

# XII. 2016

Diagnostic  
Predictive and  
Experimental  
Oncology  
Days



## ABSTRACT BOOK

November 30 - December 1, 2016

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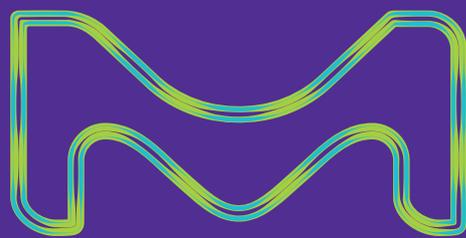
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## XII. DNY DIAGNOSTICKÉ, PREDIKTIVNÍ A EXPERIMENTÁLNÍ ONKOLOGIE / XII. DIAGNOSTIC, PREDICTIVE AND EXPERIMENTAL ONCOLOGY DAYS

STŘEDA / WEDNESDAY - 30. listopadu 2016 / November 30<sup>th</sup>, 2016

9:00-9:15 WELCOME AND INTRODUCTION

### **Mastering DNA repair.**

Chairs: Marian Hajduch, Jiri Bartek



09:15-10:00

Replication stress and DNA damage as hallmarks of cancer: From basic mechanisms to emerging clinical trials.

**Jiri Bartek**

10:00-10:45

7-(2-Thienyl)-7-Deazaadenosine (AB61), a New Potent Nucleoside Cytostatic with a Complex Mode of Action.

**Marian Hajduch**

10:45-11:00 COFFEE BREAK

### **Cellular stress and genotoxicity.**

Chairs: Martin Mistrik, Zdenek Hodny

11:00-11:20

Replication obstacles formed within common fragile sites under replication stress are targeted by the global genomic nucleotide excision repair pathway.

**Martin Mistrik**

11:20-11:40

Tumors overexpressing RNF168 show altered DNA repair and responses to genotoxic treatments, genomic instability and resistance to proteotoxic stress.

**Katarina Chroma**

11:40-12:00

Senescent tumor cells accelerate tumor growth but induce bystander senescence in vitro in a murine model.

**Milan Reinis**

12:00-13:00

**LUNCH AND POSTER PRESENTATIONS** (Note: the authors are kindly requested to present posters in between 12:45-13:00)

### **Emerging technologies in cancer research.**

Chairs: Tomas Eckschlager, Jiri Drabek

13:00-13:30

Analytical qualification of peptide arrays for tumor autoantibody profiling and resolution of batch effects in a colorectal carcinoma study.

**Peter Hettegger**

13:30-13:45

Three-dimensional cell cultures and lightsheet fluorescence microscopy in drug discovery and development.

**Viswanath Das**

13:45-14:00

Miniaturization of cytotoxic assay in HTS.

**Sona Gurska**

14:00-14:15

Decryptor: system for reliable identification of alterations by mass spectrometry of proteome.

**Miroslav Hruska**

14:15-14:30

Comparison of three genotyping methods for detection of BRAF p.Val600Glu mutation in FFPE samples.

**Jana Duchoslavova**

14:30-14:45

Specific CNV analysis from single-cell.

**Anastasiya Zidkova**

14:45-15:00

COFFEE BREAK



## **Molecular targets and cancer biomarkers I.**

*Chairs: Ondrej Slaby, Lubos Holubec*

- 15:00-15:30 Translational potential of microRNAs in oncology.  
**Ondrej Slaby**
- 15:30-15:45 Prevalence of HPV infection in oocyte donors and women treated for infertility: a prospective study.  
**Hana Jaworek**
- 15:45-16:00 Circulating glycoprotein biomarkers of gastrointestinal cancers.  
**Lakshman Varanasi**
- 16:00-16:15 Deregulation of miR-21, miR-9, miR-143 and miR-145 in sinonasal carcinoma and their value as prognostic biomarkers.  
**Helena Kovarikova**
- 16:15-16:30 Analysis of copy number variations in meningioma samples using microarrays revealed 22q deletions more frequent in higher grade tumors.  
**Josef Srovnal**
- 16:30-16:45 The mechanisms of anticancer tumor response targeted to the EGFR in colorectal carcinoma.  
**Lubos Holubec**
- 16:45-17:00 Comparison of newly prepared cisplatin-resistant and parental TGCTs cell lines.  
**Silvia Schmidtova**
- 17:00-17:15 Invasive capacity of cancer cells is enhanced by expression and catalytic performance of the pH regulating enzyme CA IX.  
**Michaela Debreova**
- 19:00 CONFERENCE DINNER** (NH hotel restaurant, for dinner-registered participants only)

**ČTVRTEK / TUESDAY - 1. prosince 2016 / December 1<sup>st</sup>, 2016**

## **Novel anticancer drugs and therapies I.**

*Chairs: Lucia Kucerova, Marian Hajduch*

- 9:00-9:45 Drug discovery for triple negative breast cancers.  
**Susan L. Mooberry**
- 9:45-10:00 Chemoresistance of cancer cells - experimental models.  
**Tomas Eckschlager**
- 10:00-10:15 Role of high-fat diet on the effect of metformin and melatonin in chemically-induced rat breast cancer models.  
**Bianka Bojkova**
- 10:15-10:30 The effect of piperlongumine on pheochromocytoma cells in vitro and in vivo.  
**Petra Bullova**
- 10:30-10:45 COFFEE BREAK**

## **Novel anticancer drugs and therapies II.**

*Chairs: Petr Dzubak, Viswanath Das*

- 10:45-11:00 The prostate cancer-targeted and nontargeted apoferritin loaded by doxorubicin and its effect on cancer and healthy cell lines.  
**Tereza Cerna**
- 11:00-11:15 Cytotoxicity and release of Paclitaxel from a PEG/PLA carrier.  
**Johana Plich**



- 11:15-11:30 Multifaceted properties of betulinic acid derivatives.  
**Jiri Rehulka**
- 11:30-11:45 Analyses of distinctive effects of ginger phenylpropanoids and quercetin on Nrf2-ARE pathway in human BJ fibroblast and HaCaT keratinocytes.  
**Ermin Schadich**
- 11:45-12:00 Novel carborane as carbonic anhydrase IX inhibitors.  
**Jana Dvoranova Stepankova**
- 12:00-13:00 LUNCH AND POSTER PRESENTATIONS**  
(Note: the authors are kindly requested to present posters in between 12:45-13:00)

## **Molecular imaging in cancer research.**

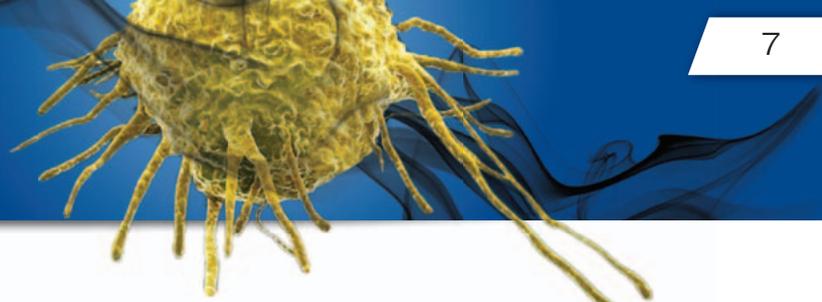
Chairs: Clemens Decristoforo, Milos Petrik

- 13:00-13:45 Translation of radiopharmaceuticals from bench to bedside - prospects and challenges in Europe.  
**Clemens Decristoforo**
- 13:45-14:00 Fusarinine C - a backbone for targeted multimodality imaging?  
**Dominik Summer**
- 14:00-14:15 <sup>68</sup>Ga and <sup>89</sup>Zr labelled RGD multimers for integrin  $\alpha_v\beta_3$  targeting based on a siderophore scaffold.  
**Piriya Kaeopookum**
- 14:15-14:30 New cholin analogs as potential diagnostics/therapeutics for prostate cancer.  
**Zbynek Novy**
- 14:30-15:00 High resolution PET insert for high field preclinical MRI: evaluation of single ring system using 7T field strength.  
**Willy Gsell**
- 15:00-15:15 COFFEE BREAK**

## **Molecular targets and cancer biomarkers II.**

Chairs: Jana Nekvindova, Josef Srovnal

- 15:15-15:45 Mesenchymal stromal cells and drug responses in breast cancer cells.  
**Lucia Kucerova**
- 15:45-16:00 Prognostic and predictive factors in primary glioblastoma multiforme WHO grade IV patients with resection: a single-institution study.  
**Ondrej Kalita**
- 16:00-16:15 Urinary cell-free microRNA panel in detection of bladder carcinoma.  
**Jaroslav Juracek**
- 16:15-16:30 Cannabinoid receptors expression affects NSCLC patients' survival.  
**Monika Vahalikova**
- 16:30-16:45 Expression of biotransformation enzymes in hepatocellular carcinoma.  
**Jana Nekvindova**
- 16:45-17:00 Identification of circulating microRNAs with diagnostic potential in colorectal cancer using next generation sequencing.  
**Petra Vychytilova-Faltejskova**
- 17:00-17:15 What is the role of peripheral double positive (CD4+CD8+) T-cells in anti-tumor immunity?  
**Jiri Sinkora**
- 17:15 CLOSING CEREMONY**



## Postery / Posters

- 1 *Deletion of 18q in Patients with Colorectal Cancer - Preliminary Data*  
**Radek Trojanec**
- 2 *DNA affinity chromatography combined with quantitative proteomics revealed role of XPC protein in replication stress and common fragile site stability*  
**Lucie Beresova**
- 3 *Application of DARTS (Drug Affinity Responsive Target Stability) for identification of the ligand-binding part of the protein.*  
**Jana Vaclavkova**
- 4 *Nuclear shuttling of microRNA hsa-miR-29b enhances etoposide toxicity in HeLa cells*  
**Zdenek Dostal**
- 5 *Surface Antibodies-Modification of Apoferritin with Encapsulated Doxorubicin Favorably Influences the Formation of Protein Corona*  
**Simona Dostalova**
- 6 *Size-Related Toxicological Aspects of PVP-Capped Platinum Nanoparticles*  
**Hana Buchtelova**
- 7 *Cross-Interactions between Trks Receptors and Neurotrophins: Insights from Molecular Docking and Molecular Dynamics*  
**Yazan Haddad**
- 8 *Sarcosine Up-Regulates Expression of Genes Involved in Cell Cycle Progression of Metastatic Models of Prostate Cancer*  
**Vladislav Strmiska**
- 9 *A role of V-ATPase in cancer cells chemoresistance*  
**Marie Belhajova**
- 10 *Effect of mutations in the post-translation modifications sites of carbonic anhydrase IX and study of their functional properties*  
**Lenka Jelenská**
- 11 *Detection of ionizing radiation biomarkers in mouse hair follicles*  
**Hanuš Slavík**
- 12 *DNA methylation changes in tumour invasivity associated genes in breast cancer*  
**Lenka Kalinkova**
- 13 *X-irradiation-induced senescence model: detection of senolytic drugs in high-throughput system*  
**Natálie Táborská**
- 14 *Metformin and melatonin administration in a rat model of breast cancer improves liver antioxidant status.*  
**Bianka Bojková**
- 15 *Importance of promoter methylation of GATA5 and THSB1 genes in malignant tumors of the sinonasal area.*  
**Marcela Chmelarova**
- 16 *The prognostic aspect of the perioperative circulating tumor cells detection in non-small cell lung cancer patients*  
**Alona Rehulkova**
- 17 *NGS technology as a way for monitoring of IgVH clones in ALL patients - pilot study*  
**Katerina Hrochová**
- 18 *Oncogenic action of S100P in cancer includes sequestration of p53 and stimulation of therapy-induced senescence*  
**Adriana Gibadulinova**
- 19 *The effect of metallothionein - doxorubicin combination approach in the targeted anticancer therapy*  
**Michaela Docekalova**
- 20 *A new type of carbon nanotransporter for doxorubicin targeted delivery in breast cancer: the effect of metallothionein*  
**Michaela Docekalova**
- 21 *Bid-dependent activation of mitochondrial pathway is essential for cisplatin- or LA-12-mediated enhancement of TRAIL-induced apoptosis in human prostate cancer cells*  
**Olga Vondalova Blanarova**
- 22 *Regulation of NEU4 sialidase expression in tumor hypoxia*  
**Mária Bartošová**
- 23 *The role of the soluble form of tumor-associated CA IX protein in vivo.*  
**Ivana Vidlickova**
- 24 *Plasma-based biomarkers of gastrointestinal cancers*  
**Martina Jakoubkova**
- 25 *Detection of HPV in lung cancer patients*  
**Jana Potockova**
- 26 *Molecular diagnostics in glioma*  
**Magdalena Houdova Megova**



# Rakovina věc veřejná

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Rakovina věc veřejná - Cancer Research Foundation was founded in 1997 to promote science and research in the field of oncological diseases. During its existence the foundation has become an initiator of many educational and prevention programs, as well as social events which followed its main mission and goals. The foundation has successfully realized the project Tell your story for cancer patients as well as the cervical cancer prevention project. Dedicated Fairy Tales are the Foundation's latest project whereby the Foundation wants to draw attention to the lack of funds in cancer research.

[www.vecverejna.cz](http://www.vecverejna.cz)

## The Book "Dedicated Fairy Tales".

Dedicated Fairy Tales are a collection of stories from the pen of personalities more or less known. Amusing and cute fairy tales are a mixture of diverse situations and characters, and their common objective is to entertain, and even teach a little. See for yourself... The Dedicated Fairy Tales project was created for the purpose of obtaining financial support for cancer research. We would like to draw your attention to the importance of public support for science and research in this field as a wise investment in the future of all of us.

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PURCHASES WILL SUPPORT SCIENCE  
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## Forthcoming fundraising event of the year 2017

Another planned project in the next year is the beneficial concert. It will take place on February 17, 2017, in the hall of the Moravian Philharmonic Orchestra and with the support of the City of Olomouc. The concert will be an attractive combination of singer and guitarist Lenka Filipova and Strings Brno. During the evening, you will not only enjoy songs of world authors, but also famous songs of Lenka Filipova NEBO and also famous songs written by Lenka Filipová.





## Mastering DNA repair.

Chairs: Marco Foiani, Jiří Bártek

středa / 30. listopadu 2016 / Wednesday / November 30, 2016 / 9:15 - 10:45

### An integrated ATR, ATM and mTOR-mechanical network controlling nuclear plasticity.

Giulia Bastianello<sup>1</sup>, Gururaj Kidiyoor<sup>1</sup>, Qinseng Li<sup>1</sup>, Martin Kosar<sup>1</sup>, Amit Kumar<sup>2, 3</sup>, Galina V. Beznoussenko<sup>1</sup>, Alexandre A. Mironov<sup>1</sup>, Dario Parazzoli<sup>1</sup>, G.V. Shivashankar<sup>4</sup>, Jiri Bartek<sup>5</sup>, Michele Mazzanti<sup>6</sup>, Giorgio Scita<sup>1,6</sup> and Marco Foiani<sup>1,6\*</sup>.

<sup>1</sup> IFOM (Fondazione Istituto FIRC di Oncologia Molecolare) Via Adamello 16, 20139 Milan, Italy.

<sup>2</sup> CSIR-Indian Institute of Toxicology Research, MG Marg 80, 226001, Lucknow, India.

<sup>3</sup> Academy of Scientific and Innovative Research (AcSIR), India

<sup>4</sup> Mechanobiology Institute and Department of Biological Sciences, NUS, Singapore.

<sup>5</sup> Danish Cancer Society Research Center, Copenhagen, Denmark.

<sup>6</sup> Università degli Studi di Milano, Italy.

