DallaPozza L.[16], Morales J.[17], Dworzak M.[18], Buldini B.[19], Basso G.[20], Campbell M.[21], Cabrera M. E.[22], Marinov N.[22], Elitzur S.[24], Izraeli S.[25], Luria D.[24], Feuerstein T.[27], Kolenova A.[28], Svec P.[28], Kreminska E.[30], Rabin K. R.[31], Polychronopoulou S.[32], da Costa E.[33], Vibeke Marquart H.[12], Kattamis A.[35], Ratei R.[36], Reinhardt D.[37], Moorman A. V[8], Schrappe M.[38], Stary J.[4]

[1]2nd Faculty of Medicine, CLIP, Charles University, Prague, Czech Republic; [2]Dutch Childhood Oncology Group (DCOG), The Hague, Netherlands; [3]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands; [4]Dept. Pediatric Hem/Onc, Charles University, Hospital Motol, Prague, Czech Republic; [6]ChristianAlbrechtsUniv. Kiel, Univ. Med.Center, Kiel, Germany; [7]Department of Pediatrics, University Medical Center SchleswigHolstein, Campus Kiel, Kiel, Germany; [8]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom; [9]Department of Pediatric Hematology, Oncology and Transplantology, University of Medical Sciences, Poznan, Poznan, Poland; [10]Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN; [12]Department of Pediatrics and Adolescent Medicine, The Juliane Marie Centre, Rigshospitalet, Copenhagen University Hospital and University of Copenhagen, Copenhagen, Denmark; [13]Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; [14]Immunology and Rheumatology, Hospital Nacional de Pediatría Prof. Dr. J. P. Garrahan, Buenos Aires, Argentina; [15]Hematology and Oncology, Hospital Nacional de Pediatria Prof. Dr. J. P. Garrahan, Buenos Aires, Argentina; [16]Australian and New Zealand Children's Haematology/Oncology Group, Melbourne, Australia; [17]Sydney Children's Hospital Network, NSW, Westmead, Australia; [18]Department of Pediatrics, Medical University of Vienna, St. Anna Children's Hospital and Children's Cancer Research Institute, Vienna, Austria; [19]Woman and Child Health Department, University of Padova, Padova, Italy; [20]Department of Woman's and Child's Health, Laboratory of Pediatric Oncohematology, University of Padova, Padova, Italy; [21]Department of Pediatric Hematology Oncology, Hosp Roberto del Rio / Universidad de Chile, Santiago, Chile; [22]Universidad de Chile, Hospital del Salvador, Santiago, Chile; [24]Pediatric Hematology Oncology, Schneider Children's Medical Center, PetahTikva, Israel; [25]Cancer Research Center, Sheba Medical Center, Ramat Gan, Israel; [27]Clalit Health Svces, Tel Aviv, Israel; [28]Commenius Univ. Children's Hospital, Bratislava, Slovakia; [30]Regional Oncologic Hospital, Kiev, Ukraine; [31]Pediatric Hematology/Oncology, Baylor College of Medicine, Houston, TX; [32]Department of Pediatric Hematology/Oncology, Athens, Greece; [33]Bone Marrow Transplantation Center, National Cancer Institute (INCA), Rio de Janeiro, Brazil; [35]First Department of Pediatrics, University of Athens, Athens, Greece; [36]HELIOS Klinikum Berlin Buch, Berlin, Germany; [37]Pediatric Hematology and Oncology, University Children's Hospital Essen, Essen, Germany; [38]Department of General Pediatrics, ALLBFM Study Group, Christian Albrechts University Kiel and University Medical Center Schleswig Holstein, Kiel, Germany

Despite attempts to improve the definitions of ambiguous leukemia (AmbL) during the last two decades, general therapy recommendations are missing. We have recently reported a large cohort of children with AmbL and propose an algorithm for treatment. A retrospective multinational study on 247 cases of pediatric patients who fulfilled AmbL definitions is presented. Survival statistics were used to compare the prognosis of subsets and types of treatment. Log rank Test was used to evaluate the results. Five-year event-free survival (5yEFS) of patients with acute lymphoblastic leukemia (ALL)-type of primary therapy (78±4·1%) was superior to that of children who started on acute myeloid leukemia (AML)-type or combined-type treatments (38±7·2% and 50±12%, respectively). When genetic syndromes and ALL- or AML-specific gene fusions were excluded, 5yEFS of $CD19^{pos}$ leukemia was 82±5·4% on ALL-type primary treatment compared to 0±0% and 28±14% on AML-type and combined-type primary treatment, respectively. Superiority of ALL-type of treatment was documented in single-population mixed phenotype AmbL (using WHO and or EGIL definitions), and in the bilineal AmbL. We present a treatment recommendation algorithm for primary treatment in AmbL. The benefit of transplantation, which might be affected by the retrospective nature of the study, was only significant in AML-type primary therapy. A poor initial response to ALL treatment was generally more frequent than reported for children with non-ambiguous ALL. We found no marker that would be a clear indication for a change to AML-type treatment. Treatment with ALL-type protocols is recommended for the majority of pediatric patients with AmbL. AML-type of treatment is recommended as a first choice for undifferentiated leukemia. AML-type of treatment is not recommended for $CD19^{pos}$ AmbL. For 30% of patients, the recommendation is not unequivocal because the difference between types of treatment was less clear-cut. The results provide a basis for a prospective multinational trial.
Supported by Ministry of Health of the Czech Republic, grant nr. 15-28525A.

CT-4

DIAGNOSTIC UTILITY OF FLOW CYTOMETRY IN MYELODYSPLASTIC SYNDROME (SINGLE CENTRE EXPERIENCE)

Zábranská T., Holubová M., Lysák D., Jindra P.

Charles University Hospital in Pilsen, Czech Republic

Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic stem-cell disorders with bone marrow cells displaying aberrant morphology and maturation, resulting in ineffective blood cell production. The diagnosis is primarily based on a combination of morphological and cytogenetic bone marrow examination.
Objective: Verification of diagnostic utility of flow cytometry (FCM) in MDS.
Patients: We performed a retrospective analysis of 28 consecutive patients examined by FCM in 2016-2017 with a suspicion of MDS based on morphological assessment of bone marrow. In our cohort of patients there were 15 women and 13 men, median age 66 years (7-90).

Methods: FCM analysis of surface antigen evolution and their aberrant expression on monocytes (CD14/ CD64/ CD11b/ HLA-DR/CD56), granulocytes (CD16/ CD10/ CD11b/ CD13/ HLA-DR) and myeloid progenitors (CD117/ CD34/ HLA-DR/ CD7/ CD19/ CD56). FCM analysis was executed on BD FACS Canto II, software Diva and the data were evaluated using FACS MDS score according to Ogata K et al.,2006 (FACS MDS score 0-4, FACS MDS status A-C).
Results: We have followed up a correlation between FCM score and morphological assessment of myelodysplastic features in bone marrow, changes in complete blood count, cytogenetic risk category and IPSS-R. We have detected a significant correlation between the grade of morphological myelodysplastic changes and FACS MDS status as well as FACS MDS score ($r = 0,85$, resp.$r = 0,61$, $p<0,05$) and also between FACS MDS status and the presence of anemia ($r=0,66$, $p<0,05$).
Conclusion: Morphological assessment of myelodysplastic features in bone marrow is influenced by inaccuracy of subjectivity, cytogenetic shows a normal karyotype in a number of cases, FCM is therefore a valuable method for reassuring the diagnosis of MDS. Our goal is to continue the follow-up and create a larger group of patients with the possibility of better statistical evaluation.
This work was supported by the grant MZ ČR-RVO (FNPl, 00669806).

CT-5

DIFFERENT APPROACHES FOR VIABILITY TESTING OF CELL THERAPY PRODUCTS

Groh T.[1], Kočí Z.[1,2], Kosnarová E.[1], Bauer P.O.[1,3]

[1]*Bioinova, Ltd., Vídeňská 1083, Prague, Czech Republic*
[2]*Institute of Experimental Medicine, Czech Academy of Sciences, V*ídeňská 1083*, Prague, Czech Republic*
[3]*Laboratory for Molecular Diagnostics, Clinical Biochemistry, Hematology and Immunology, Homolka Hospital, Roentgenova 37/2, Prague, Czech Republic*

One of the crucial attributes for efficacious cell-based therapies is a sufficient cell viability within the applied products. Two general approaches for cell viability analysis have been introduced by the European Pharmacopeia (chapter 2.7.29) including cell staining with viability dyes and subsequent manual or automated counting using flow cytometer or hemocytometer. The flow cytometry-based method relies on dyes that cross damaged membranes. Most commonly used dyes are propidium iodide and 7-AAD, while manual counting methods commonly utilize trypan blue.

We have optimized several other approaches and validated a method for viability measurement in Good Manufacturing Practice (GMP) regime. As this method lacks efficiency for some types of input biological specimens, we tested several strategies depending on specific properties of particular cell therapy products. We identified critical steps to be considered while developing new protocols for viability testing.

IMMUNOLOGY

IM-1
PATTERN RECOGNITION RECEPTORS-TRIGGERED CALCINEURIN-NFAT SIGNALLING IN HUMAN MONOCYTES

Bendickova K., Tidu F., Jose S.S., Burilova P., Fric J.

Center for Translational Medicine (CTM) ,International Clinical Research Center (ICRC) St. Anne's University Hospital Brno, CZ

The calcineurin (Cn)-NFAT signalling pathway has been recently identified as an important player in the innate immunity. Activation of NFAT proteins is induced by the engagement of receptors including pattern recognition receptors (PRRs) that are coupled to the calcium signalling pathway.
Recently we have shown that Cn-NFAT signalling in myeloid cells is essential for better survival of mice with aspergillosis. To study the role of PRRs signalling in human myeloid cells, we used monocytes isolated from blood of healthy donors or patients with sepsis. We focused on total monocyte population or their subsets ($CD14^{hi}CD16^{neg}$, $CD14^{dim}CD16^{hi}$, and $CD14^{hi}CD16^{hi}$). Confocal microscopy confirmed NFAT expression. Based on our previous experiments in mice, we identified array of genes linked to innate immunity potentially co-regulated by NFAT. To test whether these genes are associated with Cn-NFAT pathway also in human we stimulated monocytes with PRR ligands under inhibition of NFAT signalling using immunosuppressive drug cyclosporine A or tacrolimus. Subsequently, we analysed their expression both on mRNA and protein level. Pentraxin-3 was identified as candidate gene co-regulated by NFAT signalling in human.
In order to provide detail understanding of involvement calcineurin signalling in regulation of monocyte transcription in response to PAMPs we have prepared NFκB, NFAT and HIF-1α luciferase reporter lines from cell lines Thp-1 and HL-60. Observed changes in gene expressions were correlated to transcription factor activity.
Cn-NFAT dependent gene expressions upon PRRs triggers in mouse and human myeloid leukocytes showed importance of NFAT pathway during innate immune response. This research is especially pertinent for immunosuppressed patients treated with Cn/NFAT inhibitors where such data can explain part of patients' susceptibility to infections and potentially provide data leading to better infection control.

IM-2
CASPASE 8 DEFICIENCY IMPAIRS NFκB SIGNALING AND APOPTOSIS

Kanderová V.[1], Ravčuková B.[2], Grombiříková H.[2], Fejtková M.[1], Klocperk A.[3], Zentsová I.[3], Paračková Z.[3], Kalina T.[1], Šedivá A.[3], Freiberger T.[2]

[1]CLIP-Cytometry, 2nd Faculty of Medicine and University Hospital Motol, Prague, CZ

[2]*Centre for Cardiovascular and Transplantation Surgery, Brno, CZ*
[3]*Institute of Immunology, 2nd Faculty of Medicine and University Hospital Motol, Prague, CZ*

Background
Caspase 8 is a member of the cysteine-aspartic acid protease (caspase) family. It is primarily involved in the signaling leading to apoptosis but it can also behave as non-apoptotic signaling molecule. To date, only one loss-of-function mutation in one family has been described (Science 2002, J Clin Immunol 2015). It causes syndrome characterized by severe lymphoproliferation, lymphadenopathy and splenomegaly, but also immune deficiency with increased susceptibility to infections.
Methods and Results
Whole-exome sequencing has discovered novel homozygous mutation in *CASP8* (c.1276C>T) in Czech male patient with lymphoproliferation and immune deficiency. His parents and older brother are healthy, being heterozygotes for the same mutation. Using multicolor flow cytometry we have determined the immunophenotype of patient's peripheral blood cells and found highly activated T-cells with the maturation shift towards terminal effectors. In B-cell pool, naive cells prevailed and mature cells were reduced which correlated with low levels of serum immunoglobulins. Myeloid dendritic cells were also reduced as well as their capability to produce cytokines. Moreover we have proven loss-of-function characteristics of the mutation with series of functional tests. Upon anti-CD3/28 stimulation T-cells showed reduced caspase 8-dependent NFκB signaling as measured by single-cell phospho-flow. In contrast, caspase 8-independent NFκB signaling induced by TNFα was unchanged. Apoptosis measured by Annexin V positivity on effector memory T-cells and by caspase 3 cleavage was significantly impaired upon caspase 8-dependent anti-FAS stimulation but normal when induced by etoposide.
Conclusion
We identified novel loss-of-function mutation in Caspase 8 whose functional impact was validated in patient cells using flow cytometry-based methods detecting impaired non-apoptotic NFκB signaling and apoptotic signaling.
Supported by grants: 15-28541A; P302/12/G101; 64203; LO1604; 204012

IM-3
HOW TO MEASURE SELF-REACTIVITY OF A T-CELL POPULATION?

Horková V., Štěpánek O.

Institute of Molecular Genetics, Prague, Czech Republic

Our biochemical data and mathematical model indicated that the two major T-cell populations, CD8+ and CD4+ T cells, have different levels of self-reactivity. We addressed the level of self-reactivity of these populations in mice using flow cytometry. The set of our assays included detection of surface markers in the steady-state, T-cell activation markers upon in vivo stimulation of monoclonal T cells with antigens mimicing self-

antigens, proliferation during homeostasis by cell tracker dyes, phosphorylation of the TCR signaling components in the resting state, and usage of Nur77-GFP reporter mouse. Our data indicated that murine CD8+ T-cell are on average more self-reactive than CD4+ T cell. We show that the developmental dynamics of the interaction between kinase Lck and coreceptors CD4 and CD8 is at least partially responsible for this phenomenon.

IM-4

VALIDATION OF NOVEL CONTINUOUS LIVE-CELL ASSAYS FOR IMMUNE CELL ACTIVATION AND KILLING OF BLOOD CELL CANCERS

Bevan N., Campwala H., Szybut C., Patel K., Appledorn D., Dale T., Trezise D.

Essen BioScience Ltd., Welwyn Garden City, United Kingdom; [2]Essen BioScience Inc., Ann Arbor, MI

The blood cancers leukaemia, lymphoma and myeloma are expected to cause the deaths of > 55,000 people in the US in 2016. New immunological approaches afford great promise for improved therapies.

Here, we describe novel high-throughput live-cell image-based assays for immune cell activation and killing of target cells that are geared toward screening for new treatments for these malignancies. Myeloid and lymphoid cells (Jurkats, Raji, Ramos, WIL2-NS, THP-1, PBMCs) were plated on poly-L-ornithine (PLO) or fibronectin coated 96-well flat plates and monitored over time (h to days) using non-invasive live-cell analysis (IncuCyte®). The dynamics of proliferation were quantified via phase-contrast image analysis (% confluence), and validated as a robust measure of cell number by correlating to direct cell counts (Scepter, Millipore) and ATP assays (PerkinElmer). Anti-CD3/IL-2 (0.1-100/10 ng/mL) or anti-CD28 activation (1-100 ng/mL) of PBMCs evoked time-dependent proliferation (0-5d) that was sensitive to the initial cell density and concentration of stimulus. L-Kynurenine (4.69 - 300 µM), a metabolite of the amino acid L-tryptophan caused concentration and time-dependent inhibition of proliferation of PBMCs. To quantify immune cell killing in co-cultures, WIL2Ns and Ramos B-cell myelomas were first transduced with nuclear-targeted RFP (NucLight Red) to enable direct cell counting. PBMCs, either pre-activated or activated in situ (IL-2/CD3), were then added and the time-course of killing quantified through live (RFP) and dead/apoptotic (annexin-V) cell counting. Together, these protocol developments and validation data illustrate non-invasive continuous measurement of proliferation, activation, clustering and immune-cell killing of non-adherent tumour cells at industrial scale. Unlike flow cytometry, this approach follows the full time-course of the biology without perturbing the cells and allows cell-cell interactions to be visualised. These assays are amenable to testing new therapeutic antibodies, small molecules and genetic T-cell modulation such as CAR-T.

IM-5

CD47 ANTIBODY-INDUCED ENGULFMENT OF HUMAN T-CELL LEUKAEMIA CELLS BY BONE-MARROW DERIVED MACROPHAGES

Lovell G., Szybut C., Patel K., Campwala H., Bevan N., Appledorn D., Dale T.J., Trezise D.J.

Essen BioScience Ltd., Welwyn Garden City, United Kingdom
Essen BioScience Inc., Ann Arbor, MI

CD47 is a trans-membrane “don’t-eat-me” signalling protein that enables tumour cells to evade clearance by neighbouring phagocytes. Blocking CD47 allows phagocytes to identify and clear tumour cells and is a promising new approach for cancer immunotherapy.
In this study, we characterised anti-CD47 antibody-mediated engulfment of living tumour cells (CCRF-CEM) by mouse bone-marrow derived macrophages (BMDMs) or immortalised mouse macrophages (J774A.1). Phagocytosis was quantified using a pH-sensitive cell-labelling fluorescent probe, pHrodo, and automated kinetic live-cell analysis (IncuCyte®). CCRF-CEM cells were first labelled using pHrodo (250ng ml-1 for 1h), washed and then treated with antibody for 1 h. Target cells were then added to BMDMs or J774A.1 that had been seeded overnight on 96-well plates. Phase- and fluorescence images were captured and quantified every 15 min. Anti-CD47 antibody (B6H12.2, 0.04-5µg ml-1), but not IgG-control, produced time- and concentration-dependent engulfment of CCRF-CEMs by BMDMs (30’-4h), as evidenced by an increase in intracellular fluorescence as the label accumulates in the acidic phagosome. After 4h the red fluorescence area was increased by 25-fold (1975 ± 391 µm2 vs 80 ± 41 µm2). From close inspection of the time-lapse images cellular engulfment could be clearly observed, coincident with the appearance of the fluorescent signal. Similar observations were made with J774A.1 as the effector cell. Interestingly, the rate and degree of engulfment appeared effector cell-dependent. The mechanism of engulfment was not via induction of target cell apoptosis since anti-CD47 did not induce PS externalisation (Annexin V) or activate caspase 3/7. Anti-CD47 had no direct effect on CCRF-CEM proliferation for the first 4 h but upon longer exposures (>8 h) cell growth was attenuated. Our experimental findings substantiate the known pro-phagocytic effects of anti-CD47 antibodies, and provide a model system and method for quantitative functional analysis and mechanistic insight of CD47 modulators as cancer therapeutics.
In this study, we characterised anti-CD47 antibody-mediated engulfment of living tumour cells (CCRF-CEM) by mouse bone-marrow derived macrophages (BMDMs) or immortalised mouse macrophages (J774A.1). Phagocytosis was quantified using a pH-sensitive cell-labelling fluorescent probe, pHrodo, and automated kinetic live-cell analysis (IncuCyte®). CCRF-CEM cells were first labelled using pHrodo (250ng ml-1 for 1h), washed and then treated with antibody for 1 h. Target cells were then added to BMDMs or J774A.1 that had been seeded overnight on 96-well plates. Phase- and fluorescence images were captured and quantified every 15 min. Anti-CD47 antibody (B6H12.2, 0.04-5µg ml-1), but not IgG-control, produced time- and concentration-dependent engulfment of CCRF-CEMs by BMDMs (30’-4h), as evidenced by an increase in intracellular fluorescence as the label accumulates in the acidic phagosome. After 4h the red fluorescence area was increased by 25-fold (1975 ± 391 µm2 vs 80 ± 41 µm2). From close inspection of the time-lapse

images cellular engulfment could be clearly observed, coincident with the appearance of the fluorescent signal. Similar observations were made with J774A.1 as the effector cell. Interestingly, the rate and degree of engulfment appeared effector cell-dependent. The mechanism of engulfment was not via induction of target cell apoptosis since anti-CD47 did not induce PS externalisation (Annexin V) or activate caspase 3/7. Anti-CD47 had no direct effect on CCRF-CEM proliferation for the first 4 h but upon longer exposures (>8 h) cell growth was attenuated. Our experimental findings substantiate the known pro-phagocytic effects of anti-CD47 antibodies, and provide a model system and method for quantitative functional analysis and mechanistic insight of CD47 modulators as cancer therapeutics.

MICROFLUIDICS

MI-1

HIGH THROUGHPUT IMAGING FLOW CYTOMETRY

Holzner G., Anandkumar Sham R., deMello A.J., Stavrakis S.

Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

Flow cytometry, incorporating either point- or imaging-based detection schemes, is recognized to be the gold standard tool for the high-throughput manipulation and analysis of single cells in flow, but is typically limited in either the number of cells that can be interrogated per unit time or the resolution with which individual cells can be imaged. To address these limitations, we herein present a microfluidic imaging flow cytometer incorporating stroboscopic illumination, for blur-free cellular analysis at throughputs exceeding 100,000 cells per second. Specifically, we demonstrate the probing of size, multi-colour fluorescence and morphology of single cells at throughputs one order of magnitude higher than existing microfluidic flow cytometry platforms. By combining passive (inertial or viscoelastic) focusing of cells in parallel microchannels with stroboscopic illumination, the chip-based cytometer is able to extract multi-colour fluorescence, bright-field and dark-field images, perform accurate sizing of individual cells, intracellular localization and analysis of heterogeneous cell suspensions, whilst maintaining operational simplicity. To showcase the efficacy of the approach we apply the method to the rapid enumeration of apoptotic cells, the high-throughput discrimination cell cycle phases and localization of protein spots inside cellular compartments. Furthermore, I will present an image analysis workflow based on machine learning for the real time processing/morphometric classification of cells imaged using the imaging flow cytometer.

MI-2

A NANOBIOTECHNOLOGY-ADVANCED LAB-ON-A-CHIP FOR BLOOD CELL PHENOTYPING

Rothbauer M.[1], Frauenlob M.[2], Gutkas K.[2], Fischer M.B.[3,4], Sin-ner E.-K.[2], Küpcü S.[2,*], Ertl P.[1]

[1]Vienna University of Technology, Faculty of Technical Chemistry, Institute of Applied Synthetic Chemistry & Institute of Chemical Technologies and Analytics, Getreidemarkt 9, 1060 Vienna, Austria
[2]University of Natural Resources and Life Sciences, Department of Nanobiotechnology, Institute for Synthetic Bioar-chitectures, Muthgasse 11, 1190 Vienna, Austria
[3]Department of Life Science and Biomedicine, Danube University Krems, Dr. Karl Dorrekstrasse 30, 3500 Krems, Austria
[4]Clinic for Blood Group Serology and Transfusion Medicine, Medical University Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria

Identification, isolation, separation as well as analysis of subpopulations of cells from complex cellular samples such as blood is crucial for early detection of human diseases. For instance, iterations of cell phenotypes including surface receptor expression, cell size and frequency are of known diagnostic value. A major challenge in this field lies in reproducible immobilization of antibodies thus cell capture within biochips. In particular, poor antibody binding and site-oriented antibody binding is a well-known issue for state-of-the-art plastic surfaces commonly used for industrial mass production. Here, we present a microfluidic chip with a selective nanobiointerface for on-chip cell capture and in situ cell phenotyping,

MI-3

DEVELOPMENT OF A MICROFLUIDIC NANOTOXICOLOGICAL-SCREENING SYSTEM

Zirath H.[1], Rothbauer M.[1], Müller B.[2], Mayr T.[2], Ertl P.[1]

[1]Vienna University of Technology, Faculty of Technical Chemistry, Institute of Applied Synthetic Chemistry & Institute of Chemical Technologies and Analytics, Vienna, Austria
[2]Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Graz, Austria

Nanoparticles represent a growing area in industry and medicine due to their novel and distinguished properties. In healthcare, nanoparticles are applied in areas such as targeted drug delivery, diagnostics and regenerative medicine. However, possible toxic health effects associated with human exposure are unknown and therefore toxicological testing is recommended for all nanomaterials to evaluate potential hazardous effects. The conventional methods for safety testing of nanomaterials includes *in vivo* animal models. However, animal testing are time and cost intensive and the genetic differences between humans and e.g. rodents decreases the validity of the tests. Cell-based screening platforms for investigation of nanotoxicity has the potential to replace the conventional *in vivo* animal

models. The drawback of these systems are the static condition of the exposure which can lead to aggregation of nanoparticles, and thereby affect the stability and varying the cellular dosage. In recent years, microfluidic platforms are emerging as a promising technology for high throughput toxicology screening. With microfluidics, conditions such as flow rate, temperature, shear stress and delivery of nanoparticles can be precisly controlled. Due to the miniaturization, microfluidics is reducing the consumptions of sample, media and cells, but also enables a precise control of the fluidic and enables a high-throughput. By using system-integrated valves, pumps and gradient generators, microfluidic has the potential to further reduce the cost of the method compared to the robotic systems used with microplate systems. However, there is a lack of technology for real-time non-invasive control of nanotoxicity exposure. We are developing a microfluidic live-cell screening system with integrated optical O_2 and pH sensors for an *in situ* analyzing of nanotoxicity of different nanomaterials. Cells of different cell lines are cultured inside microfluidic chambers, representing the organs which nanoparticles are passing or aggregating after exposure. The sensors are label free and enables therefore a continuous monitoring of the cell-nanoparticle interaction during the entire exposure time. With this system, we are aiming to develop the next generation microfluidic live-cell assays for medium and high throughput nanotoxicity testing.

MI-4

EXPERIMENTAL DEVICE FOR FLUORESCENCE MEASUREMENT BY OPTICAL FIBER COMBINED WITH DIELECTROPHORETIC SORTING IN MICROFLUIDIC CHIPS

Ježek J., Pilát Z., Šmatlo F., Zemánek P.

Institute of Scientific Instruments of the CAS, v. v. i., Brno, Czech Republic

We present a device that combines fluorescence spectroscopy with fiber optics and dielectrophoretic micromanipulation in PDMS (poly-(dimethylsiloxane)) microfluidic chips. The device allows high speed detection (in the order of kHz) of the fluorescence signal, which is coming from the sample by an inserted optical fiber, e.g. from a micro-droplet flow in a microfluidic chip, or even from the liquid flowing in the transparent capillary, etc. The device uses a laser diode at a wavelength suitable for excitation of fluorescence, excitation and emission filters, optics for focusing the laser radiation into the optical fiber, and a highly sensitive fast photodiode for detection of fluorescence. The device is combined with dielectrophoretic sorting on a chip for sorting of micro-droplets according to their fluorescence intensity. The electrodes are created by lift-off technology on a glass substrate, or by using channels filled with a soft metal alloy or an electrolyte. This device found its use in screening of enzymatic reactions and sorting of individual fluorescently labelled microorganisms. The authors acknowledge the support from the Grant Agency of the Czech Republic (GA16-07965S) and Ministry of Education, Youth and Sports of the Czech Republic (LO1212) together with the European Commission (ALISI No. CZ.1.05/2.1.00/01.0017).

MI-5

DIRECTED EVOLUTION OF ENZYMES USING MICROFLUIDIC CHIPS

Pilát Z., Ježek J., Šmatlo F., Zemánek P.

Institute of Scientific Instruments of the CAS, v. v. i., Brno, Czech Republic

Enzymes are highly versatile and ubiquitous biological catalysts. They can greatly accelerate large variety of reactions, while ensuring appropriate catalytic activity and high selectivity. These properties make enzymes attractive biocatalysts for a wide range of industrial and biomedical applications. Over the last two decades, directed evolution of enzymes has transformed the field of protein engineering. We have previously devised microfluidic systems for directed evolution of haloalkane dehalogenases in emulsion droplets. In such a device, individual bacterial cells producing mutated variants of the same enzyme are encapsulated in microdroplets and supplied with a substrate. The conversion of a substrate by the enzyme produced by a single bacterium changes the pH in the droplet which is signalized by pH dependent fluorescence probe. The droplets with the highest enzymatic activity can be separated directly on the chip by dielectrophoresis and the resultant cell lineage can be used for enzyme production or for further rounds of directed evolution. The developed platform is applicable for ultra-fast screening of large libraries in directed evolution experiments requiring mutagenesis at multiple sites of a protein structure. The authors acknowledge the support from the Grant Agency of the Czech Republic (GA16-07965S) and Ministry of Education, Youth and Sports of the Czech Republic (LO1212) together with the European Commission (ALISI No. CZ.1.05/2.1.00/01.0017).

MI-6

CHARACTERIZATION OF HALOALKANE DEHALOGENASES SUBSTRATE SPECIFICITY BY CAPILLARY MICROFLUIDICS

Vasina M.[1], Buryska T.[1], Vanacek P.[1], Pilat Z.[2], Jezek J.[2], Zemanek P.[2], Damborsky J.[1], Prokop Z.[1]

[1]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic
[2]Institute of Scientific Instruments, The Czech Academy of Sciences, Czech Republic

Haloalkane dehalogenases have recently attracted a lot of attention in biotechnology as they are able to convert a broad range of halogenated substrates including a variety of toxic environmental pollutants. There is a growing number of haloalkane dehalogenases variants, whose substrate specificity profile is not yet characterized. In this study, we present a microfluidic platform which enables the characterization of haloalkane dehalogenase substrate specificity in 1000-fold decreased reaction volume and 100-times reduced time requirements in comparison with conventional methods. This platform utilizes a novel manner of substrate delivery: the partitioning of the substrate between the carrier

oil and the reaction aqueous phase of the droplet containing the enzyme. The system development, its validation and comparison with conventional biochemical methods will be presented.

REPRODUCTION IMMUNOLOGY

RI-1

DEVELOPMENT OF A NEW ANTIBODY AGAINST HUMAN CARBONIC ANHYDRASE II TO SUPPORT THE DISCRIMINATION BETWEEN FETAL AND MATERNAL CELLS IN CASE OF FETOMATERNAL HEMORRHAGE

Schuitemaker J. H.N.[1], Wijbenga G.[1], Schakel L.[1], Cremers T. I.F.H.[1,2]

[1]Research & Development, IQ Products bv, Groningen, Netherlands
[2]Dept. of Pharmaceutical Analysis, University of Groningen, Groningen, Netherlands

Objective: Blood cell trafficking from fetus to mother and vice versa is a normal physiological event that occurs at any stage in pregnancy. Larger transfusion might occur after for instance abdominal trauma and consequences can vary from fetal stress to fetal death and even lead to consequences for future pregnancies in the case of blood group incompatibility. The combination of hemoglobin F (HbF) and carbonic anhydrase II (CAII) can be used to discriminate fetal erythrocytes, high HbF and low CAII expression, from maternal erythrocytes, high CA II and low HbF expression, in the maternal circulation. During fetal development CAII is slowly upregulated after week 32 of gestation. The objective of this project was to develop a murine monoclonal antibody against human carbonic anhydrase II to improve the separation of the fetal and the maternal populations in an flow cytometry existing protocol to determine fetomaternal hemorraghe.
Methods: Mice have been immunized with human CAII and after the standard immunization protocol a spleen was fused with a myeloma cell line. Screening of the clones was performed by (direct) ELISA and flow cytometry and after further cloning steps the lines have been further characterized by epitope mapping (Pepscan, The Netherlands).
Results: Only four polyclonal cell lines, lines that had one round of limiting dilution after fusion, were generated against the full human CAII. One was of IgM, one of IgG2b and two were of IgG1 isotype. The IgM was discarded and all three IgG antibodies, reactive in (direct) ELISA, were cloned to monoclonal cell lines in three cloning rounds. One of the two IgG1's showed a clear increase in the ratio between the fetal signal vs adult signal for CAII from 7.9 (95%CI 7.1 – 8.6) to 17.2 (95%CI 13.3 – 21.2) in the flow cytometry protocol. Epitope mapping showed the reactivity of both antibodies to the $_{182}$GLLPESLD$_{189}$ residue in the CA II protein.
Conclusions: The significant homology between human and murine carbonic anhydrase proteins is likely to be the reason for the limited number of clones generate. After selection, cloning and conjugation of the best IgG clone, the ratio between the two target populations was improved considerably from the situation using a polyclonal antibody against CAII and was chosen to be used in the Fetal Cell Count™ kit (IQ Products, The Netherlands).

RI-2

FLOW CYTOMETRY IN FETOMATERNAL HEMORRHAGE – CASE REPORTS

Zajíc T.[1,4], Kamenická H.[2], Procházková R.[2,4], Bydžovská I.[3]

[1]*Regional Hospital Liberec, Department of Clinical Microbiology and Immunology, Liberec, Czech Republic*
[2]*Regional Hospital Liberec, Department of Transfusion Medicine, Liberec, Czech Republic*
[3]*Regional Hospital Liberec, Department of Gynecology and Obstetrics, Liberec, Czech Republic*
[4]*Faculty of Health Studies, Technical University of Liberec, Czech Republic*

Fetomaternal hemorrhage means entry of fetal erythrocytes (blood) into the maternal circulation. It occurs in a minute amounts in almost all pregnancies. Clinically significant FMH can lead to alloimunisation of mother. Excessive FMH is very rare, but can result in life threatening consequences. The most widely used test for quantifying FMH, the Kleihauer-Betke acid-elution test, has several limitations. Flow cytometry using specific monoclonal antibodies is promising alternative. We present several cases of excessive FMH determined by flow cytometry.

RI-3

NATURAL KILLER CELLS ACTIVATION IN PERIPHERAL BLOOD OF WOMEN WITH RECURRENT PREGNANCY LOSS AND UNEXPLAINED INFERTILITY

Jankovičová K., Vokurková D., Malá E.

Institute of Clinical Immunology and Allergology, Faculty of Medicine and University Hospital in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic

The regulation of natural killer (NK) cells in the peripheral blood and endometrial layers has been associated with reproductive immunopathology such as recurrent spontaneous abortions (RSA) and infertility of implantation failures (Kwak-Kim J. and Gilman-Sachs A., 2008, Sacks G. et al., 2012). Abnormalities in peripheral blood NK cells have also been reported in infertile / RSA women in presence of thyroid autoimmunity and endometriosis. Although there are some studies pointing out that the further research is needed before NK cell assessment can be recommended as a diagnostic tool in the context of female infertility or recurrent miscarriage (Seshadri S. and Sunkara SK., 2014).
We summarize our experiences with investigation of the functional activity and immunophenotypic characteristics of NK cells in women with RSA or infertility of unknown etiology using fast and simple cultivation method and flow cytometry. Briefly, the method is based on the detection of the early activation marker CD69 after JAR human trophoblast cell line and sperm cell antigens co-culture, respectively, with the peripheral heparinized blood samples of women.
In our point of view the results of the co-culture in vitro experiments reveal unbalanced

CD69 expression after antigen stimulation and the data are in concordance with the results of some other studies. These in vitro cultivation experiments seem to be the effective means for investigation of the cell mediated reactivity in women with the reproductive failure history.

References:

Kwak-Kim J. and Gilman-Sachs A.: Clinical implication of natural killer cells and reproduction. American Journal of Reproductive Immunology 59 (2008) 338-400.

Sacks G. et al.: Detailed analysis of peripheral blood natural killer cells in women with repeated IVF failure. American Journal of Reproductive Immunology 67 (2012) 434-42.

Seshadri S. and Sunkara SK.: Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. Human Reproduction Update 20 (2014) 429-38.

RI-4

DETERMINATION OF CYTOKINE PROFILE IN INFERTILE WOMEN

Luxová Š[1], Špryngar M.[2], Krátká Z.[1]

[1]*Laboratory of Immunology GENNET, Prague, Czech Republic*
[2]*EXBIO Praha, a. s., Vestec, Czech Republic*

Introduction:

Pregnancy is described as an immunological paradox. The maternal immune system accepts the fetus despite an expression of paternal alloantigens. This notable phenomenon is partly explained by a switch in Th-cell cytokine balance. T-helper lymphocytes can be classified into many subpopulations depending on their cytokine profiles. The most important ones are as follows: Th1 cells producing IFN γ , IL-2 and TNFα are involved in cellular immunity, Th2 cells producing IL-4, IL-5, IL-10 and IL-13 are involved in humoral immunity, Th17 cells which produce proinflammatory cytokine IL-17 and Treg cells that are indispensable for immune tolerance.

Aim:

The evaluation of cytokine profile in peripheral blood of infertile women.

Study group:

250 women with fertility disorder were examined.

Method:

Peripheral blood was diluted 1:1 with X-VIVO. Diluted samples were stimulated using sperm lysate or *JAR cells* for 24 hours or using PMA and ionomycin for 4 hours in 5% CO_2/ humidity air at 37°C . Brefeldin was added as an inhibitor of cytokine secretion for the last 4 hours of incubation. Prior to intracellular staining, cell surface staining with CD3, CD4 antibodies was performed. The cells were then fixed with 4% paraformaldehyde, washed and permeabilized. Subsequently, intracellular staining was performed using monoclonal antibodies (IL-4, IL-10, TNFα, IFN γ, IL-17). Flow cytometry analyses were carried out using NAVIOS flow cytometr (Beckman Coulter).

Results:

We detected an increased Th1: Th2 ratio in 14% of subfertility women. An increased

production of proinflammatory cytokines TNF-alpha and IFN-gamma after sperm lysate stimulation was found in 9.2% of women or 3,2% after JAR stimulation.

RI-5

EVALUATION OF ACROSOME REACTION BY FLOW CYTOMETRY

Dostálová P.[1], Dvořáková-Hortová K.[1,2]

[1]Group of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Průmyslová 595, 252 50, Vestec, Czech Republic
[2]Department of Zoology, Faculty of Science, Charles University, Viničná 7, Prague 2,Czech Republic

Ejaculated sperm are still not able to fertilize the egg. They first have to undergo a maturation process called capacitation. Only capacitated sperm can undergo the acrosome reaction and fertilize the egg. It is matter of debate where the sperm undergo the acrosome reaction, whether on the *zona pellucida* of the egg or whether earlier during their passage through the female reproductive tract or cumulus cells. Several detection methods for assessing the sperm maturation status have already been described, including tyrosine phosphorylation, chlortetracycline assay, PNA staining etc. All these methods require fixation of sperm. We are of possession of a transgenic mouse model that express EGFP under the acrosomal promotor and, as a result, the EGFP protein starts to be expressed in spermatids and localizes inside the acrosome in sperm. The loss of EGFP signal in the sperm acrosomal region indicates that sperm have undergone the acrosome reaction. This EGFP-sperm model is suitable for assessing the status of acrosome also on living sperm. Combining this model with flow cytometry is a great and strong tool for evaluating the impact of various substances such as pollutants or different inductors of acrosome reaction (progesterone, *zona pellucida* and calcium ionophore) on course of acrosome reaction.

This work was supported by project “BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University” (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund (www.biocev.eu), by the Grant Agency of the Czech Republic No. GA-14-05547S, and by the Institutional support of the Institute of Biotechnology RVO: 86652036.

RI-6

CYTOMETRY ANALYSES OF ANNEXIN V POSITIVE SPERM AS A PREDICTION MARKER OF MALE FERTILITY AND HEALTH

Krátká Z.[1], Luxová Š.[1], Vik V.[2]

[1]*Laboratory of Immunology GENNET, Prague, Czech Republic*
[2]*Urology and andrology GENNET, Prague, Czech Republic*

Introduction:
Basic semen analysis according to the WHO 2010 recommendation has its limitation as a predictive factor of male fertility potential. We recommend the extension of the test with flow cytometry analyses of apoptotic sperm, acrosome integrity, DNA fragmentation, and microscopic detection of sperm antibodies. These parameters better characterize the real quality of sperm.
Aim:
To verify the quality of sperm in semen by flow cytometry analyses.
Study group:
509 men from subfertile couples (271x normozoospermia, 238x abnormal spermiogram).
Method:
Following liquefaction the specimens were subjected to basic semen analyses. The number of viable and apoptotic sperm was evaluated using ApoFlowEx test. The number of sperm with permeabilized acrosome was evaluated using SpermFlowEx test. The fragmentation of DNA was evaluated using TUNEL method with ApoDirect kit.
Results:
The cytometry analysis of annexin V positive sperm was included in the routine diagnostic schema of men from subfertile couples treated in IVF clinic GENNET Prague. Normal value is > 50% of annexin V and propidium jodide negative cells. A higher percentage of apoptotic sperm was detected in 23% of normozoospermic patients. We confirmed the correlation between higher sperm apoptosis and higher acrosome permeability.
We recommended the andrology examination to all patients with a higher percentage of apoptotic sperm. Patients were clinically examined and microbiology, biochemical and genetic tests were performed with an attempt to identify the possible causes of subfertility. In the cases of successful treatment, an increase in sperm viability and sperm quality were observed.
Conclusion:
The cytometry analyses of apoptotic sperm in fresh semen is affordable test, which correlates with other qualitative parameters and might have a good predictive value regarding male fertility potential and it might be used as a marker of our successful treatment as well.

Educational Session
CLINICAL CASES IN FLOW CYTOMETRY

T-LYMPHOCYTE PROLIFERATION IN RESPONSE TO AN ALLERGEN STIMULUS

Vokurková D.[1], Jankovičová K.[1], Šrotová A.[1], Víšek P.[2]

[1]Inst.of Clinical Immunology and Allergology, Faculty of Medicine in Hradec Králove, Charles University in Prague, University Hospital, Hradec Kralove, Czech Republic
[2]Dpt.of Allergology, Litomyšl

Introduction:
Clinical and immunological studies suggest that type-I (IgE-mediated) and type-IV (T cell-mediated) pathogenic mechanisms are involved in most immediate and nonimmediate reactions, respectively. Hypersensitivity reaction to drugs can cause a variety of skin diseases like maculopapular, bullous and pustular erruption. The lymphocyte transformation test (LTT) is a method to determine which drug has caused the hypersensitivity reaction. The LTT measures the proliferation of T-cells to drugs in vitro – from which one concludes to a previous in vivo reaction due to a sensitization. In recent years, increasing evidence indicates the important role of T-cells in these drug-induced skin diseases. The main advantage of this test is its applicability with many different drugs - its main disanvantages are that an in vitro proliferation of T-cells to drugs is difficult to transfer to the clinical situation and that this test is rather hard and technically demanding.

Method:
Peripheral blood mononuclear cells are isolated from whole blood using Ficoll/Hypaque gradient centrifugation. The culprit drugs used for LTT are dissolved in distilled water, filtered and stored at -20°C. Cell suspensions are added to each culture-plate well and the cells are stimulated with drug solutions, with mitogen PWM (as positive control) and the negative control comprises the cells without any stimulation. The cultures are incubated for 6 days in humidified 37°C incubator with 5% $CO_{2.}$

Results:
Lymphocyte proliferation is measured using incorporation of PI into the blasts. The stimulation index (SI) is calculated as the mean ratio of the stimulated cells divided by the unstimulated cells. Some problems and case history of several patients will be discussed.

References:
1. Zahra Karami, Mehrnaz Mesdaghi, Parvaneh Karimzadeh, Mahboubeh Mansouri,et al.: Evaluation of Lymphocyte Transformation Test Results in Patients with Delayed Hypersensitivity Reactions following the Use of Anticonvulsant Drugs. Int Arch Allergy Immunol 2016;170:158–162

2. James Yun1, Fenfen Cai, Frederick J Lee, and Werner J Pichler.: T-cell-mediated drug hypersensitivity: immune mechanisms and their clinical relevance. Asia Pac Allergy 2016;6:77-89

3. Amy Popple, Jason Williams, Gavin Maxwell, Nichola Gellatly, Rebecca J. Dearman & Ian Kimber.: The lymphocyte transformation test in allergic contact dermatitis: New opportunities Journal of Immunotoxicology, 2017, 13:1, 84-91

Educational Session
FLOW CYTOMETRY BASICS

WHAT CAN AN APPLICATION SPECIALIST ADVICE AND RECOMMEND TO A FLOW CYTOMETRIST

Šinkora J.

BD Biosciences, Prague, Czech Republic

The first part of the presentation will be focused on optimized set up of a classical flow cytometer based on hydrodynamic focusing, laser beam excitation and fluorescence detection by a system of dichroic mirrors a band-pass filters. Manual compensation setup and its automatic calculation will be compared and modern approaches to standardization and result reproducibility will be discussed. Different up-to-date digital systems will be compared in terms of standardization of signal intensity measurements and compensation matrix maintenance. In the second part of presentation, recommendations, tips and tricks used for obtaining high quality and reliability data will be given. A rather limited time of presentation will not allow for extensive presentation of flow cytometry experience. However, experts and specialists form all sponsors will be present during the whole conference in dedicated locations where sufficient amount of possibilities for detailed discussions in all fields of flow cytometry will be available.

CELL BIOLOGY AND SIGNALLING

pCB-1

ADVANCED IN VITRO TOXICITY TESTING WITH LIVER STEM CELL SPHEROIDS - HEPATOTOXIC AND TUMOR PROMOTING EFFECTS OF CYANOTOXINS

Basu A., Dydowiczova A., Musilova V., Blaha L., Babica P.

Masaryk University, Faculty of Science, RECETOX, Kamenice 5, 625 00 Brno Brno, Czech Republic
amrita@recetox.muni.cz

Three-dimensional (3D) *in vitro* cell cultures are being increasingly used in pharmaceutical and toxicological research to predict effects of chemicals, since they better mimic *in vivo* physiological characteristics of cells and tissues than the traditional 2D monolayer cultures. We optimizeda protocol for simple, cost-effective and versatile preparation of 3D spheroid cultures using micromoldednonadhesivehydrogels, and we developeda workflow for automated microscopic image acquisition and analysis of spheroid morphological characteristics. This semi-high-throughput non-invasive, non-destructive and label-free method allows to quantitatively assessof spheroid growth, size and morphology, and can be easily combined with functional endpoints for evaluation of spheroid viability, metabolism, or specific molecular targets. Using this method, we prepared spheroid cultures of hTERT-immortalized adult human liver stem cells HL1-hT1, normal rat liver stem-like (progenitor) cells WB-F344, and their ras-oncogene transformed derivative (WB-ras), and tested the protocol with common environmental and drinking water contaminants -cyanobacterial toxin microcystin-LR (MC-LR) and cylindrospermopsin (CYN).Both MC-LR and CYN induced strong cytotoxic effects in mature HL1-hT1 spheroids at submicromolar concentrations which was comparable with the effective concentrations in primary human hepatocytes. WB-F344 cells formed only loose and unstable aggregates, which were less sensitive to cyanotoxin cytotoxicity. In contrast, WB-ras cells formed compact spheroids, whose growth was promoted by low concentrations of cyanotoxins, while higher concentrations induced cytotoxicity. These data indicate that cyanotoxins elicit strong cytotoxic effects in spheroids of normal liver stem/progenitor cells. Low cyanotoxin concentrations can promote growth of transformed cells in 3D. *In vitro* 3D models of liver stem and progenitor cells can thus provide an effective tool to study hepatotoxic and tumor promoting effects of toxic chemicals, and their impact on liver tissue homeostasis.

Support: Project GA15-12408S funded by the Grant Agency of the Czech Republic.

pCB-2

VACUOLAR-ATPASE-MEDIATED LYSOSOMAL SEQUESTRATION PLAYS ROLE IN RESISTANCE TO CYTOSTATIC DRUGS

Belhajová M.[1], Plch J.[1], Černá T.[1,2], Hraběta J.[1], Eckschlager T.[1]

[1]Department of Pediatric Hematology and Oncology, 2nd Medical Faculty, Charles University and University Hospital Motol, Prague 5, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Charles University, Albertov 2020, Prague 2, Czech Republic

The cytostatic drugs such as cisplatin, ellipticine and doxorubicin have become very significant in cancer treatment. However, they are known to induce resistance in NB cells. Vacuolar ATPase (V-ATPase), a proton pump required for the acidification of vacuoles, as a sensor of cytosolic pH, plays important role in development of drug resistance. This resistance is associated with V-ATPase-mediated vacuolar trapping of hydrophobic weak base chemotherapeutic drugs via a mechanism known as lysosomal sequestration and it can influence its anticancer action.
Sensitive neuroblastoma cells and cells resistant to ellipticine, doxorubicin and cisplatin were treated with these cytostatics and lysosomal capacity (volume) was measured by lysosomal uptake of lysosome-specific dye Lysotracker red (LTR) and expression of V-ATPase (ATP6V0D1). Subcellular localization of cytostatics (ellipticine and doxorubicin) and their colocalization with lysosomes were observed in confocal microscope. Quantitative image analysis was done by Squass, plug-in of ImageJ and with the statistical software R.
Treatment by doxorubicin and ellipticine, weak base drugs, increased V-ATPase expression significantly only in neuroblastoma cell lines resistant to doxorubicin and ellipticine. Cell lines resistant to cisplatin, showed downregulation of V-ATPase. LTR uptake and V-ATPase expression were depended on the concentration of ellipticine, but not cisplatin. We observed enlargement of lysosomal compartments in resistant cell lines compared to the sensitive and lower fluorescence of the nuclei. Fluorescence intensity measurements show that the LTR accumulates in lysosomes depending on the concentration of ellipticine, but not cisplatin. Resistance to doxorubicin and ellipticine in the tested neuroblastoma cells is associated with V-ATPase-mediated lysosomal trapping of these drugs.

Supported by GACR (grant 17-12816S) and GAUK (grant 812217)

PCB-3

NITRO-OLEIC ACID REGULATES FIBROBLAST FUNCTIONS IN DIFFERENT PROFIBROTIC MODELS

Cechova V., Koudelka A., Koreny M., Kubala L., Pekarova M.

Institute of Biophysics, AS CR, v.v.i., Brno, Czech Republic

It is becoming increasingly evidence that nitration products of unsaturated fatty acids (NO_2-FAs) represent an important class of endogenous biological mediators. Their effects are classically linked with anti-inflammatory activities. Recently, we discovered some new aspects of NO_2-FA functions in regulation of pathological states (e.g. atrial fibrosis and pulmonary hypertension) associated with increased proliferation activity and tissue remodeling, showing their high potential as pleiotropic signaling molecules in different cell types. Under physiological circumstances, the process of fibrosis is connected with formation of excess fibrous connective tissue in reparative or reactive processes. Nevertheless, fibrosis represents also a pathological process linked with scarring and thickening of the affected tissue (e.g. heart and lung), which could interferes with normal organ functions.

Therefore, the main aim of this study was to evaluate a protective role of nitro-oleic acid (NO_2-OA) in development of profibrotic responses in mouse fibroblasts. Pathologic conditions associated with tissue remodeling were simulated by exposition of fibroblasts to Angiotensin II (Ang II), TGF-β, IL-6 or their combination for different time points (10 min – 72 h). We have analyzed basic functions of fibroblasts by investigating cell viability, proliferation, and expression of fibrotic markers (e.g. α-SMA and MMPs) as well as activation of intracellular signaling cascades (e.g. Smads, MAPKs, NFκ-B, Akt, and STATs), crucially involved in the process of fibrosis. Interestingly, our results showed that physiologically relevant concentrations of NO_2-OA prevented the activation of fibroblasts by Ang II, TGF-β, and IL-6. The effect of NO_2-OA was mediated via reduced phosphorylation of Smads, STAT3, and Akt.

In aggregate, our study provided the unique results showing the protective effects of NO_2-OA in development and progression of fibrotic processes, supporting that NO_2-FAs represent new drug candidates suitable for deployment against chronic and inflammatory diseases having a complex pathogenesis. Following main signaling pathways, we also helped to clarify molecular mechanism of nitro-lipids action.

This work was supported by the Czech Science Foundation (17-08066Y).

pCB-4

APOFERRITIN NANOCAGE AS A PROMISING ELLIPTICINE OR DOXORUBICIN NANOCARRIER AND ITS EFFECTS ON NEUROBLASTOMA CELL LINES

Černá T.[1,2], Indra R.[1], Plch J.[2], Belhajová M.[2], Dostálová S.[3], Heger Z.[3], Adam V.[3], Hraběta J.[2], Eckschlager T.[2], Stiborová M.[1]

[1]Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic
[2]Department of Pediatric Hematology and Oncology, 2nd Medical Faculty, Charles University and University Hospital Motol, Prague, Czech Republic
[3]Laboratory Metallomics and Nanotechnology, Mendel University in Brno and Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

Conventional cancer treatment based on cytostatic therapy is highly toxic not only for

cancer cells, but also for normal ones. However, novel methods of targeting drugs to cancer cells are being investigated. Apoferritin is a protein composed of 24 polypeptide subunits, structurally arranged to create an internal cavity, which is naturally used for storage of iron ions; but artificially it can be employed for carrying of any molecule of interest.
Here, we have shown that apoferritin can carry and deliver high dose of doxorubicin (DOX) or ellipticine (ELLI) into neuroblastoma cancer cells. To evaluate potential application of this technology for neuroblastoma therapy, the aim of this study was to compare the cytotoxic effects of doxorubicin resp. ellipticine loaded apoferritin nanoparticles (APODOX resp. APOELLI) and free cytostatic on sensitive and resistant neuroblastoma UKF-NB-4 cell lines *in vitro* under the normoxic and hypoxic conditions. Cell viability was assessed using the alamar blue assay and the real time impedance based platform (xCELLigence). Apoptosis rates were measured using annexin V and DAPI. DNA-double strand breaks were detected by flow cytometry as phosphorylation of histone H2A variant.
We show here that the effect of APODOX on sensitive and resistant neuroblastoma cells is similar to that of free DOX. The cytotoxicity of APOELLI is lower than cytotoxicity of free cytostatic, but APOELLI causes more double strand breaks than free ELLI. Further, using fluorescence microscopy, we have shown that apoferritin can deliver drugs inside cancer cells and the drug exerts their effect thereof. Entry of APODOX and free DOX into sensitive and resistant cells was similar. However, entry of APOELLI into cells was significantly lower than entry of free ELLI. The results found in this study seem to be promising, because encapsulation does not affect toxicity of cytostatic and improves drug stability. Moreover, entry of APODOX is significantly lower into non-malignant cells than into cancer cells. We suppose that apoferritin is targeted to the several cancer cells (i.e. neuroblastoma) through TfR 1 which is expressed in many cancer cells.

Supported by GACR 17-12816S and GAUK 998217.

PCB-5

DOUBLE POSITIVE $CD4^+CD8^+$ T CELLS AND THEIR CYTOKINE PROFILE IN MELANOMA-BEARING LIBECHOV MINIPIGS (MELIM)

Čížková J.[1,2], Strnadová K.[1,2], Červinková M.[1,3], Horák V.[1], Šinkorová Z.[4], Šinkora J.[5], Šinkora M.[6]

[1]*Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic*
[2]*Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic*
[3]*Surgical Department, 1st Medical Faculty of Charles University and Hospital Na Bulovce, Prague, Czech Republic*
[4]*Department of Radiobiology, University of Defence, Hradec Kralove, Czech Republic*
[5]*Life Sciences, Becton Dickinson Czechia, s.r.o., Prague, Czech Republic*
[6]*Institute of Microbiology, Czech Academy of Sciences, Nový Hrádek, Czech Republic*

Mature double positive (DP, $CD4^+CD8^+$) T-cells have been reported in circulation and tissues of numerous species. Due to their relative scarceness, the role and function of DP T-cells is rarely studied in humans, although some reports have described their involvement in autoimmune disorders and immune response to cancer. In pigs, DP T-cells represent a wellknown population of peripheral lymphocytes with significant role in e.g. anti-viral immunity. In MeLiM model of spontaneously rejecting inherited melanoma, we have observed a significant expansion of blood-born DP T-cells in late stages of tumor regression. Cells of similar phenotype can be found in some tumor-bearing patients.
Using three-color direct and indirect surface immunophenotyping and PMA/ionomycin induced cytokine expression analysis we have characterized melanoma regression-associated circulating DP T-cells in MeLiM pigs. We also analyzed tumor-infiltrating lymphocytes (TIL) and identified DP T-cells strongly resembling circulating DP pool as the prevailing fraction of TIL. TCR CDR3 length polymorphism analysis of sorted Tcells with the $CD4^+CD8^{hi}$ phenotype indicated that such cells consist of a few clones (oligoclonal expansion). An extended immunophenotyping distinguished tumor regression-associated DP T-cells from DP T-cells naturally occurring and gradually accumulating in pig circulation. We have also identified an anti-CD45 isoform (designated as CD45R) monoclonal antibody that seems to recognize a subset of tumorassociated DP T-cells. These finding support our hypothesis that peripheral and tumor-born DP T-cells in MeLiM pigs represent cytotoxic, tumor-specific clones involved in melanoma elimination. In late stages of melanoma regression, such peripheral blood DP lymphocytes represent the re-circulating pool of activated/memory T-cells killing remaining melanoma cells and, as a side effect, melanocytes, which results in vitiligo. Our results could emphasize the importance of DP T-cells in homeostasis maintenance in mammalian species and the equivalent DP population in humans will receive more attention, namely in the field of tumor immunity.
This study was supported by the National Sustainability Programme, project number LO1609 (Czech Ministry of Education, Youth and Sports) and CIGA 20162001 project.

pCB-6

***IN VITRO* ASSESSMENT OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION FOR IDENTIFICATION AND CHARACTERIZATION OF CHEMOPREVENTIVE AND ANTICANCER COMPOUNDS.**

Ctverackova L., Brozman O., Dydowiczova A., Babica P.

RECETOX - Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic

Dysregulation of gap junctional intercellular communication (GJIC) has been linked to various adverse health effects and diseased states, including tumor promoting phase of cancer. Different anticancer compounds and cancer chemopreventive agents (CPAs) were demonstrated *in vivo* or *in vitro* to either prevent chemically-induced inhibition of GJIC in normal cells or to restore GJIC in neoplastic and transformed cells. In our study, we focused on evaluation of GJIC in response to various phytochemicals and dietary agents

using (semi-)high throughput method based on microplate-adapted scalpel loading-dye transfer technique in combination with automated cell imaging and image analysis. The effects of selected CPAs were investigated in a normal non-tumorigenic rat liver epithelial cell line WB-F344 characterized by functional GJIC, and also in ras-oncogene transformed WB cells, which exhibit neoplastic phenotype including downregulated GJIC and *in vivo* tumorigenicity. Our results showed that the tested CPAs were able to 1) prevent inhibition of GJIC induced in WB-F344 cells by chemical tumor promoters or toxicants; 2) restore GJIC in oncogene-transformed WB cells; and/or 3) induce different antiproliferative/cytotoxic effects in normal *vs*. ras-transformed WB cells. The observed effects of CPAs on GJIC were chemical-specific, and also concentration- and time-dependent. These results indicate that *in vitro* assessment of GJIC using automated microscopy and image analysis provides an efficient tool, which can be employed in phenotypic screening for anticancer and cancer chemopreventive drugs.

pCB-7

3-D TIME LAPSE ANALYSIS OF THE GROWTH OF CULTURED EMBRYONIC STEM CELL COLONY AND THE EXPRESSION OF A PLURIPOTENCY REGULATOR PROTEIN WITH YOKOGAWA CONFOCAL IMAGE CYTOMETER CQ1

Endo T.[1], Nomura Y.[1], Pasculli R.[2], Dintner R.[2], Ota A.[1]

[1]Yokogawa electric corporation, 2-3 Hokuyodai Kanazawa, Japan
[2]Accela s.r.o., Služeb 4 108 00 Prague 10, Czech Republic

Embryonic stem cells (ES cells) have been a fundamental resource in gene engineering and regenerative medicine. As ES cells are increasingly essential tools in life science, there are growing demands for 3-dimensional analysis of the dynamic regulation of physiological functions in living ES cells.
The CQ1 is an all-in-one confocal quantitative imaging cytometer based on the confocal scanner unit (CSU) from Yokogawa Electric Corporation. The core of the CSU is a microlens-enhanced dual Nipkow disk confocal optical system. In this system, laser beams with moderate power scan the sample multiple times during image acquisition. This scanning method results in drastically lower-phototoxicity and lower-photobleaching compared to conventional single beam scanning instruments. Therefore, the CSU has long been an ideal solution for live cell imaging since the first model was launched in 1996.
In this presentation we will report the results of 3D time lapse analysis of the growth of cultured mouse ES cell colony. Time lapse imaging was conducted with CQ1 equipped with an internal incubation chamber to control culture environment. We successfully tracked the proliferation of the cells and consequent increase of the volume of the colony for over two days. In addition, we were able to quantify the change of the expression of a pluripotency regulator protein Nanog as well. The present results indicate that CQ1 is a powerful tool for studies on living ES cells.

PCB-8

CHK1 INHIBITOR SCH900776-MEDIATED ENHANCEMENT OF PLATINUM DRUG-INDUCED CYTOTOXICITY IS ACCELERATED IN THE ABSENCE OF P21

Herůdková J.[1,2], Paruch K.[3,4], Souček K.[1,2,4], Sova P.[5], Hyršlová Vaculová A.[1]*

[1]Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, v.v.i., Brno, Czech Republic
[2]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]Department of Chemistry, CZ Openscreen, Faculty of Science, Masaryk University, Brno, Czech Republic
[4]Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic
[5]Platinum Pharmaceuticals, a.s., Brno, Czech Republic
**correspondence to Alena Hyršlová Vaculová: vaculova@ibp.cz*

Checkpoint kinase 1 (Chk1) is a serine/threonine kinase essentially involved in regulation of the cell cycle progression, DNA repair or cell death. Chk1 kinase inhibitors are currently under clinical investigation as effective sensitizers of numerous cancer cell types to the cytotoxic effects of various chemotherapeutics. However, there is still a considerable uncertainty over the role of Chk1 in modulation of anticancer potential of platinum-based drugs.
We newly demonstrated the outstanding ability of one of the most specific Chk1 inhibitors, SCH900776 (MK-8776), to enhance human colon cancer cell sensitivity to the cytotoxic effects of platinum(II) – cisplatin – and platinum(IV) – LA-12 - complexes. The combined treatment with SCH900776 and cisplatin or LA-12 resulted in apparent increase in DNA damage, G1/S phase-related apoptosis, and stimulation of mitotic slippage, accompanied by loss of important G2/M regulators. Importantly, the cooperative anticancer action of the SCH900776 and cisplatin/LA-12 was significantly accelerated in p21-deficient cells, which was associated with significant stimulation of caspase-dependent apoptosis beyond G2/M cell cycle phase, and increase in cell polyploidy, while the level of crucial mitotic regulators remained preserved. Our findings suggest that p21 status is an important determinant of colon cancer cell response to combined action of platinum-based drugs and Chk1 inhibitor SCH900776, which warrants further investigation.
This work was supported by Czech Science Foundation, project No. 15-06650S, and HistoPARK (CZ.1.07/2.3.00/20.0185) from the European Regional Development Fund.

pCB-9

BINDING OF OXALIPLATIN IMPAIRS THE FUNCTION OF NEURONAL Na^+/K^+-ATPase

Huličiak M.[1,2], Souček K.[1,2]

[1]Department of Cytokinetics, Institute of Biophysics, Academy of Science of the Czech

Republic, v.v.i., Brno, Czech Republic
[2]International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic

Na^+/K^+-ATPase (sodium potassium pump) is a member of P-type ATPases which generates concentration gradients of sodium and potassium ions across the plasma membrane of all animal cells. This protein pumps three sodium ions out and two potassium ions into the cell for each ATP molecule and maintains their electrochemical potential gradients, required for electrical excitability and transport of other ions, nutrients, and neurotransmitters, as well as for regulation of cell volume and intracellular pH. Change of activity of this enzyme can influence these mechanisms and can potentially result in variety of diseases.
Oxaliplatin (eloxatin) is a cancer medication used to treat colorectal cancer. The way that oxaliplatin operates is by forming a platinum complex inside of a cell which binds to DNA and impairs DNA replication and cell division. However, treatment by the oxaliplatin has many side-effects and the most serious is neurotoxicity which leads to chemotherapy-induced peripheral neuropathy.
We tried to verify the hypothesis that neurotoxic effect during the treatment by the oxaliplatin can be caused by interaction of the oxaliplatin with the neuronal isoform of the sodium potassium pump. According to our results we can conclude that the oxaliplatin significantly inhibits activity of the neuronal isoform of the sodium potassium pump and moreover experiments with neurons showed that the oxaliplatin impairs transportation of sodium ions clearly indicating interaction between Na^+/K^+-ATPase and oxaliplatin.