\* e-mail: marco.foiani@ifom.eu

ATR and ATM control chromosome integrity, chromatin dynamics and cell cycle events. mTOR exhibits similarities to ATR and ATM and coordinates nutrient sensing pathways and cytoskeleton dynamics.

We recently found (A.Kumar et al. Cell, 2014) that ATR, ATRIP and Chk1 associate to the nuclear envelope during S phase and prophase, and in response to mechanical stimulation of the plasma membrane. The ATR-mediated mechanical response occurs within the range of physiological forces, recovers rapidly, and is not influenced by RPA or DNA damage. ATR defective cells exhibit aberrant chromatin condensation and nuclear envelope breakdown.

We found that this pathway is influenced by mTOR, actin dynamics and calcium levels. We used electron microscopy to visualize the nucleus morphology of the nucleus in ATR and Chk1-defective cells and found aberrant condensation events and nuclear envelope anomalies that may contribute to micronuclei formation and chromosome fragmentation. Using mechanobiology approaches we measured the stiffness of wild type, ATR, ATM, Chk1 and mTOR defective cells and found significant differences that influence cell plasticity and interstitial migration. These and other observations implicate ATR, ATM and mTOR in the control of genome integrity, nuclear dynamics and cell plasticity and suggest the existence of an integrated mechanical network involving different PI3-kinases.

### Replication stress and DNA damage as hallmarks of cancer: From basic mechanisms to emerging clinical trials.

Jiri Bartek, Jirina Bartkova.

Danish Cancer Society, Copenhagen, Denmark & Karolinska Institute, Stockholm, Sweden

Replication stress (RS) induced by activated oncogenes and loss of some tumor suppressors is emerging as one of the hallmarks of cancer. The RS-induced DNA damage and the ensuing activation of cell cycle checkpoints commonly induce cellular senescence or cell death of the nascent tumor cells, providing an inducible intrinsic barrier to cancer progression. On the other hand, this scenario creates an environment that favors outgrowth of tumor cell clones featuring p53 mutations and other checkpoint defects such as those in ATM or Chk2 kinases, events that

allow tumor growth at the expense of enhanced genomic instability. The ongoing enhanced RS, on the other hand, unmasks higher dependence of tumor cells on RS-support pathways such as those provided by the ATR-Chk1 axis and replication fork protective mechanisms, a vulnerability that can be targeted by inhibitors of ATR, Chk1, MK2 and Wee1 kinases, for example.

The lecture will briefly outline this concept, provide background information and then focus on our new data relevant for three of the open questions in this field:

I) How are the molecular obstacles such as RNA-DNA hybrids and aberrant intermediates resulting from RS-causing collisions between replication and transcription resolved in cells?;

II) What is the impact of RS and the ensuing ATR signaling on the mutation spectra (mutation signatures) in major types of human solid tumors such as breast carcinomas?; and

III) How do human aggressive cancers such as glioblastomas cope with the excessive endogenous RS, how do such mechanisms promote tumor cell survival and how could this knowledge help in designing innovative treatment strategies.

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## Cellular stress and genotoxicity.

Chairs: Martin Mistrik, Zdenek Hodny

středa / 30. listopadu 2016 / Wednesday / November 30, 2016 / 11:00 - 11:45

### Replication obstacles formed within common fragile sites under replication stress are targeted by the global genomic nucleotide excision repair pathway.

Martin Mistrik<sup>1</sup>, Lucie Beresova<sup>1,2</sup>, Eva Vesela<sup>1</sup>, Rene Lenobel<sup>2</sup>, Ivo Chamrad<sup>2</sup>, Jiri Voller<sup>1</sup>, Masayuk Yamada<sup>1</sup>, Tomas Furst<sup>1</sup>, Katarina Chroma<sup>1</sup>, Jan Gursky<sup>1</sup>, Jiri Bartek<sup>1,3</sup>

<sup>1</sup> Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomuc, Czech Republic,

<sup>2</sup> Department of Protein Biochemistry and Proteomics, Centre of the Region Hana for Biotechnological and Agricultural Research, Faculty of Science, Palacky University, Olomouc, Czech Republic,

<sup>3</sup> Danish Cancer Society Research Center, Copenhagen, Denmark,

<sup>4</sup> Science for Life Laboratory, Division of Translational Medicine and Chemical Biology, Department of Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

#### Introduction

A brief Introduction into the biology of common fragile sites (CFSs) will be followed by our recent discoveries related to this topic. CFS's are genomic loci present in higher vertebrates that are particularly sensitive to various forms of replication stress and suffer from increased breakage and rearrangements in tumors. They have rather enigmatic evolutionary role and employ various DNA repair and checkpoint mechanisms promoting their stability. We used an original approach for identification

of CFS's associated factors based on DNA probe designed to match the high flexibility island sequences typically present in some of the highly expressed CFS's. This probe was used as affinity bait for fishing specifically enriched proteins which were further identified using SILAC and quantitative mass spectrometry. Among already known CFS's stabilizers we identified also hits so far not implicated in CFS's maintenance. Interestingly, most of these novel hits are components of the global genomic nucleotide excision repair pathway (GG-NER). Knock down-based functional experiments revealed that GG-NER works most likely as an important trigger turning the CFS's-associated replication obstacles into DNA lesion further recognized by the ATR-promoted cellular checkpoint which function is to block an escape of DNA replication intermediates into mitosis and the next cell generation.

#### Materials/methods

DNA Affinity Chromatography, Mass Spectrometry, Fluorescence Microscopy, Immunochemistry, Gene silencing

#### Results and conclusions

In the presented study we performed the first unbiased proteome-wide screen to identify new putative proteins responsible for maintenance of CFS stability. Besides previously characterized WRN and MSH2 proteins, we also identified several additional candidates whose role in CFS maintenance warrants deeper characterization. Because of the fact that almost half of the identified proteins are implicated in NER, the XPC protein as the main initiator of the NER pathway was chosen for a follow-up functional study. On the basis of our results, we propose a hypothesis for the role of XPC

in preventing CFS expression by promoting checkpoint signaling under replication stress.

### Tumors overexpressing RNF168 show altered DNA repair and responses to genotoxic treatments, genomic instability and resistance to proteotoxic stress.

Katarina Chroma<sup>1</sup>, Martin Mistrik<sup>1</sup>, Pavel Moudry<sup>1,2</sup>, Jan Gursky<sup>1</sup>, Martin Liptay<sup>1</sup>, Robert Strauss<sup>2</sup>, Zdenek Skrott<sup>1</sup>, Radek Vrte<sup>3</sup>, Jirina Bartkova<sup>2</sup>, Juraj Kramara<sup>1</sup>, Jiri Bartek<sup>1,2</sup>

<sup>1</sup> Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomuc, Czech Republic,

<sup>2</sup> Danish Cancer Society Research Center, Copenhagen, Denmark,

<sup>3</sup> Department of Clinical Genetics, Olomouc, Czech Republic

#### Introduction

DNA double-strand break (DSB) signaling and repair is crucial to preserve genomic integrity and maintain cellular homeostasis. During the DNA damage response (DDR), histone ubiquitination by the apical RNF168 ubiquitin ligase is a critical event, which orchestrates the recruitment of downstream effectors, e.g. BRCA1 and 53BP1. Under conditions of ubiquitin starvation, mostly resulting from proteotoxic stress, the ubiquitin dependent accrual of DDR proteins at the sites of damage is impaired and the ubiquitin mediated DDR is attenuated or lost. A common manifestation of the attenuation is disappearance of the 53BP1 and BRCA1 proteins from the irradiation induced foci (IRIF). However, we have identified diverse



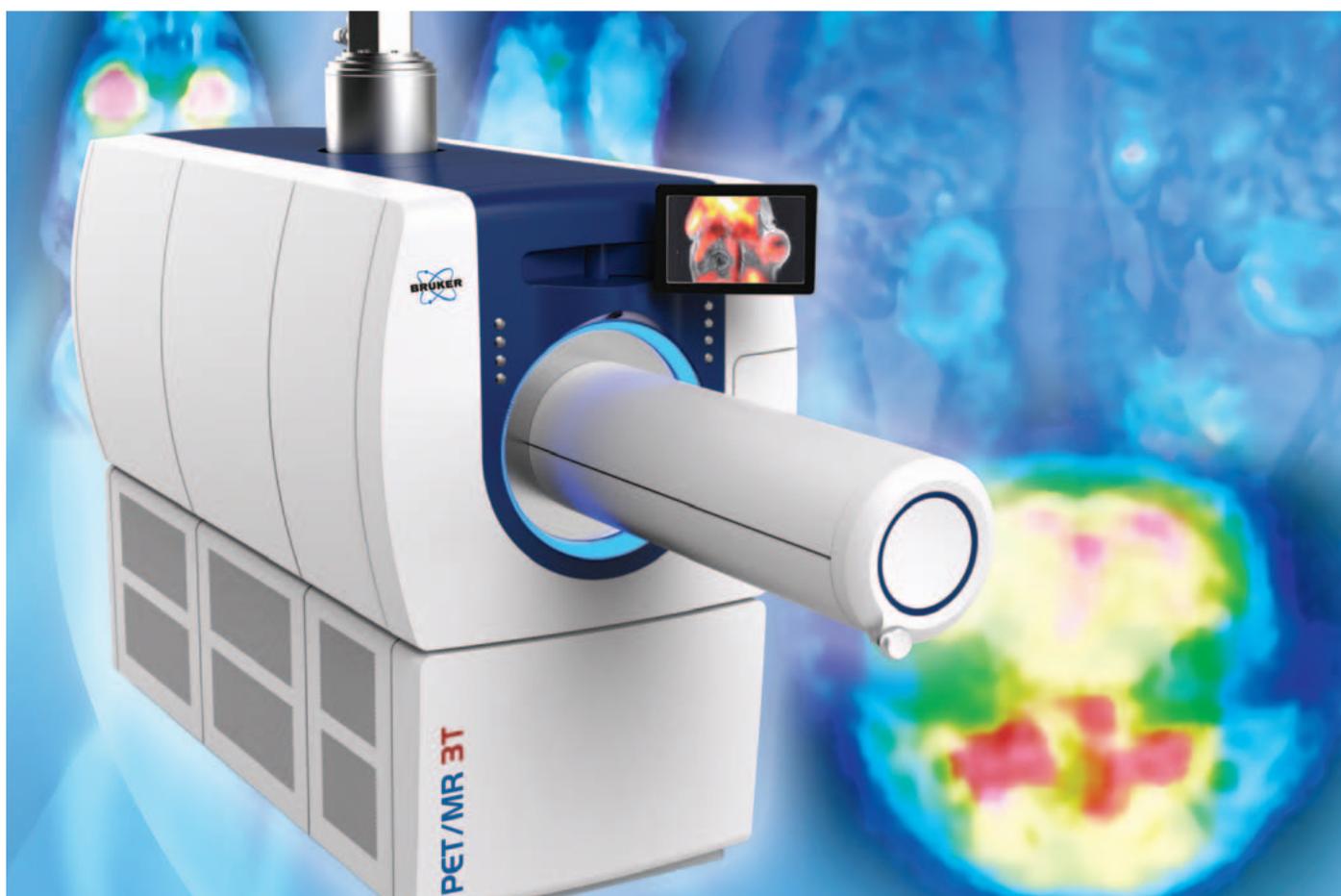
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human cancer cell lines that display 53BP1 recruitment to IRIFs even under the conditions of proteasome-inhibitor induced proteotoxic stress *that is* under substantial depletion of nuclear free ubiquitin levels.

We set out to investigate mechanism and the potential relevance of the phenotype in cancer biology.

#### Materials/methods

The number and intensity of 53BP1 IRIF after 5 $\mu$ M MG132 treatment followed by X ray was quantified in the primary BJ cell line and several cancer cell lines (MDA-MB-231, MDA-MB-436, MCF7, Hela U2OS, U2OS RNF168-GFP, AMO1, MM.1S) using the ScanR imaging workstation. Laser micro-irradiation performed on a Zeiss Axioimager Z.1 instrument was used for subsequent immunofluorescent detection of recruited proteins to localized DNA damage sites. Cellular level of selected ubiquitin proteasome and DDR pathways related proteins (53BP1, RNF168, RNF8, UBC13) was detected by standard Western blot using ImageJ for band quantification. The abundance of RNF168 was verified by quantitative PCR using a Nano Light Cycler instrument. For probing of the DNA repair pathway choice the Traffic light system with subsequent flow cytometry analysis was employed

#### Results and conclusions

We show that central to this phenotype is an elevated level of the RNF168 ligase that enables more efficient exploitation of the residual free ubiquitin. Elevated RNF168 levels harboring cells are more resistant to combined treatment by gamma irradiation and proteasome inhibitor which implies that the RNF168 upregulation may have arisen as an adaptation to constant proteotoxic stress experienced by tumor cells. Moreover, the overabundance of RNF168 E3 ligase causes a boost in 53BP1 recruitment thus shifting the repair pathway balance towards the non-homologous end-joining (NHEJ), a scenario accompanied by enhanced chromosomal instability/micronuclei formation

and sensitivity under replication stress-inducing treatments with camptothecin or poly(ADP-ribose) polymerase (PARP) inhibitor. As tumors often display heterogeneity in RNF168 expression, upregulation of the RNF168/53BP1 pathway could provide a useful biomarker for assessment of tumor sensitivity to PARP1 and topoisomerase inhibitors.

#### **Senescent tumour cells accelerate tumour growth but induce bystander senescence in vitro in a murine model.**

*Milan Reiniš, Jana Šimová, Olena Sapega, Romana Mikyšková, Terezie Imrichová, Ivan Štěpánek, Lenka Kyjacová, Marie Indrová, Jana Bieblová, Jiří Bártek, Zdeněk Hodný*

*Institute of Molecular Genetics AS CR, v.v.i., Prague, Czech Republic*

#### Introduction

Genotoxic therapy, as well as certain cytokines, can induce cell senescence in tumour cells. Senescence is considered to be a principal barrier against tumorigenesis. However, senescent cells can influence their environment through specific secretory phenotype. Paradoxically, induction of bystander senescence in adjacent cells, as well as a promotion of tumour development can be observed.

#### Materials/methods

In our study we have shown that tumour growth of proliferating mouse TC-1 HPV-16-associated cancer cells in syngeneic mice became accelerated by co-administration of TC-1 or TRAMP-C2 prostate cancer cells that were made senescent by pre-treatment with docetaxel. In a therapeutic experiment, accelerated tumour growth was effectively inhibited by cell immunotherapy using irradiated TC-1 cells producing interleukin-12. Further, conditioned medium obtained from the docetaxel-treated cells induced bystander senescence when applied on cultured proliferating tumour cells. In

a control experiment, medium from proliferating TC-1 tumour cell culture did not induce proliferation arrest and senescence induction. Further, we compared the phenotype and the biological effects of senescent tumour cells in which senescence was induced either by docetaxel or by a combination of IFN $\gamma$  and TNF $\alpha$ .

#### Results and conclusions

Collectively, we have documented that senescent tumour cells produce senescence-associated secretory phenotype (SASP) capable to mediate bystander senescence. On the other hand, the same senescent cells accelerate the tumour growth when co-injected with proliferating cells. So we can speculate that tumour infiltrating cells, such as leukocytes, or stromal cells are necessary to mediate the tumour growth-accelerating effects. Our results also suggest the although both docetaxel or a combination of IFN $\gamma$  and TNF $\alpha$  induced senescence in B16 cells, remarkable differences were observed in their capacity to induce bystander senescence, as well as to accelerate tumour growth.