Acknowledgement
This work was supported by funds from Ministry of Health of the Czech Republic, grant nr. 15-33999A, 15-28628A, projects no. LQ1605 from the National Program of Sustainability II (MEYS CR).

pCB-10

PLATINUM-BASED DRUG-MEDIATED INCREASE OF TRAIL-INDUCED PROSTATE CANCER CELL APOPTOSIS DEPENDS ON MITOCHONDRIA BUT NOT CASPASE-10

Vondálová Blanářová O.[1], Šafaříková B.[1,2], Herůdková J.[1,2], Krkoška M.[1,2], Anděra L.[3], Sova P.[4], Hyršlová Vaculová A.[1,*]

[1]Department of Cytokinetics, Institute of Biophysics, Czech Academy of Sciences, v.v.i., Brno, Czech Republic
[2]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]Institute of Biotechnology, Czech Academy of Sciences, v.v.i., Praha, Czech Republic
[4]Platinum Pharmaceuticals, a.s., Brno, Czech Republic
**Correspondence to: vaculova@ibp.cz*

TRAIL (tumor necrosis factor-related apoptosis inducing ligand) is a cytokine with promising

anticancer potential, known for a high level of molecular specificity to trigger apoptosis in many cancer but not normal cells. However, in some cancer cell types, therapeutic relevance of TRAIL is hampered due to their resistance to its cytotoxic effects. We demonstrated a notable ability of cisplatin or LA-12 to enhance the sensitivity of several human prostate cancer cell lines to TRAIL-induced cell death via an engagement of mitochondrial apoptotic pathway. This was accompanied by augmented Bid cleavage, Bak activation, loss of mitochondrial membrane potential, activation of caspase-8, -10, -9, and -3, and XIAP cleavage. RNAi-mediated silencing of Bid or Bak suppressed the drug combination-induced cytotoxicity, further underscoring the involvement of mitochondrial signaling. The caspase-10 was dispensable for enhancement of cisplatin/LA-12 and TRAIL combination-induced cell death and stimulation of Bid cleavage. Our results indicate that anticancer strategies employing tumor-specific TRAIL combined with platinum drugs to enhance its efficacy can contribute to more successful killing of prostate cancer cells compared to the individual application of the drugs, and suggest novel molecular mechanisms.
This work was supported by the Czech Science Foundation (15-06650S), grant HistoPARK (CZ.1.07/2.3.00/ 20.0185), and Brno City Municipality (Brno Ph.D. Talent).

pCB-11

BCL2 AND BCL11A IN PREDICTION OF RELAPSE IN TRIPLE-NEGATIVE BREAST CANCER TREATED WITH ADJUVANT ANTHRACYCLINE-BASED CHEMOTHERAPY.

Kharaishvili G.[1], Svoboda M.[3], Gachechiladze M.[1], Vrbkova J.[2], Slavkovsky R.[2], Navratil J.[3], Koudelakova V.[2], Trojanec R.[2], Cwiertka K.[4], Hajduch M.[2], Kolar Z.[1], Bouchal J.[1], Bouchalova K.[2]

[1]Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[2]Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic
[3]Masaryk Memorial Cancer Institute, Department of Comprehensive Cancer Care, Brno, Czech Republic
[4]Department of Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic

Background: Patients with aggressive triple-negative breast cancer (TNBC) cannot benefit from targeted treatment and predictive markers for chemotherapy are needed. The objective of this study was to determine whether BCL2 expression and BCL11A status can predict outcome in TNBC patients treated with adjuvant anthracycline-based regimens.
Methods: The BCL2 study included 187 patients with TNBC, 164 of whom were treated with adjuvant anthracycline chemotherapy. BCL2 expression was evaluated by immunohistochemistry. For BCL11A study, fresh-frozen tumor tissues were collected from 148 patients with TNBC, 123 were treated by anthracycline-based adjuvant chemotherapy. Genomes were profiled by Affymetrix SNP6.0 arrays. Arrays were normalized using Aroma and GISTIC 2.0 was used to identify copy number changes. Survival data were analyzed

with software R.
Results: High BCL2 expression predicted poor relapse free survival (RFS) (logrank p=.035, HR 2.37, 95%CI 1.04-5.41) and a trend to poor overall survival (OS) in patients treated with adjuvant anthracycline-based regimens (logrank p=.075, HR 2.31, 95%CI 0.90-5.97). High levels of BCL2 were associated with poor OS in basal-like TNBC patients treated with adjuvant anthracycline-based regimens (log-rank p=0.033, HR 3.04, 95 %CI 1.04–8.91).
Segments with BCL11A locus were amplified in 11.6%, normal status was found in 71.7%, and deletion in 16.7% patiens. TNBC patients with BCL11A deletion treated with anthracycline-based chemotherapy had worse outcome (breast cancer specific survival, BCSS, logrank p=.017; RFS, logrank p=.021) than those with normal or amplified status.
Conclusions: High BCL2 expression and BCL11A deletion predicted poor outcome in TNBC treated with adjuvant anthracycline-based chemotherapy. BCL2 expression and BCL11A copy number status could facilitate decision making on adjuvant treatment in TNBC patients.
Grants: National Sustainability Programme LO1304 and Research Support Foundation Vaduz 991100531/39.

pCB-12
TESTING OF POTENTIAL HIV-1 INHIBITORS USING A LENTIVIRUS SYSTEM

Křížová I., Rumlová M.

UCT, Technická 5, 155 28, Prague 6, Czech Republic

Current therapy of HIV-1 infection i.e. Highly Active Antiretroviral Therapy: HAART, is based on a combination of several inhibitors targeting viral enzymes: mostly reverse transcriptase and protease. However, due to development of multidrug resistance, there is a continuing demand for identification of novel anti-HIV compounds that would target another critical steps in HIV-1 life cycle. This novel mechanisms of action would allow to expand potential treatment options and overcome the problems with the resistant strains.
Recently we have developed the assay FAITH, for *in vitro* testing of compounds interfering with the assembly of both, immature and mature HIV-1 particles (Hadravova et al., 2015). To extend the *in vitro* screening of potential HIV-1 inhibitors toward the *in vivo* experiments, we established a flow cytometric method based on a lentiviral system of second-generation. The lentiviral system provides the possibility to produce VSV-G-pseudotyped HIV-1 particles that are composed of HIV-1 Gag and Gag-Pol polyproteins, VSV-G envelope glycoproteins and two copies of RNA encoding reporter GFP. Once these VSV-G pseudotyped HIV-1 particles enter the cells, the RNA is reverse transcribed into DNA and level of GFP expression is quantified using flow cytometry. Functional lentiviral particles were prepared using three various vectors: a packaging vector (psPAX2) encoding HIV-1 structural and enzymatic Gag and Pol polyproteins, respectively; a transfer vector (pWXLDp-GFP) encoding LTR sequences and GFP reporter protein and an envelope vector (pHEV-VSV-G) encoding envelope proteins of vesicular stomatitis virus VSV-G. This system allowed us to test compounds with potential inhibitory activity in the late as well

as early phase of HIV-1 life cycle. To test the effect of inhibitory compounds on the HIV-1 uncoating during the early phase, HEK-293 cells were infected by prepared lentiviral stock in the presence of relevant inhibitors. To evaluate the effect of tested compounds during late phase of HIV-1 life cycle, the inhibitors were added immediately after transient transfection of HEK 293 cells. In both cases the number of infected cells, i.e. number of GFP-producing cells, was measured after 48 hours. Using combination of both, *in vitro* and *in vivo* methods, we identified several compounds inhibiting the early as well as late phase of HIV-1 life cycle.

This work was supported by projects: GAČR 17-25602S, CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) NPU I (LO1601 - No.: MSMT-43760/2015).

pCB-13

TOTAL BODY IRRADIATION AND LUNG: STUDY OF EARLY RESPONCE

Lierová A.[1], Jeličová M.[1], Filipová A.[1], Pejchal J.[2], Zárybnická L.[1], Němcová M.[1], Šinkorová Z.[1]

[1]Department of Radiobiology, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

[2]Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

The lung is complex organ, consisting of the large central airways (the trachea and mainstem bronchi), the smaller conducting airways and the alveolar sacs, where gas exchange occurs. Biological effect of ionizing radiation (IR) is irreversible damage to various cell types, and may thus have implications for the level of the whole tissue[1]. Radiation induced lung injury (RILI) can disrupt multiple aspects of normal pulmonary physiology, including efficient gas exchange, epithelial and endothelial integrity leading to edema and a cascade of molecular events altering the microenvironment. Simultaneously, the lung is slowly proliferating system and any radiation effects are delayed (months, till years)[2,3]duration, and severity of the complex reactions of the lung to ionizing radiation. These reactions have been assayed biochemically, cell kinetically, physiologically, and pathologically. Clinical and experimental data are used to describe the acute and late reactions of the lung to both external and internal radiation including pneumonitis, fibrosis and carcinogenesis. Acute radiation pneumonitis, which can be fatal, develops in both humans and animals within 6 months of exposure to doses greater than or equal to 8 Gy of low LET radiation. It is divisible into a latent period lasting up to 4 weeks; an exudative phase (3-8 weeks.

Our aim was to determine the effects of IR at early time intervals: 4, 8 and 24 hours, as well as at time intervals: 7, 21 and 30 days. This time period, also known as radiation pneumonitis associated with early response, is the most critical phase for preventing and possible treating radiation damage[4]accumulating evidence suggests that various cells, cytokines and regulatory molecules are involved in the tissue reorganization and immune response modulation that occur in RILF. In this review, we will summarize the

general symptoms, diagnostics, and current understanding of the cells and molecular factors that are linked to the signaling networks implicated in RILF. Potential approaches for the treatment of RILF will also be discussed. Elucidating the key molecular mediators that initiate and control the extent of RILF in response to therapeutic radiation may reveal additional targets for RILF treatment to significantly improve the efficacy of radiotherapy for lung cancer patients.","ISSN":"1389-4501","note":"PMID: 23909719\ nPMCID: PMC4156316","journalAbbreviation":"Curr Drug Targets","author":[{"family ":"Ding","given":"Nian-Hua"},{"family":"Li","given":"Jian Jian"},{"family":"Sun","given" :"Lun-Quan"}],"issued":{"date-parts":[["2013",10]]}}],"schema":"https://github.com/ citation-style-language/schema/raw/master/csl-citation.json"} . The selected model for this purpose; female C57BL/6 mice, were whole body irradiated by dose 8 Gy. This dose is the limited value for a development of RILI. We studied the changes in major cell populations in the peripheral blood and in the lung (lymphocytes, monocytes and granulocytes), lymphocyte subpopulations (T, B and NK cells) and the myeloid subtypes (monocytes, granulocytes and in the lung - macrophages) by multi-color flow cytometry. Also, variations in cytokine levels were investigated, as possible mediators of lung toxicity by cytometric bead assay. Second set of experiments were histopathological analysis and immunofluorescent staining. Sections of lung tissue were fixed and processed by microscopic analysis.

We had observed changes in all time intervals in experiment schedule; although, the most significant changes were observed at 21 day. These changes included significant infiltration of T – lymphocytes (CD3+ CD4+) in the lung tissue, increased level of MCP- 1 and obvious histological changes in alveolar – capillary complex. The results of this study clearly demonstrated a time - dependent response of the whole body after gamma irradiation.

References:

1. Ghafoori, P., Marks, L. B., Vujaskovic, Z. & Kelsey, C. R. Radiation-induced lung injury. Assessment, management, and prevention. *Oncol. Williston Park N* **22,** 37-47; discussion 52-53 (2008).
2. Coggle, J. E., Lambert, B. E. & Moores, S. R. Radiation effects in the lung. *Environ. Health Perspect.* **70,** 261–291 (1986).
3. Almeida, C. *et al.* The Role of Alveolar Epithelium in Radiation-Induced Lung Injury. *PLOS ONE* **8,** e53628 (2013).
4. Ding, N.-H., Li, J. J. & Sun, L.-Q. Molecular Mechanisms and Treatment of Radiation-Induced Lung Fibrosis. *Curr. Drug Targets* **14,** 1347–1356 (2013).

pCB-14

THE LEVEL OF CYCLIN-DEPENDENT KINASE INHIBITOR p27 ^{Kip1} AFTER DNA DAMAGE IS RETAINED IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

Raskova Kafkova L.[1], Navrkalova V.[3], Jarosova M.[2], Loja T.[3], Chovancova J.[3], Fialova Kucerova J.[1], Kriegova E.[4], Prochazka V.[2], Novak Z.[5], Simkova D.[1], Pospisilova S.[3], Divoky V.[1]

[1]Department of Biology, Faculty of Medicine and Dentistry, Palacky University, Olomouc,

Czech Republic,
[2]Department of Hemato-oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic,
[3]Center of Molecular Medicine, CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic,
[4]Department of Immunology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic,
[5]Department of Surgery, University of Alabama at Birmingham, Birmingham, AL, United States
E-mail: vladimir.divoky@upol.cz

CLL cells display enhanced activity of p38MAPK, with concomitant upregulation of $p27^{Kip1}$. The Cdk inhibitor $p27^{Kip}$ is essential for the maintenance of cells in G0/G1 phase in response to persistent DNA damage. In particular, stabilization of $p27^{Kip1}$ plays a key role in maintaining cell cycle arrest after persistent chemotherapy-induced genotoxic stress. Since it has been reported earlier that decreased $p27^{Kip1}$ level by caspase cleavage represents a key step in chemotherapy-induced apoptosis in CLL cells, we hypothesized that $p27^{Kip1}$ function may represent a marker link between functionality of the canonical DDR pathway and non-DDR cell cycle regulatory proteins in CLL, and therefore, can serve as a surrogate marker for *ATM*/*TP53* inactivating mutations. Our hypothesis was supported by the fact that in some cell types, degradation of $p27^{Kip1}$ during unperturbed S-phase was impaired upon ATM inhibition. Since association of $p27^{Kip1}$ degradation with ATM activity and arrested/cycling status in B-CLL was not studied, we characterized changes in $p27^{Kip1}$ levels in untreated and IR-treated samples of our patients' cohort. To assess degradation or accumulation of $p27^{Kip1}$ after DNA damage, we used flow cytometry (FACS) and western blotting analyses.

pCB-15

ANALYSIS OF HENOTYPIC, MOLECULAR AND FUNCTIONAL ALTERATIONS ON MODEL OF METASTATIC COLORECTAL CARCINOMA

Ďuriníková E., Poturnajová M., Kvokačková B., Kozovská Z., Miklíková S., Plavá J., Kučerová L., Matúšková M.

Cancer Research Institute of Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

Metastatic dissemination of tumour cells represents a serious problem in oncology, and it has negative impact on quality of life and survival of patients suffering from cancer. It is known that tumour microenvironment (TME) is crucial for behaviour of tumour cells, and mesenchymal stromal cells (MSC) as an integral part of CME interact with malignant compartment, and influence their characteristics. We developed several metastatic models of colorectal carcinoma including chemoresistant spontaneous metastatic model. We focused on analysis of phenotypic, molecular and functional changes in these tumour

cells as well as on alterations caused by their interactions with MSC.
We used flow cytometry for immunophenotyping, aldehyde dehydrogenase (ALDH) activity and apoptosis detection, gene expression arrays and real time kinetic imaging to characterize malignat cells. Immunomagnetic separation was used for isolation of subpopulations positive for CSC-related surface markers or for separation of tumour cells and MSC at direct coculture experiments. *In vivo* studies were performed on athymic or SCID/bg mice.
We have shown that chemoresistance is in correlation with expression of cancer stem-like markers, and it is accompanied with increased activity of ALDH. Our study proved for the first time the role of ALDH1A3 isoform in chemoresistance of colorectal carcinoma. MSC or MSC-derived soluble factors increased the invasiveness of tumour cells and supported expression of markers associated with cancer stem cells.
We prepared valuable model for study of tumor biology and metastatic process.
Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contracts No. APVV- 0052-12, APVV-15-0697; by VEGA grants No. 2/0087/15, 2/0128/17 and 2/0124/17; by the RFL2009 and WAC2003 587programs funded by the Slovak Cancer Research Foundation and by 588TRANSMED2, ITMS: 26240120030 supported by the Research & Development Operational Programme funded by ERDF.

pCB-16

ANALYSIS OF FUNCTIONAL CHARACTERISTICS OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STROMAL CELLS ISOLATED FROM HEALTHY DONORS AND ONCOLOGICAL PATIENTS

Miklíková S.[1], Plavá J.[1], Matúšková M.[1], Boháč M.[2], Kučerová L.[1]

[1]*Cancer Research Institute of Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Department of Plastic, Aesthetic and Reconstructive Surgery, University Hospital, Bratislava, Slovakia*

Tumors are considered as an organ-like structures where the stroma is recognized as a major regulator of tumor progression. In breast cancer, tumor stroma is recognized to have a critical role in development of invasive breast cancer, progression of the disease as well as in a resistance to the therapy. An important component of tumor stroma are mesenchymal stromal cells (MSC) displaying homing and engraftment potential to tumor stroma. As adipose tissue is the most abundant component surrounding breast cancer cells and it is a rich source of mesenchymal stromal cells, we want to analyse four groups of adipose tissue patient´s samples. Adipose tissue-derived MSC from healthy women undergoing reduction mammoplasty, from resection of premalignant and malignant lesions of breast cancer patients, and from BRCA positive patients.
Mesenchymal stromal cells, as a part of tumor microenvironment, are exposed to therapy together with tumor cells and we can´t ignore the effects of therapy on MSC. Our previous experiments showed that cisplatin exposed healthy MSC were able to produce factors that

turn on the changes in stemness and resistance of tumor cells. We have shown, that these factors increase both the ALDH positivity and expression of CD24-/CD44+/EpCAM+ cell surface markers in tumor cells, which were previously connected with stem cell properties. We want to analyse and correlate the changes in secretory phenotype and functional characteristics of MSC from healthy donors with oncological patients.

This study was supported by the VEGA grants No.1/027/17 (M.B., S.M.) and 2/0087/15 (L.K.), by the Slovak Research and Development Agency under the contract No.APVV-0052-12 (M.M.) and with the kind help and the financial support from the Cancer Research Foundation.

pCB-17

NOVEL CYTOTOXIC ANTICANCER DRUGS (1,10-PHENANTROLINE)$_2$Cu(II) COMPLEXES INDUCE CELL DEATH BY MODULATION OF UNFOLDED PROTEIN RESPONSE IN OVARIAN CANCER CELLS

Moráň L.[1], Pivetta T.[2], Vašíčková K.[1], Hampl A.[1], Vaňhara P.[1]

[1]Masaryk university – Department of Histology and Embryology, Faculty of medicine, Brno, Czech Republic
[2]Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Cagliari, 09042 Monserrato (CA), Italy

Endoplasmic reticulum (ER) is the major cellular organelle for protein synthesis, posttranslational modifications, membrane biosynthesis and calcium ions management. The state of ER stress induces various cell responses to intrinsic and extrinsic homeostasis disturbing factors and plays role in a huge number of civilization diseases including cancer. A dominant significant response to ER stress is UPR (Unfolded Protein Response), containing several pathways activated in the case of accumulation of misfolded proteins in the lumen of ER. The final cell response is then dependent on a specific activity of individual pathways and can lead to adaptation or apoptosis.
In this work we described effects of recently synthesized (1,10phenantroline)$_2$Cu(II) complexes on UPR. These (1,10phenantroline)$_2$Cu(II) compounds are promising anti-cancer therapeutics, however, the precise molecular mechanism of action is unclear. We show, that these complexes affect the UPR signaling pathways on protein as well as on mRNA level and also influence the cell morphology and ultrastructure of ovarian cancer cells. Importantly, we demonstrated that modulation of UPR led to synergistic and/or antagonistic effects of (1,10phenantroline)$_2$Cu(II) complexes. In summary, we characterized cytotoxic effects of new class of anti-cancer candidates and revealed cell structures and molecular pathways involved.
Supported by the project no. LQ1605 from the National Program of Sustainability II (MEYS CR) and by Masaryk University (MUNI/A/1369/2016). We acknowledge the core facility Cellular Imaging (CELLIM), institution CEITEC MU supported by the Czech-BioImaging large RI project (LM2015062 funded by MEYS CR) for their support with obtaining scientific data presented in this paper.

pCB-18

SCAVENGER RECEPTOR B1 AS A TARGET OF OUTDOOR STRESSORS AFFECTS CUTANEOUS HOMEOSTASIS

Muresan X.M.[1,2], Belmonte G.[2], Gruber F.[3], Valacchi G.[2], Souček K.[1]

[1]International Clinical Research Center FNUSA-ICRC; Institute of Biophysics of the CAS, Department of Cytokinetics, Brno, Czech Republic
[2]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
[3]Department of Dermatology, Medical University of Vienna, Anna Spiegel Gebäude; Christian Doppler Laboratory for Biotechnology of Skin Aging, Vienna, Austria

Scavenger receptor B1 (SR-B1) is a membrane protein, identified as HDLs main receptor and therefore involved in tissue reverse cholesterol transport. Several studies have demonstrated that SR-B1 is also implicated in other processes, such as regulation of intracellular antioxidant levels and recognition of bacteria and apoptotic cells. Although this receptor is localized mostly in the liver and steroidogenic tissues, it is significantly expressed also in human skin, especially in the epidermis. *In vitro* studies on cultured keratinocytes have shown that SR-B1 protein expression is down-regulated by environmental stressors, while this down-regulation can be prevented by natural radical quenchers. Our purpose was to investigate SR-B1 regulation by outdoor stressors in 3D human epidermis models and to assess the role of the receptor in epidermal structure. We have demonstrated that outdoor pollutants, such as ozone, air particles and cigarette smoke, down-regulate SR-B1 protein levels in *in vitro* reconstructed epidermis. Moreover, we developed SR-B1 knockdown organotypic skin equivalents, which showed changes in the epidermal structure, correlated with changes in lipid distribution. Overall, our findings suggest that SR-B1 loss upon environmental oxidants influences keratinocytes ability of differentiation and lipid homeostasis within the epidermis.
This work was supported by grants AZV ČR 15-28628A and by GAČR 15-11707S, and by project project no. LQ1605 from the National Program of Sustainability II (MEYS CR).

pCB-19

FUNCTIONAL STATUS OF CD47 MOLECULAR SPECIES ON THE SURFACE OF PRIMARY BLADDER CANCER CELLS

Rajtmajerová M., Novák J., Drbal K.

Laboratory of Molecular Dynamics of the Immune Response, Faculty of Science, Charles University, Czechia

Broadly expressed CD47 molecule, known as a “don´t eat me signal“ on the surface of all healthy and tumor cells, binds the inhibitory receptor SIRPA expressed on macrophages preventing phagocytosis. On the other hand, enabling phagocytosis of tumor cells via CD47 blockade is a novel powerful therapeutic approach. Recently, a novel biological therapy

using anti-CD47 blocking monoclonal antibodies (mAbs) and blocking soluble recombinant SIRPA molecule is being tested in several clinical trials in multitude of tumor types.
Numerous subtle aspects of CD47 biology regulate the cell fate with respect to engulfment by a macrophage. These comprise: the level of expression of CD47, molecular conformation and stoichiometry as well as the local density and its functional ability to bind SIRPA. During the cell lifespan in healthy tissues there is a gradual CD47 loss during aging process and moreover a change in distribution/conformation has been reported. Still, it is a very controversial topic whether the critical SIRPA binding is preferentially confined to any of the CD47 variants.
For the future success of CD47 biological therapy it is essential to stratify cancer patient responders. Therefore, we are interested in detailed analysis of CD47 molecular species on the surface of bladder cancer (BCa) cells obtained from urine and bladder washouts collected before and after transurethral resection of the bladder (TURB). The assessment of soluble SIRPA binding as well as various CD47 mAbs will be shown and flow cytometry data will be compared to superresolution microscopy and microscale thermophoresis.

pCB-20

TOWARDS HIGH BIOCOMPATIBILITY OF ORGANIC CONDUCTIVE MATERIALS FOR HEART ON CHIP APPLICATIONS

Šafaříková E.[1], Víteček J.[1,2], Stříteský S.[3], Vala M.[3], Salyk O.[3], Omasta L.[3], Weiter M.[3], Kubala L.[1,2]

[1]Institute of Biophysics AS CR, Královopolská 135, 612 65 Brno, Czech Republic
[2]International Clinical Research Center-Center of Biomolecular and Cell Engineering, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, Czech Republic
[3]Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 612 00 Brno, Czech Republic

Current trend involves the use of organic conductive materials in biosensors since they have a potential to form an excellent biointerface to determine electrochemical signals arising from living cells and tissues. Though organic materials are generally regarded to be biocompatible, detailed insights into their biocompatibility are sparse as well as the knowledge if they can support cardiomyocytes. In this study multiple aspects of biocompatibility of triisopropylsilyethynyl pentacene (TIPS), diketopyrrolopyrrole (DPP), poly 3-hexylthiophene-2,5-diyl (P3HT) and poly 3,4-ethylenedioxythiophene (PEDOT:PSS) which show high potential for a biosensor construction were investigated. Stability of these materials in physiological environment and wetting properties which are preconditions for biocompatibility were determined. TIPS DPP and P3HT were stable but PEDOT:PSS produced some leachates. Native TIPS DPP and P3HT showed lowered wettability. The biocompatibility was studied by means of 3T3 fibroblasts. Native TIPS DPP P3HT and PEDOT:PSS showed limited biocompatibility compared to standard cell culture plastics. Further trials to manipulate biocompatibility were carried out. Among other procedures tested the biocompatibility could be improved remarkably by means of collagen IV coating.

Finally a culture of spontaneously beating cardiomyocytes differentiated out of murine embryonic stem cells was established at TIPS DPP P3HT and PEDOT:PSS. The collagen IV coating procedure allowed construction of highly biocompatible PEDOT:PSS based sensor. This was sensor successfully seeded with spontaneously beating cardiomyocytes. The electrical characteristic of the sensor enabled detection of an electrical signal of spontaneously beating cardiomyocytes, however, the noise to signal ratio was significant.
This work was supported by grants GACR 13-29358S and GACR 17-24707S

pCB-21

INHIBITION OF SCF^{SKP2} AFFECTS CHARACTERISTICS OF CANCER STEM-LIKE CELLS

Šimečková Š.[1,3], Fedr R.[1,2], Kahounová Z.[1,2], Remšík J.[1,2,3], Slabáková E.[1], Mičková A.[4], Kharaishvili G.[4], Král M.[5], Bouchal J.[4], Souček K.[1,2,3*]

[1]*Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic;*
[2]*Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne´s University Hospital Brno, Brno, Czech Republic*
[3]*Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic;*
[4]*Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic*
[5]*Department of Urology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic*
**correspondence to: ksoucek@ibp.cz*

S-phase kinase-associated protein 2 (Skp2) protein is a crucial component of SCF^{Skp2} complex that functions as an E3 ubiquitin ligase involved in protein degradation. Skp2 is essential for regulation of many cellular processes, such as cell cycle, apoptosis, differentiation and others. Moreover, Skp2 is often overexpressed in various cancers including prostate cancer. The cancer stem-like cells (CSCs) represent an attractive target for anticancer therapy. However, the role of Skp2 in prostate CSCs is not well described yet. Therefore, we aimed to investigate the role of Skp2 in prostate cancer stem-like cells. We hypothesized that inhibition of SCF^{Skp2} activity will affect properties of the prostate cancer cells characterized by expression of cancer stem-like cell markers.
We found that pharmacological inhibition of Skp2 activity affected clonogenic ability of single cells in 2D condition. Besides that, Skp2 downregulation diminished spheroid formation ability in anchorage independent growth in 3D, abrogated tumor xenograft growth *in vivo* and decreased activity of ALDH1 was detected. Finally, we elucidated effects of Skp2 downregulation on expression of CSC markers. We found that expression of CD24 and CD44 surface markers was increased in cells with downregulated Skp2. Conversely, in cells overexpressing Skp2 protein, expression of CD24 was downregulated. In conclusion, we showed that pharmacological inhibition or downregulation of Skp2 affect cancer stem-like cells properties of the cells and change their phenotype regarding to expression of CSC

markers. This work was supported by grants AZV ČR 15-28628A, 17-28518A & 15-33999A, HistoPARK (CZ.1.07/2.3.00/20.0185), and by project project no. LQ1605 from the National Program of Sustainability II (MEYS CR), ICRC-ERA-HumanBridge a.k.a. „REGPOT"(Grant agreement no. 316 345) from the European Regional Development Fund and MZČR 17-28518A.

pCB-22

FLOW CYTOMETRY NO-WASH ANALYSIS OF FLUORESCENTLY LABELED EXOSOMES AND MICROVESICLES IN THE URINE OF BLADDER CARCINOMA PATIENTS

Šťastná E., Drbal K.

Laboratory of Molecular Dynamics of the Immune Response, Faculty of Science, Charles University, Czechia

Exosomes (ES) and microvesicles (MV) are cell-derived vesicles from endosomal compartment and plasma membrane representing the cellular function including the surface protein expression. These particles are present in all body fluids in much higher amounts compared to the original cells themselves. Their size ranging from smaller ES at (80-100 nm) to larger MV (200-1000 nm) makes it a suitable object for sensitive flow cytometric analysis. Clinical samples have been tested for a specific biomarker (RNA, protein) presence in ES/MV in various diseases such as cancer.
To detect ES/MV, we are using a modified flow cytometer CytoFLEX S. We will present a simple protocol for no-wash staining to minimize sample manipulation. Non-invasive urine sampling of bladder carcinoma (BCa) patients provides us with a continuous monitoring ability for comparison with standard cytology and other clinical parameters. Therefore, we focus mainly on the staining protocol using fluorescence dyes specific for DNA/RNA/protein/lipid/sugar components as well as the identification of the specific markers of cells present in the bladder wall – epithelia, endothelia and leukocyte subsets. We are fully aware of numerous challenges to overcome such as the vesicles size and heterogeneity, precipitated urine proteins or the presence of bacterial components, which might interfere with the assay. Fast ES/MV enrichment using magnetic beads will be compared to total urine samples. Our vision for the future would be a fast and simple test suitable for better patient stratification and management.

pCB-23

RADIATION INDUCED APOPTOSIS IN RATS SPERMATOZOA

Tsukanova E.V., Chueshova N.V., Veyalkina N.N., Cheshik I.A.

Institute of Radiobiology of National Academy of Sciences of Belarus, Gomel, Belarus

Well known that acute radiation influence first affects reproductive system function. Spermatogenic epithelium is one of the most radiosensitive tissues of the body.

The cytometric methods become a technology of choice in diverse studies of cellular demise. Thus, when using Annexin V in conjunction with plasma membrane permeability marker a distinction can be made between live, apoptotic, and late apoptotic/secondary necrotic cells.

The aim of this study was to estimate the influence of acute single ionizing radiation dose of 1.0 Gy on the number of apoptotic and necrotic epididymal sperm of rats on the 3, 15 and 30th day after exposure.

Male Wistar rats (4,5 - weeks-old, weighing 344,85±2,01g) were irradiated once at a dose of 1 Gy to the whole body. The rats were then sacrificed 3 days, 2 and 4 weeks after irradiation and rats, which were not exposed to irradiation were used as controls. At each time point, six rats of each group were sacrificed and their epididymal sperm samples were harvested for analysis.

Analysis using Annexin V and propidium iodide (PI) was carried out by means of the cytofluorimeter.

Mann–Whitney U test was used to investigate the significance of difference between the groups. Analyzes were performed at significance level of 0.05 using SPSS 21 package.