## Emerging technologies in cancer research.

Chairs: Tomas Eckschlager, Jiri Drabek

středa / 30. listopadu 2016 / Wednesday / November 30, 2016 / 13:00 - 14:45

### Analytical qualification of peptide arrays for tumor autoantibody profiling and resolution of batch effects in a Colorectal Carcinoma study.

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#### Introduction

A common challenge in biomarker research is the influence of adverse covariates on high-dimensional data which substantially reduce data quality, impair reproducibility or even make data uninterpretable. Adverse covariates arise e.g. from collecting samples in different regional collection centers or from processing samples in batches. We propose a resampling based global significance test for testing such covariates in high dimensional data and performing model selection for subsequent analyses.

#### Materials/methods

Antibody-profiling platforms (1.400 peptide microarray and 140plex Luminex bead peptide array) were analytically qualified using mixtures of IgG from two plasma samples for assessment of linearity and inter-/intra- platform reproducibility. Then IgG of 172 clinical plasma samples (Colorectal Carcinoma, Polyps, Healthy Controls) from three different collection centers were processed on planar peptide microarrays.

#### Results and conclusions

Assessment of analytical performance on both peptide-based immuno-profiling platforms shows high linearity (50% of significant features have correlation coefficient  $\rho > 0.77$ ) and

high inter-platform reproducibility ( $\rho = 0.92 \pm 0.02$  for replicates vs.  $\rho = 0.7 \pm 0.05$  between samples) and good cross- platform reproducibility ( $\rho = 0.75$  for significant features vs.  $\rho = 0.5$  for all features). Knowing the good analytical performance of our assays we could show a significant reversal of phenotype effects ( $p \leq 0.01$  of interaction terms) in one of three collection centers which eventually led to exclusion of these samples ( $n = 20$ ) from the study. Phenotype effects and center specific effects of remaining samples were highly significant ( $p < 0.005$ ). Null distribution of the test statistic is calculated from the data by resampling (200 iterations) and thus any dependence structure in the data or unequal sample sizes are inherently considered. We propose a significance test for testing association of high-dimensional data with known continuous or discrete covariates and demonstrate how this aids to avoid relevant adverse covariates in biomarker-studies.

### Three-dimensional cell cultures and lightsheet fluorescence microscopy in drug discovery and development.

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#### Introduction

In recent years, methodological approaches to study cancer have been deeply influenced by two major emerging concepts: a) cancer is not a single disease but rather a heterogeneous composition of multiple disorders; b) the growing

importance of the cancer stem cell theory. These two concepts have led investigators to shift their focus from well-established cancer cell lines to primary tumor cells and three-dimensional cell cultures for target validation and lead optimization. Multicellular spheroids of cancer cells model the three-dimensional architecture of in vivo tissues, including multicellular arrangement, hypoxia, cell-cell interactions, and extracellular matrix deposition. Such arrangements, which are absent in two-dimensional cultures, can better model drug delivery and drug-target under physiological conditions. These properties, therefore, make spheroids excellent models to study the tissue penetration potentials of new therapeutics and their carriers for structural modifications. Typical confocal microscopes are yet not compatible for deep-tissue imaging of samples, such as spheroids, that are several microns thick and highly scattering. However, lightsheet fluorescence microscopy (LSFM) is a promising approach for detailed visualization of whole three-dimensional samples, which is not achieved with any other microscopy techniques. In this talk, I will present a brief overview of our unified spheroids- and spheroids-derived cell-based screening method, along with LSFM studies, for lead optimization in the preclinical stages of drug discovery.

#### Materials/methods

Spheroids of human colorectal HCT116 cancer cells were generated using the liquid-overlay culture technique and maintained according to our established laboratory protocol. Compounds were screened from LOPAC commercial library that consist of cc. 1300 compounds. The effects of selected compounds were studied in whole spheroids using a Zeiss Lightsheet Z.1 Fluorescence



Microscope.

### Results and conclusions

A number of compounds were identified that showed increased cytotoxic effects in spheroids and spheroids-derived cultures of HCT116 cells. Four these compounds were found to have significant spheroid size reducing effects. LSFM results showed that the compound YC-1, among the four potent spheroid-specific compounds, significantly reduced hypoxia in spheroids. We believe that this unified approach has tremendous potential to achieve effective and reliable cell assessment standards by linking two-dimensional/three-dimensional in vitro tumor model-based data in preclinical drug development studies.

### Miniaturization of cytotoxic assay in HTS.

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### Introduction

The HTS (high throughput screening) technique was developed to evaluate the biological activity of thousands of chemicals to identify potential drug candidates in very short time. This system requires automation, data processing and control software, precise liquid handling devices, and sensitive detectors. Besides listed characteristics, HTS is connected with miniaturization: the 96-well plates have been replaced by higher density microplates (e.g. 384-, 1536-, 3456-well plates). We tested suitability of 1536-well plates for our cell based studies in HTS.

### Materials/methods

Ten cell lines plated on both 384- and 1536- well plates were treated with conventional drugs and 90 compounds from LOPAC library. Cytotoxic effect of compounds was measured by MTS assay. For

selected active compounds the IC50 values were calculated. To quantify the suitability of cytotoxic assay in a HTS the Z-factor was determined for each plate and cell line.

### Results and conclusions

The IC50 values obtained from testing on 384- and 1536-well plates were compared. Obtained results, as well as advantages and disadvantages of 1536- well plates will be presented and discussed.

### Decryptor: system for reliable identification of alterations by mass spectrometry of proteome.

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### Introduction

Identification of peptides using mass spectrometry poses additional challenges when identification of non-reference peptides is of interest. The problems are, however, natural consequence of restricted ability to model the peptide measurement process and can be overcome to practically sufficient degree.

### Materials/methods

To obtain deeper understanding of the structure of identification, the peptide identification problem was studied, formalized and generalized. Furthermore, optimal interpretation of measurement—given particular assumptions—was derived and implemented as a submodule in decryptor. The main advantage of the approach is its clarity and straightforward applicability.

### Results and conclusions

Decryptor was used to identify altered peptides from bottom-up proteomics data in variety of samples; both

internal and external. In case of the former, decryptor identified 97 unique SNVs (20 fractions, one technical replicate) in colorectal cancer cell line HCT116 measured on Orbitrap Elite. The evaluation on NCI60 panel showed on average 23 unique SNVs (12 fractions, one replicate), maximum of 56 for colon cancer cell line HCC-2998. In general, the identified alterations correspond to highly expressed genes. The system was intentionally designed to work with standard bottom-up proteomics data and as such provides additional insights into sample of interest without additional requirements.

### Comparison of three genotyping methods for detection of BRAF p.Val600Glu mutation in FFPE samples.

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### Introduction

BRAF mutation p.Val600Glu is a validated positive predictive factor for melanoma treatment with vemurafenib and a suspected negative predictive factor for colorectal carcinoma treatment with EGFR inhibitors. Thus, analytical performance of a mutation detection method crucially affects the clinical decision about treatment of cancer patients. In this study, we tested



three molecular genetics methods based on qPCR for BRAF mutation assessment.

#### Materials/methods

FFPE samples from 72 patients with melanoma were tested for presence of p.Val600Glu mutation in BRAF gene using three kits. BRAF mutation detection kit from IntellMed (Czech Republic) uses duplex reaction aimed at BRAF mutation hotspot in FAM fluorescence channel and at globin in JOE channel. Thus, globin signal serves as an internal standard of DNA and pipetting quality. The second BRAF mutation detection kit, gB ONCO BRAF V600E (Generi Biotech) is quantitative and uses two PCR tubes per sample, where the first tube detects BRAF mutant and the second tube BRAF wildtype. The third kit is home-made based on CADMA (Competitive Amplification of Differentially Melting Amplicons) principle. Consensus result for each sample was established when at least two methods agreed. Sensitivity, specificity, and percentage of typable samples were calculated.

#### Results and conclusions

We show here that three molecular genetics methods perform

satisfactorily regarding robustness, sensitivity and specificity of BRAF mutation testing. One sample (1.3%) was untypable by Generi-Biotech kit that reached 96.9% specificity and 100% sensitivity. BRAF mutation detection kit from IntellMed typed all samples with 90.6% sensitivity and 100% specificity. CADMA kit typed all samples with 100% sensitivity and 95% specificity.

#### Acknowledgements:

This project was supported by grants LO1304, CZ.1.05/3.1.00/14.0307, AZV 16-32198A, and TE02000058. gB ONCO BRAF V600E kit was a generous gift from Generi-Biotech. Jiří Drábek is a partner of IntellMed. Part of this presentation was presented also at Forensica 2016 conference.

#### Specific CNV analysis from single-cell.

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#### Introduction

Copy Number Variation (CNV) can involve gains and losses of genomic

DNA on submicroscopic level that are not visible on karyotyping. It is assumed that at least 10% of human genome contains CNV. While majority of CNVs is not associated with pathological phenotype, several CNVs were identified as “driver” alterations in oncological patients. Currently, non-invasive analysis of cell-free DNA (cfDNA) is proven to be suitable for cancer patients diagnostics. However, cfDNA analysis means that most of the time single-cell is analyzed, which brings high risk of not specific results.

#### Materials/methods

Here, we would like to present two technologies (10x genomics and PicoPLEX DNA-seq) focused on specific and efficient preparation of library for NGS from a single-cell with subsequent data-analysis for CNV detection. Part of the lecture will be focused on explaining algorithm for optimal CNV detection from NGS data.

#### Results and conclusions

10x genomics and PicoPLEX DNA-seq are promising technologies for CNV diagnostics especially for low-amount DNA samples.



## Molecular targets and cancer biomarkers I.

Chairs: Ondrej Slaby, Lubos Holubec

středa / 30. listopadu 2016 / Wednesday / November 30, 2016 / 15:00 - 17:15

### Translational potential of microRNAs in oncology.

#### Ondrej Slaby

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MicroRNAs (miRNAs) are small non-coding RNAs 18–25 nucleotides in length that downregulate gene expression during various crucial cell processes such as apoptosis, differentiation and development. Changes in the expression profiles of miRNAs have been observed in a variety of human solid tumors and hematological malignancies. Functional studies indicate that miRNAs act as tumor suppressors and oncogenes and are involved in hallmarks of cancer. These findings significantly extend concept of molecular pathogenesis of cancer and have shown great potential for miRNA as a novel class of therapeutic targets. Several investigations have also described the ability of miRNA expression profiles to predict prognosis and response to selected treatments in cancer patients, and support diagnosis of origin among cancer of unknown primary site. miRNAs' occurrence has been repeatedly observed also in blood serum, plasma and urine, and miRNAs as novel minimally invasive biomarkers have indicated reasonable sensitivity for cancer detection. The knowledge regarding miRNAs functioning in pathogenic signaling pathways and their translational potential to serve as disease biomarkers and novel therapeutic targets in cancer will be summarized and demonstrated on several examples based on our

recent observations.

### Prevalence of HPV infection in oocyte donors and women treated for infertility: a prospective study.

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#### Introduction

HPV infection is a known oncogenic agent associated with about 5% of all cancers in men and women. High-risk HPV (hrHPV) can cause malignant transformation predominantly in the ano-genital and aero-digestive tract. Recent studies also suggests HPV could alter human fertility however, the exact role of HPV infection remains uncertain.

#### Materials/methods

Cervical smears of oocyte donors (n=207) and women from infertile couples (n=945) were taken from March 2013 to October 2015 and analyzed for the presence of 14 hr HPV genotypes. Prevalence of HPV in both groups was compared. In those who underwent IVF treatment

within the next 6 months after sampling, number of pregnancies and abortions was evaluated in relation to their HPV status. Only embryotransfers (ET) with one or two fresh embryos were included. All participants signed written consent, filled a questionnaire focused on their health status and sexual behaviour. Study was approved by ethical board of the Faculty of Medicine and University hospital.

#### Results and conclusions

The median age of women from infertile couples (IW) was 33 years (range, 19-48 years) and of oocyte donors (OD) was 26 years (range, 18-39 years). HrHPV prevalence was significantly higher in OD than in IW (28.0%, [58 of 207] vs. 16.1 % [152 of 945], P <0.001). The pregnancy rate was lower in women treated by IVF (106 of 362, 29.3%) than in recipients of donated oocytes (35 of 87, 40.2%, P = 0.001). The abortion rate increased from spontaneously pregnant women (2 of 46, 4.35%), by IVF (24 of 106, 22.6%), to recipients of donated oocytes (15 of 35, 42.9%, P = 0.006). However, we have not find any association between hrHPV infection and lower pregnancy rate or higher abortion rate in recipients who obtained oocyte donated by hrHPV positive OD, in hrHPV+ IW undergoing IVF or IUI as well as in hrHPV positive spontaneously pregnant women. Despite this study did not find any association between HPV infection in women and pregnancy/abortion rate there are certain evidence that HPV could affect fertility outcome. Due this finding more attention should be payed to HPV infection in men and its influence on fertility outcome.



## Circulating glycoprotein biomarkers of gastrointestinal cancers.

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### Introduction

Biomarkers are physiological indicators of diseased tissue and have value in diagnosis, prognosis and in the evaluation of a treatment regimen. The larger goal of this project is to identify and validate serum-based N-Glycoprotein markers of human GI cancers early enough for a treatment to be effected successfully. N-Glycosylation is frequently indicative of perturbations in cellular physiology and this makes the glycosylated proteins clinically valuable. Many of these proteins are secretory or cell-surface proteins and are more likely to be secreted or shed into serum than proteins in the cytoplasm. The biomarker discovery phase is being performed in a mouse xenograft model, for a couple of reasons. First, the mouse has a blood volume that is several times lesser than a human's and this makes detection of a marker that much easier, and second, because any human protein observed in the mouse serum necessarily comes from the tumor.

### Materials/methods

Cell-culture and Xenograft Cancer cell-lines were purchased from DSMZ (Germany) and cultured as per the company's instructions. These were then grafted in immunocompromised (SCID) mice and allowed to grow. When the xenograft/tumors reached a size

of approximately 1 cubic cm, the mice were sacrificed, and blood and tumor tissue collected for analysis of protein, RNA and DNA.

Purification of N-Glycopeptides, LC-MS/MS and computational data analysis

N-Glycopeptides are isolated from mouse serum using a solid-phase purification procedure (Solid-phase extraction of N-glycoproteins, Tian et al, 2007, Nature Protocols). The N-Glycopeptides are then separated by liquid chromatography and analyzed in an Orbitrap Fusion mass spectrometer (Thermo). Raw data is analyzed by software developed in-house for the identification of normal and mutant human N-Glycopeptides.

### Results and conclusions

Thirty different cell lines and some patient derived tumors, representing a spectrum of cancers of the GI-tract, were grafted in immunodeficient mice. More than one cell-line of each cancer type were chosen. Sera from these mice was prepared and analyzed using an established proteomic workflow. Preliminary analyses yielded several candidate biomarkers, some of which are novel and some of which have been previously reported. Two of these N-glycoproteins have been reported in a recent diagnostic signature, suggesting that our workflow is sound. A few hundred sequence alterations were also identified in the data from the discovery screen using a software developed in house. The biomarker candidates will be assayed in a pool of xenograft sera and subsequently in sera from pancreatic cancer patients, using a targeted proteomic method called selected reaction monitoring.

### Deregulation of miR-21, miR-9, miR-143 and miR-145 in sinonasal carcinoma and their value as prognostic biomarkers.

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### Introduction

Sinonasal carcinomas (SNCs) are malignant tumors arising from nasal and paranasal sinuses and make up 3 % of all cancers of head and neck area. Risk factors for developing SNC include cigarette smoking professional exposure to cancerogenous substances and HPV infection. MicroRNAs (miRNAs) are short (18 – 25 nt) noncoding RNA molecules that are part of gene expression and their primary role is negative regulation of translation as part of the RNAinduced silencing complex (RISC). The aim of this study was to investigate relative expression levels of selected miRNAs in sinonasal carcinoma samples and to compare the results with recorded clinicopathological data.

### Materials/methods

Formalin fixed, paraffin embedded samples of sinonasal carcinoma (70) and normal sinonasal tissue (17)



were analyzed. Relative expression of miR21, miR9, miR143 and miR145 were measured by realtime PCR with specific TaqMan® Advanced miRNA Assays and calculated using the  $\Delta\Delta C_t$  method. Oneway analysis of variance and regression analysis were used to analyze the correlation between expression levels of miRNA and clinicopathological data. The KaplanMaier method and Logrank test were used to determine survival rates.

### Results and conclusions

Real-time PCR data show statistically significant upregulation of miR21 (5.50fold,  $p < 0.0001$ ), miR9 (6.31fold,  $p < 0.0001$ ) and miR143 (1.79fold,  $p = 0.044$ ) in SNC samples in comparison to control tissue. On the other hand miR145 was significantly downregulated (1.92fold,  $p = 0.027$ ). We found correlation between shorter overall survival interval of the patients with higher upregulation of miR21 ( $p = 0.0030$ ) and downregulation of miR145 ( $p = 0.036$ ). After comparing relative expression levels of the miRNAs with clinicopathological data, we found out that smokers and former smokers had significantly higher upregulation of miR9 than nonsmokers ( $p = 0.021$ ). Additionally, miR143 was significantly more upregulated in male patients than in female patients ( $p = 0.036$ ) and the upregulation was also higher in patients with risk occupations ( $p = 0.026$ ). Our data show that miR21, miR9, miR143 and miR145 may represent important regulatory molecules involved in development and progression of the disease and survival time of sinonasal carcinoma patients. On top of that, they could be potentially used as valuable prognostic biomarkers of the disease. The study was supported by the program MH CZ - DRO (UHHK, 00179906), by the program PRVOUK P37/11 and by the project BBMRI\_CZ LM2015089.

### Analysis of copy number variations in meningioma samples using microarrays revealed 22q deletions more frequent in higher grade tumors.

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### Introduction

Meningiomas represent one of the most common intracranial tumors. They are generally thought to progress from low to high-grade lesions. However, the molecular mechanisms underlying their pathogenesis remain still uncertain. We suppose the existence of a correlation between the parameters that will help to predict more precisely their biological behavior and response to therapy. Identification of meningioma molecular subgroups may have significant potential to improve clinical management, through molecular disease risk stratification strategies and the identification of patients who could benefit from targeted molecular therapeutics.

### Materials/methods

Formalin-fixed paraffin embedded tumor samples were obtained from 45 patients and 5 healthy controls (dura mater). Comprehensive clinical-pathological data were

mined. There were 15 males and 30 females; mean age was 54 years, range 28 - 91 years. Total DNA was purified from FFPE samples after pathological verification using proteinase K treatment followed by QIAmp DNA FFPE Tissue Kit (Qiagen). Microarray analysis was performed using the OncoScan FFPE Assay Kit (Affymetrix), raw data were obtained using Chromosome Analysis Suite (Affymetrix) in default manner. Subsequently, the data were analyzed using Nexus Express software (Biodiscovery).

### Results and conclusions

In total, 65 OncoScan arrays were performed for copy number variants (CNV) analyses in meningioma samples. Our results confirm that chromosome 22 deletion and del(1p) are the most common (55%, resp. 47% of cases) deletions in meningioma. In this study, we revealed chromosomal gains not as rare as was published previously. The most common duplication was dup(3q) present in 31% of cases. Three meningioma molecular patterns were identified based on CNV profiling - "normal-like profile", "deletion profile" and "complex profile". Deletion profile is characterized by loss of 1p and monosomy of chromosomes 6, 10, 14, 18 and 22. The complex profiles combines gains (3q, 8q, chromosomes 12, 15 and 20) and losses (1p, chromosomes 13, 21 and 22). The primary tumor samples of patients with meningioma recurrence (20 patients) had different CNV profile in comparison with patients without recurrence (25 patients). Recurrent tumors had statistically significant higher frequencies of losses on chromosomes 1, 9, 10, 11, 13 and 21 and gains on chromosomes 12, 19 and 20.

We have identified the CNV profiles in meningioma patients allowing for better knowledge of pathological pathways and tumor progression. Del(22q) is frequently present in higher grade tumors probably altering NF2 a TP53 pathways. However, it will require further validation.

This work was financially supported



by Ministry of Health of the Czech Republic, grant nr. 15-29021A, IGA UP LF 2016\_010 and NPU LO1304.

### The mechanisms of anticancer tumor response targeted to the EGFR in colorectal carcinoma.

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#### Introduction

Colorectal carcinoma has generally been regarded as an immunoresistant tumor. In the light of recent research findings, it has become clear that this presumption is not true. The possibility for specific immune system modulation appears to be crucial not only for prognosis but also for the prediction of response to therapy in patients with colorectal cancer and other tumors. The combination of anti-EGFR monoclonal antibodies with other immunotherapeutic treatment modalities certainly brings new opportunities for targeted therapy in patients with colorectal carcinoma.

#### Materials/methods

In this review lecture, we discuss the mechanisms of immunomodulation together with the anticancer immune response to the monoclonal antibodies targeted to the EGFR.

#### Results and conclusions

The clinical significance of cetuximab treatment in patients with colorectal cancer or other cancer types (head and neck tumors, lung tumors) has been associated so far with the combined chemotherapy or with radiation. It was shown that these treatment strategies have their clinical limitations and do not fully exploit the immunomodulatory effect of cetuximab, particularly in the induction of ADCC response. The combination of cetuximab with other immunotherapeutic treatment

modalities certainly brings the new opportunities for the targeted therapy in patients with colorectal cancer.

### Comparison of newly prepared cisplatin-resistant and parental TGCTs cell lines.

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#### Introduction

More than 80% of patients with testicular germ cell tumors can be cured by using cisplatin-based chemotherapy, but acquired chemoresistance is associated with poor prognosis of patients. Better understanding of the mechanisms causing this chemotherapy failure may represent progress in the development of new treatment modalities or might improve currently used therapies.

#### Materials/methods

Newly prepared cisplatin-resistant counterparts of TGCTs cell lines were cultivated in standard DMEM (JEG-3, NTERA-2 cells) or RPMI medium (NOY-1 cells) and exposed to increasing cisplatin concentrations for 5 months. Chemosensitivity to various chemotherapeutics was evaluated by luminometric assay. Proliferation and morphology were analyzed by live-cell kinetic imaging.

#### Results and conclusions

In our laboratory we have been trying to prepare cisplatin-resistant counterparts of TGCTs cell lines (NOY-1, JEG-3, NTERA-2). In the preliminary data we observed changes in cell proliferation, morphology and chemosensitivity to different chemotherapeutics compared to parental cell lines. For better prediction of the therapeutic outcome we will also use the 3D

multicellular spheroids and mouse xenograft models. The expression of chosen candidate genes associated with cisplatin chemoresistance will be inhibited by small-interfering RNA. Newly prepared cisplatin-resistant TGCTs cell lines might be suitable for the evaluation of the anticancer therapeutic strategies. They might also serve for the future studies to target specific genes responsible for the acquired chemoresistance to cisplatin of TGCTs.

This work was supported by Slovak Research and Development Agency under the contract No. APVV-0230-11 and VEGA 2/0087/15; Cancer Research Foundation RFL 2009, RFL 2012 and Foundation of Tatra bank – grant “Študenti do sveta”.

### Invasive capacity of cancer cells is enhanced by expression and catalytic performance of the pH regulating enzyme CA IX.

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#### Introduction

The aggressive behavior of malignant tumor cells is reflected by the formation of proteolytically active actin-rich membrane protrusions termed invadopodia. Production of focal pH gradient at both sides of membrane is crucial for invadopodia formation and proper functioning. Cancer-associated carbonic anhydrase IX (CA IX) facilitates generation of pH gradient thus promotes invasive properties of tumor cells.

#### Materials/methods

We analyzed gelatin-entrapped invadopodia and demonstrated CA IX presence in these structures by Western blot and immunofluorescence. Effect of reduced CA IX expression on the capacity of cancer cells to cleave



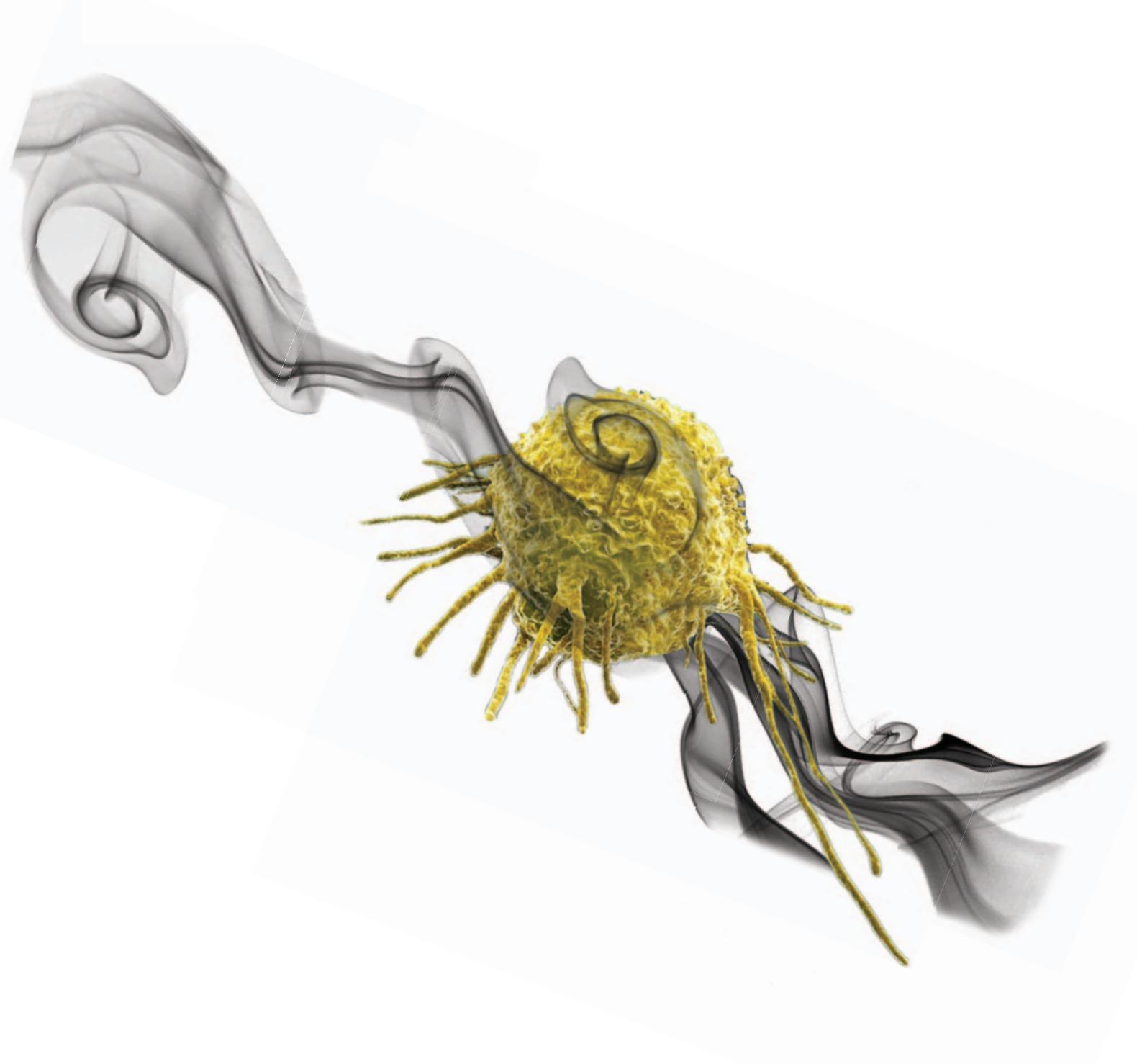
extracellular matrix (ECM) was also evaluated. Transient CA9 silencing followed by Western blot was used to explore the impact of CA IX suppression on the invadopodia signalosome.

### Results and conclusions

We showed that pH controlling CA IX distributes to invadopodia with ECM digesting activity. We further observed that CA IX is functionally involved in the invadopodia assembly, maturation and focal ECM proteolysis since downregulation of

its expression as well as inhibition of its enzymatic activity disrupts invadopodia functioning. CA IX-depleted cells performed impaired ECM degradation with reduced number, size and depth of formed invadopodia. Moreover, loss of CA IX expression caused the decrease in protein level of several invadopodia-bound molecules including pH regulator  $\text{Na}^+/\text{H}^+$  exchanger (NHE1) and Actin-related protein 2 (Arp2). Taken together, CA IX is an active player of the cell invasion machinery

regulating invadopodial biology. The pro-invasive capacity of CA IX and its tight relation to aggressive tumor subtypes constitute CA IX as an enzyme favoring cancer cell dissemination. Therefore, it might be beneficial to block CA IX in the anticancer therapy to prevent metastatic spread of tumor cells. We thank Research and Development Support Agency (APVV-14-0816) and Slovak Scientific Grant Agency (VEGA 2/0139/15).





## Novel anticancer drugs and therapies I.

Chairs: Lucia Kucerova, Marian Hajduch

čtvrtek / 1. prosince 2016 / Thursday / December 1, 2016 / 9:00 - 10:30 hod.

### Drug discovery for triple negative breast cancers.

Susan L. Mooberry

University of Texas Health Science Center, San Antonio, USA

Triple negative breast cancers (TNBC) are devoid of estrogen and progesterone receptors and lack gene amplification of HER2. These are commonly aggressive cancers they are more common in young women and in African American and Hispanic women. There are no molecularly targeted therapies for TNBC and patients diagnosed with these types of cancers have a lower 5 year survival than patients with other types of breast cancers. There is a major need to identify targeted therapies for TNBC. TNBCs are highly heterogeneous cancers and recently genetic subtyping of 587 TNBC patients was accomplished and 5 different subtypes of TNBC with distinct genetic alterations were defined. Cell lines representing these subtypes were also identified. We initiated a screening program to identify extracts from diverse fungi and plant extracts that have selective activities against one subtype of TNBC. Extracts with excellent selectivity were detected and compounds isolated using bioassay-guided fractionation. Five compounds and/or compound classes with highly selective actions have been isolated and the mechanisms of actions of the compounds are being determined. These studies have yielded new molecular targets for the subtypes of TNBC. In one case a combination of two FDA approved drugs has the same effects as the natural product and allows rapid translation into clinical trials. These results demonstrate that compounds with potential therapeutic value and new molecular targets for the treatment of TNBC subtypes can be identified from nature. Funding

support was provided by a grant from the National Cancer Institute (USA) (UO1CA182740).

### Chemoresistance of cancer cells - experimental models.

Johana Plch, Jan Hraběta, Ashraf Mohamad Khalil, Tomas Eckschlager

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#### Introduction

The resistance of cancer cells to cytostatics represents a big problem in oncology. The development of drug resistance is commonly explained by the selection of resistant mutant cancer cells. However, non-genetic heterogeneity of cell populations produces phenotypic variants, some of which are chemoresistant - epigenetic mechanisms of resistance. Moreover hypoxia which is a common in micro-environment of a solid tumors plays a role in several signaling pathways which among others induce chemoresistance.

#### Materials/methods

As model of genetically based chemoresistance we use long term cultivation in medium with increased doses of cytostatic and as model of "epigenetic chemoresistance" we use small population which survives single high dose of cytostatic. Moreover we are able to cultivate cells in hypoxic conditions.