The apoptotic and necrotic sperm cells were determined by flow cytometry (Fig. 1).

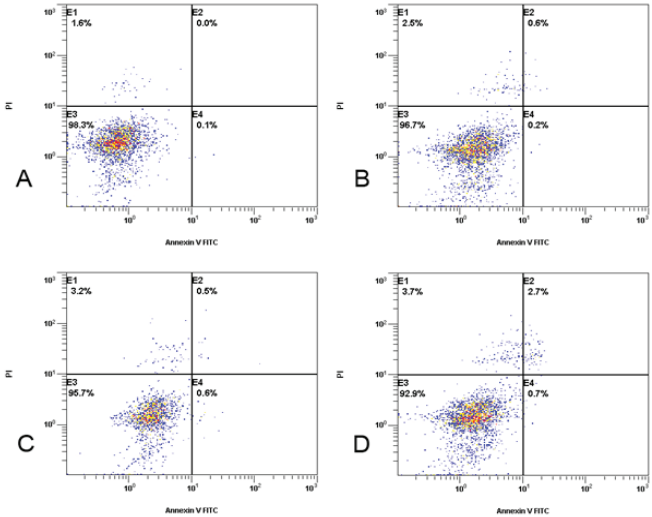


Fig. 1. Detection of apoptosis by concurrent staining with Annexin V and PI. Rats epididymal sperm cells, A – control group, B – 3 day after whole body radiation, C and D – 2 and 4 weeks after whole body radiation.

Whole body radiation of rats caused a significant increase in the level of epididymal spermatozoa at the stage of early and late apoptosis (0.88 ± 0.23%) compared to the control group (0.18 ± 0.03%). In the more distant periods after irradiation (4 weeks), the level of apoptotic cells increased significantly to 0.92 ± 0.38% at the stage of early apoptosis and to 2.77 ± 0.57% at the stage of late apoptosis.

The level of necrotic cells increased significantly already on day 3 after irradiation to 2.4

± 0.15% from 1.78 ± 0.12 in the control group. At 2 and 4 weeks after irradiation, this indicator was 3.05 ± 0.32% and 3.85 ± 0.55, respectively.
This study demonstrates that whole body radiation in dose of 1.0 Gy significantly increase percentage of apoptotic and necrotic cells in the rat sperm.

pCB-24

n-3 POLYUNSATURATED FATTY ACIDS ALLEVIATE BENZO[A]PYRENE TOXICITY IN HUMAN COLON CANCER CELL LINES

Tylichová Z.[1], Neča J.[2], Machala M.[2], Topinka J.[3], Milcová A.[3], Hofmanová J.[1], Kozubík A.[1], Vondráček J.[1]

[1]Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic
[2]Veterinary research institute, Brno, Czech Republic
[3]Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic

Diet rich in n-3 polyunsaturated fatty acids (PUFAs) has been proposed to provide anti-tumor protection and several epidemiologic studies have suggested a decrease in colorectal cancer risk among individuals consuming diets high in PUFAs. The primary aim of this study was to investigate the effect of two major PUFAs, eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), on genotoxic effects of benzo[a]pyrene (BaP), a carcinogenic polycyclic aromatic hydrocarbon that can be found at significant levels in processed food. We hypothesized that addition of physiological dose of EPA and DHA might interfere with BaP metabolism/bioactivation and/or alter its toxic effects. Using human colon carcinoma cell lines HCT-116 and HT-29 we analyzed the impact of PUFAs on BaP metabolism and induction of DNA damage. Colon cancer cells are able to metabolize and bioactivate BaP, via the cytochrome P450 family 1 (CYP1) enzymes, which in turn leads to formation of covalent DNA adducts. We observed that cells treated with either EPA or DHA exhibited significantly lower CYP1 activity, reduced formation of covalent DNA adducts and decreased formation of major BaP metabolites. BaP treatment resulted in phosphorylation of H2A.X in HT-29 cells, suggesting formation of DNA double strand breaks. However, only EPA seemed to decrease H2A.X induction, while DHA had no apparent effect on its level. In conclusion, both PUFAs may limit bioactivation and genotoxic effects of BaP in colon epithelial cell models, although they may act through different mechanisms. [Supported by the Czech Science Foundation, project no. 13-09766S.]

pCB-25

SIDE POPULATION IS NOT AN UNEQUIVOCAL MARKER OF CANCER STEM CELLS IN A549 CELL LINE AND CAN BE NEGATIVELY AFFECTED BY HYPERICIN, AN ACTIVE METABOLITE OF ST. JOHN'S WORT

Vargová J.[1], Mikeš J.[1], Jendželovský R.[1], Mikešová L.[1], Kuchárová B.[1], Čulka Ľ.[1], Remšík J.[2,3,4], Fedr R.[2,3], Souček K.[2,3,4], Kozubík A.[2,4], Fedoročko P.[1]

[1]Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University in Košice, Košice, Slovak Republic
[2]Department of Cytokinetics, Institute of Biophysics of the CAS, v.v.i., Brno, Czech Republic
[3]Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic
[4]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Side population (SP) phenotype is based on elevated activity of BCRP and MDR1 transporters, thus designating more resistant subset of cells. However, status of SP as credible marker of cancer stem cells (CSC) remains controversial.
Naphtodianthrone hypericin (HYP) holds great potential as natural photosensitizer for photodynamic diagnosis and therapy. HYP is the bioactive constituent of St. John's Wort plant (*Hypericum perforatum*, SJW), which is widely utilized as a custom remedy for self-treatment of mild depression. However, natural origin of drugs does not ensure their inertness and HYP is not an exception, since it was found to enhance level of certain transporters.
The aim of presented study was to characterize SP derived from A549 lung carcinoma cells and to determine the impact of HYP on these cells.
Our results showed that SP cells possessed higher level of all studied transporters, formed bigger spheroids and were coupled with $CD24^{low}$ phenotype, while exhibited equal rate of clonogenicity, tumorigenicity and similarly overlapped with $ALDH^+$ subpopulation when compared to nonSP or parental counterparts. HYP increased level of BCRP, inversely accompanied by reversible decrease of SP. However, small subset of SP selected by HYP acquired some additional traits of resistance, including the lowest intracellular content of HYP, higher $ALDH^+$ and bigger spheroid size, while overall viability or proliferation was not affected. Interestingly, pre-treatment with HYP slightly inhibited growth of cells *in vivo*.
Altogether, our results suggest that SP phenotype designates cells with several favorable features but at the same time contravene SP as *bona fide* CSC-marker in A549 cells. Furthermore, despite its reported anticancer effects, HYP *per se*, whether as a part of SJW-supplements or as a photosensitizer, could encourage selection of a subset of resistant cancer cells, primarily due to its stimulatory effect on transporters and assumed competitive inhibition of BCRP, confirming our previous conclusions.

Acknowledgements: Special thanks go to Lucia Bino and Zuzana Kahounová for their help with experiments conducted at the IBP of the CAS, v.v.i., Brno.
Funding: APVV-14-0154 and VEGA 1/0147/15.

pCB-26

GAP JUNCTION INTERCELLULAR COMMUNICATION AS A FUNCTIONAL BIOMARKER TO ASSESS TESTICULAR TOXICANTS

Yawer A., Laboha P., Dydowiczova A., Brozman O., Sychrova E., Kubincova P., Babica P., Sovadinova I.

RECETOX - Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic

Gap junction intercellular communication (GJIC) plays a central and critical role in cellular and tissue homeostasis in male reproductive system and consequently in male fertility. Untimely dysregulation of these connexin (Cx)-based junctions during critical stages of development may result in male reproductive dysfunctions such as inadequate and decreased sperm motility, impaired spermatogenesis, low sperm count, hyperplasia of androgen-producing Leydig cells, impairment blood-testis barrier integrity etc. Therefore, GJIC in testes is widely discussed as an important target for testicular toxicants through which they disturb male reproductive health.

To evaluate testicular toxicity of well-recognized endocrine disruptors, we optimized a semi-high throughput cell-imaging assay for a simultaneous assessment of GJIC, cell viability and cell density/growth in adherent cultures of mammalian testicular cells. The assay is based on the traditional scrape-loading dye transfer (SL/DT) for GJIC evaluation but combining three different fluorophores and adapted to a microplate format in order to allow automated microscope image acquisition and analysis to assess more samples within a shorter time. We confirmed that the environmentally relevant contaminants affecting male fertility are able to dysregulate GJIC in the key somatic testicular cells such as Leydig and Sertoli cells *in vitro* and *ex vivo*. In addition, we studied testicular Cxs as a molecular target for these GJIC inhibitors. We focused on the localization and expression of relevant Cxs affected by recognized endocrine disruptors dysregulating testicular GJIC. Finally, we identified intracellular signal transduction pathways, such as MAPK ERK1/2 pathway, activated in response to these endocrine disruptors leading to dysregulation of testicular GJIC. Acknowledgment: This research is supported by Czech Science Foundation Project No.GA16-10775Y.

pCB-27

SHORT CHAIN FATTY ACIDS ALTER EXPRESSION OF XENOBIOTIC-METABOLIZING ENZYMES IN COLON EPITHELIAL CELLS

Zapletal O.[1,2], Tylichová Z.[1,2], Dubec V.[1,2], Neča J.[3], Kohoutek J.[3], Machala M.[3], Milcová A.[4], Topinka J.[4], Hofmanová J.[1,2], Kozubík A.[1,2], Vondráček J.[1,2]

[1]*Department of Cytokinetics, Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic*

[2]*Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech*

Republic
[3]Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, Czech Republic
[4]Department of Genetic Ecotoxicology, Institute of Experimental Medicine, Czech Academy of Sciences, ASCR, Prague, Czech Republic

Dietary contaminants are suspected to contribute to the development of colorectal carcinoma, one of the most prevalent forms of cancer in the Western world. Benzo[a] pyrene (BaP), IARC group 1 human carcinogen, is the best characterized polycyclic aromatic hydrocarbon (PAH) found in the processed food. Cytochrome P450 family 1 (CYP1) enzymes, regulated by the aryl hydrocarbon receptor, play a major role in bioactivation of BaP; however, additional enzymes and further regulatory events contribute to metabolism of BaP and other dietary carcinogens within gut epithelium. The short-chain fatty acids produced by gut microflora, such as butyrate, serve as a major source of energy for gut enterocytes and contribute to maintenance of gut homeostasis, partly via inhibition of histone deacetylases, which may alter chromatin structure and gene expression. Our data suggest that butyrate may alter metabolism/toxicity of PAHs through modulation of expression of xenobioticmetabolizing enzymes (XMEs) involved in BaP metabolism. Using in vitro models of colon epithelial cells, we found that butyrate alters metabolism/toxicity of BaP through modulation of CYP1 expression. In human HCT116 cells, butyrate reduced binding of HDAC1 to the enhancer region of *CYP1A1* gene, which was linked with upregulation of CYP1A1 expression/activity, enhanced metabolism of BaP and increased formation of covalent DNA adducts by anti-BaP-dihydrodiolepoxide. In addition, butyrate also altered expression of further XMEs, like aldo-keto reductase 1C1, Nacetyltransferases 1 and/or 2, UDP glucuronosyltransferase 1A1 and sulfotransferase 1A1. The effects of butyrate to selected XMEs varied among differentiating and nondifferentiating colon cell lines. These results indicate that butyrate may interact with dietary toxic compounds, such as BaP, in regulation of multiple XMEs. The mechanisms underlying the butyratedependent regulation of XMEs within colon epithelium needs further clarification. Supported by the Czech Science Foundation (project no. 13-09766S).

CLINICAL HEMATOLOGY & CELL THERAPY

pCT-1

RITUXIMAB-MEDIATED RELEASE OF CLL CELLS FROM LYMPH NODES TO PERIPHERY: USE OF CD62L MOLECULE AS A MARKER

Borský M., Osičková J., Brychtová Y., Doubek M., Mayer J., Trbušek M.

Department of Internal Medicine – Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic

Regimens consisting of rituximab (RTX) and chemotherapy represent important therapeutic

option for chronic lymphocytic leukemia (CLL) patients. RTX eliminates B cells by complex mechanisms involving complement dependent cytotoxicity, antibody dependent cellular cytotoxicity and apoptosis. RTX efficacy in a particular patient is variable and depends on parameters such as tumor burden, CD20 antigen density and activity of complement and macrophage system. It is not entirely clear how CLL cells from peripheral blood (PB) and lymph nodes (LNs) respond to initial RTX administration. CD62L (L-selectin) plays an important role in lymphocyte homing to the LNs.
We analyzed CLL cell counts and CD62L expression in PB samples from 25 CLL patients with WBC >50. The cells were taken before RTX administration, immediately after its completion and 20 hours thereafter (Day 1). At the end of RTX administration, CLL cell numbers in PB were reduced in all tested patients (median 40.6% of pre-therapy values). The depth of this response was independent on CD20 density (range 57 753 – 207 920 molecules per cell). The testing of complement activity in plasma obtained immediately after the RTX administration revealed a partially or fully depleted complement in majority of patients (73%). This inability to further eliminate PB CLL cells highly likely contributed to a reverse increase in total CLL cell counts observed on Day 1 in 56% of patients. In 13.5% of the patients, this absolute count even exceeded the starting value (109 – 198%). We hypothesized that this newly emerged CLL cells came to the PB from LNs. To support this assumption, we measured CD62L expression on CLL cells taken immediately after the RTX administration and on Day 1. The expression was prominently diminished on Day 1 in the patients manifesting the reverse increase in leukemic cell numbers, while it showed only negligible changes compared to the starting point in remaining cases. Conclusively, during its administration RTX significantly reduced PB leukemic cells in most of the studied patients. At the same time, in a significant proportion of them this initial drop was followed by the CLL cells´ release from the LNs. This phenomenon, which may have significant consequences for successive chemotherapy, can be monitored through CD62L expression.
Supported by grant AZV 16-32743A.

pCT-2

FUNCTIONALIZATION OF NANOFIBER MEMBRANE BY AMINE- AND CARBOXYL-RICH LAYERS

Cernochova P.[1], Medalova J.[1], Michlicek M.[2], Obrusnik A.[2], Zajickova L.[2]

[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[2]RG Plasma Technologies, CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czech Republic

For many years, it has been an effort of tissue engineering to develop tissue replacements that would be natural to the body, absorb themselves and have been the carrier for the body's own cells. Synthetic polymers are gaining popularity especially when they are modified by relatively simple, economically and ecologically suitable plasmachemical processes, which make them a suitable carrier for the cells. This project focuses on

polycaprolactone nanofibrous membranes plasmachemically coated with either non-toxic cyclopropylamine (CPA), which leads to the creation of layers rich in positively charged amine groups or by copolymerization of maleic acid with acetylene to form negatively charged carboxylated layers (BioMA). Our goal was to test these layers in practice, i.e. to determine their effect on human keratinocytes of the HaCaT line. We assessed the effect on the adhesion, proliferation, and differentiation of these skin cells. The method for estimating the rate of division was to determine the amount of ATP in the culture of cells growing on plasmachemically treated materials in contrast to the control layer without plasma-chemical treatment either after 24 hours or 72 hours of cultivation. Using immunocytochemical detection of structural proteins, we tested whether the cells were subject to significant morphological alterations or were subject to remodeling associated with any of the cytokinetic processes. Differentiation was tested using qRT-PCR and keratinocyte differentiation markers Keratin1 and involucrin. Unlike in preliminary experiments performed on the C2C12 myoblast line, HaCaT cells grew equally fast on non-functionalized membranes as the functionalized. It should also be noted that their adhesivity rate to the surface was lower than that of C2C12 cells. This corresponds to minimal changes in cytokinetic parameters and the expression and localization of structural proteins (F-actin, vimentin, vinculin, pancadherin, etc.).

Acknowledgement:
This project was supported by following grants CEITEC 2020 (LQ1601) and COST (LD15150) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II., grant TRIO (FV10342) by Ministry of industry and business, SCOPES (IZ73Z0_152661/1) Swiss National Science Foundation / SCOPES - Scientific Co-operation between Eastern Europe and Switzerland, and MUNI/C/1639/2016 of Masaryk univerzity / GA MU.

pCT-3

NOVEL MASS SPECTROMETRY AND FLOW CYTOMETRY-BASED APPROACH FOR CHARACTERIZATION OF NEURAL DIFFERENTIATION

Červenka J.[1,2], Poliakh I.[1,2], Žižková M.[1,2], Tylečková J.[1], Suchá R.[1], Vodička P.[1]

[1]Laboratory of Applied Proteome Analyses and Research Center PIGMOD, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, v.v.i., Libechov, Czech Republic
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

Differentiation of human multipotent neural stem cells (NSC) into mature neurons or glial cells is at the forefront of interest because of its potential in cell replacement therapies of nervous system injuries and neurodegenerative disorders. However, a differentiation efficacy and population purity has to be strictly controlled to prevent potential safety issues, such as a presence of undifferentiated stem cells with a carcinogenic potential.
In our study we aimed to develop a specific and rapid selected reaction monitoring (SRM) mass spectrometry screening approach to assess quantitatively a proliferative, gliogenic

and neurogenic potential of NSC. Our approach complements antibody-based methods which can be constrained by an insufficient specificity and limited capacity for multiplexing. Based on a literature search we have chosen 22 proteins that are widely accepted as neural cell markers to characterize their changes during neural differentiation (e.g. Nestin and Pax6 for NSC or GFAP and S100 for astrocytes etc.). We detected increase in neuronal markers and decrease in NSC multipotency markers in the course of neural differentiation. However, we observed some unexpected changes in abundances of the studied proteins, which suggest a non-homogeneity of the analysed cell populations. This presents a safety concern for therapeutic use of these cells, as some may remain as undifferentiated stem cells with a malignant potential. Thus, a characterization of cells on a single cell level remains crucial. To reach this level, we validated the results of our SRM assay using flow cytometry analyses of selected cell surface and intracellular markers.
Our SRM assay may be useful as a specific screening method and for identification of new population specific cell surface markers. However, fluorescence activated cell sorting (FACS) remains so far the best available method for isolation of cell populations with a high purity.
Research was supported by the MEYS (MŠMT, LO1609).

pCT-4

TCF/LEF SIGNALING SUPPRESSES PROLIFERATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

Daněk P., Kardošová M., Zjablovskaja P., Janečková L., Kořínek V., Alberich-Jorda M.

Institute of Molecular Genetics of the ASCR, Prague, Czech Republic

The canonical Wnt signaling pathway has been shown to be an essential regulator of body plan formation and cell fate decision. While well described in various tissues, the function of Wnt signaling pathway remains unclear in the hematopoietic system. Activation of Wnt-target genes is mediated by the interaction of β-catenin with the TCF/LEF family of transcription factors. In order to investigate the role of Wnt/ β-catenin signaling in hematopoiesis, we employed a mouse model expressing a dominant negative form of human TCF4 (dnTCF4) that inhibits β-catenin interaction with TCF/LEF factors and, consequently, abrogades transcription of Wnt-target genes. Flow cytometry analysis demonstrated that introduction of dnTCF4 results in expansion of hematopoietic progenitor cell populations but not long-term hematopoietic stem cells. Expanded progenitors also showed higher BrdU incorporation *in vivo*, indicating their enhanced proliferation. Subsequent colony forming assays confirmed that both dnTCF4-expressing bone marrow and c-kit-enriched cells present an increased growth potential compared to wild type. Further, when transplanted into lethally irradiated mice, dnTCF4-expressing bone marrow cells displayed a significantly augmented engraftment compared to control, while preserving self-renewal abilities of long-term hematopoietic stem cells. Our data therefore suggest that Wnt/β-catenin signaling pathway suppresses extensive generation of hematopoietic progenitors in the adult murine hematopoietic system.

pCT-5

NATURAL KILLER CELL CYTOTOXIC ACTIVITY: A COMPARISON BETWEEN TWO CELL POPULATIONS

Dolejšová M.[1], Vištejnová L.[1], Jindra P.[2], Lysák D.[2], Holubová M.[2]

[1]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Czech Republic
[2]Department of Hemato-oncology, University Hospital in Pilsen, Czech Republic

Natural killer (NK) cells can recognize malignant and viral transformed cells with altered expression of HLA-I molecules. There are many receptors on the surface of NK cells which activate or inhibit their particular functions. NKp44 and CD25 (IL-2R) show contradictory expression during *in vitro* activation by IL-2 (NKp44 positive cell don't express CD25 and vice versa). CD25 should be associated with increased proliferation in contrast with NKp44 which is more associated with cytotoxic function.
Considering the different function of CD25 and NKp44 proteins, we decided to compare the cytotoxic activity of both populations. Peripheral blood samples were obtained from 18 healthy donors. NK cells were isolated by magnetic separation from cryopreserved mononuclear cell fraction. The cells were cultured for 7 days and IL-2 was supplemented each 2-3 days. Afterwards the cells were harvested and stained for detection of CD25 and NKp44 positive NK cells. The subsets were sorted (15x10e4 of each subset) using FACS ARIA sorter and recovered overnight. CFSE labelled K562 cell line was added next day in a ratio 1:10 (K562:NK subset). Cells were co-cultured for 4 hours and then the proportion of dead and viable K562 cells was evaluated using 7AAD staining and flow cytometry
Higher cytotoxic potential of NKp44 subset was detected in 7 donors (in average 1.4x higher compared to CD25 subset; range 1.07x-1.48x). In case of 8 samples, cytotoxic activity of NKp44 subset reached only 0.74x the value of CD25 positive cells. 3 samples were recognized as samples with the same cytotoxicity in both groups (range 0.98x-1.04x). Surprisingly, according to our results, expression of CD25 or activation marker NKp44 does not indicate the antitumor cytotoxicity of NK cells and cannot be used as a substitute for the functional cytotoxic testing.

This work was supported by the project of Ministry of Health number 15-30661A.

pCT-6

FLOW CYTOMETRY PANEL DESIGN - FROM FUNDAMENTALS TO MULTICOLOR IMMUNOTYPING USING THE ZE5

Hedlund S.

Bio-Rad Laboratories | Life Science Group

In multicolor flow cytometry a bad fluorochrome panel can severely reduce the resolution which potentially makes it difficult to identify dim positive populations, thus stating the

critical role of panel design. The general rule of thumb is that the more colors used in the panel, the more effort needs to be put into the panel design as well. The ZE5™ cell analyzer from Bio-Rad carries 5 spatially separated lasers and 30 detectors allowing analysis of up to 27 colors simultaneously, which makes it an excellent instrument for multicolor flow cytometry. Its Everest software has built in tools to assist with the panel design, and the aim of this presentation is to discuss the parameters that come in to play in multicolor flow cytometry, give a concrete example of data acquired with the ZE5 spanning from an initial bad panel up to a good fluorochrome panel, and finally look at a 21 color panel for quantifying major and minor cell types in human peripheral blood.

pCT-7

EXPRESSION OF INHIBITORY KIRs AFTER HEMATOPOIETIC STEM CELLS TRANSPLANTATION

Holubová M.[1], Houdova L.[2], Jindra P.[3], Lysak D.[1]

[1]Hemato-oncology dept., Faculty Hospital in Pilsen, [2]NTIS – West Bohemia University; [3]Czech bone marrow registry, Czech Republic

NK cells are part of a non-specific immune response and play a key role in anti-tumour immunity. They express spectrum of activation and inhibitory receptors and the balance between them ensures the right activity.

The donors for allogeneic hematopoietic stem cell transplantation are chosen not only by the HLA match but also by KIR-HLA mismatch. Inhibitory KIR receptors (iKIRs) are most important group of inhibitory receptors on NK cells and their ligands are HLA molecules. The missing HLA ligands for KIRs on malignant cells (KIR-HLA mismatch) improve graft vs. leukaemia response.

KIR-HLA mismatch is standardly determined with molecular genetics. In our study, we evaluated the expression of iKIRs on donor NK cells before transplantation and on recipient´s NK cells after 1-2-3 months after transplant ("same NK cells in different body"). Peripheral blood was stained with mAbs for detection of NK cells - CD3PB/CD16PCP/CD56PC7; Exbio, CZ) in combination with anti iKIRs antibodies - 2DL1-PE, 2DL2/2DL3-APC, 2DL3-FITC or 3DL1/DL2-PE, 3DL3-APC, 3DL5-FITC (Miltenyi, USA). Samples were measured on CANTO II flow cytometer, analysis was performed in FlowJo.

We compared expression before transplant and changes after transplant. Expression of KIR2DL1 receptor decreased by more than 50% at 80% of patients. This receptor reacts with HLA-C molecules just like KIR2DL2/DL3 which also showed lower expression at 1 month after transplant at 67% of patients and about 50% of patients retained lower expression for next both months. In contrast, iKIRS binding HLA-A or B (KIR3DL1/3DL2) showed higher expression at about 70% of pts. at first two months after transplant than the expression returned to original value. We did not find any correlation with presence of relapse.

We detected dynamic behaviour in KIRs expression depending on many unknown factors and the understating KIRs behaviour needs deeper research.

This work was supported by the project of Ministry of Health number 15-30661A.

pCT-8

NOVEL ANTIGEN COMBINATION FOR MINIMAL RESIDUAL DISEASE DETECTION IN HAIRY CELL LEUKEMIA USING FLOW CYTOMETRY APPROACH

Chovancová J.[1,2], Hrabčáková V.[1], Dvořáková D.[1], Mayer J.[1,2], Doubek M.[1,2]

[1]*Department of Internal Medcine-Hematology and Oncology, University Hospital, Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic*

Background: Hairy cell leukemia (HCL) is a rare B cell chronic lymphoproliferative disorder manifesting itself by pancytopenia and infiltration of typical "hairy" leukemic cells in liver, spleen and bone marrow. The mainstay of HCL therapy comprises of purine analogues cladribine and pentostatin. Crucial diagnostic step resides in distinguishing HCL from HCL-like disorders, since these do not show therapeutic response to these agents. The BRAF V600E mutation is now recognized as a reliable molecular marker of typical HCL. In this study, we propose suitable combination of CD markers that could lead to effective differential diagnosis of HCL as well as detection of minimal residual disease (MRD). **Methods:** Using flow cytometry, the expression of surface antigens CD11c, CD20, CD25, CD27, CD48, CD103, CD123, CD200, CD305 and ROR1 were analyzed on peripheral blood B cells within three groups of samples: i) typical HCL (patients with detected BRAF V600E mutation), ii) variant HCL (vHCL) (patients without BRAF V600E mutation) and iii) healthy controls. **Results:** We confirmed significantly higher expression of commonly used antigens in differential diagnosis (CD11c, CD20, CD25, CD103 and CD123) on HCL cells compared to healthy controls ($p<0.01$). Moreover we found significantly higher expression of CD48, CD200, CD305 and ROR1 on HCL cells compared to healthy controls ($p<0.01$). Although various phenotypes were described in vHCL with negative findings in at least one above mentioned antigen, all vHCL patients showed significantly lower expression of CD200 and ROR1 compared to typical HCL ($p<0.01$). **Conclusion:** Various immunophenotypes of HCL have been observed, which complicates both diagnostics and MRD detection. Combination of proposed antigens could help in differential diagnostics of B lymphoproliferations and determine suitable MRD flow cytometry panel. **Acknowledgement:** The work was supported by MZ ČR-RVO (FNBr, 65269705).

pCT-9

IMMUNOPHENOTYPIC CORRELATIONS WITH RECURRENT GENETIC ABERRATIONS DETECTED IN T- CELL ACUTE LYMHPOBLASTIC LEUKEMIA

Kulis J.[1], Słota Ł.[1], Sędek Ł.[2], Sonsala A.[1], Twardoch-Pyka M.[1], Pierzyna-Świtała M.[1], Szczepański T.[1]

[1]*Department of Pediatric Hematology and Oncology, Medical University of Silesia, Zabrze, Poland*
[2]*Department of Microbiology and Immunology, Medical University of Silesia, Zabrze, Poland*

Acute lymphoblastic leukemia (ALL) is the most frequent leukemia in children. There are two main types of ALL, one of them is T-cell acute lymphoblastic leukemia (T-ALL), comprising about 15-17% of all ALL cases. There are several genetic aberrations related with T-ALL that have evidenced prognostic significance.
Our main focus was to find correlations between known genetic aberrations and blast cells phenotype to determine if it is possible to detect potential mutations by immunophenotype only.
In our study we tested bone marrow samples from 60 pediatric T-ALL patients. We performed 8-color flow cytometric (FC) immunophenotyping of blast cells using EuroFlow T-ALL monoclonal antibody panel. The expression of all antigens on leukemic blasts measured as median fluorescence intensity (MFI) was transformed into scores of a normalized nMFI scale. Four genetic aberrations (formation of fusion genes *NUP214-ABL* and *SIL-TAL*, and mutations in *Notch1* and *FBXW* genes) were detected with PCR or FISH techniques.
Preliminary data analysis showed that elevated expression of CD13 and CD10, as well as cytoplasmatic CD3 antigens correlated with *NUP214-ABL* mutation. Higher than normally seen on T-cells expression of CD4 can be related to *Notch1* gene mutation. *SIL-TAL* aberration correlated with higher than on normal T-cells expression of TCRαβ, CD2 and lower than normal expression of cytoplasmatic CD3 antigens. So far we did not find any correlation between blast cell phenotype and *FBXW* gene mutation.
Multiparametric FC could be a potential tool to detect the most common aberrations in T-ALL on early stage. Using this method, patients can be qualified to the specific risk group before obtaining the molecular test results, although further studies are necessary to confirm our initial findings. We plan to extend our study group and apply more advanced data mining techniques to find multivariable relationships.

pCT-10

FORWARD SCATTER (FSC) AND SIDE SCATTER (SSC) IDENTIFIES LSK CELLS FROM REGENERATING BONE MARROW AS LATE MYELOID PROGENITORS IN THE MOUSE

Molik M., Savvulidi F., Necas E.

Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

The hematopoietic tissue is hierarchically organized with stem cells (HSCs) at its apex, followed by multipotent progenitors (MPPs), common myeloid and lymphoid progenitors (CMPs and CLPs), late myeloid and lymphoid progenitors, the later giving rise to differentiated myeloid and lymphoid cells. We studied the spontaneous regeneration of the mouse bone marrow arising from a small number of repopulating cells surviving in their natural microenvironment after total body sublethal irradiation, or after transplantation of a tiny part of normal bone marrow to lethally irradiated mice. With the use of forward scatter (FSC) and side scatter (SSC), two basic flow-cytometric parameters, we could distinguish between the late myeloid committed progenitor cells and their less mature precursor cells, CMPs, MPPs, and HSCs. By applying this approach to the analysis of the regenerating

bone marrow, we could strongly support the late myeloid progenitor character of lineage negative (L), Sca-1 positive (S), c-Kit positive (K) LSK cells. This is important because the LSK cells in normal bone marrow contain almost exclusively MPPs and HSCs. The application of FSC and SSC analysis into the characterization of immature hematopoietic cells thus helped to reveal a unique state of the hematopoietic tissue in the course of its regeneration, based on the activation of developmentally restricted late myeloid progenitors. Often neglected (due to its „simplicity"), FSC and SSC cell characteristics are powerful flow-cytometric assets which might be used in studies into the hierarchical organization of murine hematopoiesis.

pCT-11

NOVEL METHOD FOR THE MANUFACTURE OF CAR-T-CELLS: EFFECTS OF DIFFERENT CYTOKINES IN CULTURE MEDIA ON THE PHENOTYPE OF CAR-T-CELLS

Musil J., Ptáčková P., Otáhal P.

Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Current manufacture of clinical CAR-T-cells is primary based on lentiviral/retroviral transduction of CD3/CD28 activated T-cells and subsequent cultivation in the presence of IL2. This well-established approach, however has few weak points such as that it induces polyclonal T cells activation resulting in low frequency of transduced T cells and that the anti-CD3/CD28 activation is supra-physiological and modifies the differentiation of T cells. Recently, alternative methods for efficient T cells expansion were developed such as those described by Gerdemann *et al* which is based on T cell stimulation with antigen followed by cultivation in the presence of cytokines IL4 and IL7. Interestingly, the authors have shown that the expanded T cells were TH1-polarized despite exposure to IL4, which is a TH2 signature cytokine, and that the combination of IL4+IL7 supports the retention of a central memory phenotype and promotes T-cell survival by upregulating anti-apoptotic molecules. This procedure has been developed to manufacture GMP-grade multivirus specific T cells which were then successfuly used for treatment of viral infections such as CMV, EBV, AdV in patients after allogeneic bone marrow transplantation. We have hypothesised that similarly to the manufacture of antiviral T-cells, we could efficiently prepare CAR19 T-cells by electroporation of plasmid DNA into followed by cultivation in the presence of cytokines IL2, IL4, IL7 and/or IL21 without any additional artificial T-cell activation step. This method leads to a spontaneous activation of transfected T-cells most probably through recognition of endogenously present B cells. We have observed profound effects of these cytokines on the phenotype of cultured CAR19 T-cells. We found that the cultivation of CAR19 T cells in the presence of IL4 and IL7 preferentially expands CD4+ CAR19 T cells. Addition of IL2 into this cytokine cocktail enhances expansion of CD8+ CAR19 T cells and increases the overall CAR19+ cell yield (>90% CAR+) which surpasses the standard methods after three weeks of culture.

Both CD4+ and CD8+ CAR19 T-cells prepared in the presence of IL2+IL4+IL7 are cytolytic and produce extensive amounts of effector cytokines TNFα and IFNγ. Similarly to IL-2 addition of IL21 to IL4 and IL7 enhances CD8 expansion. However, IL21 has a more profound effect

on the distribution of memory T-cell populations. IL-21 preferentially expands CD8+ central memory T-cells compared to IL2 alone, IL4+IL7 or IL2+IL4+IL7. Furthermore, it enhances expression of costimulatory molecules CD28 and CD27 on CD8+ CAR-T-Cells and to a lesser extent on CD4+ CAR-T-cells. On the other hand, it decreases expression of inhibitory receptor Tim3.
In the presented work we present a novel method of manufacturing CD19+ CAR-T-Cell and show how different combinations of cytokines can influence the phenotype of cultured CAR19-T-Cells.
This project is supported by grant 15-34498A of the Ministry of Health, Czech Republic.

pCT-12

EXPRESSION OF CD64 ON GRANULOCYTES AS MARKER OF SEPSIS IN NEONATES

Novák M.[1], Hálek J.[2], Medková A.[2], Juráňová J.[1], Kantor L.[2]

[1]Department of Hemato-Oncology, University Hospital Olomouc, Czech Republic
[2]Neonatal Department, University Hospital Olomouc, Czech Republic

Neonatal sepsis is a serious, potentially life-threatening complication. Its occurrence is frequent, especially in the population of preterm newborns. Neonatal sepsis is associated with significant mortality and long-term morbidity. Complete early diagnosis and therapy is absolutely essential for the prognosis of children with neonatal sepsis. Diagnosis of sepsis is based on two basic pillars: clinical symptoms accompanying systemic inflammatory response (SIRS) and evidence of pathogens in the bloodstream. Both of these diagnostic pillars are very problematic in the neonatal period, especially in premature babies: the clinical symptoms are nonspecific, the established SIRS criteria meet only a small part of the newborns, the yield of hemoculture is low - especially in the case of early postnatal sampling. Due to the considerable unreliability of these diagnostic methods, antibiotic therapy is being initiated in the treatment of neonatal sepsis already on suspicion of infection or in the presence of risk factors. The problem, therefore, is the late initiation of treatment and the treatment of children who do not have neonatal sepsis.
For all the above reasons, it is necessary to rely on other diagnostic markers in diagnosis. None of the markers available and used is ideal. The yield of common biochemical and haematological markers is problematic in the neonatal period. The search for new, reliable diagnostic markers that would be able to confirm or exclude the presence of systemic bacterial infection in preterm infants with high certainty is a priority task of contemporary medicine.
One of the promising and tested markers is the proportion of leukocytes with the high affinity FcR1 receptor for IgG, referred to as CD64. It is a single-chain transmembrane glycoprotein that is used in antibody mediated cellular cytotoxicity.
We assume that expression of CD64 on neutrophil granulocytes reflects the presence of early forms of sepsis in neonates and complex analysis of immunocompetent lymphocyte subpopulations together with the determination of CD64 expression on neutrophilic granulocytes by multicolor flow cytometry can serve as a reliable and sensitive identifier

of early forms of sepsis in neonates.
A prospective study was attended on 125 newborns hospitalized at the Newborn Department of University Hospital Olomouc with suspicion of neonatal sepsis - regardless of the gestational and postnatal age of the child. Analysis of the obtained samples was performed by a multicolored flow cytometry method with CD3, CD4, CD14, CD16/CD56, CD14, CD15, CD163, CD19, CD138, CD45RO, CD64, CD45RO, CD64 antibodies (EXBIO, Biolegend, BD Pharmingen) and appropriate isotype controls using FACS Canto II cytometer (Becton Dickinson) and analytical software FACS Diva ver. 8.0.
The cut-off CD64 on granulocytes was determined to be over 10%, and its expression was evaluated in the range 0-2 hours after birth, 12-24 hours after birth, and in newborns with late sepsis. Newborns with congenital malformations and children born significantly before the physiological date of birth were evaluated in separate categories. In addition, IL-6, CRP, RET, leukocyte and neutrophil count, I / T index, CTP, HCT and clinical status were determined.
Determination of sepsis markers by CD64 expression on granulocytes showed a sensitivity of 83.33%, specificity 100%, positive predictive value 100% and negative predictive value 94.74% (all according to Wilson's score) in the group of children 12-24 hours post-partum. In 0-2 hours postpartum group sensitivity 27.27%, specificity 97.3%, positive predictive value 75% and negative predictive value 81.82% were determined (all according to Wilson's score).
In newborns with suspected sepsis, CD64 expression on granulocytes appears to be a potentially useful marker of septic status, especially within 12-24 hours after birth. We are currently processing a significantly larger cohort to create a scoring system to predict neonatal sepsis.
Supported by Ministry of Health, Czech Republic – conceptual development of research organization (FNOl, 0098892)

pCT-13

COMPARISON OF VB EXPRESSION IN MATURE T-CELL LEUKEMIA DETECTED BY FLOW CYTOMETRY AND NEXT GENERATION SEQUENCING

Nováková M.[1]*, Kotrová M.[1]*, Oberbeck S.[2], Mayer P.[2], Schrader A.[2], Knecht H.[1], Herling M.[2], Brüggemann M.[1]
*contributed equally

[1]Medical Department II, Unit for Hematological Diagnostics, University Hospital Schleswig-Holstein, Kiel, Germany
[2]Dept. of Medicine I, Cologne University, Cologne, Germany

Background: T-cell prolymphocytic leukemia (T-PLL) is the most common mature (post-thymic) T-cell leukemia. T-cell receptor (TR) clonality can either be assessed by flow cytometry (FCM) employing Vβ antibody panels covering ~70% of the normal human TR Vβ repertoire or by molecular techniques like next generation sequencing (NGS) with primers that amplify virtually all possible Vβ-Jβ rearrangements.

Aims: To compare the results of parallel TRB-based clonality analyses by FCM and NGS in T-PLL.
Methods: We investigated diagnostic peripheral blood (PB) leukocytes of 73 T-PLL patients. FCM of surface Vβ expression was assessed by the IOTest Beta Mark kit (Beckman Coulter). Libraries for NGS were prepared using 100ng of DNA via a 2-step PCR and sequenced on the Illumina MiSeq (2x250bp, v2) with a median coverage of 17,908 reads. Annotation of V, (D)- and J-regions of TRB sequences was done using ARResT/Interrogate (Bystry et al, Bioinformatics 2016).
Results: In all samples one or two dominant clonal TRB rearrangements were detected by NGS. In 36/73 of these cases, also FCM demonstrated clonality. Interestingly, in 8/36 of cases the dominant Vβ by FCM differed from the molecular clonotype. In 5 of these cases the discrepancy was most likely accountable to a non-functional TRB clonotype detected by NGS corresponding to a bi-allelic TRB rearrangement with the second non-functional allele being preferentially identified by NGS. In 37/73 of cases no reactivity with one of the Vβ antibodies was seen. In 16 of these cases this could be attributed to expression of a TRB rearrangement for which the appropriate Vβ antibody was not present in the FCM panel. In another 12 of these cases a non-productive TRB rearrangement represented the dominant NGS clonotype. However, in further 9 cases (24%), the functional TRB clonotype was not detected by FCM despite theoretical coverage. Of note, 10/73 T-PLL lacked surface TRα/β chain expression.
Conclusion: T-cell clonality is detected by TRB NGS in all T-PLL, whereas FCM-based Vβ repertoire analysis identifies a dominant single Vβ domain expression in only 49%. A substantial proportion of such failures of FCM-based clonality detection can be best explained by lost surface TR expression and the limited coverage of the Vβ antibody panel.

pCT-14

USEFULNESS OF CHEMOKINE RECEPTOR CCR4 FOR DIAGNOSIS OF CUTANEOUS T-CELL LYMPHOMAS BY FLOW CYTOMETRY

Osickova J.[1], Fialova P.[2], Vasku V.[2], Doubek M.[1]

[1]*University Hospital Brno, Czech Republic*
[2]*St. Anne´s University Hospital Brno, Czech Republic*

Cutaneous T-cell lymphomas (CTCL) is a large group of extranodal non-Hodgkin lymphomas characterized by malignant skin-homing CD4+ T cells. The most common form of CTCL is Mycosis fungoides which typically manifests as skin patches or plaques and malignant T cell primarily concentrated in the skin. The leucemic variant of CTCL, Sezary syndrome (SS), is more agressive than MF and the malignant T cells are found in peripheral blood predominantly (Xiao et al. 2015). Diagnosis of MF and SS is established based on clinical symptoms as well as histological analysis (Xiao et al. 2015). Flow cytometry is not used routinely for establishing diagnosis because of lack highly specific antigens for malignant CD4+ T cells in patients with MF and SS (Wojdylo et al. 2005; Wandersee et al.2015). The chemokine receptor CCR4 (CD194) is expressed on malignant CD4+ T cells in skin lesions

of MF and SS patients and it can be potential specific marker for detection of malignant T lymphocytes by flow cytometry. The aim of this study is to find combination of markers highly specific for malignant T cells by flow cytometry. Peripheral blood of 29 patients with MF, 12 patients with SS and 12 healthy controls were analyzed for CD2, CD3, CD4, CD5, CD7, CD8, CD26, CD27, HECA-452 and CCR4 (CD194) antigens by flow cytometry. 83 % of patients were positive for CCR4 (36,5-75%), whereas only 31 % of 29 MF patients were positive for CCR4 (22,7 %-59,5 %). All the 12 healthy were negative for CCR4. Our results show that combination of CCR4 positivity and loss of CD26, CD7 and CD8 on malignant CD4+ T cells could be a valuable markers for detection of tumor Sezary syndrome cells.

Acknowledgements: Supported by grant MZ CR-RVO (FNBr, 65269705)

References:

Sokolowska M, Wenzel J, Gaffal E, Lenz J, Speuser P et al., Circulating clonal CLA+ and CD4+ T cells in Sezary syndrome express the skin-homing chemokine receptors CCR4 and CCR10 as well as the lymph node-homing chemokine receptor CCR7, British Jurnal of Dermatology 2005, 152:258-264.

Xiao N, Jeffrey L, Goswami M, Challagundla P, Decker WK, Kim YH, Duvic MA, Reduction of Regulatory T cells by Mogamulizumab, a Defucosylated Anti-CC Chemokine Receptor 4 Antibody, in Patients with Aggressive/Refractory Mycosis Fungoides and Sezary syndrome, Clinical Cancer Research, 21(2),2015, 274-285.

pCT-15

ASSESSING CMV SPECIFIC T CELLS IN DONORS FOR ADOPTIVE IMMUNOTHERAPY: MULTIMER OR INTRACELLULAR CYTOKINE STAINING IS MORE APPROPRIATE?

Pelák O.[1,2], Říha P.[1], Hubáček P.[1], Keslová P.[1], Sedláček P.[1], Formánková R.[1], Hrušák O.[1,2], Kalina T.[1,2]

[1]Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University, Prague, Czech Republic
[2]CLIP – Childhood Leukaemia Investigation Prague

Hematopoietic stem cell transplantation (HSCT) is potentially curative, but associated with post-transplant immunodeficiency. Cytomegalovirus (CMV) reactivation is one of the most common post-transplant viral complications, which correlates with deficient CMV specific T cell responses. Adoptive immunotherapy with third party CMV specific T cells can prevent CMV post-transplant complications. In order to make the adoptive transfer possible, it is essential to facilitate the donor screening for the presence of suitable CMV specific T cells. We have screened 55 parents of 33 pediatric patients after HSCT for eligibility either for solation by CMV specific streptamer technology (73%, n=40) or for isolation through the IFNγ capture system (87%, n=48). Screening was done by HLA matched CMV specific Pentamers or Dextramers or by stimulation of T cells by CMV antigens (pp65, IE-1 and whole CMV lysate) and the CMV specific T cell responses were detected by flow cytometry intracellular cytokine staining assay (ICS) for production of interferon-γ.

In 35 parents, in whom we were able to perform ICS and multimer assessments

simultaneously, CMV specific T cells were detected in 29 cases by ICS and in 26 cases by multimer staining. Overall we detected more CMV specific CD8+ T cells using ICS (median 1.073% of CD8+) than by usage of multimers (median 0.45% of CD8+ cells; $p<0.05$). This discrepancy is most probably due to the usage of CMV antigens mixture for T cell stimulation in ICS rather than to a lower accuracy of multimer staining. To examine the proliferative potential of multimer positive CD8+ T cells, we performed a 6 days proliferating assay followed by multimer staining in three healthy donors. In response to stimulation by pp65 we detected proliferation of not only multimer positive cells but also of multimer negative ones. These results correspond with more CMV specific cells detected by ICS and demonstrate that there are clones of T cells that respond to pp65 antigen but do not stain positive with the corresponding multimer.
Response detected by ICS is generally broader than by multimer, nevertheless both methods showed to be appropriate for detection of CMV specific T cells. In total we found a suitable donor by at least one isolation method for 85% of our patients.
Supported by grant 15-26588A Ministry of Health Czech Republic

pCT-16

COMPARISON OF 8-COLOR IMMUNOPHENOTYPIC PANEL WITH 7-COLOR B CELL CLONALITY ASSAY IN DETECTION OF MINIMAL RESIDUAL DISEASE IN MANTLE CELL LYMPHOMA

Řádek M.[1,2], Špaček M.[1,2], Karban J.[1,2]

[1]*General University Hospital, Prague, Czech Republic*
[2]*First Faculty of Medicine, Charles University, Prague, Czech Republic*

Background: Mantle cell lymphoma (MCL) is a B-cell neoplasia characterized by the chromosomal translocation t(11;14) and cyclin D1 overexpression. It constitutes approximately 3-10% of non-Hodgkin lymphomas and is associated with poor prognosis. It has been demonstrated that the level of minimal residual disease (MRD) after therapy has a great prognostic impact in several hematological malignancies. The reference method for MRD analysis is mostly real-time quantitative PCR. However, a standardized flow cytometry method is widely used in chronic lymphocytic leukemia MRD monitoring. An 8-color combination represents a very fast and sensitive approach suitable for clinical practice. Recently a similar standardized flow-cytometric procedure in MCL has been published, using the multicolor combination for sensitive and specific immunophenotypic detection of MRD. The aim of our study was to compare the published 8-color MRD panel with 7-color B cell clonality assay.
Methods: Peripheral blood (PB) and bone marrow (BM) samples from MCL patients were acquired at diagnosis and after treatment. Multiparameter flow cytometry immunophenotyping was performed in a Navios (Beckman Coulter, Miami, FL) and FACSVerse (Becton Dickinson) flow cytometers, and analyzed using the Kaluza software (Beckman Coulter). Flow cytometer was set according to the EuroFlow instrument setup Standard Operating Protocol (1). All samples were stained with7-color (CD45/sIgLambda/

sIgKappa/CD5/CD19/CD3/CD10) and 8-color assay (CD20/CD45/CD23/CD62L/CD5/CD19/CD200/CD3), using a modified Euroflow SOP for bulk lysis for MRD panels. PB and BM specimens were incubated with antibodies purchased from eBioscience (CD200), Beckman Coulter (CD3, CD10, CD19, CD45, CD62L), Biolegend (CD5, CD20) and DAKO (CD23, sIg). All samples were immediately measured and at least 500,000 leukocytes were acquired per tube. Identification of MCL cells population was based on light chain (Kappa or Lambda) positivity and the gating strategy published by Chovancova et al. (2).
Results: Flow-cytometric MRD monitoring was performed on 480 samples from patients after treatment. Using the 7-color clonality panel and 8-color immunophenotypic panel, 20.4% and 24.6% of samples were MRD positive, respectively. The overall concordance between the panels was 95%.
Conclusions: The published flow-cytometric MRD method in MCL patients has several limitations, e.g. in CD5 negative MCL cases. In our study, we compared previously published 8-color panel with the 7-color panel based on B-cell clonality testing. We confirmed previous reports that 8-color tool has a high sensitivity and specificity in MCL MRD detection. However, in MCL cases with atypical immunophenotype (CD5 negative or CD5dim expression), residual MCL cells are better detected by determining the B cells clonality using our 7-color panel. Therefore our recommendation is to use both panels to achieve the accurate results in most cases of MCL.

This work was supported by grant RVO VFN64165.

1. Kalina T, Flores-Montero J, van der Velden VHJ, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 2012;26:1986–2010.
2. Chovancová J, Bernard T, Stehlíková O, et al. Detection of minimal residual disease in mantle cell lymphoma. Establishment of novel 8-color flow cytometry approach. Cytometry Part B: Clinical Cytometry 2014;88B:92–100.

pCT-17

POSSIBILITIES IN FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA

Bezdekova R., Stejskalova M., Kralova R., Penka M., Rihova L.

Dept. of Haematology, University Hospital Brno, Brno, Czech Republic

Multiple myeloma (MM) is a hematologic malignity characteristic by presence of clonal bone marrow plasma cells (PC). Effective treatment comes along with the need for more sensitive approaches to compare the efficacy of different treatment strategies, and implementation of individualized therapy monitoring strategies to prevent both under- and overtreatment. Minimal residual disease (MRD) information can be used as biomarker to evaluate the efficacy of treatment, help on treatment decisions, and act as surrogate for overall survival. It is obvious, that valid and/or even better standardized MRD detection will

ensure superior uniform assessment of response and clinical prognostication. Confirmation of the elimination of myeloma residual cell clones resistant to the therapy should be the way to cure MM.
There are available techniques on cellular and/or molecular level including imaging methods which proved that persistent MRD means worse survival in MM. Multiparametric flow cytometry (MFC) seems to be the most effective of existing approaches. Development of 8 colour protocols for MFC-MRD followed technical progress of cytometry itself, availability of new antigens and fluorochromes together with standardization requirements. Clinical laboratories usually use home-made methods, semi-standardized Duraclone Rare Event PC product, Euroflow-like and standardized Euroflow approach.
Our laboratory is focused on MM phenotyping for almost 15 years including MRD assessment. As Czech central laboratory for EMN02 HOVON study we have repeatly analysed 29 patients achieving complete remission (CR) by Euroflow 1st generation protocol and MRD negativity was confirmed in 44.8% (13/29) of them. Nowadays, we are testing Euroflow 2st generation protocol for research purposes.
Comparison of different assays for high-sensitive and fast quantification of MRD in treated MM will be presented.

Supported by Ministry of Health of the Czech Republic, grant nr. 17-30089A.

pCT-18

ISOLATION OF BLOOD MICROVESICLES BEFORE ANALYSIS BY FLOW CYTOMETRY LEADS TO CONTROVERSIAL RESULTS IN RELAPSING PATIENTS WITH MULTIPLE SCLEROSIS

Soukup J.[1,2], Kostelanska M.[1], Havrdova E.[3], Holada K.[1]

[1]Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University, Prague, Czech Republic
[2]Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic
[3]Department of Neurology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Raised numbers of blood microvesicles (MVs) were reported in patients with multiple sclerosis. We performed flow cytometry analysis of endothelial, platelet, erythrocyte, B – lymphocyte and T- lymphocyte MVs to examine their diagnostic potential. We collected blood of MS patients in relapse of the disease and blood of healthy controls (HC) (n = 16) in BD Vacutainer tube with K_2EDTA and proceeded it within 20 minutes from collection by centrifugation to obtain platelet free plasma. The MVs were sedimented by centrifugation (14 000 g, 70 min) and labelled by antibodies for endothelial cells (CD105), erythrocytes (CD235a), platelets (CD36 and CD41), leukocytes (CD45, CD19 and CD3) or for phosphatidylserine (Annexin V). Analysis of MVs was performed on BD FACSCanto II flow cytometer. MVs gate was set up to exclude particles smaller than the resolution of

cytometer and larger than 1 µm (Figure 1A). MS patients had lower relative number of endothelial (CD105+) MVs than HC (4.5% vs. 7.6%; p= 0.0098), lower relative numbers of T – lymphocyte (CD3+) and B – lymphocyte (CD19+) MVs 14.3% vs. 6.9% (p= 0.0037) and 6.7% vs. 3.4% (p= 0.0268), respectively. In other analysed populations of MVs were no significant differences. Plasma deprived of MVs (14 000 g, 70 min supernatant) was analysed in a similar manner. It contained similar number of events in Microvesicle gate but showed more phosphatidylserine positive MVs in MS patients than in HC (260 MVs/µl vs. HC 175 MVs/µl (p= 0.0249)). The previously published results demonstrating elevated numbers of endothelial and platelet MVs in plasma of MS patients, were not reproduced by our study on isolated MVs. In contrast, the relative numbers of MVs were lower or unchanged in MS patients which indicates that the analysis of MVs crucially depends on preparation of the sample.

The study was supported by the Ministry of Health of the Czech Republic, grant no. NV15-32961A and the Charles University, project GA UK No. 360216.

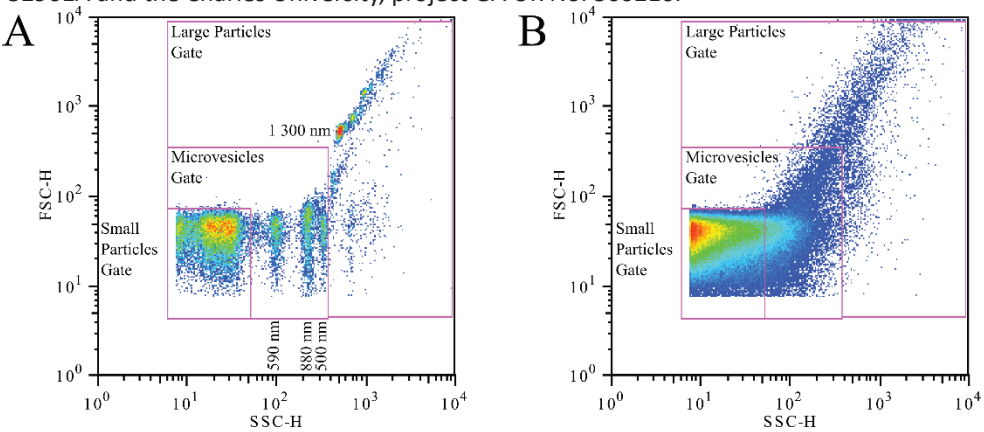


Figure 1: A) ApogeeMix beads used for definition of microvesicle gate. The Mix contains 8 populations of beads – 6 silica beads (refractive index 1.43) and 2 fluorescent latex beads (refractive index 1.59). B) A representative scattergram of vesicles isolated from blood plasma.

pCT-19

ACUTE LEUKEMIAS PHENOTYPE DETERMINATION USING MASS CYTOMETRY

Stančíková J.[1,2], Vášková M.[1,2], Pelák O.[1,2], Pavlištová T.[3], Thürner D.[1,2], Kalina[1,2], Hrušák O.[1,2]

[1]CLIP, Childhood Leukemia Investigation Prague, Czech Republic
[2]Department of Pediatric Hematology/Oncology, Charles University, 2nd Faculty of Medicine, Hospital Motol, Prague, Czech Republic
[3]Faculty of Science, Charles University, Prague, Czech Republic

Acute leukemias are defined by malignant blast phenotype present in patient´s bone

marrow. Fluorescent cytometry enables to distinguish the atypical cells using their characteristic markers. In some cases, the presence of lymphoid and myeloid markers in the same blast cells, or, in other cases, observing multiple distinct blast populations, are diagnosed as hybrid leukemias. Although the hybrid leukemias are rare, their progress is complicated, so as the successful therapy determination. Contemporary knowledge of this malignity is frequently restricted to case reports and the studies targeting to the disease origin or the therapy possibilities are sporadic. One way how to broad the knowledge of the disease principles and support the development of "tailored" therapy leads through deciphering particular phenotype of leukemia blasts. Unfortunately, the task seems to be unfeasible by use of standard fluorescent cytometry, which is restricted to measurement of only few cell markers in one tube. The aim of our study was to establish the panel of mass cytometry markers that will cover the phenotype of bone marrow cells in hybrid leukemia. Mass cytometry is a relatively new method with an advantage of measuring the large spectrum of antibody-tagged antigens in the same tube, as the antibodies are metal-conjugated. We established a panel of 35 markers expressed in normal and leukemic bone marrow. In pilot experiment we used a restricted panel (25 antibodies) for evaluation of four bone marrows. Specific expression of markers was visualized by viSNE analysis, which is suitable for expression relations in cell populations according to their phenotype. In meanwhile, the panel contains surface as well as intracellular and nuclear markers. We plan to evaluate about 20 leukemias, including hybrid, and control bone marrows. As an outcome, we aim for broadening the knowledge of hybrid leukemias, in particular determining the phenotype of blast lineages.

pCT-20

ERYTHROID LINEAGE SHOWS QUALITY OF A SAMPLE DURING ANTILEUKEMIC THERAPY

Vakrmanová B.[1], Nováková M.[1], Vodičková E.[2], Houdková A.[2], Vášková M.[1], Janotová I.[3], Starý J.[3], Trka J.[1,3], Hrušák O.[1,3], Mejstříková E.[1,3]

[1]Charles University Prague, Fac Med 2, CLIP, Dept. Pediat Hematol Oncol, Prague 15006 5, Czech Republic
[2]Univ Hosp Motol, Prague, Czech Republic
[3]Charles Univ Prague, Sch Med 2, Dept. Pediat Hematol & Oncol, Prague, Czech Republic

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood. Successful therapy leads to decrease of disease burden assessed either by optical microscopy or by more sensitive techniques allowing assessment of minimal residual disease (MRD). According to the AIEOP BFM 2009 protocol, we use flow cytometry (FC) to quantify the level of MRD in bone marrow (BM) at day 15 (D15). Patients categorized as high risk (HR) have ≥10% of malignant cells (blasts) in BM, patients categorized as standard risk (SR) have < 0.1% of blasts and the rest of the patients is classified as medium risk (MR) group. Evaluation of MRD at D15 is often complicated by low cellularity and potential contamination by peripheral blood (PB). The key question is, how to recognize non-representative sample. In 2008 we, together with another 4 national laboratories, defined

standard operation protocol. We used SYTO-16 and SYTO-41 dyes to define nucleated cells. In this protocol we defined representative sample as a sample containing ≥2% erythroid precursors (EP) defined as nucleated cells with immunophenotype $CD19^{neg}CD45^{neg}$ in BCP ALL and $CD7^{neg}CD45^{neg}$ in T ALL respectively.
We tested whether marker CD71 (transferrin receptor) and viability dye DAPI (4',6-Diamidino-2-Phenylindole) will increase the specificity of cells defined as EPs.
We valuated 332 samples of BM D15 (294 BCP ALL and 38 T ALL), 36 patients were classified as HR, 178 as MR and 112 as SR, 6 samples were non-evaluable due to low amount of EPs. Median of EPs is 5.2% (0.4-43%). We find out that EP in SR patients is significantly lower than in MR (6.9%) and HR (5.4%). There was no significant difference in level of EPs between BCP ALL and T ALL.
According to FC, 49 patients had low EPs (<2%), of which 27 were SR, 14 MR and 2 HR and 6 non-evaluable.
We find out that population $CD19^{neg}CD45^{neg}$ includes important subpopulation of dead cells according to DAPI (6.5-97%, median 58%). Population defined as $CD19^{neg}CD45^{neg}CD71^{pos}$ is according to viability more specific (dead cells 0-67%, median 8.9%).Addition of CD71 and DAPI increases specificity of the evaluation of EPs in BM, however cut-offs for evaluation of representativeness of BM sample has to be prospectively assessed.

IMMUNOLOGY

pIM-1

THE EFFECTS OF CYTOKINES ON DEVELOPMENT AND PHENOTYPE OF B LYMPHOCYTES

Bohacova P., Javorkova E., Holan V.

Institute of Experimental Medicine AS CR, Prague, Czech Republic
Faculty of Science, Charles University, Prague, Czech Republic

Cytokines represent a broad group of small molecules that are indispensable for the communication among cells of the immune system. Cytokine environment strictly influences the development of individual subpopulations of immune cells and their phenotype. We analysed the effects of different cytokines on B cell development *in vitro*. We were aiming to describe modulatory effects of selected cytokines on the activation of suppressive action of B cells stimulated with lipopolysaccharide. It was shown that the percentage of interleukin (IL)-10-producing B cells is significantly enhanced by IL-12 and interferon-γ and negatively regulated by IL-21 and transforming growth factor-β. The number of IL-10-producing B lymphocytes is under a strict regulatory control of cytokines and that individual cytokines differently regulate the development and activity of regulatory B lymphocytes. The cytokine environment also influences the differentiation of IL-10-producing B cells from distinct B-cell subpopulations. Altogether, cytokines play a decisive role in the diversification of lymphocyte subpopulations and their action. The study of cytokine effects on development and phenotype of immune cells leads to better understanding of reciprocal regulation and cross-talk among cells of the immune systems.

pIM-2

USEFULNESS OF CD200 ASSESSMENT IN DISCRIMINATION OF CD5-POSITIVE B-CELL NEOPLASMS

Čandová J., Manďáková P., Kalinová M., Mrhalová M., Campr V., Kodet R.

Department of Pathology and Molecular Medicine, Charles University in Prague, 2nd Medical Faculty, Prague

Immunophenotypic characterization of lymphoid neoplasms is important for diagnosis, subclassification, staging and it also plays a role in monitoring minimal residual disease. The differential diagnosis may be difficult in some cases, especially in distinguishing B-cell neoplasms with CD5 expression. Molecule CD200 has been identified as a potentially useful antigen for flow cytometric distinction between B-CLL and MCL.
In our flow-cytometric laboratory we analyzed 93 samples from 80 patients with CD5-positive B-cell neoplasms. We confirmed bright expression of CD200 molecule in patients with typical B-CLL (expression of CD200 molecule was detected in 58 - 100 % of CD5+ pathologic B-cells) and less frequent expression was found on atypical B-CLL (expression of CD200 molecule was detected in 35 - 95 % of CD5+ pathologic B-cells). On the other hand, tumor cells of MCL were CD200 negative in most cases (expression of CD200 molecule was detected in 0,1 - 5,9 % of CD5+ pathologic B-cells). The diagnosis of MCL has been confirmed by histology and immunohistochemistry (expression of cyclin D1 and SOX11 molecules) and also by presence of t(11;14) by means of FISH and/or PCR.
The assesment of CD200 seems to be a powerful and very helpful tool in the distinction between B-CLL and MCL. However, the still remain single controversial cases like one of our patients with typical cytometric B-CLL immunophenotype together with bright expression of CD200 molecule, but with proved t(11;14) and a high expression of cyclin D1 molecule.

Supported by the Ministry of Health, Czech Republic - Conceptual Development of Research Organization, Motol University Hospital, Prague, Czech Republic 00064203.

pIM-3

TRANSEPITHELIAL ELECTRICAL RESISTANCE (TEER): IMPLICATION TO ANTIINFECTIOUS IMMUNITY

Machát R.[1,2], Zemánková N.[1,2], Smržová Z.[1], Faldyna M.[1]

[1]*Veterinary Research Institute, Brno, Czech Republic*
[2]*Faculty of Science, Masaryk University, Brno, Czech Republic*

Epithelial barrier represents border between inner and outer environment and forms the first line of defence of body against negative external influences. In this light, it is not surprising that one of the most important pathology associated with many infectious diseases is a damage of epithelial cell-mediated barrier functions. On the other hand,

maintenance of the barrier functions is a target for many preventive or therapeutic measures.
Transepithelial electrical resistance (TEER) is a laboratory approach how to assess the integrity of the cellular barrier *in vitro*. Relevance of that technique will become more visible as *in vitro* alternatives to animal experiments will play more relevant role, mainly during early stages of research.
We have introduced TEER-based measurements for experiments testing role of two different feed additives for farm animals - lactoferrin and dried egg melange. Preliminary results have showed that variability of TEER values can be affected by many factors, incl. cell line used or composition of cultivation media.
This work was supported by Ministry of Agriculture of the Czech Republic (QJ1310258, QJ1510128, QJ1510127, RO0517) and Ministry of Education, Youth and Sports of the Czech Republic (LO1218).

pIM-4

FLOW CYTOMETRY AS A POWERFUL METHOD FOR TREATMENT MONITORING IN A PATIENT WITH CHRONIC MUCOCUTANEOUS CANDIDIASIS CAUSED BY GAIN-OF-FUNCTION MUTATION OF STAT1

Fejtková M.[1], Kanderová V.[1], Paračková Z.[2], Maródi L.[3], Svatoň M.[1], Froňková E.[1], Kalina T.[1], Bloomfield M.[2], Šedivá A.[2]

[1]CLIP-Cytometry, 2nd Faculty of Medicine and University Hospital Motol, Prague, CZ
[2]Institute of Immunology, 2nd Faculty of Medicine and University Hospital Motol, Prague, CZ
[3]Department of Dermatology, Faculty of Medicine, Semmelweis University, Budapest, HU

Background
Signal transducer and activator of transcription 1 (STAT1) is a molecule activated in immune cells in response to diverse cytokines such as IFNα, IFNγ, or IL-6. The activation results in formation of homo- or hetero-dimers followed by translocation to nucleus where it functions as a transcription factor. There are several activating so called „gain-of-function (GOF)" mutations of STAT1 described in the literature causing chronic mucocutaneous candidiasis (CMC). GOF mutations cause hyperactivation of STAT1 signaling, decrease of STAT3 signaling, and in consequence reduction of TH17 cells and anti-fungal immunity. Targeted inhibition of the hyperactive STAT1 signaling using ruxolitinib has been tested as a possible treatment of CMC.
Methods and Results
Using Sanger sequencing novel mutation in *STAT1* (c.617T>C, L206P) was found in Czech male patient (12 yo) with CMC. To clarify the activating status of the mutation we stimulated peripheral blood cells of the patient and healthy controls with IFNa and IFNg at several timepoints and found hyperphosphorylation of STAT1 (Tyr701) in patient's T-cells, B-cells, and monocytes using single-cell phospho-flow. Moreover the delay in dephosphorylation of activated STAT1 was detected. Ruxolitinib caused dephosphorylation of STAT1 *in vitro*

advocating the treatment option.
After final approval, ruxolitinib treatment was started in Feb 2017. We have optimized flow cytometry-based method for phospho-STAT1 (Tyr701), phospho-STAT3 (Tyr705), and phospho-STAT5 (Tyr694) detection in whole blood as a single-tube platform to monitor treatment efficacy. All STAT molecules have been gradually dephosphorylated throughout the treatment which correlated with clinical improvement. Nevertheless, T_H17 cells remained unchanged in the peripheral blood.

Conclusion

We confirmed GOF characteristics of novel STAT1 mutation and developed single-tube test for monitoring activity of STAT1, STAT3, and STAT5 molecules in a patient with CMC on targeted treatment.

Supported by grants: 15-28541A; P302/12/G101; 64203; LO1604; 204012; The J Project

pIM-5

CAN IMMUNOPHENOTYPING OF SYNOVIAL FLUID CELLS HELP DISTINGUISH BETWEEN PATIENTS WITH OSTEOARTHRITIS?

Gabcova G.[1], Gallo J.[2], Mikulkova Z.[1], Manukyan G.[1,3], Gajdos P.[4], Kriegova E.[1]

[1]*Department of Immunology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*
[2]*Department of Orthopaedics, Palacky University, University Hospital Olomouc, Olomouc, Czech Republic*
[3]*Group of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia*
[4]*Department of Computer Science, Faculty of Electrical Engineering and Computer Science, Technical University, Ostrava, Czech Republic*

Background: Osteoarthritis (OA) is highly heterogeneous group of synovial joint diseases and leading cause of pain and functional disability in elder people. Many therapeutic interventions have not led to clinical outcome or fail to relief from pain. The comprehensive immunophenotyping of cells in synovial fluid (SF) might therefore advance our understanding of a particular type/stage of OA.
Aim: To characterize major immune cell populations in SF from OA patients.
Methods: We immunophenotyped cells in SF obtained from 53 OA patients using 6-colour flow cytometry (FACSCanto II, BD). Data acquisition and analysis were performed using FACSDiva and FlowJo vX0.7. In all experiments a minimum of 10,000 events was counted. Radiographic severity of osteoarthritis was evaluated according to Kellgren and Lawrence classification. Results are expressed as the percentage and median fluorescence intensity (MFI) for each examined marker. Statistical tests were performed using GenEx and R statistical software.
Results: Lymphocytes were the predominant immune cells in SF from OA patients (Th/Tc = 1.25). Tc cells expressed higher HLA-DR than Th cells. 22% of lymphocytes expressed activation marker CD69. 1.2% of all gated lymphocytes comprised regulatory T cells (Treg).

Higher percentage of Treg was detected in 3rd grade compared with 1 and 2 grades (1.6% vs 0.5%, P< 0.05, respectively). NK cells presented 8% within lymphocyte gate, out of which 14% expressed CD69. Within monocyte/macrophage gate we identified 34% monocytes ($CD68^{dim}/CD14^{+}/CD163^{-}/CD86^{-}/CD206^{-}/HLA\text{-}DR^{dim}$), 15% macrophages ($CD68^{hi}/CD14^{+}/CD163^{+}/CD86^{+}/CD206^{+}/HLA\text{-}DR^{hi}$) and 0.6% myeloid dendridic cells ($CD68^{+}/CD14^{-}/CD11c^{+}/HLA\text{-}DR^{+}$). Third largest population of CD45+ cells was represented by neutrophils (8 %).
SF from patients with more severe joint damage showed higher percentage of neutrophils than SF from patients with milder joint damage (9.8% vs 4.3%, respectively).
Conclusions: We characterized major immune cell populations in SF in OA using flow cytometry. Lymphocytes were the most prevalent population in SF. We found an association between the severity of a disease and the number of neutrophils. The findings give a rational starting point for the future research of clinically useful biomarkers associated with OA development and progression.
Acknowledgements: Grant support: MZ CR VES16-31852A, MZ CR VES15-27726A, IGA_LF_2017_009
Disclosure of Interest: None declared

pIM-6

MESENCHYMAL STEM CELLS IN COMBINATION WITH DIFFERENT IMMUNOSUPPRESSIVE DRUGS MODULATE THE BALANCE AMONG Th17/Treg CELLS

Hájková M.[1,2], Heřmánková B.[1,2], Javorková E.[1,2], Jabůrek F.[1], Holáň V.[1,2], Krulová M.[1,2]

[1]*Faculty of Science, Charles University, Prague, Czech Republic*
[2]*Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic*

Mesenchymal stem cells (MSCs) represent a heterogenous population of multipotent stem cells that can be isolated from various tissues. Due to their immunomodulatory, anti-apoptotic, cytoprotective and differentiation capabilities, MSC hold a great promise for cell-based therapy. Especially MSCs applied in combination with immunosuppressive drugs offer a promising alternative approach that enables the reduction of immunosuppressant doses and thus dampen their unwanted side effects. Using flow cytometry, we aimed to determine the effects of four widely used immunosuppressive drugs with different mechanisms of action (cyclosporine A, mycophenolate mofetil, rapamycin and dexamethasone) in combination with MSCs on mouse $CD4^{+}$ lymphocyte viability and activation, T helper 17 (Th17) ($ROR\gamma t^{+}$) and regulatory T (Treg) cells ($Foxp3^{+}$) balance and on the intracellular production of corresponding cytokines (IL-17 and IL-10). We demonstrated that MSCs modulate the adverse effects of immunosuppressants on $CD4^{+}$ T cells. Furthermore, MSCs in combination with immunosuppressive agents influence immune balance by harnessing proinflammatory Th17 cells while preserving the anti-inflammatory Treg phenotype. Understanding these mechanisms is essential to improve the efficacy of MSCs-based therapy combined with application of immunosuppressive drugs in treatment of many inflammatory diseases and transplantation reactions.

pIM-7

IMMUNOMODULATORY POTENTIAL OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF RETINAL DISORDERS

Hermankova B.[1,2], Kossl J.[1,2], Javorkova E.[1,2], Bohacova P.[1,2], Hajkova M.[1,2], Zajicova A.[1], Krulova M.[1,2], Holan V.[1,2]

[1]*Department of Transplantation Immunology, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic*
[2]*Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic*

Retinal disorders such as diabetic retinopathy, age-related macular degeneration and retinitis pigmentosa, are the most common cause of decreased quality of vision or even blindness. There are currently no effective treatment protocols for these diseases. A promising approach for patients suffering from retinal disorders is represented by stem cell-based therapy which could support regeneration. Mesenchymal stem cells (MSCs) are a perspective candidate due to their ability to migrate, differentiate into multiple cell types and suppress inflammatory response in the site of injury. In this study we analysed the effect of mouse bone marrow-derived MSCs in the early phase of retinal inflammation caused by intravitreal injected inflammatory cytokines. The infiltration of immune cells in the retina and the migration of intravitreal injected fluorescent-dye-labeled MSCs were measured by flow cytometry. The analysis of cell suspensions prepared from the eyes revealed that MSCs specifically migrated into the posterior segment of the eye and the infiltration of immune cells in the retina was suppressed in the eyes treated with MSCs. The results thus show that MSCs could represent a promising tool for the therapy of various retinal disorders.

pIM-8

THE EFFECT OF INTRANASAL IMMUNISATION BY DELIPIDATED *BACILLUS FIRMUS* ON CELLULAR RESPONSE IN NALT

Hrdý J., Hnilicová Š., Novotná O., Petrásková P., Prokešová L.
Institution of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Influenza infection is one the most common cause of the death in developed countries. Current vaccine against influenza are not fully efficient especially in preventive vaccination of young children and elderly people. Moreover, there is a need to identify a novel safe adjuvant capable to promote Th1 immune response. It seems that delipidated *Bacillus firmus* (DBF) could be used as such promising adjuvant. In our study, we have tried to clarify the mechanism of DBF in nasal-associated lymphoid tissue (NALT) during intranasal vaccination. The effect of DBF on secretion of IFN-gamma in CD4+ and CD8+ T cells was evaluated using flow cytometry. Proportion of T-regulatory cells (Tregs) was followed as well. We have observed a huge induction of Tregs 2 days after intranasal immunisation

being followed by decrease and induction of IFN-gamma secretion by CD4+ T cells on day 3 after intranasal immunisation. We have detected the capacity of DBF to promote Th1 immune response which was preceded by temporal increment of Tregs probably as a result of nonspecific transient activation after DBF administration. This work was supported by Charles University program Progres Q25/LF1, UNCE 204017 and SVV 260 369.

pIM-9

WHOLE BLOOD CULTURES IN 12x75 MM CYTOMETRY TUBES FOR MITOGEN INDUCED PROLIFERATION ASSAY

Jinoch P., Prouza M.

EXBIO, Vestec, Czech Republic

Introduction:
Mitogen induced proliferation of lymphocytes is a test that evaluates lymphocyte ability to expand when stimulated with plant lectins. Reduced response to stimulation indicates immune deficiency or immune suppression. The test requires a short term *in vitro* culture (2-3 days) and the response detection system. The original ^{3}H-thymidine uptake detection system was replaced in routine immunological laboratories with flow cytometry methods (e.g. Ki67 expression, CFSE dilution, DNA content) because of its demands for processing radioactive labels. Our intention was to adapt the stimulation part of the test with regards to flow cytometry detection methods.
Aim:
To simplify and directly link the *in vitro* cultures with flow cytometry sample processing and analysis by mitogen stimulation of whole blood within cytometer compatible polystyrene tubes.
Methods:
Heparin anticoagulated blood samples were diluted 1:10 with culture medium and stimulated with various amounts of plant lectins (PHA, PWM, ConA) in 12x75 mm polystyrene tubes. The samples were fixed and permeabilized with a new set of buffers developed for intracellular staining with CD3 APC and Ki67 PE antibodies.
Results and Summary:
The Ki67 detection in CD3 cells have high signal to noise ratio for all lectins tested. The proportion of Ki67 expressing CD3 cells was dependent on the amount of blood put into the culture and on culture media (serum free or bovine serum supplemented medium). The mentioned variability will be addressed in future experiments by coating the tubes with appropriate amounts of lectins that would match predefined volumes of blood and culture media. The combination of whole blood cultures in cytometry tubes and intracellular CD3/Ki67 detection is a feasible approach how to set up a simple lymphocyte proliferation assay without substantial equipment expenses and extra staff training.

pIM-10

IMMUNOPHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF PBMC AND FIBROBLASTS IN PATIENTS WITH SCLEROMYXEDEMA

Kalli F.[1], Parodi A.[1], Nasi G.[1], Marini C.[1], Rongioletti F.[3], Fenoglio D.[1,2], Filaci G.[1,2]

[1]*Center of Excellence for Biomedical Research, University of Genova, Italy*
[2]*Department of Internal Medicine, University of Genova, Italy*
[3]*Department of Medical Sciences and Public Health, University of Cagliari, Italy*

Scleromyxedema (SME) is a chronic connective tissue disease characterized by diffuse skin sclerosis due to abnormal proliferation of fibroblasts and accumulation of mucopolysaccharides. It is associated with monoclonal gammopathy and severe systemic disorders, leading to a guarded prognosis. The association of the disease with the presence of a monoclonal gammopathy and the therapeutic effect shown in some cases by intravenous immunoglobulin administration suggest that immunological alterations may be involved in disease pathogenesis. Hence, we decided to characterize peripheral T cell subpopulations (Th1, Th2, Th17, Treg cells) and study the function of skin fibroblast primary lines (proliferation and mucin secretion) derived from ten SME patients matched by age and sex to ten healthy donors. The results showed any significant difference in the frequencies of CD4+ and CD8+Treg populations between patients and controls but an increased effector and terminal effector memory phenotype in SME patients both in CD4+ and in CD8+ T cells. Regarding the functional aspect, cytokine analysis in SME patients showed in CD4+ T cells an increase of Th2 profile both ex-vivo and after in vitro expansion with a polyclonal stimulus or Candida bodies. Regarding fibroblast data, SME fibroblast growth was higher in presence of autologous plasma and only SME fibroblasts produce mucin at high level. Collectively, the study provides at the first time a comprenhensive picture of peripheral T cell populations showing the existence of Th2 prevalence in inflammatory compartment that is could correlated with altered functions of fibroblasts.

pIM-11

MYELOPEROXIDASE IN THE REGULATION OF POLYMORPHONUCLEAR NEUTROPHIL CELL DEATH

Kocurkova A.[1,2], Kremserova S.[1,3], Klinke A.[3,4], Kubala L.[1,2,3]

[1]*Institute of Biophysics, AS CR, Czech Republic*
[2]*Department of Experimental Biology, Faculty of Science MU, Brno, Czech Republic*
[3]*ICRC, St. Anne's University Hospital Brno, Czech Republic*
[4]*Heart Center, University of Cologne, Germany*

Polymorphonuclear neutrophils (PMNs) play a key role in host defense. However, their massive accumulation at the site of injury can initiate chronic inflammatory processes, thus the clearance of PMN mediated by regulated cell death is a key process. Myeloperoxidase

(MPO), highly abundant enzyme in PMN granules, primarily connected with PMN defense machinery is suggested to be involved in PMN regulated cell death. Nevertheless, mechanisms how MPO affects PMN cell death remain incompletely characterized.
MPO-deficient PMNs revealed significantly decreased the rate of cell death characterized by phosphatidylserine surface expression in response to activation of oxidative burst by 12-myristate 13-acetate (PMA). An inhibitor of MPO activity 4-ABAH (50 μM) showed only limited effect on PMA induced cell death compared to MPO deficiency. This can be related to the remaining peroxidase activity determined by luminol-amplified chemiluminescence, both in resting and activated PMNs, even in the presence of 4-ABAH even up to 500 μM. Interestingly, PMA stimulated PMNs do not present activation of other markers characteristic for apoptotic cell death including activation of caspase 3 and 8 and DNA fragmentation. In contrast, markers characterizing autophagy such as cleavage of LC3 protein and increased expression of p62 were observed in PMA stimulated PMNs.
The important role of MPO in the regulation of the course of inflammation, independent of its putative microbicidal functions, can be potentially linked to MPO ability to modulate the life span of PMN accumulated at the site of inflammation.

pIM-12

PHENOTYPIC CHANGES OF PERIPHERAL BLOOD MONOCYTES AFTER KIDNEY ALLOGRAFT TRANSPLANTATION

Kopecka I., Svachova V., Slatinska J., Fialova M., Sekerkova A., Viklicky O., Striz I.

Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Blood monocytes may be divided into different subsets based on the expression of membrane antigens. Our pilot data suggested that after the kidney transplantation, the proportion of proinflammatory intermediate/nonclassical CD14+CD16+ monocytes was downregulated while the percentage of CD14+CD163+ monocytes (related to immunosuppressive M2 macrophages) was increased.
The aim of our study was to monitore changes of monocyte expression of CD16, CD163, CD206, CD209, HLA-DR, and CD47 in kidney allograft recipients. In total, 88 patients who underwent renal transplantation were enrolled. The phenotype was evaluated by a multicolor flow cytometry in defined time points and in the case of complications requiring fine needle aspiration biopsy procedure.
The proportions of peripheral CD14+CD16+ monocytes were downregulated during the first week after the kidney transplantation while the percentage of CD14+CD163+ monocytes were dramaticaly increased. The expression of CD206 (marker of M2 macrophages) was limited only to a less than 5% of monocytes but the receiver operating characteristic (ROC) curve analysis showed its potential importance to distinguish between patients with and without acute rejection with a sensitivity of 70% and specificity of 80.33% (area under the ROC curve 0.7787, p-value: 0.004973). The expression of CD209 (DC-SIGN) was low and did not show any time or rejection related changes. HLA-DR (MHC II) and CD47 (integrin associated protein) were constitutively expressed.

We assume from our data that kidney allograft transplantation is associated with early reciprocal modulation of monocyte subpopulations (CD14+CD16+ and CD14+CD163+). Higher percentage of CD206 positive monocytes seems to have a relationship to an increased risk of acute rejection.
Supported by MZCR grant NR 15-26883A.

pIM-13

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF DIFFERENT MONOCYTE SUBPOPULATIONS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Koudelka A.[1], Kudova J.[2], Cechova V.[1], Koreny M.[1], Perecko T.[2], Pekarova M.[1]

[1]*Institute of Biophysics, AS CR, v.v.i., Brno, Czech Republic*
[2]*Institute of Experimental Pharmacology & Toxicology, Bratislava, Slovak Republic*

For many decades myeloid cells have been regarded as anti-microbial and immunostimulatory cells. However, additional regulatory functions of different myeloid cell types (e.g. monocytes/macrophages and neutrophils) have been described quite recently. Importantly, it was found that these regulatory subsets are important players in the pathology of immune-related diseases, which cause a major socio-economic burden to the world community. Our group is currently participating in the COST Action - Mye-EUNITER, which aimed to form a unique European scientific network investigating and comparing different myeloid subpopulations in several common major immune-related disorders. In this study we aimed to identify and functionally define monocyte subpopulations in patients with rheumatoid arthritis (RA). RA is representing long-term autoimmune disorder that primarily involves the immune system attacking the joints but is also associated with systemic inflammation. In our experiments we have optimized the isolation procedure and cultivation of monocytes from healthy donors (HD) and RA patients. Beside that we have an access to RA patients that were treated with either classical or biological therapy. Isolated monocytes from HD and RA patients were further activated with pro-inflammatory mediators – TNF-α or IL-6 and treated with biological therapies *in vitro*. We were searching for expression of surface molecules (e.g. chemotactic molecules, CD115, CD11c, and CD206), molecules involved in monocyte polarization and activation of intracellular signaling pathways (e.g. STATs, MAPKs, NFκ-B, Arginase I, and iNOS) as well as for their basic physiological functions (e.g. viability, production of reactive mediators, and adhesion). We discovered that there exist different monocyte subpopulations in RA patients, characteristic of specific molecule expression and respective functional responses, which are sensitive to classical and biological therapy. Our results will help to standardize the tools, which will improve the diagnosis and the development of therapeutic options for RA

This work was supported by the Ministry of Education, Youth and Sports (LD15069) and is based upon work from the COST Action BM1404 Mye-EUNITER (www.mye-euniter.eu), supported by COST.

pIM-14

ROLE OF SRC FAMILY KINASES IN IMMUNOLOGICAL SYNAPSE OF ANTIGEN PRESENTING CELLS

Kralova J., Kotlabova K., Fabisik M., Glatzova D., Brdicka T.

Laboratory of Leukocyte Signaling, Institute of Molecular Genetics of the ASCR, Prague, Czech Republic (kralovaj@img.cas.cz)

Src family kinases (SFKs) are key regulators of cellular processes such as cell growth, differentiation, cell division and apoptosis. In immune cells, SFKs transduce signals downstream of various immunoreceptors (i.e. T cell receptor, B cell receptor, Fc receptors, Dectins etc.) and initiate signaling cascades leading to responses to the original triggers.
SFKs were also implicated in the signaling through MHCII glycoproteins on antigen presenting cells. The process of antigen presentation is a crucial process during the initiation of the adaptive immune response. Antigen presenting cells (most typically dendritic cells) present fragments of foreign antigens on their MHCII molecules on the cell surface. If antigen specific T cell recognizes these foreign peptide-MHCII complexes, crosslinking of its T cell receptors (TCR) triggers activation of SFKs. SFK activation and function in T cells after TCR crosslinking is relatively well understood, while the role and activity of these kinases on the side of dendritic cells is far more elusive.
The activity of SFKs is negatively regulated by phosphorylation of their inhibitory tyrosines by Csk kinase. This effect can be potentiated by artificially targeting Csk to the plasma membrane, resulting in strong inhibition of SFK activity. We decided to use constructs coding for membrane targeted Csk to investigate the role of SFKs in antigen presenting cells. For targeting Csk to the plasma membrane, we generated inducible constructs, where Csk is fused to transmembrane adaptor proteins LAT and SCIMP or their membrane-anchoring sequences. SCIMP brings Csk directly to the immunological synapse, while LAT is targeting Csk to lipid rafts and LAT-Csk construct was shown to be a potent SFK inhibitor. These constructs allow us to study SFK activity in the dendritic cells during the process of antigen presentation to the T cell. Furthermore, we focused on the role of the transmembrane adaptor protein SCIMP in the signaling in dendritic cells and on its contribution in the processes of antigen-presentation, fungal antigen recognition and ensuing signaling cascades.
Acknowledgements: This study was supported by GAUK
Key words: Src family kinases, Csk kinase, Antigen Presenting Cells, transmembrane adaptor proteins

pIM-15

EFFECTS OF PSEUROTIN ALKALOIDS ON SELECTED IMMUNE CELL FUNCTIONS

Vasicek O.[1,2], Rubanova D.[1], Fedr R.[1,2], Kubala L.[1,2,3]

[1]*Institute of Biophysics, Academy of Sciences of the Czech Republic, v. v. i., Brno, CZ*

[2]*ICRC, St. Anne's University Hospital, Brno, CZ*
[3]*Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, CZ*

Pseurotin A is a secondary metabolite produced by many species of fungi, mainly by *Aspergillus sp.* and *Penicillium sp*. During the pseurotin A biosynthesis, a large number of closely related bioactive compounds, such a pseurotin D or synerazol are also formed. Natural pseurotins have antimicrobial and antiparasitic activity. Interestingly a few studies suggested effects of pseurotins in eukaryotes e.g. antiangiogenic activity in chick chorioallantoic membrane assay.
In this study, we focused on effects of natural pseurotins on physiological functions of B-lymphocytes. Mouse B-lymphocytes were isolated by based on CD19 positivity by sorter Aria II. Interestingly, we show inhibition of IgE production by B-lymphocytes activated by a combination of endotoxin *E. coli* and IL-4. These effects were also related to changes in proliferation of B-lymphocytes. Modulation of selected signaling pathways from STAT family by pseutorin was shown.
It can be concluded that natural pseurotins are able to modulate B-lymphocyte immune response.
The study was supported by the GACR of the Czech Republic (17-18858S).

pIM-16
IMMUNE CHANGES DURING DEVELOPMENT OF CIRRHOTIC RAT MODEL WITH HEPATOCELLULAR CARCINOMA

Kurma K.[1,2], Macek Jilkova Z.[1,2], Marche P.[1,2], Decaens T.[1,2,3]

[1]Université Grenoble Alpes, France
[2]Institute for Advanced Biosciences, Research Center UGA / Inserm U 1209 / CNRS 5309, Grenoble, France
[3]Clinique Universitaire d'Hépato-gastroentérologie, Pôle Digidune, CHU Grenoble, France

Hepatocellular carcinoma (HCC) is the second most common cause of cancer related death mortality worldwide. Immune system of the liver contributes to the severity of the necrotic-inflammatory tissue damages, to the establishment of the fibrosis and cirrhosis and to the disease progression towards HCC. Therefore, it is crucial to understand the mechanisms of the immune and inflammatory responses of liver microenvironment. HCC therapy represents a growing challenge due to increasing morbidity and mortality of HCC. The newly developed therapies should be pre-clinically tested in an appropriate animal model which represents essential tools in cancer research. One of the models that most faithfully reproduces human scenario of advanced HCC is diethyl nitrosamine-injured rats (DEN).
The aim of this project is to deeply characterize DEN-induced HCC rat model in several time points during development of cirrhosis and HCC. 6-weeks-old Fischer 344 male rats were treated weekly during 14 weeks with intra-peritoneal injections of 50 mg/kg DEN. 9 rats are sacrificed before starting DEN-injections at (0 week), after 8 weeks of injections

(8 weeks), after 14 weeks of injections (14 weeks) and at 20 weeks after start of DEN-injections (20 weeks). FACS analysis were performed by following markers: CD45, CD3, CD161, CD8, CD4, CD152 (CTLA-4), CD25, CD90 on the tumor and non-tumor samples and on whole fresh blood. FACS samples were measured using BD-LSRII flow cytometry (BD Biosciences), data were collected with BD FACS Diva 6.3.1 software and analyzed using FCS Express V6 software.
We observed significant increase in $CD8^+$ T lymphocytes and decrease in $CD4^+$ in both the blood (p= 0.001) and liver tissue (p= 0.0001 & p=0.0137) at later time points compared to week 0. CD152 (CTLA-4) and CD25 expression was significantly increased in blood (p=0.0001) and in liver tissue (p=0.0001) compared between 0 weeks to 8, 14 and 20 weeks.
In conclusion, our work helps to understand the role of T cell subpopulation in DEN induced HCC model. Particularly CTLA-4, the only immune check point discovered in rat, is involved in DEN induced tumor initiation and progression.

pIM-17

MONITORING CD39+TREGS IN THE PROGNOSIS OF SIRS IN CANCER PATIENTS

Mrázová V.[1], Zuzulová M.[1], Záhorec R.[2]

[1]*St. Elizabeth Cancer Institute, Bratislava, Slovak Republic*
[2]*II.KAIM LF UK and St. Elizabeth Cancer Institute, Bratislava, Slovak Republic*

Sepsis is a disease defined as the systemic inflammatory response of the organism to an infection associated with high mortality. The onset of the inflammatory process is accompanied by the rapid triggering of an anti-inflammatory response that ultimately exhausts the immune system and consequently leads to suppression of immunity. Septic conditions often lead to multiple organ failure. In early treatment, it is important to assess which organ is first affected, the degree of disability. Phase when organ failure occurs. Therefore, it is appropriate to capture the passage of SIRS (systemic inflammatory response syndrome) into the sepsis by selecting the appropriate markers. A combination of markers specific to the diagnosis that would allow rapid intervention and prevent the progression of sepsis.
The inflammatory process leads gradually to the depletion of the immune system and subsequent immunosuppression. Exactly, by inducing immunosuppression are T lymphocytes (CD4 + CD25 +) involved in the pathogenesis of sepsis. Specifically, there is a significant subpopulation of CD39 + regulatory T lymphocytes that have been shown to have a significantly higher ability to induce immunosuppression compared to CD39- regulated T lymphocytes. Increased expression of the CD39 marker was recorded e.g. in HIV-positive patients, closely related to disease progression, but also in case of autoimmune and cancerous diseases. Recent studies suggest that in the case of septic patients, increased CD39 expression in regulatory T lymphocytes is associated with poor prognosis of disease and increased mortality.
Determination of the number of peripheral blood CD39+ regulatory T cells can serve as

a simple and useful biomarker of sepsis/sirs as well as a prediction marker of survival in septic/sirs in cancer patients.

pIM-18

IMMUNOSTIMULATORY AND GUT-PROTECTING EFFECT OF FUNGAL GAMMA-LINOLENIC ACID AND BETA-CAROTENE CONTANING PREFERMENTED FEED FOR BROILER CHICKEN

Mudroňová D.[1], Karaffová V.[1], Koščová J.[1], Bartkovský M.[1], Marcinčáková D.[1], Klempová T.[2], Mačanga J.[1], Čertík M.[2], Marcinčák S.[1]

[1]*University of Veterinary Medicine and Pharmacy, Košice, Slovakia*
[2]*Institute of Biotechnology, Slovak University of Technology, Bratislava, Slovakia*

At present, emphasis is put on the production of healthy and safe foods, especially so-called "functional foods" containing ingredients with health-promoting or disease-preventing actions. Gamma-linolenic acid (GLA) is an essential fatty acid from the omega-6 family delivering a range of health benefits arising from its anti-inflammatory effects. Insufficient supply of GLA from agricultural and animal sources resulted in discovery of fermentation technique using lower filamentous fungi – *Umbelopsis isabellina* CCF 2412 able to accumulate high concentrations of GLA and beta-carotene during solid state fermentation of cereals. The aim of this study was to evaluate the effect of addition of prefermented cereal product containing a high amount of GLA and carotene into the feed of broiler chicken on their immune response and numbers of lactic acid bacteria and enterobacteria in gut content. Eighty COBB 500 one day old broiler chicks were randomly divided into 2 treatment groups and fattened for 42 days. During the first 21 days, all broilers consumed the starter diets. After three weeks, broilers were fed the grower diet. Control chicks were fed without the addition of prefermented cereal product. Chickens of the experimental group were fed with commercial feed, and from the 10th day of age until the time of slaughter (39th day), 10 % of commercial feed was replaced by fermented product. Phagocytic activity was not significantly influenced however oxidative burst activity of phagocytes was significantly increased in GLA-supplemented group ($p<0.001$). Immunostimulation in GLA group was manifested by a significant increase of CD4+CD8- lymphocytes in blood and CD4:CD8 ratio. Significant increase of relative expression of gene for IgA in caecum of GLA supplemented chicks shows evidence of B-lymphocyte stimulation on the local gut level. In the caecum, we also noted increased mRNA expression for mucin-2 and insulin-like growth factor in GLA group what contributes to the protection and reparation of the intestinal mucosa and also to the better development, growth and regeneration of skeletal muscle in poultry. Immune activation and increased protection of the intestinal mucosa was subsequently manifested in the microbial composition of the intestinal contents, where significant reduction in enterobacterial counts occurred after GLA administration. We can conclude that prefermented cereals containing fungal GLA and carotene represent a low-cost supplement for broiler diet having a health beneficial effect on chicken.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-14-0397.

pIM-19

ENTRY OF MYCOBACTERIUM BOVIS BCG INTO B CELLS

Plzakova L., Krocova Z.,

Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

Mycobacterium bovis bacillus Calmette-Guérin (BCG), rod-shaped, Gram positive, intracellular pathogen, is the most widely used vaccine against tuberculosis. *Mycobacterium bovis* BCG can cause tuberculosis in humas and other mammals. Both *in vitro* and *in vivo*, infect and proliferate inside phagocytic cell types. *M. bovis* BCG also infects non-phagocytic hepatocytes, epithelial cells, and B cell lines. Information available about immune response to *Mycobacterium bovis* BCG comes from studies on natural human infections or immunizations as well as from animal model studies.
M. bovis BCG enters into B cells. B cells have a wide range of functions within the immune response, including recognition of antigens, antigen presentation to competent T cells, production of cytokines and antibodies, and contribution to the development of immunological memory. The entrance of M*. bovis* BCG into B cells requires both the active participation of bacteria and engagement of the B cell receptor and the other receptors we analyzed. Entrance of the bacteria into B cells occurs through ligation of B cell receptor. Our experiments have shown that B cell receptor is involved in *ex vivo* recognition and engulfment of bacteria into B cells. The complement receptors (CR1/2, CR3 and CR4) and the FcγR receptor are not involved in these processes. But the effect of entry of the blocking B cell receptor on CD19+ cells or separate B cell subsets demonstrated that B cell receptor alone is sufficient for the entry of *M. bovis* into B cells. Intracellular trafficking of *M. bovis* BCG inside B cells is distinct from intracellular trafficking within other antigen-presenting cells. *Mycobacterium bovis* BCG was found in the cytosol and enclosed in the vacuoles inside B cells, *in vitro* experiments. On the other hand, *in vivo* experiments the most of *M. bovis* BCG was found in the cytosol of B cells. In both of the experiment (*in vitro* and *in vivo*) *M. bovis* BCG do not proliferate there. The trafficking of *M. bovis* BCG was determined by detecting colocalization of the bacteria with the early endosome antigen EEA-1, late endosomal/lysosomal membrane marker LAMP-1, and cathepsin D.