#### Results and conclusions

Cells which survive single high dose of vincristine were genetically identical with sensitive cells and produced vincristine sensitive cells. Surviving cells show significant differences in histone methylations and acetylations specific lysine side, moreover, modifications levels differ in time. On

the other hand, cells with resistance induced by long term cultivation with cytostatic demonstrated several genetical changes e.g. MDR1 amplification and corresponding P-glycoprotein overexpression in neuroblastoma cells resistant to vincristine or doxorubicin. These types of chemoresistance are not mutually exclusive and may be simultaneously present in one population of tumor cells. Cancer cell drug resistance is caused by a combination of mechanisms and, to overcome this problem, will require a complex approach.

### Role of high-fat diet on the effect of metformin and melatonin in chemically-induced rat breast cancer models.

Bianka Bojková<sup>1</sup>, Karol Kajo<sup>2</sup>, Peter Kubatka<sup>3</sup>, Desanka Výbohová<sup>4</sup>, Soňa Báľentová<sup>5</sup>, Terézia Kisková<sup>1</sup>, Martin Péc<sup>3</sup>, Marián Adamkov<sup>5</sup>

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## Introduction

The effect of chemopreventive substances in carcinogenesis may be modulated by various factors including diet. Data collected from *in vitro* and *in vivo* studies show that both metformin, a peroral antidiabetic from biguanide group, and pineal hormone melatonin inhibit growth of many cancers including breast cancer. However, most *in vivo* studies used standard-type diet with relatively low fat content. Therefore, in this study we have evaluated the impact of high-fat diet on the efficacy of metformin and melatonin in two rat models of breast cancer, a NMU (N-methyl-N-nitrosourea) model and DMBA (7,12-dimethylbenz[a]anthracene) model.

## Materials/methods

Female Sprague-Dawley rats (Velaz, Prague, Czech Republic) aged 28-30 days were used in the experiment. The animals were fed the high-fat diet (10% total fat) and drank tap water or melatonin solution, respectively, *ad libitum*. Mammary carcinogenesis was induced by NMU (50 mg/kg i.p.) administered on the 42nd postnatal day, experiment A) and DMBA (20 mg per rat administered on the 48th postnatal day, experiment B), respectively. Chemoprevention with metformin and melatonin was initiated 12 (experiment A) and 20 days (experiment B) before the carcinogen administration, respectively, and lasted until the termination of the experiment. Animals were assigned randomly to one of five experimental groups: CONT, control group without chemoprevention; MF, chemoprevention with metformin; MEL, chemoprevention with melatonin; MF+MEL, chemoprevention with combination of metformin and melatonin; and INT, intact group. Metformin was administered in a diet (2000 ppm), melatonin was administered in tap water (20 mg/L, between 3 p.m. to 8 a.m., only water from 8 a.m. to 3 p.m.). During the experiments the animals were weighed weekly and palpated to register the presence, location and size of each palpable tumour.

The experiments were terminated 16 weeks (experiment A) and 14 weeks (experiment B), respectively, after carcinogen administration. Mammary tumour samples were obtained for histopathological and immunohistochemical analysis (Ki67, BCL-2, caspase-3). Basic parameters of mammary tumour growth were evaluated in each group (tumour incidence, latency period, tumour frequency, average and cumulative tumour volume). Data were evaluated using the Mann-Whitney *U*-test (tumour incidence, high-grade tumour incidence), one-way analysis of variance or the Kruskal-Wallis test, respectively (other parameters).

## Results and conclusions

Metformin and melatonin had no significant effect on tumour growth in NMU model although lower incidence in the MEL group (by 22%) and a decrease in average and cumulative tumour volume in the MF group (both by 51%) compared with the CONT group was recorded. Histopathological analysis showed a decrease in the high-grade/low-grade carcinoma ratio towards low-grade lesions in the MF and MEL group. Immunohistochemical staining did not prove changes in proliferation and apoptosis markers but a significant positive correlations between histological grade and *Ki67* expression in the MF group and MEL group were recorded. In DMBA model, combination of chemopreventive agents decreased tumour incidence (by 29%), the decrease in tumour frequency recorded in the MEL and MF+MEL group (by 44% and 47%, respectively) was not significant. Cumulative tumour volume was lower in all groups with chemoprevention. Histopathology did not show significant changes in high-grade/low-grade tumour ratio. Altogether, metformin and melatonin were more effective in the DMBA model than in NMU model which may result from reduction in DMBA metabolic activation.

## The effect of piperlongumine on pheochromocytoma cells in vitro and in vivo.

*Petra Bullova*<sup>1,2</sup>, *Antony Cougnoux*<sup>3</sup>, *Juraj Kopacek*<sup>2</sup>, *Silvia Pastorekova*<sup>2</sup>, *Karel Pacak*<sup>1</sup>

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## Introduction

P h e o c h r o m o c y t o m a s / paragangliomas (PHEO/PGL) are neuroendocrine tumors, for which the effective treatment is still missing. Thus, much effort is being made to identify molecular mechanisms leading to the disease and participating in its progression. We tested piperlongumine (PL), a compound known to increase reactive oxygen species (ROS) levels, in PHEO models. Strong oxidative stress promotes cancer cell death, which was the focus of our study. Since hypoxia is often involved in cancer cell resistance to a treatment, and it plays an important role in PHEO/PGL, effects of PL were also investigated in hypoxic conditions and in mouse models.

## Materials/methods

To evaluate the effect of PL *in vitro*, we performed MTT assay and migration and invasion assays on mouse pheochromocytoma cells (MPC and MTT). For hypoxia experiments, the cells were incubated at 1% O<sub>2</sub>. Western blot and co-immunoprecipitation were used to specify pathways involved in PL-mediated cytotoxicity in these cells. To analyze the effect of PL *in vivo*, nude mice carrying MTT-Luc tumors were injected daily with 24 mg/kg PL. Immunofluorescence was used



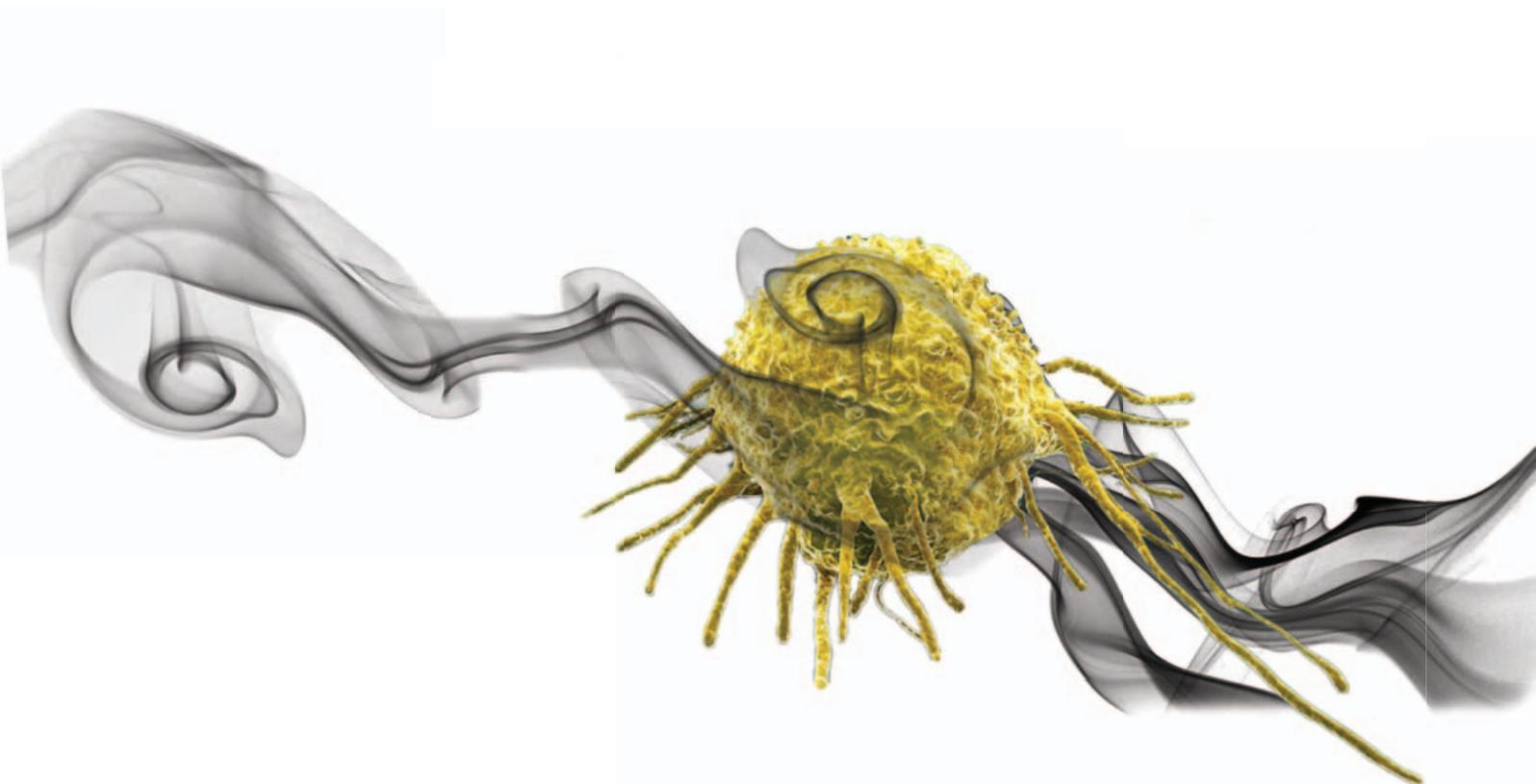
to analyze markers of proliferation, angiogenesis, EMT and apoptosis in tumors.

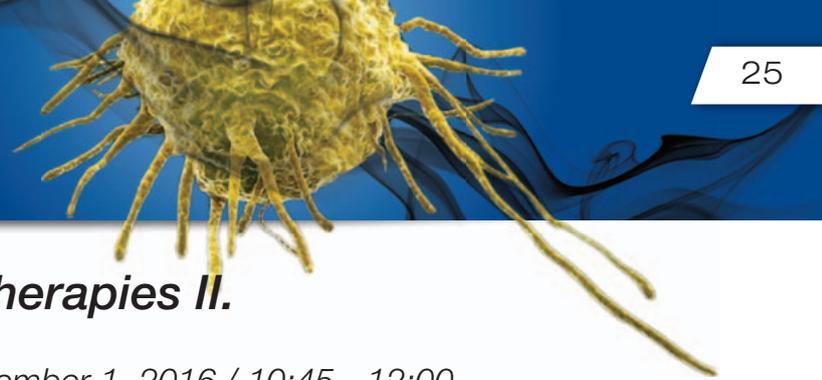
### Results and conclusions

In our study, PL exhibited cytotoxic effect on MPC and MTT cells ( $IC_{50}$ =6.04 $\mu$ M and 4.8 $\mu$ M, respectively), and the concentration necessary to induce death of 50% of the cells was lower in hypoxic conditions. PL also inhibited migration and invasion of MPC cells after 24h. To identify which pathways were involved in PL-mediated cytotoxicity,

we treated the cells with PL for 24h with increasing doses, as well as with 10 $\mu$ M for 0, 8, 16 and 24h. We observed the increase in levels of apoptosis-associated proteins cleaved caspase 3 and cleaved PARP. The presence of necrosome indicated that necroptosis was occurring in these cells after addition of PL as well. We also observed that the effect of PL on PHEO cells was magnified in hypoxia, which was associated with already increased ROS levels in hypoxic conditions. *In vivo* results showed that PL effectively

inhibited tumor growth, decreased angiogenesis and suppressed metastatic formation. In conclusion, we showed that PL induced apoptotic and necroptotic PHEO cell death via ROS increase *in vitro* and *in vivo*, inhibited metastatic propensities and angiogenesis, and therefore, it might be a potential therapeutic option for patients with metastatic PHEO/PGL. This work was supported by the Intramural Research Program of the Eunice Kennedy Shriver NICHD, NIH.





## Novel anticancer drugs and therapies II.

Chairs: Petr Dzubak, Viswanath Das

čtvrtek / 1. prosince 2016 / Thursday / December 1, 2016 / 10:45 - 12:00

### The prostate cancer-targeted and nontargeted apoferritin loaded by doxorubicin and its effect on cancer and healthy cell lines.

*Tereza Cerna*<sup>1,2</sup>, *Jan Hrabeta*<sup>2</sup>, *Simona Dostalova*<sup>3</sup>, *Vojtech Adam*<sup>3</sup>, *Tomas Eckschlager*<sup>2</sup>, *Marie Stiborova*<sup>1</sup>

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<sup>3</sup> Laboratory metallomics and nanotechnology, Mendel University in Brno and Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

#### Introduction

Conventional cancer treatment based on cytostatic therapy is highly toxic not only for cancer cells, but also for normal ones. However, side effects and organ damage can be reduced by using nanocarriers and targeted drug delivery. 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. To enhance the targeting ability of apoferritin to cancer cells, it is possible to modify its surface with antibodies.

#### Materials/methods

We compare the cytotoxic effects of doxorubicin encapsulated into apoferritin (APODOX) with and without targeting antibody against prostate specific membrane antigen (PSMA) on its surface and free doxorubicin (DOX). The prostate

cancer-targeted and nontargeted nanocarriers were tested using LNCaP and HUVEC cells. Cell viability was assessed using the MTS assay and the real time impedance based platform (xCELLigence). DNA double-strand breaks and entry of targeted and nontargeted nanocarriers were detected by flow cytometry.

#### Results and conclusions

We show here that the effect of APODOX on prostate cancer cells and HUVEC is similar to that of free DOX. Similar cell viability after treatment of cells with targeted, nontargeted APODOX and free doxorubicin (MTS assay) was found. Likewise, xCELLigence proved similar sensitivity of LNCaP cancer cell line to both forms of doxorubicin, but APODOX was not as toxic as free DOX to HUVEC. Percentage of LNCaP and HUVEC cells with DNA double-strand breaks after APODOX treatment was essentially not different from that after doxorubicin treatment. Entry of free doxorubicin was higher than entry of APODOX and APODOX with targeting antibody into HUVEC. Entry of APODOX and APODOX+anti-PSMA into HUVEC was unlike free DOX significantly lower than into LNCaP. The APO encapsulation mechanism ensures applicability using a variety of chemotherapeutic drugs, and the presented surface modification enables targeting to various tumors. The results seem promising to begin animal experiments, where it is hoped that in vivo studies will demonstrate higher therapeutic utilization of APODOX.

This work was supported by GACR 14-18344S in panel P301.

### Cytotoxicity and release of paclitaxel from a PEG/PLA carrier.

*Johana Plich*<sup>1</sup>, *Jan Hrabeta*<sup>1</sup>, *Tomas Eckschlager*<sup>1</sup>, *Radka Hobzova*<sup>2</sup>, *Jakub Sirc*<sup>3</sup>, *Marcela Munzarova*<sup>4</sup>, *Olga Janouskova*<sup>5</sup>, *Kristina Venclikova*<sup>5</sup>

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<sup>5</sup> Department of Biological Models, Academy of Science of the Czech Republic, Prague, Czech Republic

#### Introduction

In this work, we present in vitro study of a nanofibrous carrier in which the drug molecule is dispersed throughout the polymer matrix.

#### Materials/methods

Nanofibers for local drug delivery were prepared from polylactide (PLA) using Nanospider™ technology. Polyethylene glycols (PEG) with molecular weights of 6 000, 20 000 and 35 000 g/mol were added to the solution of polymers and were incorporated in nanofibers during electrospinning. Antineoplastic agent paclitaxel was dispersed throughout the polymer matrix of the nanofibrous carrier and the drug release was investigated. The kinetics



of drug release was characterized by in vitro analysis. To test the effect of Paclitaxel on cancer cell lines (UKF-NB-3, UKF-NB-4, SH-SY5Y, MCF-7) we measured viability by AlamarBlue assay.

### Results and conclusions

These findings can be applied to develop nanofibrous drug carriers for the local delivery of pharmacologically active compounds. The addition of PEG molecules significantly increased the drug release from PLA matrix and increased cytotoxicity. The release of drug and cytotoxicity correlated over time with the rising molecular weight of the PEG molecules. The PLA carriers induced cytotoxicity even after 48 hours of preincubation in medium, suggesting a gradual release of cytostatic from the carrier.

### Multifaceted properties of betulinic acid derivatives.

*Jiri Rehulka, Milan Urban, Petr Dzubak, Marian Hajduch*

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Olomouc, Czech Republic*

### Introduction

Betulinic acid derivatives are extensively studied and show a wide range of biological activities. The parental compound, betulinic acid, is a natural product with anticancer, antiinflammatory and antiviral properties. Its antitumor activity is mainly associated with the ability to trigger apoptosis by permeabilization of mitochondrial outer membrane. In addition to apoptosis induction, betulinic acid is known to modulate pathways essential for cell survival, protein degradation and angiogenesis.

### Materials/methods

Cytotoxicity was determined by 72-hour MTT assay against panel of normal human cell lines and cell lines derived from different cancer types. Biological activities were monitored by reporter cell lines or by

western blot. Cell cycle analysis was performed using flow cytometry.

### Results and conclusions

The structure activity relationship study indicate that even minor change on triterpenic skeleton results in altered mode of action. Using different substitutions, we got insight into pharmacophores responsible for particular biological activities. Moreover, the data from these studies enable design of new active compounds with improved solubility and pharmacological profile.

**Acknowledgement:** This work was supported by grant from the Ministry of Education, Youth and Sports of the Czech Republic (No. CZ.1.07/2.3.00/30.0041), Czech Science Foundation (15-05620S) and internal grant of Palacky University IGA\_LF\_2016\_019. The chemical part was supported by Technology Agency of the Czech Republic (TE01020028 and LK21310). The infrastructural part (Institute of Molecular and Translational Medicine) was supported by the grant LO1304 from the National Program of Sustainability II.

### Analyses of distinctive effects of ginger phenylpropanoids and quercetin on Nrf2-ARE pathway in human BJ fibroblast and HaCaT keratinocytes.

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*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Olomouc, Czech Republic*

### Introduction

Among different dietary plant phenolic compounds considered as chemopreventive candidates are ginger phenylpropanoids, 6-gingerol, 6-shogaol and their derivatives, and plant flavonoid quercetin. Our study aimed to determine their effects on Nuclear related factor 2 (Nrf2) antioxidant response signalling pathway (Nrf2-ARE) in BJ

foreskin fibroblasts and skin HaCaT keratinocytes.

### Materials/methods

BJ and HaCaT cells and their corresponding Nrf2-ARE luciferase reporter cells were treated by ginger phenylpropanoids and quercetin for 10 h. The level of Nrf2 activity in reporter cells was determined by luciferase assay. The level of glutathione-S-transferase P1 (GSTP1) of BJ cell and HaCaT cells was determined by western blot analyses of proteins.

### Results and conclusions

The average luciferase activity of BJ and Nrf2-ARE reporter cells treated by ginger phenylpropanoids and quercetin was significantly greater than that of corresponding controls. The average levels of GSTP1 protein in the BJ cells treated with ginger phenylpropanoids and quercetin were greater than that of their controls. The average level of GSTP1 protein was not significantly increased in HaCaT cells treated by ginger phenylpropanoids and quercetin. Thus, both ginger phenylpropanoids and quercetin have property of activating the Nrf2 pathway in BJ cells. On the other hand, while both ginger phenylpropanoids and quercetin can activate Nrf2 in HaCaT cells, their effects on expression of the GSTP1 were not mediated. This finding shows a constitutive expression of their GSTP1.

### Novel carborane as carbonic anhydrase IX inhibitors.

*Jana Dvoranova Stepankova<sup>1</sup>, Pavlina Rezacova<sup>2</sup>, Bohumir Gruner<sup>3</sup>, Jiri Brynda<sup>2</sup>, Viswanath Das<sup>1</sup>, Viteslav Masek<sup>1</sup>, Martina Michalova<sup>1</sup>, Alice Nova<sup>1</sup>, Vaclav Sicha<sup>3</sup>, Klara Pospisilova<sup>2</sup>, Dalibor Dolezal<sup>1</sup>, Petr Dzubak<sup>1</sup>, Marian Hajduch<sup>1</sup>*

<sup>1</sup> *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry Palacky University Olomouc, Olomouc, Czech Republic,*



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### **Introduction**

Carborane-based compounds have emerged as promising lead structures for the development of inhibitors of carbonic anhydrases (CAs). The aim of this study is to evaluate the selectivity of new carboranes with functional sulfonamides toward the cancer-specific CAIX isoenzyme.

### **Materials/methods**

To investigate the potency of carboranes to inhibit CAIX, at cellular level, promising carboranes were chosen based on their enzymatic activity against CAIX and CAII, in vitro from a library of over 50 new carboranes with sulphonamide

residues (IOCB, ASCR, v.v.i., Czech Republic). 2 carboranes, viz., CB-30, CB-31, showed high binding constant (10 and 16 nM, respectively) and high selectivity for CAIX, and were studied further. Extracellular pH in cell cultures was measured, in parallel with measurements of cellular cytotoxicity, in both 2D and 3D culture systems. Further, we developed a new method of Raman spectroscopy, to locate the distribution of carboranes in cells, and determined the distribution pattern of CB-30 and CB-31 in HT-29 cells, under hypoxia conditions. The highest Raman signal was detected on the cell membrane. Furthermore, we investigated the pharmacodynamics and pharmacokinetics profiles of carboranes. Pharmacokinetics of tested carboranes was assessed according to their plasma and microsomal stability, plasma protein binding, and presence of passive and active transport in cells (ADME

methods). The rapidity of drug metabolism, suitability of carboranes for per-oral administration, and the ability of carboranes to penetrate through the blood brain barrier were also evaluated by ADME methods. In vivo study showed promising pharmacokinetic profile of studied carboranes and confirmed results obtained from ADME methods. Moreover, in vivo testing of female BALB/c mice with implanted 4T1 breast cancer cells confirmed significant carborane associated antitumoral effect.

### **Results and conclusions**

Our results show the ability of tested carboranes to inhibit CAIX at enzymatic and cellular level. In vivo study suggests that novel carboranes with functional sulfonamide residues may serve as not only selective inhibitors of CAIX, but also as potential drugs in anticancer treatment.



## Molecular imaging in cancer research.

Chairs: Clemens Decristoforo, Milos Petrik

čtvrtek / 1. prosince 2016 / Thursday / December 1, 2016 / 13:00 - 15:00

### Translation of radiopharmaceuticals from bench to bedside - prospects and challenges in Europe.

Clemens Decristoforo

Medical University Innsbruck,  
Innsbruck, Austria

#### Introduction

Radiopharmaceuticals are increasingly important in oncology for Molecular Imaging (PET) also in the context of imaging biomarkers or companion diagnostics. The application of the tracer principle injecting microdosing amounts makes them a very safe group of medicinal products, but translation from preclinical development to patient application requires to adhere to the complex pharmaceutical regulatory framework.

#### Materials/methods

Basically the same procedures have to be fulfilled for radiopharmaceuticals as compared to standard drugs. For novel applications, clinical trial regulation is applicable, which has recently changed with a new EU regulation. In this context new opportunities arise with exemptions for academic centres related to pharmaceutical production. Professional organisations have released recommendations how to comply with the regulatory requirements related to documentation, GMP and preclinical (e.g. toxicity) studies.

#### Results and conclusions

There is an increasing awareness for translational requirements of radiopharmaceuticals in the regulatory process. Examples will be given how this translational process is realized in an academic setting.

### Fusarinine C - a backbone for targeted multimodality imaging?

Dominik Summer<sup>1</sup>, Miloš Petrik<sup>2</sup>, Leo Großrubatscher<sup>1</sup>, Christine Rangler<sup>1</sup>, Hubertus Haas<sup>3</sup>, Elisabeth von Guggenberg<sup>1</sup>, Clemens Decristoforo<sup>1</sup>

<sup>1</sup> Department of Nuclear Medicine, Medical University, Innsbruck, Austria,

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<sup>3</sup> Division of Molecular Biology/ Biocenter, Medical University, Innsbruck, Austria

#### Introduction

The cyclic siderophore Fusarinine C (FSC) known for excellent chelating abilities towards <sup>68</sup>Ga has three binding sites allowing the conjugation of targeting vectors as well as additional signalling moieties. In this proof of principle study two bioconjugates were evaluated regarding their potential as multimodality agents with nuclear and optical signalling for oncological applications.

#### Materials/methods

Two targeting vectors (minigastrin 11 and cyclo(RGDfK)) were attached to the FSC backbone followed by the conjugation of a near infrared fluorescent dye (SulfoCy7). *In vitro* evaluation including determination of receptor binding affinity, cell binding studies, serum stability, logD, protein binding as well as *in vivo* biodistribution,  $\mu$ PET/CT and fluorescence imaging were carried out.

#### Results and conclusions

Synthesis was straightforward and both bioconjugates were obtained

in moderate yield but in high purity. Radiolabelling with <sup>68</sup>Ga could be achieved within minutes at room temperature in quantitative yields at high specific activities and complexes resulted to be highly stable over four hours in human serum. IC<sub>50</sub> values in the low nanomolar range reveal high binding affinity and radio- as well as optical- cell binding studies indicate highly specific receptor targeting. Early timepoint (1-2 h p.i) *in vivo*  $\mu$ PET/CT and fluorescence studies showed specific tumour uptake but also high kidney and liver retention partly attributed to the increased lipophilicity related to the fluorescent dye whereas late timepoint fluorescence imaging (4-48h p.i) showed a much better tumour to non-targeted tissue ratio. In summary we could show that FSC can be utilized as a backbone for -high affinity and highly specific targeting- multimodality imaging.

### <sup>68</sup>Ga and <sup>89</sup>Zr labelled RGD multimers for integrin $\alpha_v\beta_3$ targeting based on a siderophore scaffold.

Piriya Kaeopookum<sup>1</sup>, Chuangyan Zhai<sup>1</sup>, Christine Rangler<sup>1</sup>, Hubertus Haas<sup>1</sup>, Dominik Summer<sup>1</sup>, Roland Haubner<sup>1</sup>, Peter Laverman<sup>3</sup>, Milos Petrik<sup>2</sup>, Clemens Decristoforo<sup>1</sup>

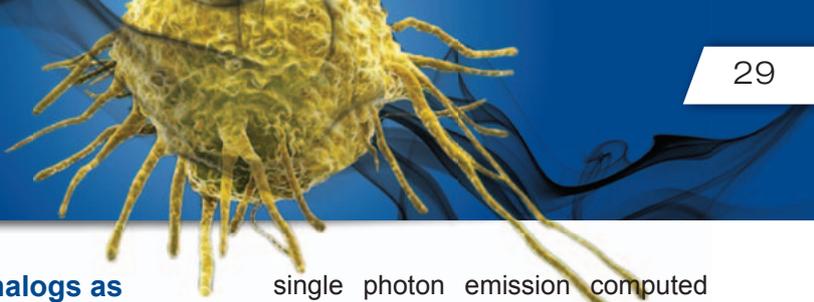
<sup>1</sup> Medical University Innsbruck, Innsbruck, Austria,

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<sup>3</sup> Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

#### Introduction

Fusarinine C (FSC), a natural cyclic siderophore, consisting of



three hydroxamic acid moieties to coordinate iron, was shown to be a good bifunctional chelator for  $^{68}\text{Ga}$  and  $^{89}\text{Zr}$ . Three amine functionalities in the backbone can be functionalized with biomolecules targeting receptors expressed on tumours. Here we present a comparison of trimeric RGD-FSC bioconjugates targeting  $\alpha_v\beta_3$  integrin labelled with  $^{68}\text{Ga}$  and  $^{89}\text{Zr}$  for Positron Emission Tomography (PET) applications.

#### Materials/methods

FSC was conjugated with c-RGDYK using different coupling strategies and linkers and labelled with Ga-68 and Zr-89. Stability, protein binding and binding to  $\alpha_v\beta_3$  integrin was assessed *in vitro*. *In vivo* biodistribution and small animal PET/CT imaging was performed using murine  $\alpha_v\beta_3$  integrin expressing tumour models.

#### Results and conclusions

Various conjugation strategies were successfully applied to prepare of FSC-RGD conjugates and labelling with  $^{68}\text{Ga}$  and  $^{89}\text{Zr}$  revealed excellent complexation properties. [ $^{68}\text{Ga}/^{89}\text{Zr}$ ] FSC-RGD conjugates exhibited high stability in examined media. *In vitro* and *in vivo* studies as well as PET/CT imaging showed enhanced  $\alpha_v\beta_3$  integrin binding affinity, receptor-selective tumor uptake, rapid predominantly renal excretion and excellent imaging properties independent of RGD conjugation strategy. From a series of FSC conjugates [ $^{68}\text{Ga}$ ]FSC(succ-RGD)<sub>3</sub> showed superior *in vivo* imaging properties compared to [ $^{68}\text{Ga}$ ]NODAGA-RGD. This work demonstrates the feasibility of FSC as  $^{68}\text{Ga}$  and  $^{89}\text{Zr}$  bifunctional chelator for targeted bioconjugates with FSC-RGD trimers exhibiting advantages compared to [ $^{68}\text{Ga}$ ]NODAGA-RGD, therefore being a promising basis for development of targeted tumour imaging agents with PET.

#### New cholin analogs as potential diagnostics/therapeutics for prostate cancer.

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*Pavel Svec*<sup>2</sup>, *Barbora Liskova*<sup>1</sup>,  
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#### Introduction

Cholin is a physiological substrate for synthesis of lecithin which is the crucial component of cell membranes. As tumor cells are dividing rapidly their uptake of cholin is significantly increased. Due to this phenomenon fluorine-18 labelled cholin is already used as a positron emission tomography (PET) tracer for imaging of prostate cancer in clinics. We are testing various cholin analogs as new potential tumor tracers based on this knowledge. Our goal is to reveal suitable candidates according their *in vitro* IC50s for *in vivo* testing and then verify the suitable properties of these candidates under *in vivo* conditions. In case of these new choline analogs the fluorine-18 labelling is replaced with iodine isotopes, what allows us to use such analogs as diagnostic tracers as well as therapeutics or even as so called theranostic agents.

#### Materials/methods

We have performed *in vitro* experiments to establish IC50 values for all studied choline derivatives using PC-3 prostate cancer cell line. In this assay the compounds competed in uptake with tritium labelled choline (as natural substrate) which was measured using beta scintillation analyzer. The best candidates with IC50 comparable to choline were transferred into *in vivo* biodistribution study and to

single photon emission computed tomography (SPECT) imaging of prostate cancer mouse model too. Additionally four preclinical ADME tests have been done to determine plasmatic and microsomal stability, plasma protein binding and permeability of cholin analogs.