Keywords: B cells; *Mycobacterium bovis* BCG

pIM-20

IMMUNOMODULATORY PROPERTIES OF SERTOLI CELLS AFTER XENOGENEIC TRANSPLANTATION

Porubská B.[1], Hájková M.[1,2], Vegrichtová M.[1], Krulová M.[1,2]

[1]Department of Cell Biology, Faculty of Science, Charles University, *Prague, Czech Republic*
[2]Department of Transplantation Immunology, Institute of Experimental Medicine, AS CR, *Prague, Czech Republic*

Original concept, that Sertoli cells (SCs) support and nourish gametes in seminiferous tubules of testes has been expanded by emphasizing the immune function of SCs that actively create the immuno-protective environment and locally supress response of the immune system against sperm cells. Immunomodulatory function of SCs is expressed also outside the testes, as co-transplantation studies have shown that SCs actively supress immune reaction against allogeneic and xenogeneic grafts. Our study was focused on capability of SCs to modulate host immune system after xenogeneic transplantation. We use a model of amphibian progenitor Sertoli cells (PSCs), derived from the testes of juvenile male *Xenopus tropicalis*. To monitor their fate in vivo, PSCs were transfected with Katushka-RFP (K-RFP) and were transplanted into new-born mice, using the neonatal tolerance phenomenon to increase xenogeneic cell survival in the host. By flow cytometry we examined the presence of K-RFP positive cells in various organs (lungs, liver, thymus etc.). We also detected $CD4^+$ T cells in spleen, monitor their activation status, as well as the balance between T helper 17 (Th17) (RORγt) and regulatory T cells (T-regs) (FoxP3). By intracellular staining we measured several pro- and anti-inflammatory cytokines (IFNγ, IL-2 and IL-4, IL-10, TGFβ, respectively). In our study we confirmed presence of xenogeneic PSCs in mouse recipient more than one month after transplantation. Furthermore these cells modified immune balance of the host by preserving the anti-inflammatory T-reg phenotype while harnessing pro-inflammatory Th-17 cells. Our results showed that SCs modulate immune system by evolutionary conserved mechanisms. Better understanding of such mechanisms will enable the new approaches in transplantation immunology.

pIM-21

STUDY OF IMMUNE RESPONSE IN BLOOD, SPLEEN AND CECUM TO LACTOBACILLUS FERMENTUM CCM 7514 IN CHICKENS INFECTED WITH CAMPYLOBACTER SPP

Revajová V.[1], Gancarčíková S.[1], Karaffová V.[1], Nemcová R.[1], Bobíková K.[1], Šefcová M.[1], Herich R.[1], Levkut M. Jr.[1], Letnická A.[1], Ševčíková Z.[1], Levkutová M.[1], Žitňan R.[2], Levkut M.[1,3]

[1]*University of Veterinary Medicine and Pharmacy, Košice, Slovakia*
[2]*National Agriculture and Food Centre-Research Institute for Animal Production, Nitra, Slovakia*
[3]*Neuroimmunological Institute, Slovak Academy of Sciences, Bratislava, Slovakia*

The immune response in blood, spleen and cecum to *Lactobacillus fermentum* CCM 7514 in chickens infected with *Campylobacter* spp. was assessed by flow cytometry. A day-old chicks (120) were divided into four groups (n=30): control (C), L. fermentum CCM 7514 (LB), Campylobacter (CB), and combined *L. fermentum*+*Campylobacter* (LBCB). *L. fermentum* was individually per os administrated to chickens during 7 days and *Campylobacter* infection was done at day 4. Direct immunofluorescence and double staining of isolated lymphocytes with mouse anti-chicken antibodies were used in combination CD3PE/IgM FITC, CD4PE/CD8FITC, CD45PE/IgAFITC (Southern Biotech, USA). White blood cell counts were determined by using of Fried-Lukáčová solution in Burker chamber and Hemacolor kit (Merck, Germany). Samplings were done 4 and 7 days post infection (dpi). Examination of white blood cell counts showed 4 dpi the lowest values in CB infected chicks and higher number in LBCB group. However, 7 dpi CB group presented the highest values in determined subtypes, except of heterophiles that was outnumbered by LBCB group. Immunophenotyping of peripheral blood T (CD3, CD4, CD8) and B lymphocytes (IgM, IgA) reproduced the situation in leukocytes, but 7 dpi the significant increase of CD3+, CD8+ and CD45+ lymphocytes was found in CB groups comparing to controls. Monitoring of mucosal immunity by determination of cecal IEL and LPL indicated improve of CD8+ IEL at 4 and 7 dpi in LBCB groups. Intraepithelial CD3+, CD4+, and IgA+ lymphocytes raised in combined LBCB groups. Cecal LPL showed the highest values of IgA+ at 4 and 7 dpi together with IgM+ cells. Splenic CD4+ and IgA+ lymphocytes were increased at 7 dpi. The preliminary results suggest beneficial effect of *L. fermentum* mainly by improving of humoral response.
Study was supported by grants APVV-15-0165 and VEGA 1/0562/16.

pIM-22

EFFECTS OF PSEUROTIN ALKALOIDS ON SELECTED IMMUNE CELL FUNCTIONS

Rubanova D.[1], Vasicek O.[1,2], Kubala L.[1,2,3]

[1]*Institute of Biophysics, Academy of Sciences of the Czech Republic, v. v. i., Brno*
[2]*ICRC, St. Anne's University Hospital, Brno*
[3]*Department of Experimental Biology, Faculty of Science, Masaryk Univeristy, Brno*

Pseurotin A is a secondary metabolite produced by many species of fungi, mainly by Aspergillus sp. and Penicillium sp. During the pseurotin A biosynthesis, a large number of closely related bioactive compounds, such a pseurotin D or synerazol are also formed. Natural pseurotins have antimicrobial and antiparasitic activity. Interestingly a few studies suggested effects of pseurotins in eukaryotes e.g. antiangiogenic activity in chick chorioallantoic membrane assay or inhibition of IgE production by activated B-lymphocytes. In this study, we focused on effects of natural pseurotins on physiological functions of main myeloid cell populations.
Our results employing endotoxin-activated myeloid RAW264.7 cells (murine peritoneal macrophages) show that natural pseurotins (Pseurotin A, Pseurotin D and some structure analogs) were able to significantly reduce NO production in a concentration-dependent manner, both at the level of NO production and at the level of iNOS expression. These

pseurotins also inhibited early response cytokines (e.g. IL-6). We did not see any cytotoxic effects of pseurotins on these cells.
It can be concluded that natural pseurotins are able to reduce oxidative stress, inhibit production of cytokines and the expression of receptors markers on professional phagocytes.
The study was supported by the GACR of the Czech Republic (17-18858S)

pIM-23

MYCOTOXIN DEOXYNIVALENOL TRANSFERED FROM SOW TO PIGLETS CHANGED THEIR T LYMPHOCYTE PROPORTION

Stepanova H., Hlavova K., Šťastný K., Faldyna M.

Veterinary Research Institute, Brno, CZ

Deoxynivalenol (DON), also known as vomitoxin, is produced by *Fusarium*. Pigs are one of the most sensitive farm animals to DON exposure. During years 2014 and 2015, vast part of feed was contaminated with moulds and simultaneously increased mortality of newborn piglets occurred in many Czech farms. It is the reason why we have paid attention to transfer of DON from sow to an offspring and its potential impact on piglet's immune system.
Five sows and their litters were included in the study. Deoxynivalenol was applied to 3 sows intravenously daily within 2-3 days before delivery (one dose 300μg). Concentration of DON was analyzed in piglet's blood using LC-MS/MS with high resolution from delivery to total elimination of DON from a blood. The most interesting finding was that DON was detectable in piglet's blood up to eight weeks after birth. This is in contrast to the data obtained on models with older pigs were DON was eliminated within 48 hours after administration.
Impact of DON persistence in a blood on T lymphocyte subsets was monitored by immunophenotyping one and three weeks after birth. Blood T lymphocytes were analysed using six-color flow cytometry protocol for surface markers CD3/CD2/CD4/CD8/γδTCR/ propidium iodide and regulatory T cells (T-regs) were analyzed using protocol combining a staining of surface markers and transcription factor: CD4/CD8/CD25/FoxP3/live-dead. Significant differences were found at both time points. Piglets from DON group showed higher percentage of γδTCR+ lymphocytes, lower percentage of CD4+ Th lymphocytes and lower percentage of T-regs in comparison to control group.
In conclusion, short time exposure of sows to DON has significant impact on the offspring. DON transferred intrauterine persists in piglet's blood up to eight weeks and affects T lymphocyte proportional representation.
The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (project COST-CZ LD15055).

pIM-24

OPTIMIZATION OF FACS PANEL FOR EFFECTIVE SCREENING OF RABBIT ANTIGEN-SPECIFIC PLASMA CELLS

Motycakova E.[1], Stepanova H.[2], Machat R.[2], Faldyna M.[2], Sevcik M.[1]

[1]BioVendor-Laboratorní medicína a.s., Research and Diagnostic Division, Brno, CZ
[2]Veterinary Research Institute, Brno, CZ

Recently, the technology of recombinant monoclonal antibodies production has become thoroughly developed by many laboratories and rapidly included into the processing of both diagnostic and therapeutic antibodies. The method is based on recognition of immunoglobulin sequences directly from **antibody-secreting cells or plasma cells (ASCs)** of immunized animals. This study led to optimization of crucial step of the process – the FACS strategy for effective screening of **ASCs** from rabbit blood and spleen.
We have introduced step-by-step staining panel that consists of ER-tracker / IgG-BV510 / antigen-FITC / dump channel: pan-T + CD14-dylight405. The efficiency of sorting was evaluated with ELISPOT assay. Two antigens (mouse recombinant transcortin - TK and human recombinant MMP3) were used in this study.
The **ASCs** from blood of rabbits immunized with TK were concentrated up to 6.51 % while **ASCs** from blood of rabbits immunized with MMP3 were concentrated up to 4.78 %. The labelling of cells from rabbits immunized with TK with fluorochrome conjugated antigen caused the most positive effect on sorting efficacy probably due to lower unspecific staining of TK when compared to staining with antigen MMP3.
When samples of rabbit splenocytes underwent FACS application, the proportion of **ASCs** among unsorted splenocytes was 2 times higher than their proportion in total isolated lymphocytes from a blood. However, it was harder to concentrate them effectively because of higher unspecific binding of the antigen. The sorting for **ASCs** from rabbit samples requires combination of various gating/staining strategies to obtain good-class population of active plasma cells that are producing antigen specific immunoglobulin. Type of an antigen seems to be critical point for the method because unspecific binding significantly decreases the efficiency of **ASCs** isolation.
Instruments used in the study were obtained thanks support of projects CZ.1.05/2.1.00/01.0006 and **ED2.1.00/19.0385.**

pIM-25

EXPRESSION OF TNFR2 CHARACTERIZES IL-10 PRODUCING HUMAN B CELLS

Ticha O., Bekeredjian-Ding I.

Paul-Ehrlich-Institut, Langen, Germany

B cell-derived IL-10 production has been described as a hallmark for regulatory function of B lymphocytes. However, there is an ongoing debate on the origin of IL- 10 secreting B cells

and lack of specific surface markers has turned into an important obstacle for studying these cells. TLR9 ligand – CpG DNA is known to induce the formation of IL-10 secreting B cells and our original data show that TNFR2 expression coincided with IL-10 production in CpG DNA-stimulated B cells.
Tumor Necrosis Factor Receptor 2 (TNFR2) preferentially interacts with membrane bound TNF (TNFR1 binds soluble TNF). TNFR2 is known for playing role in signaling pathways supporting cell survival, but there also exists evidence for its function in mediating apoptosis. So far, most attention was focused on the role of TNFR2 and soluble TNFR2 in T cells, but it is also expressed on B cells and its function in B cell differentiation is unknown. In this project we investigated expression and function of TNFR2 on human peripheral blood B cells after stimulation by TLR9 ligand - CpG DNA. The data obtained reveal that stimulation with CpG DNA induces expression of TNFR2on peripheral blood B cells. Interestingly, the TNFR2 negative B cell fraction consisted of mainly naïve B cells while TNFR2 positive B cells were found among all common B cell subpopulations upon CpG DNA stimulation. Expression of TNFR2 was absent on plasma cells in peripheral blood and lost upon terminal differentiation of CpG DNA stimulated B cells.
Notably, exposure to a TNFR2 agonist mimicking membrane bound TNF further increased IL-10 release, thus demonstrating a positive regulatory function of TNFR2 in promoting IL-10 production in B cells. Moreover, broader analysis then showed that supernatants from TNFR2-expressing B cells not only contain more IL-10 but also more IgM and IgG when compared to TNFR2 negative B cells, indicating that TNFR2 expression might precede terminal B cell differentiation. Subsequent experiments confirmed that stimulated TNFR2 positive B cells develop into plasmablasts and antibody-secreting cells in the absence of re-stimulation with CpG DNA.
Taken together, our data show that IL-10 secreting B cells express TNFR2, which, thus, might serve as a marker for B regulatory cells. Our results further describe a novel role of TNFR2 in B cell-derived IL-10 production.

pIM-26

CROSSREACTIVITY OF DIFFFERENT CD34 ANTIBODIES TO RABBIT AND HUMAN CELLS

Vašíček J.[1,2], Baláži A.[1], Chrenek P.[1,3]

[1]NAFC - Research Institute for Animal Production in Nitra, Lužianky, Slovak Republic
[2]Research Centre AgroBioTech, Slovak University of Agriculture, Nitra, Slovak Republic
[3]Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Nitra, Slovak Republic

The aim of this preliminary study was to assess the possible corssreactivity of anti-CD34 antibodies with different specificity to rabbit and human cells. Briefly, fresh rabbit PBMCs or human KG-1 cells were stained with different clones of anti-human, anti-mouse or anti-rat CD34 monoclonal and polyclonal antibodies as follows: unconjugated CD34 (Aviva Systems Biology, USA), unconjugated CD34 (Bioss Antibodies, USA), FITC-conjugated CD34 (Biorbyt, UK), FITC-conjugated CD34 (QBEnd-10, Thermo Fisher Scientific, USA),

FITC-conjugated CD34 (AC136, Miltenyi Biotec, Germany), FITC-conjugated anti-mouse CD34 (RAM34, eBioscience, USA), FITC-conjugated anti-mouse CD34 (MEC14.7, Bio-Rad Antibodies, USA), PE-conjugated anti-rat CD34 (ICO-115, Acris Antibodies, Germany), and unconjugated mouse anti-rabbit CD45 (L12/201, Bio-Rad Antibodies, USA) according to the producer's manual. As a secondary antibody PE-conjugated rat anti mouse IgG1 (Miltenyi Biotec, Germany) and FITC-conjugated goat anti rabbit IgG (Bio-Rad Antibodies, USA) were used. Inactivated rabbit or human AB serum was used in order to exclude non-specific labeling to Fc receptors. Viability dye (7-AAD, eBioscience, USA) was used to exclude dead cells from the analysis. Labeled cells were analysed using the flow cytometer FACSCalibur (BD Biosciences, USA). At least 100,000 events (cells) were analysed in each sample. The experiment was replicated three times. Results were statistically evaluated using One-way ANOVA. The origin of rabbit PBMCs were proved by high positivity of CD45 (98.77±0.22 %), whereas KG-1 cells were almost negative for this marker (4.62±1.75 %). Clones ICO-115, RAM34 and MEC14.7 revealed significantly higher (<0.001) percentage of $CD34^+$ cells in rabbit PBMCs samples (0.48±0.04 %, 0.35±0.06 % and 0.25±0.06 %, respectively) in comparison to other tested polyclonal (Aviva, Bioss and Biorbyt) or monoclonal antibodies (QBEnd-10 and AC136), which values varied from 0.01 to 0.06 %. On the other hand, more than 99 % of KG-1 cells were positive for CD34 detected by clones (QBEnd-10 and AC136). These clones detected significantly higher (<0.001) percentage of $CD34^+$ cells within KG-1 samples than the other tested antibodies as follows: 38.42±0.26 %, 7.64±1.60 % and 0.46±0.21 % by polyclonal antibodies (Aviva, Bioss and Biorbyt, respectively), and 19.41±4.48 %, 3.22±2.59 % and 1.98±1.60 % by clones ICO-115, RAM34 and MEC14.7, respectively. In conclusion, only clones ICO-115, RAM34 and MEC14.7 shown possible crossreactivity to rabbit cells, but their specificity is still doubtful.

This work was supported from the grants of Slovak Research and Development Agency: APVV-14-0348 and by Research Centre AgroBioTech built in accordance with the project Building Research Centre „AgroBioTech" ITMS 26220220180. KG-1 cell line was a courtesy of Dr. Medhat Shehata (Medical University of Vienna, Austria).

pIM-27

THE LYMPHOCYTE TRANSFORMATION TEST - MONITORING THE EFFECTIVENESS OF IMMUNOSUPPRESSIVE THERAPY.

Vlas T.[1], Tymkivová L.[2], Velková A.[2], Lysák D.[3]

[1]Department of Immunology and Allergology, Faculty Hospital, Pilsen, Czech Republic
[2]Charles University, Faculty of Medicine in Pilsen, Pilsen, Czech Republic
[3]Department of Hemato-onkology, Faculty Hospital, Pilsen, Czech Republic

The lymphocyte transformation assay (LTT) determines the proliferation of lymphocytes in vitro after their stimulation with different products - antigens, mitogens. LTT monitors the ability of the lymphocytes to proliferate. Proliferation is a response to stimulation subjects (lymphocyte activation). With the development of new technologies, we have new proliferation indicators and their measurement methods. Flow cytometry methods using

Ki-67 or propidium iodide (PI). Ki-67 is a nuclear protein interfering with p53 binding to DNA. It is a specific marker of proliferation. Its expression is limited to the G1, S, G2 and M cell cycle phases. Protein KI67 is undetectable in the G0 phase. KI67 is extensively studied in the context of tumor cell proliferation. The purpose of immunosuppressive therapy is to induce a condition in the body that leads to a reduction in the pathological manifestations of immune reactions while maintaining the patient's defenses against infections.
The monitored group has 42 patients, 27 of whom are on immunosuppressive therapy and 15 represent a control group. Whole blood of healthy donors or patients treated with immunosuppressive therapy stimulated PHA (5 μg / ml) for 72 hours at 37 ° C and 5% CO2. After incubation, the cells were labeled with PI or KI67 and measured on a Navios flow cytometer. In the Ki-67 method, we designated a mixture of cells using the monoclonal antibodies CD45 KO, CD4 FITC, CD3 PB and Ki-67 PE, and the measurements were made on a Navios flow cytometer. By labeling the Ki-67 protein with the monoclonal anti-Ki-67 antibody at intracellular levels, we have reliably achieved a distinction between inactivated and proliferating (activated) cell populations.
In determining proliferative activity, we found statistically significant differences between the control group and the group of immunosuppressed patients. A statistically significant difference was also between the groups with different immunosuppressive corticosteroid or corticoid + Cyclosporin A (CsA) combination.

	Detection with Ki67		
	control/corticoids	control/corticoids+CsA	corticoids/ corticoid-s+CsA
difference	45,23	63,4	18,17
p	<0,01	<0,01	<0,01

	Detection with PI		
	control/corticoids	control/corticoids+CsA	corticoids/ corticoid-s+CsA
difference	15,26	29,14	13,85
p	<0,01	<0,01	<0,01

The blast transformation assay using the Ki-67 protein can serve as a means of evaluating the success of treatment of patients by immunosuppressive therapy.
The work was supported MZ 15-30661A.

pIM-28
ALTERED dsDNA SIGNALING PATHWAY OF T1D PATIENTS

Zentsová I., Kayserová, Paračková Z., Rataj M., Klocperk A., Vrabcová P., Bloomfield M., Šedivá A.

Department of Immunology, University Hospital Motol, Prague, Czech Republic.

Recently, pathogen-induced innate cells activation has been suggested to play a significant role in type I diabetes (T1D) pathogenesis. Professional antigen presenting cells, such as dendritic cells (DC) and monocytes recognize pathogens via set of pathogen-associated molecular patterns (PAMPs), for example TLRs on cellular surface and in endosomal compartment, cytosolic NOD-like receptors and various intracellular signal receptors. Activation of these PAMPs leads to robust inflammatory response. In mouse models, specific defects in TLR2, TLR9 and MyD88 result in delayed T1D development

In our study, we investigate response of peripheral blood mononuclear cells (PBMC) of T1D patients to lipopolysacharide (LPS), ssRNA (Loxoribin) and dsDNA (CpG2216) and their intracellular and extracellular production of selected cytokines. We detected elevated production of proinflammatory cytokines IL-1b (p=0,0224), TNFa (p=0,004) and IL-6 (p< 0.0001) in supernatants in T1D patients' PBMCs (n=33) after dsDNA stimulation compared to controls (n=62). Flow cytometry analysis demonstrated significant production of IL-1b and IL-6 by both plasmacytoid dendritic cells (p=0,0013) and myeloid dendritic cells (p=0,001), as well as by monocytes (p=0,0005), while TNFa was exclusively produced by plasmacytoid dendritic cells (p=0,0012). Remarkably, significant dsDNA-induced release of cytokine IL-1b and IL-6 by monocytes and myeloid dendritic cells was noted despite their weak expression of TLR9. Endocytosis study demonstrated 96% and 95% ability of monocytes and myeloid dendritic cells to transport FITC-labelled CpG2216 intracellularly. Monocyte production of proinflammatory cytokines was not reduced by pretreatment of cells with bafilomycin (endosomal TLR signalling blocker), indicating involvement of other cytosolic molecules in pathogen sensing by these cells.

Our data further support the hypothesis that altered pathogen recognition of T1D patients, especially involvement of other lymphocyte populations, might be associated with the disease mechanisms.

IMAGING CYTOMETRY

pIT-1

3D IMAGING OF ADULT LIVER STEM CELL SPHEROIDS IN TOXICITY TESTING

Dydowiczova A., Musilova V., Basu A., Babica P., Bláha L., Sovadinová I.

Masaryk University, Faculty of Science, RECETOX - Research Centre for Toxic Compounds in the Environment, Brno, CZ

Multicellular spheroids of mammalian cells have become of the most commonly used 3D cell culture techniques. Spheroid cultures confer higher degree of biological relevance than traditional 2D monolayer cell cultures, and can be effectively used in toxicology and drug screening to more reliably predict effects of toxic chemicals and drug candidates. 3D/4D bioimaging microscopic techniques represent a valuable tool for efficient evaluation of chemically-induced changes in spheroid formation, morphology, growth or viability, especially when combined with automated microscopy/HCA readers and

automated image analysis into a high-throughput setup. In our study, we developed a microplate-based in vitro model of multicellular spheroids of hTERT-immortalized adult human liver stem cells HL1-hT1 grown in micromolded agarose hydrogels, which allows rapid, non-invasive, non-destructive and label-free quantitative assessment of spheroid morphological characteristics using automated brightfield microscopic imaging and image analysis using in-house developed ImageJ macro. Next, we combined this in vitro multicellular spheroid model with a 3-fluorophore labeling for the total, viable and dead cells. The labeled spheroids were evaluated using automated multichannel fluorescence imaging, when a series of images was acquired at different planes along the focal axis (Z-stack) and combined in ZEN image processing software. The method enabled 3D visualization and quantitation of viable and dead cells within the spheroid structure. Qualitative and quantitative changes in the distribution and numbers of live and dead cells were evaluated in response to the model hepatotoxicants, such as cyanobacterial toxins microcystin-LR and cylindrospermopsin. In summary, we demonstrated that our in vitro model of multicellular spheroids of adult human liver stem cells HL1-hT1 is suitable for automated image acquisition and 3D microscopy, and provides a human-relevant in vitro system for assessment of hepatotoxic and hepatocarcinogenic effects of environmental and food toxicants.

Acknowledgment: Project GA15-12408S funded by the Grant Agency of the Czech Republic.

pIT-2

TIME LAPSE IMAGING AND ANALYSIS OF CELL MIGRATION, PROLIFERATION AND CELL CYCLE IN SCRATCH ASSAY WITH YOKOGAWA CONFOCAL QUANTITATIVE IMAGE CYTOMETER CQ1

Endo T.[1], Nomura Y.[1], Pasculli R.[2], Ota A.[1]

[1]Yokogawa electric corporation, 2-3 Hokuyodai Kanazawa, Japan
[2]Accela s.r.o., Služeb 4 108 00 Prague 10, Czech Republic

Wound healing assays are conducted to evaluate cell migration, cell invasion and the influence of culture conditions onto these abilities. The scratch assay is one of the most commonly used method for this purpose. Considering that cell proliferation, and underlying regulatory mechanisms of cell division, are also critical for cell invasion, it is would be beneficial to monitor cell cycle phase during wound healing assays using fluorescent cell cycle markers. However, repeated illumination of excitation light often result in severe damages to the cells and significant photobleaching as well. The CQ1 is an all-in-one confocal quantitative image cytometer based on the Yokogawa confocal scanner unit (CSU), which has been an ideal solution for live cell confocal imaging because of low phototoxicity and low photobleaching during laser scanning by the system. Here we report the time lapse live cell imaging and analysis of cell migration, proliferation and cell cycle in scratch assay using CQ1. The effects of a genotoxic substance mitomycin C (MMC) was examined.

MICROFLUIDICS

pMI-1

MICROFLUDIC DEVICE FOR DIELECTROPHORETIC DROPLET SORTING

Křivánková J.[1], Foret F.[1], Michálek T.[2], Hurák Z.[2]

[1]*Institute of Analytical Chemistry, The Czech Academy of Sciences, v. v. i., Brno, CZ*
[2]*Czech Technical University in Prague, Faculty of Electrical Engineering, Prague, CZ*

Technologies for producing of monodisperse microdroplets, encapsulation and manipulation of bio-particles or living cells are important for many bioanalytical applications. Here, we present a new simple and effective droplet-based microfluidic device for generating, trapping and sorting of aqua's droplets using dielectrophoretic (DEP) forces. The chip fabrication method relies on traditional polydimethylsiloxane (PDMS) casting against the SU-8 replication master. DEP platform with gold electrodes is fabricated separately and subsequently coated with pre-polymerized PDMS to seal the PDMS chip and DEP platform with oxygen plasma treatment. Water droplets in fluorinated oil stabilized by commercial surfactant are sorted in the microfluidic chamber with 8 pairs of electrodes with triangular tips by positive dielectrophoresis (pDEP). When harmonic electrical signals of opposing phases are applied to the two neighboring electrodes, the droplets are attracted towards location in between the triangular tips (into the trap). By controlling the voltages applied to electrodes, positions of these traps are changed. As a consequence the droplets move to new locations. Described microfluidic device will be used for trapping and further manipulation of objects encapsulated into droplets.

This work was funded by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601) and by the Czech Science Foundation (P206/12/G014) and Institutional support RVO: 68081715.

pMI-2

PREPARATION OF A SIMPLE MICROFLUIDIC MODEL TO STUDY VASCULAR INFLAMMATION WITH IMPROVED PHYSIOLOGICAL RELEVANCE

Víteček J.[1,2], Černík M.[1], Šafaříková E.[1], Sochová L.[1], Kolářová H.[1], Kubala L.[1,2]

[1]*Institute of Biophysics AS CR,Brno, Czech Republic*
[2]*International Clinical Research Center-Center of Biomolecular and Cell Engineering, St. Anne's University Hospital, Brno, Czech Republic*

Tissue inflammation results in rolling and firm adhesion as well as rapid extravasation of leukocytes to the inflamed locus. We focus on phenotype of endothelial cells and their response to inflammatory stimuli, subsequent neutrophil adhesion and the impact of shear stress on these processes. For this purpose we have chosen a microfluidic system based

on the concept of parallel plate chamber. This way we have proven that myeloperoxidase, an abundant enzyme released by phagocytes, can act as an inflammation activator. On the other hand, the parallel plate concept based microfluidic channels suffered from flow disturbances in channel edges resulting in disarrangement of endothelial cells and preferential margination of leukocytes in these places. Such flare hampered precise analysis of the behavior of endothelial cells and leukocytes in this in vitro model. Thus a concept of physiologically relevant circular cross-section microfluidic channels that would allow for a uniform shear stress along whole channel wall was adopted. The channels were made in a silicone chip by means of removable or sacrificed molding elements. Since native silicone is not biocompatible in terms of supporting cell adhesion a set of surface modification was tested. These included oxidation of inner surface of channels, silanization, protein coating, etc. The combination of surface oxidation and collagen I coating provided the most stable support for cell adhesion. The microfluidic chip was successfully colonized with endothelium cells under flow conditions.

Supported by the projects no. 17-24707S (Czech Science Foundation), no. LQ1605 (MEYS CR, NPU II) and by European Union - project ICRC-ERA-HumanBridge (No. 316345).

NEW METHODS

pNM-1

FLOW CYTOMETRIC ANALYSIS OF NUCLEOSIDE TRANSPORTERS ACTIVITY

Drápela S.[1,2,3], Fedr R.[1,3], Khirsariya P.[3,4] , Paruch K.[3,4], Souček K.[1,2,3*]

[1]Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Brno, Czech Republic
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]International Clinical Research Center, Center for Biomolecular and Cellular Engineering, St. Anne´s University Hospital Brno, Brno, Czech Republic
[4]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic
**correspondence to ksoucek@ibp.cz*

Nucleoside analogues represent a relevant class of antimetabolites used for therapy of various types of cancer. However, their effectivity is limited by drug resistance. The nucleoside transport capability of tumours cells is considered to be a determinant of the clinical outcome of treatment regimens using antimetabolites.
Due to hydrophilic properties of antimetabolites, its transport across the plasma membrane is mediated by two families of transmembrane proteins, the SLC28 family of cation-linked concentrative nucleoside transporters (CNTs) and SLC29 family of energy-independent equilibrative nucleoside transporters (ENTs). Loss of functional nucleoside transporters has been associated with reduced efficacy of antimetabolites and their derivatives and

treatment failure in diverse malignancies including acute myeloid leukaemia.
Here we established flow cytometric assay for analysis of nucleoside transporter activity employing fluorescent nucleoside analogue, uridine-furane. We focused on the description of long-time kinetic of uridine-furane incorporation and endpoint analysis in the parental prostate cancer cell line PC3 and its docetaxel-resistant derivative.
In summary, our protocol serves as an effective tool for detection of nucleoside transporter activity which could contribute to the selection of suitable chemotherapy for diverse malignant diseases.

Acknowledgements: This work was supported by funds from Ministry of Health of the Czech Republic, grant nr. 15-33999A, 15-28628A, all rights reserved (KS), The Czech Science Foundation, grant nr. 15-11707S, projects no. LQ1605 from the National Program of Sustainability II (MEYS CR) and by the project FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123 (OP VaVpI) by European Union - project ICRC-ERA-HumanBridge (No. 316345) (to RF), and by project HistoPARK (CZ.1.07/2.3.00/20.0185; KS). We would like to thank Iva Lišková, Martina Urbánková and Kateřina Svobodová for technical assistance.

pNM-2

FLUORESCENTLY LABELLED MOUSE SYNGENEIC MODEL FOR CIRCULATING TUMOUR CELLS RESEARCH

Dvořák V.[1,2], Slabáková E.[1], Binó L.[1,3], Remšík J.[1,2,3], Fedr R.[1,3], Souček K.[1,2,3]

[1]Department of Cytokinetics, Institute of Biophysics, Czech Academy of Science, v.v.i., Brno, Czech Republic
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic

Cancer progression and formation of distant metastasis are mainly responsible for cancer-related mortality. Release of circulating tumour cells (CTCs) from primary tumor into the bloodstream has a key role in this complex process. It is known that population of CTCs could be highly heterogeneous and plastic, which could not only affect their invasiveness but also complicate their detection and isolation. To overcome this obstacle in experimental condition, we decided to develop fluorescently labelled mouse cell line, syngeneic to BALB/c background. Usage of this model brings advantage of fluorescent visualisation and isolating all CTCs subset, using multiple techniques, such as cell sorting and micromanipulation, without missing any highly plastic subpopulation, which could have an important role in the metastatic process.
The spontaneously metastasizing murine mammary cancer cell line 4T1 12B with firefly luciferase expression was transduced with lentiviral vector encoding fluorescent protein mCherry under constitutively active promoter. Cells were then orthotopically transplanted into fat pad of female BALB/c mice, where they formed primary tumors and, at later time

point, also distant metastasis. Presence of fluorescent protein in cancer cells enables non-invasive *in vivo* monitoring of primary tumor growth and dissemination and also detection and isolation of cancer cells from collected blood. This approach thus allows us to isolate viable CTCs for a variety of downstream analyses, including single cell PCR.
4T1 12B mCherry model is a valuable tool for cancer research, due to its spontaneous dissemination, ease of cancer cell tracking and similarity to stage IV human breast cancer. Hence it could help clarify processes behind cancer dissemination.

Acknowledgement
This work was supported by funds from Ministry of Health of the Czech Republic, grant nr. 15-33999A, 15-28628A, all rights reserved (KS), projects no. LQ1605 from the National Program of Sustainability II (MEYS CR) and by the project FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123 (OP VaVpI) by European Union - project ICRC-ERA-HumanBridge (No. 316345) (to LB, RF), and by project HistoPARK (CZ.1.07/2.3.00/20.0185; KS). We would like to thank Iva Lišková, Martina Urbánková and Katka Svobodová for technical assistance, to MVDr. Nina Charvátová and Pavla Řezníčková for maintenance of animal facility.

pNM-3

THE FLOW CYTOMETRIC DETECTION OF HLA-B27 USING DURACLONE B27 KIT

Prajzlerová K., Hulejová H., Kryštůfková O.

Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University, Prague, CZ

Background: HLA-B27 antigen is associated with seronegative spondyloartritides (SpA) and its positivity is included in the classification criteria of SpA. The molecule share common epitopes with HLA-B7 and belongs to the the cross-reacting group CREG-B7. The use of two different clones of monoclonal antibodies (MoAb) is recommended for exclusion of cross-reactivity interference in flow cytometric HLA-B27 assay. Here we compare the DuraClone B27 kit (Beckman Coulter) and three different clones of anti-HLA-B27 MoAbs, included in our diagnostic algorithm which is based on the median fluorescence intensity cut off values and presence of HLA-B7.
Method: Three clones of FITC-conjugated anti- HLA-B27 MoAbs were sequentially used for whole blood direct staining. The clone ABC-m3 (IOTest, Beckman Coulter) was used for screening. Because of its cross-reactivity, the assay includes the anti-HLA-B7-PE antibody (clone BB7.1). The HLA-B7 positive or HLA-B27 borderline samples were further evaluated with clones GS145.2 (HLA-B27 Kit, Becton Dickinson) and FD705 (HLA-B27-FITC; One Lambda). The DuraClone B27 kit contains two PE-labeled diagnostic MoAbs anti-HLA-B27 (clones ABC-m3 and FD705) and an unlabeled anti-HLA-B7 antibody (clone BB7.1), which blocks the B7 antigen cross-reactivity. Fifty patients were evaluated and the percentage of agreement between results of DuraClone kit and diagnostic algorithm or individual antibodies was determined.
Results: The algorithm allowed final conclusion of all samples with 100% agreement with

DuraClone kit. The IOTest was conclusive in 78% of samples. The further step using two other antibodies (GS145.2 a FD705) gave conclusive result in 96% and 92% of patients with 100% and 93% match to DuraClone kit. Detection of HLA-B27 with clone FD705 resulted in 6% with false positivity compared to diagnostic algorithm.

Conclusion: The advantage of the IOTest is the information about B7 positivity. However it requires experience with the interpretation of staining patterns. For laboratories with less sample number requests, the DuraClone kit could be preferred as only numeric values are interpreted. The nonspecific reactivity of clone FD705 with non-CREG-B7 alleles should be considered.

Acknowledgement: Grant of Ministry of Health Czech Republic 0002372801

pNM-4

TEM EXOSOME ANALYZER: A NOVEL SOFTWARE TOOL FOR DETECTION AND ANALYSIS OF EXOSOMES IN TRANSMISSION ELECTRON MICROSCOPY IMAGES

Kotrbová A.[1*], Štěpka K.[2*], Maška M.[2*], Pálenik J. J.[2], Ilkovics L.[3], Klemová D.[3], Hampl A.[3], Bryja V.[1], Matula P.[2], Pospíchalová V.[1#]

[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlarska 2, 611 37, Brno, Czech Republic
[2]Centre for Biomedical Image Analysis, Faculty of Informatics, Masaryk University, Botanická 68a, 602 00, Brno, Czech Republic
[3]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
**equal contribution*
#corresponding author: Vendula Pospíchalová, Ph.D.
Address: Complex University Campus at Bohunice
Kamenice 753/5, building A36
625 00 Brno
Czech Republic
Tel. +420 54949 6811
Fax: +420 54121 1214
Email: pospich@sci.muni.cz

Introduction: Exosomes (exs) are nano-sized extracellular vesicles that function as conveyers of information between cells. Their content reflects the cell of origin as thus can serve as promising disease biomarkers. Different cargo of vesicles underlines their function and may have effect on morphological characteristics (shape, size) of exs. So far, there has been a lack of studies correlating morphological characteristics of exs with their content and possible function. This is caused partially by the fact, that analysis of individual exs in transmission electron microscopy (TEM) images is time-consuming if performed manually. Therefore we present here a software for computer-assisted evaluation of exs in TEM images.

Methods: Exs were isolated using differential centrifugation followed by a purification

step in sucrose/D_2O cushion. Morphology of exs was observed using negative contrasting and visualization by TEM. Exs morphological characteristics were analyzed by TEM ExosomeAnalyzer software based on their shape and edge contrast criteria. The exs segmentation was carried out using morphological seeded watershed on gradient magnitude image, with the seeds established by applying a series of hysteresis thresholding, followed by morphological filtering and cluster splitting.
Results: We developed a software tool capable of analyzing morphological features of exs (size and roundness) in often not so clear TEM images. Our software is superior to the existing ones as even images with exs both lighter and darker than the background, or containing artefacts or precipitated stain, can be successfully processed. If the fully-automatic processing fails to produce correct results, the program allows the user to adjust the detection seeds as well as exosome boundaries manually.

Summary/conclusion: Our software is an easy to use tool that might be of high interest to the exosomal community. It is publicly available at: http://cbia.fi.muni.cz/projects/tem-exosome-analyzer.html.

pNM-5

EFFECT OF THE FÖRSTER RESONANCE ENERGY TRANSFER ON FLUORESCENCE INTENSITY CELL MARKERS STUDIED BY FLOW CYTOMETRY

Slanina P.[1], Vlk D.[2], Vlková M.[3]

[1]Faculty of Science, Masaryk University Brno, Czech Republic
[2]Department of Biophysics, Faculty of Medicine, Masaryk University Brno, Czech Republic
[3]Department of Clinical Immunology and Allergology , st. Anne Faculty Hospital in Brno, Czech Republic

Introduction:
Förster resonance energy transfer (FRET) is used to determine a relation between nearby molecules at the nanometer scale. Its main purpose is to study protein and intermolecular interactions in fluorescence microscopy, flow cytometry and spectrofluorometry. This study addresses the effect of FRET on fluorescence intensity measurements of the cell surface receptors marked by monoclonal antibodies.
Material and Methods:
Three sets of measurements were done for the observation of FRET by flow cytometry, two times with the same combination of monoclonal antibodies on peripheral blood mononuclear cells (PBMC nonstimulated/stimulated), once with another combination of monoclonal antibodies while was marked whole blood.
Results:
For the first combination of monoclonal antibodies which consisted of three pairs of donor-acceptor molecules was measured FRET on 5 samples of PBMC without stimulation and subsequently after stimulation in water bath. These measurements didn't confirm FRET between these molecules. Third combination of monoclonal antibodies consisted of 2 pairs

donor-acceptor molecules was used for labelling 35 whole blood samples. Under specific conditions characteristic for FRET a decrease in the fluorescence intensity of donors was proved in the presence of acceptors. The result is statistical evaluation of fluorescence intensity of donor-acceptor pairs that are characteristic for FRET. The consequence was false decrease in the rate of expression of the measured sign.

Conclusion:

Obtained data confirmed that Förster resonance energy transfer could influence routine measurements by flow cytometry.

pNM-6

FLOW CYTOMETRY DETECTION OF FIBROBLAST ACTIVATION PROTEIN USING INHIBITOR-DECORATED POLYMER CONJUGATES

Stollinová Šromová L.[1], Bušek P.[1], Dvořáková P.[2,3], Knedlík T.[2,4], Šácha P.[2,4], Schimer J.[2,4], Etrych T.[5], Kostka L.[5], Šubr V.[5], Konvalinka J.[2,4], Šedo A.[1]

[1]Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, U Nemocnice 5, 12853, Prague 2, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, The Czech Academy of Sciences, Flemingovo n. 2, 16610, Prague 6, Czech Republic
[3]Department of Cell Biology, Faculty of Science, Charles University, Viničná 7, 12843, Prague 2, Czech Republic
[4]Department of Biochemistry, Faculty of Science, Charles University, Hlavova 8, 12843, Prague 2, Czech Republic
[5]Institute of Macromolecular Chemistry, The Czech Academy of Sciences, Heyrovského n. 2, 16206, Prague 6, Czech Republic

Fibroblast activation protein alpha (FAP, seprase) is a membrane bound serine protease. Its expression is typical for activated fibroblasts in epithelial cancers and other states associated with the remodeling of the extracellular matrix. iBodies are water-soluble and biocompatible N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers containing functional groups that serve as targeting ligands, affinity anchors, and imaging probes.
The aim of this work was to optimize the detection of FAP+ cells using a FAP inhibitor-decorated iBody. The anti-FAP iBody has two tags that can be used for visualization – Atto488 and biotin. Direct detection of Atto488 fluorescence is quick and has sufficient resolution for FAP overexpressing cells. The use of an amplification step with Streptavidin –PE (or APC) improves the sensitivity of the methodology and can be used to visualize endogenous levels of FAP expression. Our results show that the anti-FAP iBody is useful for flow cytometry detection of both mouse and human FAP.
This work was supported by Grant No. 15-31379A from the Ministry of Health of the Czech Republic, UNCE 204013 and Progres Q28 projects from the Charles University, Grant No. LM2015064 of the EATRIS-CZ from the Ministry of Education of the Czech Republic

pNM-7

CYTOKINE ANALYSIS OF MESENCHYMAL STEM CELL SECRETOME MEASURED BY LUMINEX® TECHNOLOGY ADAPTED TO FLOW CYTOMETRY

Turnovcová K.[1], Vacková I.[1], Kupcová Skalníková H.[2], Kočí Z.[1], Kubinová Š.[1]

[1]Institute of Experimental Medicine AS CR v. v. i., Prague, Czech Republic
[2]Institute of Animal Physiology and Genetics AS CR v. v. i., Liběchov, Czech Republic

Mesenchymal stem cells (MSCs) have been known to produce broad spectrum of bioactive molecules involved in tissue regeneration and modulation of inflammation. These features suggest the MSC as a promising therapeutic agent in various inflammatory and degenerative diseases. The Luminex multiplex bead-based assay presents the analytic method with wide spectra of chemokines, cytokines and growth factors to be investigated in one sample. However, the availability of Luminex® Instrument might be a limiting factor for such kind of analysis. Here, we describe an adaptation of bead-based multiplexing Luminex kit to flow cytometer (BD FACSAria).
Human MSCs from different origin (adipose tissue, umbilical cord and bone marrow) were cultivated under normoxic and hypoxic conditions to stimulate cytokine production. Cell supernatants were collected, aliquoted and incubated with the Luminex bead-based assay kit according to the manufacturer's recommendations. All samples were then run on the Luminex® 100™ instrument and on BD FACSAria Flow Sorter. Obtained Luminex data were analysed using Luminex Software. Data from BD FACSAria were processed using the FlowLogic™ and BeadLogic™ Software (Inivai Technologies). The values obtained from both types of measurements were then compared.
We conclude that data obtained from FACS and calculated using dedicated software were comparable to those from Luminex®. Precise calibration curves are crucial to get accurate results from both tested methods.

Supported by GAČR 17-03765S and MŠMT LO1309.

PLANT CYTOMETRY

pPC-1

APPLICATION OF SINGLE CHROMOSOME GENOMICS IN PLANTS

Cápal P.[1], Blavet N.[1], Vrána J.[1], Endo T. R.[2], Karafiátová M.[1], Doležel J.[1]

[1]Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, 78371 Olomouc, Czech Republic
[2]Faculty of Agriculture, Ryukoku University, 1-5 Yokotani, Seta Oe-cho, Otsu, Shiga 520-2194, Japan

The analysis of complex plant genomes which have high repeat content and very often are polyploid, may be considerably simplified by dissecting them into their natural subunits – chromosomes. This can be done by flow cytometric sorting. However, sorting of chromosomes in plants is often hampered by similar size and DNA content, which renders them undistinguishable on flow karyotypes. To overcome this limitation a method for obtaining DNA from single copies of chromosome was developed. Each individual copy of a chromosome is amplified 10^6-fold to obtain microgram quantities of chromosome-specific DNA that is suitable for various downstream applications, including next-generation sequencing. This approach is suitable for localization of genic sequences to particular chromosomes, development of chromosome-specific DNA markers, verifying assignment of DNA sequence contigs to individual pseudomolecules, and validating whole-genome assemblies. The protocol expands the potential of chromosome genomics, which may now be applied to any plant species from which chromosome samples suitable for flow cytometry can be prepared, and opens new avenues for studies on chromosome structural heterozygosity and haplotype phasing in plants. This work was supported by the Ministry of Education, Youth and Sports (grant award LO1204 from the National Program of Sustainability I).

pPC-2

FLOW CYTOMETRIC CHARACTERIZATION OF NUCLEAR GENOME AND CHROMOSOME SORTING IN *AGROPYRON CRISTATUM*, A WILD RELATIVE OF WHEAT

Said M.[1], Vrána J.[1], Karafiátová M.[1], Čížková J.[1], Cabrera A.[2], Doležel J.[1]

[1]Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, 78371 Olomouc, Czech Republic
[2]Department of Genetics, ETSIAM, University of Córdoba, Apartado 3048, 14080 Córdoba, Spain

Crested wheatgrass, *Agropyron cristatum* (L.) Gaertn., is a wild relative of wheat which exhibits large genetic variation and is an attractive source of novel genes for wheat improvement. The species comprises a complex of diploid (2n=2x=14, PP), tetraploid (2n=4x=28, PPPP) and hexaploid (2n=6x=42, PPPPPP) forms. In the first part of this work, we characterized 80 accessions collected from different parts of the world. Flow cytometric analysis classified 26 accessions as diploid, 51 accessions as tetraploid and 3 accessions as hexaploid. The results were confirmed by chromosome counting. Mean nuclear 2C DNA content of diploid plants was estimated as 12.83 pg DNA (1C genome size = 6.27 Gbp). Some diploid genotypes exhibited slightly higher DNA content, and were identified by FISH analysis as B-chromosome carriers. With the aim to facilitate genome mapping and sequencing, we explored a possibility of developing flow cytogenetics for the species. Flow karyotypes obtained after the analysis of DAPI-stained suspensions of mitotic metaphase chromosomes of diploid *A. cristatum* consisted of three peaks representing the seven chromosomes pairs of the species. The chromosomes could only be sorted in groups of two or three. However, chromosomes 1P - 6P could be sorted individually from wheat-*A.*

cristatum addition lines at purities ranging from 81 to 98 %. B chromosomes could be easily discriminated and sorted at purities higher than 95%. These results provide an opportunity to investigate the structure and evolution of the P genome of *A. cristatum* and to develop molecular tools to facilitate the exploitation of this wild species and support alien introgression breeding of bread wheat. This work has been supported by the Czech Science Foundation (award 315160-2015) and the Ministry of Education, Youth and Sports (award LO1204 from the National Program of Sustainability I).

pPC-3

FLOW CYTOMETRIC SORTING FACILITATES THE ANALYSIS OF PLANT NUCLEAR PROTEOME

Petrovská B.[1], Blavet N.[1], Uřinovská J.[2], Jeřábková H.[1], Chamrád I.[2], Vrána J.[1], Lenobel R.[2], Beinhauer J.[2], Šebela M.[2], Doležel J.[1]

[1]Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, 78371 Olomouc, Czech Republic
[2]Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů *27, 78371 Olomouc, Czech Republic*

The organization of DNA in three-dimensional space of cell nuclei, DNA synthesis and repair as well as gene expression and nuclear and cell division depend on a variety of proteins. However, with the exception of histones and a few other proteins, only a fraction of nuclear proteins has been identified in plants. Nuclear proteome analysis is hampered by several factors, the most critical being the contamination of nuclei by cytosolic material during their isolation. Moreover, the conventional multi-step fractionation procedure is laborious and prone to contamination. In order to overcome these difficulties, we have developed a nuclei isolation protocol based on flow sorting. The method allows isolation of nuclei at different phases of cell cycle and minimizes the risk of contamination by cytoplasmic proteins. We have applied the protocol in barley (*Hordeum vulagre* L.) and developed a dedicated barley nuclear proteome database called UNcleProt http://barley.gambrinus.ueb.cas.cz. Its current version contains 2,429 proteins identified *via* MALDI-MS (matrix assisted laser desorption ionization – mass spectrometry) and ESI-MS (electrospray ionization – mass spectrometry) from G1, S and G2 cell cycle phase nuclei. To complete this protein set, a total of 34,675 peptides were identified and assigned to corresponding protein sequences. UNcleProt is the first database containing plant nuclear proteins identified in nuclei during different stages of the cell cycle. The database is an important resource for plant cell biologists and will contribute to the efforts aiming at understanding the nuclear architecture and its relationship to genome function. This work was supported by the Czech Science Foundation (award 14-28443S) and the Ministry of Education, Youth and Sports of the Czech Republic (award LO1204 from the National Program of Sustainability I).

pPC-4

FAOS TECHNIQUE AS A SUITABLE TOOL FOR DETERMINATION OF AUXIN METABOLIC PROFILES AT THE SUBCELLULAR LEVEL OF ISOLATED INTACT PLANT NUCLEI

Vojtková T.[1], Skalický V.[1], Vrána J.[2], Pěnčík A.[1], Novák O.[1], Kubeš M.[3]

[1]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science of Palacký University & Institute of Experimental Botany AS CR, Olomouc, Czech Republic
[2]Centre of the Region Haná for Biotechnological and Agricultural Research, Centre of Plant Structural and Functional Genomics, Faculty of Science of Palacký University & Institute of Experimental Botany AS CR, Olomouc, Czech Republic
[3]Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Chemical Biology and Genetics, Faculty of Science of Palacký University, Olomouc, Czech Republic

The aim of the study is to determine naturally occurring subcellular levels of auxins in Arabidopsis nuclei using optimized isolation method combined with LC-MS analysis. Current knowledge about the role, function and occurrence of auxins in plants is quite broad, however, nowadays the main focus of plant scientists moves from organ and tissue level to cellular or rather subcellular levels. So far described methods of subcellular fractionation are mainly based on differential or density gradient ultracentrifugation, albeit the resolution power of these approaches is low and contends with co-purifying contaminants comparing to the emerging methods like immunoaffinity purification or fluorescence-assisted organelle sorting (FAOS) (Lee et al., 2010). Since in our study we focus on effective organelle (nuclei) isolation combining approaches, we have exploited highly accurate FAOS method that separates fluorescently positive organelles based on binding of fluorescent selective dye or protein tag. This method allows us to obtain nuclear fraction of high-purity and intactness in an adequate yield suitable for future analytical assessment. Our preliminary data from LC-MS analysis point out to the fact that auxin profiles in plant nuclei are quite complex and do not include just expected active molecule of indole-3-acetic acid (IAA) but also other key representatives that covers auxin biosynthesis, conjugation and degradation. Moreover, detected auxin levels are not negligible and they suggest us to think about existing scientific hypotheses in the new light.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic through the National Program of Sustainability I (No. LO1204).

pPC-5

AM I REALLY SO SMASHED?

Sadílek D.[1], Urfus T.[2], Vilímová J.[1]

[1]Department of Zoology, Faculty of Science, Charles University, Praha, Czech Republic

[2]*Department of Botany, Faculty of Science, Charles University, Praha, Czech Republic*

The human bed bug (*Cimex lectularius* Linnaeus, 1758) is currently the most serious insect ectoparasite in temperate zone around the world. This blood feeding pest spread enormously thanks to the mass transport development and insecticide resistance. However, this little annoying fellow possesses a very interesting cytogenetic features assembly. Chromosomes are holokinetic, that means with no centromere, sex chromosomes divide in different way from autosomes and the most amazing is the intraspecial chromosome number variation.
The additional sex X chromosomes in *C. lectularius* karyotype were described already in the first half of the 20. century and the hypothesis of their origin through sex X chromosome fragmentation has been posted. The basic karyotype consist of 2n = 26+XXY and known variation ranges up to 2n = 26+20XY.
In the current study, we want to clearly verify origin of the additional chromosomes using the flow cytometry. The karyotypes in 213 *C. lectularius* specimens from 51 localities were analyzed, subsequently the nuclear DNA volume was estimated with *Solanum pseudocapsicum* plant as an inner standard through FCM method.
The results show that karyotypes with more chromosomes have much broader range of nuclear DNA volume than the specimens with basic karyotype. Among specimens with additional chromosomes, we found individuals with 28% higher (2C = 2.72 pg) nuclear DNA volume and also specimens with 12% lower (2C = 1.87 pg) nuclear DNA volume than is average (2C = 2.12 pg) among specimens with basic karyotype. That suggests occurrence of more chromosomal rearrangements like duplication and deletion beside simple fragmentation during development of karyotypes with high chromosome number.
This project was supported by the grants no. 277815/2015 of the Grant Agency of Charles University and no. SVV 260434/2017 of Ministry of Education, Youth and Sports of the Czech Republic.

pPC-6

POLYPLOIDIZATION OF ANEMONE SYLVESTRIS L.

Zahumenická P.[1], Fernández E.[1], Šedivá J.[2]

[1]*Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Department of Phytoenergy and Biodiversity, The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech republic*

The objective of this research was to induce mitotic polyploidization in *Anemone sylvestris* L. The mitosis inhibitor (oryzalin) was directly added to the induction medium at 1, 2, 5, 10 and 15 µM for 8, 10 or 12 weeks of cultivation. Three tetraploid plants (2n = 4x = 32), 0,8 % (polyploidization efficiency), were obtained from diploid plants (2n = 2x = 16) in three treatments (1 µM for 10 weeks, 5 µM for 8 weeks and 8 µM for 10 weeks). Ploidy level was confirmed by flow cytometry. Morphological characteristic (e.g. flower diameter, total

plant height, leaf area) were observed.
New genotype with different morphological characteristics was obtained through somatic polyploidization. The tetraploid plants were stronger, more vigorous and had an early flowering, which is essential for its use as an ornamental plant.

REPRODUCTION IMMUNOLOGY

pRI-1

LYMPHOCYTES IN PREGNANT WOMEN WITH GESTATIONAL DIABETES MELLITUS: DIFFERENCE BETWEEN SUBCUTANEOUS AND VISCERAL ADIPOSE TISSUE

Cinkajzlová A.[1,2], Anderlová K.[3,4], Šimják P.[4], Lacinová Z.[1,2], Kloučková J.[1,2], Kratochvílová H.[1,2], Krejčí H.[3,4], Mráz M.[1,5], Haluzík M.[1,2,5,6], Kršek M.[3,7]

[1]Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czech Republic
[2]Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[3] 3rd Department *of Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czech Republic*
[4]Gynaecology and Obstetrics Department, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czech Republic
[5]Department of Diabetes, Diabetes Centre, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[6]Department of Obesitology, Institute of Endocrinology, Prague, Czech Republic
[7]2nd Department of Internal Medicine, 3rd Faculty of Medicine, Charles University and University Hospital Královské Vinohrady, Prague, Czech Republic

Gestational diabetes mellitus (GDM) is accompanied by a changed lymphocyte profile in peripheral blood; however, little is known about lymphocyte content in adipose tissue. The aim of this study was to analyse lymphocyte populations in subcutaneous (SAT) and visceral adipose tissue (VAT) in patients with GDM. Furthermore, relative gene expression of selected inflammatory factors was explored between SAT and VAT.
Anthropometric and biochemical analyses were performed in 21 pregnant GDM women in 26th-30th and 38th-39th gestational week. GDM was diagnosed according to IADPSG criteria. Lymphocytes were assessed as % of CD45+ cells measured by flow cytometry in SAT and VAT samples taken during delivery.
In GDM subjects CD45+ cells were higher in VAT compared to SAT (19.19 ± 2.23 vs. 6.54 ± 0.67 %, p<0.001). The ratio of CD3- and CD3+ cells did not differ between both compartments. T helper (CD4+) cells were higher in SAT compared to VAT (37.61 ± 2.36 vs. 28.63 ± 2.57 %, p=0.019), while no difference was seen in T cytotoxic (CD8+) cells resulting in a higher ratio of CD4+/CD8+ cells in SAT. Similarly, the amount of B (CD19+) and NKT (CD16/56+CD3+) cells was higher in SAT relative to VAT (1.32 ± 0.25 vs. 0.58 ± 0.14

%, p=0.010 for B cells; 9.67 ± 1.07 vs. 5.45 ± 0.92 %, p<0.001 for NKT cells). In contrast, NK (CD15/56+CD3-) cells were higher in VAT compared with SAT (17.92 ± 3.07 vs. 9.14 ± 1.30 %, p=0.015). Of all measured cytokines only the expression of IL-10 was increased in VAT relative to SAT (1.063 ± 0.084 vs. 0.750 ± 0.106, p=0.026), while other selected inflammatory factors did not differ.
Compared with SAT the visceral compartment of GDM subjects showed an almost 3-fold increase in lymphocyte content suggesting a more proinflammatory milieu despite the absence of difference in mRNA expression of the main inflammatory cytokines. These findings were further stressed by the elevated ratio of NK cells in VAT.
Acknowledgements: Supported by AZV 15-27630A.

pRI-2
REGULATORY T CELLS AND THEIR ROLE IN REPRODUCTIVE PROCESSES

Onderová B.

MEDIREX,a.s., Bratislava, SK

Regulatory T cells (Tregs) are a distinct population of T cells, which play a role in immune tolerance. Disturbance of their population has been linked to multiple immunopathologies, including allergies, autoimmunity diseases and cancer. Human Tregs have also been identified in the process of maintaining the pregnancy. Successful pregnancy is a challenge for the immune system of a mother, as tolerance mechanisms have to protect the semiallogeneic fetus against immune attacks from the mother. Among these factors providing tolerance during pregnancy, Tregs may play an important role. Several functional studies have shown that unexplained infertility, miscarriage and preeclampsia are often related to deficit in Treg cell number and function. However, successful pregnancy in humans is associated with increased numbers of Treg cells, at least locally in decidua, whereas Treg cells changes in blood have recently been questioned. Many studies suggest their importance in peripheral blood and therefore also in fetal tolerance. Our aim was to measure Treg cells in peripheral blood throughout the menstrual cycle in order to determine their importance in reproductive health.

pRI-3
UBIQUITIN RESPONSE TO EPIGENETIC CHANGES IN SPERM

Štiavnická M.[1,2], García-Álvarez O.[1,2], Abril-Parreno L.[1,2,3], Nevoral J.[1,2], Dolejšová M.[2], Hošek P.[1,2], Lošan P.[4,5], Ulčová-Gallová Z.[4,5], Králičková M.[1,2]

[1]Charles University, Faculty of Medicine in Pilsen and Department of Histology and Embryology, Pilsen, Czech Republic
[2]Biomedical centre in Pilsen, Czech Republic
[3]Department of Biological Sciences, Faculty of Science and Engineering. University of

Limerick, Ireland
[4]Genetika Plzeň, s.r.o. (Ltd.), Pilsen – Černice, Czech Republic
[5] Department of Gynecology and Obstetrics, Charles University, Pilsen, Czech Republic

Sperm extracellular ubiquitination is well known phenomenon and its role is indispensable during sperm maturation in epididymis. Assumably, ubiquitin as sperm quality marker is able to reflect inner sperm condition, including epigenetic code of residual histones, and negative correlation with fertilization success was proven. Based on our knowledge, we expect correlation of extracellular ubiquitin with DNA status and selected histone code markers.

In accordance with the Ethical Committee of Faculty of Medicine in Pilsen, 26 semen samples from males with different sperm quality were collected in cooperation with laboratory Genetika Pilsen Ltd. and processed routinely according to World Health Organisation criteria. Subsequently, there was made analysis called Sperm Chromatin Structure Assay (SCSA®), providing us DNA fragmentation index (%DFI) and high DNA stainability index (%HDS) by means of flow cytometry (FACSVerse BD). In addition, the phosphorylated histone H2A (ϒH2AX), histone H3 dimethylation on lysine 4(H3K4me2) and extracellular ubiquitination level was measured by flow cytometry.

Acquired results point out strong correlation between the level of extracellular ubiquitin and ϒH2AX (R=0.42). This observation corresponds to ubiquitin ability to reflect on DNA damage. However, our experiments did not prove a relationship of ϒH2AX and DFI. Furthermore, no association of extracellular ubiquitin and H3K4me2 in sperm head has been observed. Nevertheless no relation of H3K4me2 with extracellular ubiquitination has been examined, positive correlation of H3K4me2 and HDS (R=0.49) was detected and this histone modification seems to be usable marker of immature sperm.

Achieved results support our idea that histone code represents unique tool for advanced sperm analysis and further selection. Moreover, we found out connection of ubiquitin and histone code through ϒH2AX, and based on this we can expect that ubiquitin is able to response not only to physiological changes but also to epigenetic variation.

This work was supported by Charles University (PROGRES Q-39) and the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education, Youth and Sports of the Czech Republic.