#### Results and conclusions

Fifty different choline analogs were tested for their IC50 values. Ten of them revealed suitable IC50s i.e. they had the same order as natural substrate (choline). These candidates were tested *in vivo* for their biodistribution in tumor model and finally ratio of accumulation tumor to blood was calculated. In case of compound PS152 this ratio was 2,09 and this compound was also used as SPECT tracer in tumor model. The ADME properties of all tested compounds were in favorable values i.e. high plasma and microsomal stability, low plasma protein binding and low artificial membrane permeability (PAMPA). We can conclude that these ten compounds are promising candidates for further testing as prostate cancer tracers/therapeutics.

#### High resolution PET insert for high field preclinical MRI: evaluation of single ring system using 7T field strength.

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### Introduction

Positron Emission Tomography (PET) is a very sensitive molecular imaging modality, but it suffers from a lack of anatomical information. We designed a novel PET insert based on monolithic LYSO crystals and high density arrays of SiPMs. The aim of this work was thus to characterize the performance of such a system for simultaneous PET-MRI acquisitions.

### Materials/methods

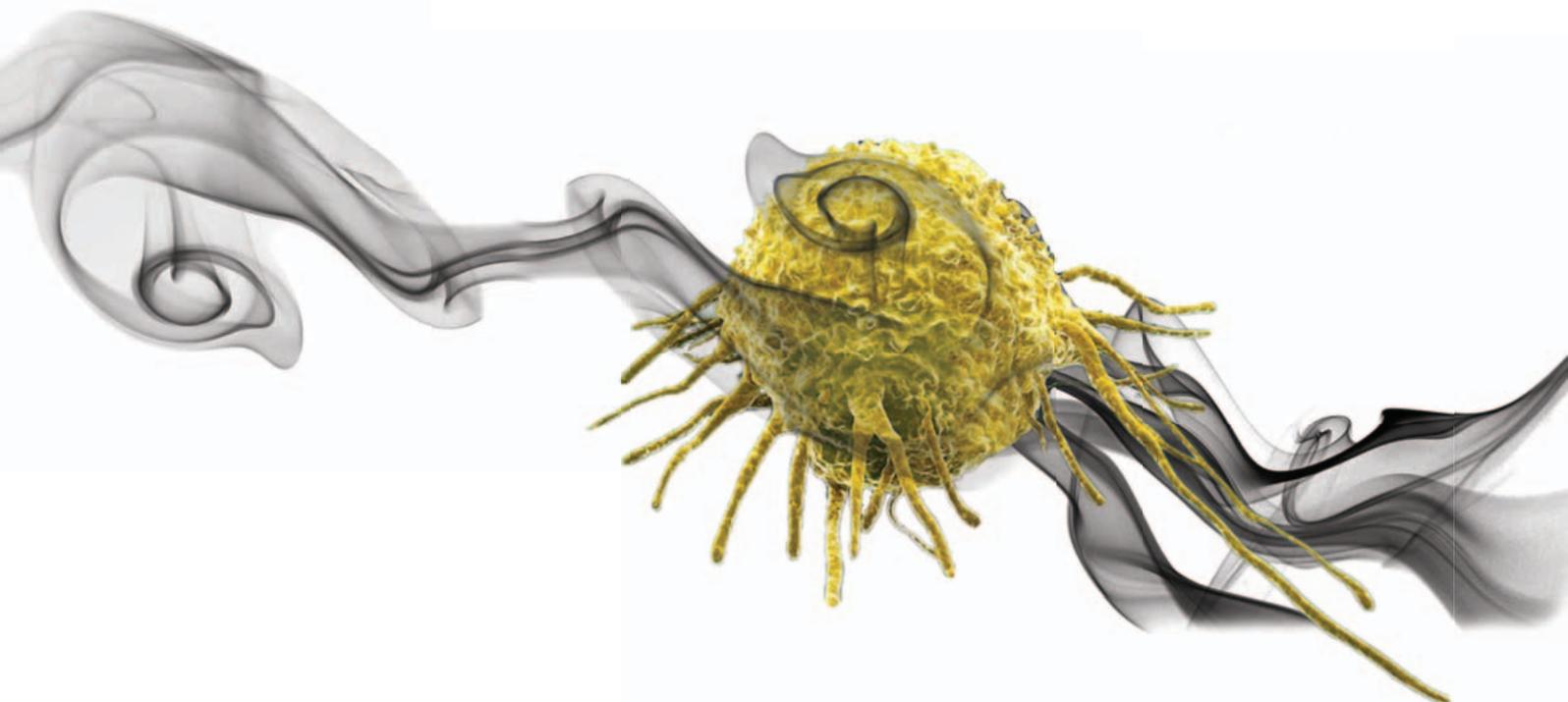
For the RF shielding, we implemented Carbon Fiber structures with tubular shape, surrounding the PET electronics. Fast Spin echo and EPI sequences were tested simultaneously with the PET insert. We assessed potential eddy currents induced by fast switching of the gradient, field homogeneity through B0 maps and PET/MR image quality (resolution, SNR, image homogeneity

etc.). For PET characterization, we followed the NEMA protocol for sensitivity, and use mini-Derenzo like phantom (filled with 150 uCi of <sup>18</sup>F-FDG) for estimation of resolution and image quality. For all tests, PET data were reconstructed using Maximum Likelihood Estimation Method (MLEM) with either voxel size of 0.5 or 0.29 mm<sup>3</sup> and at least 12 iterations. Several animal models (mouse glioma, mouse stroke, xenografts in mice, rat heart, see Figures) were evaluated to provide real in-vivo data for the quality assessment of the simultaneous PET/MRI acquisitions.

### Results and conclusions

We tested different RF pulses (20ms and 630W, with 51us and 1ms duration) and MRI sequences (RARE, EPI, etc...) without observing PET degradation of the PET image quality or eddy currents that could produce a sub-optimal MR performance. The FieldMap sequences showed in the present study no change in the B0

field with a 55mm spherical phantom when the PET insert was inside the MR scanner (SNR variation with/without PET <6%). Both RARE and EPI sequences showed ghost levels of about 2.4 to 3.6%. The PET geometry and performance were almost identical to the current in-line Albira Si system<sup>6</sup>. Sub-millimeter image resolution (between 0.9 and 0.7mm) and homogeneous-FOV spatial resolution were reached, as shown in Fig.1. The sensitivity for the one-ring PET, following the NEMA protocol, was determined to be beyond 3.5%. In-vivo evaluation demonstrated the added value of using simultaneously high resolution PET and MRI (Fig. 2). For example, a glioma as small as 0.6mm<sup>3</sup> were visualized on the MRI and small SUV differences with contralateral side were demonstrated by PET. Dynamic data were acquired using cardiac PET and MRI acquisition in the rat heart.





## Molecular targets and cancer biomarkers II.

Chairs: Jana Nekvindova, Josef Srovnal

čtvrtek / 1. prosince 2016 / Thursday / December 1, 2016 / 15:15 - 17:15

### Mesenchymal stromal cells and drug responses in breast cancer cells.

Lucia Kucerova

Cancer Research Institute BMC  
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Cells of the tumor microenvironment including mesenchymal stromal cells (MSCs) are recognized as important determinants in the tumor biology. The MSCs alter cell morphology, induce epithelial-to-mesenchymal transition, increase mammosphere formation, cell confluence and migration in breast cancer cells SKBR3. These features were attributed to molecular changes induced by MSC-secreted cytokines and chemokines. MSCs also alter drug responses of the cancer cells both by secreted paracrine factors and direct interactions with tumor cells. We have shown that the secretory phenotype and behavior of MSCs after exposure to cisplatin differs from that of the naïve MSCs. MSCs were more resistant to cisplatin in comparison to tumor cells. Cisplatin did not induce apoptosis in MSCs, but a part of MSCs population underwent senescence. Nevertheless, MSCs pretreatment with cisplatin led to changes in phosphorylation profiles of many kinases and also increased secretion of IL-6 and IL-8 cytokines which in combination with alteration in phosphorylation profile of the MSCs led to increased chemoresistance and stemness of breast cancer cells. We suggest that the exposure of the chemoresistant cells in the tumor microenvironment leads to substantial alterations and might lead to promotion of acquired microenvironment-mediated chemoresistance and stemness. We will discuss the potential mechanisms how MSCs blunt therapeutic responses in our presentation.

### Prognostic and predictive factors in primary glioblastoma Multiforme WHO grade IV patients with resection: A single-institution study.

Ondrej Kalita<sup>1,2</sup>, Marian Hajduch<sup>1,2</sup>, Radek Trojanec<sup>1,2</sup>, Magdalena Houdova Megova<sup>2</sup>, Miroslav Vaverka<sup>1,2</sup>, Lumir Hrabalek<sup>1,2</sup>, David Vrana<sup>1,2</sup>, Zuzana Sporikova<sup>2</sup>, Jiri Drabek<sup>2</sup>, Lucie Tuckova<sup>1</sup>, Jiri Ehrmann Jr.<sup>1,2</sup>, Jana Vrbkova<sup>2</sup>

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#### Introduction

Patients with GBM continue to have a dismal prognosis, with a median survival of about 12 months.

#### Materials/methods

This prospective population-based study is focused on the relation among the selected gene aberrations and overall survival of primary Glioblastoma Multiforme patients only with resection. We collected clinical data of 140 patients treated in our hospital from July 2006 to June 2014. All tumour samples were submitted to histologic analysis and were investigated for the aberrations of TP53, EGFR1, PTEN, MDM2, RB1, CCND1, BCR, 9p21 (CDKN2A, p16), 10p11, 19q13, 1p36, IDH1 mutation, and MGMT promoter methylation.

#### Results and conclusions

The younger age, Karnofsky score an chemoradiotherapy (14.6 vs 5.3 months in radiotherapy alone) at diagnosis was a positive and smoking was a negative prognostic

factor. Temporal lobe tumour origin was associated with a shorter period of Performance free status in the group with chemoradiotherapy. Relation with OS according to univariate Cox regression model: p53 high copy number (HCN), CCND1 HCN, 10p11HCN and partly MGMT promoter methylation were linked to prolonged OS. Cox proportional regression models for survival revealed that TP53 HCN was associated with a prolonged OS of all patients and the chemoradiotherapy group. CCND1 HCN, 10p11HCN and partly MGMT promoter methylation significantly extended OS in the group with chemoradiotherapy. The effect of these gene changes on OS was reduced in the group of all patients. Authors made the efforts to gain clinical and genetic factors, which easily usable in the clinical practice. Contrary literature data, there were confirmed TP53, CCND1 as predictive and prognostic factors.

### Urinary cell-free microRNA panel in detection of bladder carcinoma

Jaroslav Juráček<sup>1,2</sup>, Barbora Peltanová<sup>2</sup>, Hana Mičochová<sup>1,2</sup>, Michal Staník<sup>3</sup>, Táňa Macháčková<sup>2</sup>, Michal Fedorko<sup>4</sup>, Jan Doležel<sup>3</sup>, Ondřej Slabý<sup>1,2</sup>

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Urothelial carcinoma of the urinary bladder (UCUB) is the most common malignancy of the urinary system. Although about 80% of cases is a non-muscle invasive form of UCUB a high rate of local recurrence and progression to invasive form is observed. Early-stage tumors have very good prognosis, but current diagnostic methods (cystoscopy and urine cytology) suffer from low sensitivity. This reflects a large number of relapse, which occurs in almost 70% of superficial UCUB. This has led to the development of multiple molecular urinary biomarkers, but none are sufficiently robust to

enter clinical practice. In this study we aimed to develop a clinically applicable, specific and sensitive panel of urine microRNAs enabling early detection of UCUB and prediction of risk of progression to muscle-invasive form.

In the first phase of study we have analyzed expression profiles of 1733 miRNAs in urine supernatant of 16 UCUB patients (6 invasive, 5 high-grade non-invasive, 5 low-grade non-invasive), 17 controls, 10 RCC patients and 4 urinary tract infections (UTI) using Affymetrix miRNA microarrays. MicroRNAs able distinguish between UCUB and control groups were further validated using specific TaqMan assays and qRT-PCR method on independent cohort of 100 UCUB patients, 40 controls and 25 RCC patients (training phase – 40 UCUB, 15 controls, 10 RCC; validation phase – 60 UCUB, 25 controls, 15 RCC, 20 UTI).

Global expression profiling revealed set of 76 miRNAs significantly differentially expressed in urine of UCUB patients ( $P < 0,01$ ) compared to healthy controls, thereof 64 highly up-regulated and 12 down-regulated. These miRNAs showed specificity for UCUB even when compared to other examined cohorts (RCC, UTI). Moreover 23 miRNAs were able distinguish invasive and non-invasive forms of UCUB ( $P < 0,01$ ) and 18 miRNAs high-grade and low-grad non-invasive ( $p < 0,01$ ). Set of

12 miRNAs with highest specificity and expression level was validated in training phase of study. Based on training phase results the panel of three miRNAs was profiled. Validation phase confirmed diagnostic potential and ability of this urine miRNA-based panel to differentiate between UCUB patients and controls with high sensitivity and specificity.

Our data have shown that urinary microRNAs could serve as sensitive and specific biomarkers of UCUB and could be useful tool to increase sensitivity of standard cytological examination and to decrease high costs for long-term follow-up of UCUB patients.

### Cannabinoid receptors expression affects NSCLC patients' survival.

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### Introduction

Lung cancer is one of the leading causes of cancer death in the world. Tumor progression and patients survival depends on a lot of factors e.g. overexpression of receptors and growth factors, oncogene activation, tumor suppressor gene inactivation etc. Epidermal growth factor receptor (EGFR) is frequently overexpressed or mutated in non-small cell lung cancer (NSCLC). EGF pathway can be regulated through other receptors including cannabinoid receptors 1 and 2 (CNR1, CNR2). In a several studies there was demonstrated that synthetic cannabinoids inhibit cell proliferation, migration and

invasion in NSCLC. In our study we investigated the CNR1 and CNR2 gene expression in the NSCLC patients' tumor tissue and analyzed relationship between their expression and patients survival.

### Materials/methods

The CNR1 and CNR2 gene expression was analyzed in RNA purified from tumor tissues in 98 NSCLC patients. RNA purification was done using precipitation method from TRIzol lysates. Expression of CNR1 and CNR2 was detected using real-time RT-PCR on LightCycler 1536 from Roche. B-actin gene expression was used for gene expression normalization. Specific cut-off values were calculated for each marker using maxstat R software, ver. 3.3.1. Relationship between expression of CNR1 and CNR2 in tumor tissue and patients survival was analyzed using COX regression, Wald's test, Firth's method, Kruskal-Wallis/ANOVA test and Kaplan-Meier method.

### Results and conclusions

We investigated 98 patients (31 females and 67 males), IA-IIIa stages, with histology type adeno in 38,8 %, adeno-like in 8,2 %, non-adeno in 53,0 %. CNR1 expression was detected in 50 % of all patients, 14,3 % was bellow and 85,7 % was above the cut-off value. CNR2 expression was detected in 100 % of patients, 20,4 % was bellow and 79,6 % was above the cut-off value. We found out that patients with higher CNR1 and CNR2 gene expression have significantly better cancer-specific survival ( $p=0,018$ ;  $p<0,001$ , respectively) and disease-free survival ( $p=0,049$ ;  $p<0,001$ , respectively) than patients with low cannabinoid gene expression. Patients with CNR2 expression positivity have also significantly longer overall survival ( $p<0,001$ ) than the negative patients. This could be caused by affecting EGFR pathway. Novel approaches for targeting CNR1/2 are promising and requires further validation.

**Acknowledgement:** The work was supported by grants IGA LF UP 2016\_010, TACR TE02000058 and



NPU LO1304.

## Expression of biotransformation enzymes in hepatocellular carcinoma.

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<sup>5</sup> Masaryk Memorial Cancer Institute, Brno, Czech Republic

### Introduction

The objectives of this study are aimed at better understanding of the changes of biotransformation capacity of malignantly transformed liver tissue of hepatocellular carcinoma (HCC) with the perspective of a safer and more efficient pharmacotherapy for the patients and further uncovering of genetic and epigenetic mechanisms regulating the expression of biotransformation enzymes in general.

### Materials/methods

Pilot analyses included assessment of broad spectrum of genes coding for major enzymes of both phase I and II of biotransformation at the mRNA (qPCR, TaqMan Low Density Arrays) and protein level (immunoblotting) in paired samples of HCC and non-cancerous surrounding tissue; and correlation of their expression with clinico-pathological characteristics of the tumors and patients.

### Results and conclusions

Paired samples from 17 patients with hepatocellular carcinoma were collected from the biobank of Masaryk Memorial Cancer Institute. For biotransformation phase

I, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2S1, CYP2U1, CYP2W1, CYP3A4 (metabolizing sorafenib), CYP3A5 and their major regulators, nuclear receptors PXR, CAR and AhR were included, phase II was represented by GSTA1, GSTA2, GSTM1, GSTT1, NAT1, NAT2, SULT1A1/1A2, SULT1A1, SULT1E1, SULT2A1, UGT1A1, UGT1A4, UGT1A9 (metabolizing sorafenib) and UGT1A10. qPCR analysis revealed a significant down-regulation of expression of most of the genes/enzymes in approximately half of the patients. The data was confirmed at the protein level. Expression of the cytochromes P450 correlated with expression of their nuclear receptors and clinically with histological grade of the tumors (namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4, CYP3A5, GSTA1, NAT1, NAT2, SULT1E1, SULT2A1 and UGT1A10 at  $p=0.05$ ). The findings suggest that patients with larger high-grade tumors could be at a higher risk of adverse drug reactions (toxicity) and/or ineffective pharmacotherapy compared to common population, especially for drugs metabolized by cytochromes P450.

Supported by MH CZ - DRO (UHHK, 00179906)

### Identification of circulating microRNAs with diagnostic potential in colorectal cancer using next generation sequencing

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<sup>2</sup> Molecular Oncology II – Solid

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### Introduction:

Colorectal cancer (CRC) is one of the most common types of tumors worldwide and the second leading cause of cancer related death. The main reason of high mortality is the non-existence of appropriate methods and biomarkers for the diagnosis of early stages of CRC. Therefore, new noninvasive biomarkers for early detection are sorely needed. MicroRNAs (miRNAs) are small noncoding RNAs that post-transcriptionally regulate gene expression. Several studies have proved their presence and stability in different body fluids, thus, they could serve as novel molecules for early diagnosis and prediction of prognosis and therapeutic response of CRC patients.

### Patients and Methods:

Using next generation sequencing, expression of miRNAs has been analyzed in blood serum of 144 CRC patients, 96 healthy donors and 36 patients with polyps. Total RNA enriched for small RNAs was isolated (miRNeasy Serum/Plasma kit, Qiagen) and concentration and quality of RNA were measured (NanoDrop ND-1000, Agilent Bioanalyzer 2100). Further, cDNA library was prepared using TruSeq Small RNA Sample Preparation Kit and sequenced using MiSeq (Illumina). Finally, selected miRNAs were validated using qRT-PCR (QuantStudio, Applied Biosystems). Data was analyzed using standard and multidimensional biostatistical methods.

### Results:

In total, 54 miRNAs were identified to be differently expressed between CRC patients and healthy donors (P



< 0.01). In addition, 26 miRNAs were found to be significantly deregulated in serum samples of patients with hyperplastic, tubular or tubulovillous polyps ( $P < 0.005$ ). Subsequently, 13 miRNAs (miR-186-5p, miR-148a-3p, miR-223-3p, miR-21-5p, miR-146a-5p, miR27a-3p, miR-23a-3p, miR-126-5p, miR-4419a, miR-484, miR-142-5p, miR-376c-3p, and miR425-3p) were chosen for validation phase of the study on independent cohort of 280 CRC patients and 180 healthy donors. Using miR-93 as an endogenous control, expression levels of miR376c-3p ( $P < 0.0001$ ), miR-27a-3p ( $P < 0.0001$ ), miR-21-5p ( $P < 0.0001$ ), miR-142-5p ( $P < 0.0001$ ), miR23a3p ( $P < 0.0001$ ), miR-148a-3p ( $P < 0.0001$ ), miR-126-5p ( $P < 0.0001$ ), and miR-223-3p ( $P = 0.0316$ ) were found to be significantly higher in CRC patients compared to healthy donors. Importantly, ROC analyses showed that based on the expression of miR-142-5p we are able to differentiate between these two groups of samples with the sensitivity of 97 % and specificity of 98% (AUC = 0.9917).

### Conclusions:

Expression profiling of miRNAs in blood serum or plasma using next generation sequencing is one of the most promising approaches for identification of new non-invasive biomarkers enabling early diagnosis and prediction of prognosis and therapy response. Moreover, new miRNAs and their isoforms can be identified.

### What is the role of peripheral double positive (CD4+CD8+) T-cells in anti-tumor immunity?

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### Introduction

Peripheral double positive (DP, TCRab+CD4+CD8+) T-cells have been described in numerous species including birds (chicken) and ungulates (specifically pigs, a widely used large-animal experimental model). DP T-cells exist in humans, too, and they are generally (and unfortunately) neglected in lymphoid compartment analysis both in clinical assays and research. The number of DP T-cells in men significantly increases with age and, in patients suffering from specific cancer disorders, such T-cells may represent a significant, thus easily recognizable T-cell subset. Interesting enough, in the pig melanoma model (MeLiM), circulating DP T-cells appear to be associated in melanoma regression status. We have thus investigated the clonality of such DP T-cells in the pig melanoma model and we have tried to characterize their immunophenotype in human patients suffering from solid tumors.

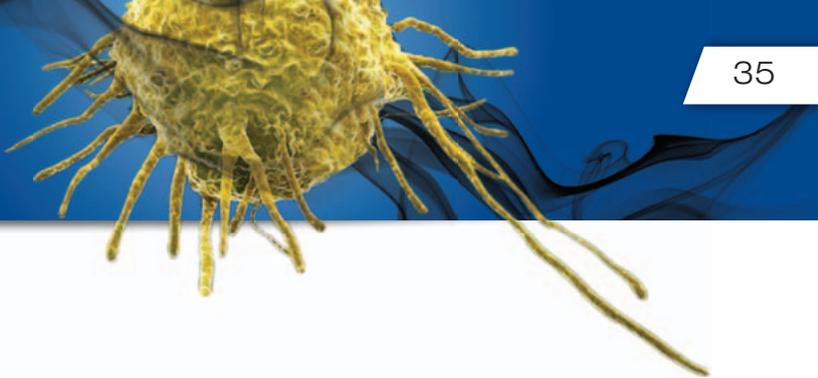
### Materials/methods

Peripheral blood lymphocytes MeLiM pigs have been characterized by multicolor immunophenotyping using directly-conjugated BD monoclonal antibodies and flow cytometry analysis on the BD FACSAria flow cytometer at different stages of melanoma progression. Dynamics

of DP T-cells subset(s) have been recorded on the signal animal level. Cells with the DP T-cell phenotype have been sorted and the TCRab receptor spectra-typing has been determined. In parallel, the DP T-cell subset have been characterized in patients suffering from solid tumors at different staged of progression/ treatment.

### Results and conclusions

Our results indicate that DP T-cells in MeLiM pigs play a significant role in anti-melanoma immunity. Sorting for CD4+CD8+ T-cells has proven the existence of clonal expansion within the DP T-cell compartment during the latest stages tumor regression. In parallel, our unpublished data suggest the involvement of peripheral DP T-cells in the anti-PRV (pseudorabies) immunity in pigs. Last but not least, human DP T-cells are an indispensable part of the lymphoid compartment of the immune system in mature (elderly) individuals. Interestingly, such cells are more frequent in patients who have suffered or are suffering from an oncological disorder. Taken together, so far underestimated DP T-cells should receive much more attention in terms of their capacity to maintain homeostasis in long-living organisms including humans, namely in terms of anti-virus / anti-tumor adaptive immunity.



## Posterová sekce / Poster section

1

### Deletion of 18q in patients with colorectal cancer - preliminary data.

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#### Introduction

Chromosomal instability (CIN) is one of the typical pathways, which lead to malignant transformation of colorectal cancer (CRC). It is characterized by widespread imbalance in chromosome number (aneuploidy) and genetic aberrations. Chromosomal instability is associated with poor prognosis and drug resistance and it is thought to be associated with errors in mitosis. As the most common genetic aberration, 18q deletion has been described. This loss is a marker for poor prognosis. As a potential tumor suppressor gene was proposed DCC (*Deleted in Colorectal Carcinoma*) gene, but its influence has never been conclusively proven. Recently, it was found that there are other three CIN – suppressor genes located on chromosome 18q, whose silencing leads to the replication stress, structural chromosome abnormalities

and chromosome missegregation during mitosis. These genes are ZNF516 (*Zinc-finger Protein 516*), PIGN (*Phosphatidylinositol Glycan Anchor Biosynthesis Class N*) a MEX3C (*MEX-3 RNA Binding Family Member C*).

#### Materials/methods

Fluorescence *in situ* hybridisation (FISH) in 50 patients with CRC was used

#### Results and conclusions

Deletions of 18q have been observed in different range, the individual patients with 18q deletions may therefore have the loss of one or more of these genes, including DCC. The aim of the study was to determine the effect of a range of 18q deletion (accordingly deletion of DCC, ZNF516, PIGN and MEX3C genes) to disease progression and whether any of these genes could be used as a prognostic marker or as a target for future biological treatment.

**Dedication:** The project was supported by Project of Sustainability of the Institute of Molecular and Translational Medicine NPU LO1304; Center of Competence for Molecular Diagnostics and Personalized Medicine TE02000058 and VES 16-32198A project.

2

### DNA affinity chromatography combined with quantitative proteomics revealed role of XPC protein in replication stress and common fragile site stability.

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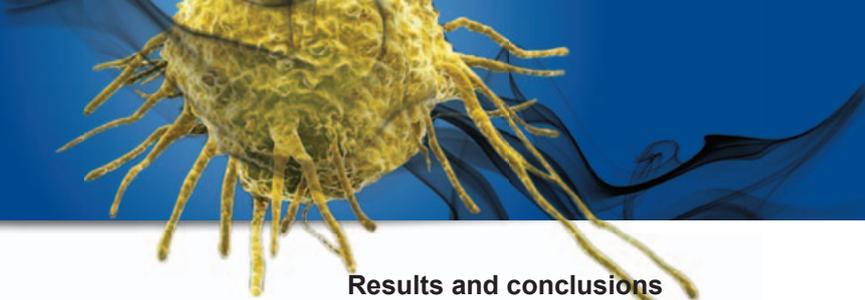
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#### Introduction

Common fragile sites (CFSs) are DNA sequences that exhibit breaks, gaps and constrictions visible on metaphase chromosomes especially under conditions of partial replication stress. CFSs are conserved regions in genomes of diverse mammalian species and are prone to chromosomal rearrangements found in many cancer cells. Molecular basis of CFSs expression was partially explained by their structural analyses. The CFSs are characterized by long distances of A-T –rich sequences forming unusual secondary structures that preclude effective replication and can be easily damaged upon replication stress. The role of previously characterized proteins (ATR, BRCA1, WRN, MSH2, SMC1 etc.) in maintenance of CFSs stability was mostly discovered using methods based on the absence or loss of functional activity of the protein and subsequent visual detection of chromosomal breaks and gaps with/without induction of replication stress directly on mitotic spreads. Several papers also utilized chromatin immunoprecipitation (ChIP) followed by quantitative PCR that allowed the detection of the studied protein at CFSs sequences. Here, we present new strategy, an unbiased proteome-wide screening, for identification of novel protein candidates responsible for CFSs maintenance.



## Materials/methods

DNA-affinity chromatography at FRA16D-fragment as a method for isolation of potential CFSs protein partners coupled to quantitative proteomics was performed. Stable isotope labeling in cell culture (SILAC) was used for protein quantification and allowed us not only to identify specific CFSs interactors but also to distinguish between those bound under normal conditions and those enriched upon aphidicolin (APH) treatment. The role of XPC protein in CFSs maintenance was verified by immunofluorescence microscopy with detection of DNA damage markers in XPC silenced cells with and without APH treatment.

## Results and conclusions

Our proteomic screening confirmed previously characterized CFSs keepers Werner helicase (WRN) and Mismatch protein 2 (MSH2) that clearly validate this approach. The most interesting candidate, DNA damage response factor XPC, whose connection to the CFSs stability has not been explored yet, was chosen for deeper characterization. XPC silenced U-2-OS cells show decrease in recruitment of ATR and its partner ATRIP to the chromatin and subsequent phosphorylation of Chk1 kinase upon replication stress. In addition to defective checkpoint response, escape of DNA intermediates into mitosis was detected by increased number of 53BP1 bodies in next generation of G1 cells. Based on our results, we suggest the role of XPC protein in recognition/resolution of hard to replicate structures arising at CFSs and in prevention of CFSs expression by promoting checkpoint signaling under replication stress. Besides important biological insight into the function of XPC protein in maintenance of CFSs stability, we also introduced new strategy of DNA affinity chromatography using DNA-structure specific bait which, combined with quantitative proteomics, provides valuable data for studying important biological processes.

## 3

### Application of DARTS (Drug affinity responsive target stability) for identification of the ligand-binding part of the protein.

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#### Introduction

The aim of the presented research is to determine the optimal conditions for identification of drug targets using DARTS (Drug affinity responsive target stability). This method is based on the fact that a ligand (such as a bioactive substance) binds specifically to a target protein and prevents complete digestion of protein by a protease. The ligand-binding part of the protein is then identified by analysis of digested fragments separated by polyacrylamide gel electrophoresis (SDS-PAGE) in combination with mass spectrometry (MS). DARTS is a useful method to identify a small molecule native protein interaction. It can be a great tool for identification or validation of molecular targets of anticancer drugs. The experiment validating the method and adapting the workflow to our conditions was performed to determine the binding sites of ibuprofen to Cyclooxygenase-2.

#### Materials/methods

Samples were treated with the said drug and then subjected to proteolytic digestion by Pronase. Digested protein fragments were electrophoresed and resolved by SDS-PAGE. Ideal drug treatment, digestion and electrophoresis conditions were determined in the process. Protein fragment bands in the electrophoretic gel were further digested by trypsin and analyzed on a Dionex UltiMate 3000 RSLC Nanoliquid chromatograph coupled to an OrbitrapElite™ mass spectrometer (Thermo).

## Results and conclusions

Peptide band intensities, as quantified by the mass-spectrometer, of drug-treated samples were compared with those of untreated controls and based on 2D and 3D map the binding site was suggested. Our data have shown that it is possible to identify the ligand binding part of the protein by SDS-PAGE and mass spectrometry but mass spectrometry analysis revealed some other sequences as well which need further study. Project is supported by IGA\_LF\_2016\_19 grant.

## 4

### Nuclear shuttling of microRNA hsa-miR-29b enhances etoposide toxicity in HeLa cells.

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#### Introduction

Etoposide, a semisynthetic derivative of podophylotoxin, is a broadly used cancerostatic agent. We investigated whether the cytotoxic effect of etoposide could be enhanced by the miR-29 family, a well-known microRNA family that affects apoptosis and DNA methylation status.

#### Materials/methods

xCELLigence system was used for evaluating cell viability in real time (96 hours). MicroRNA and its artificial counterparts were introduced into HeLa cells by transfection. The influence of miR-29 and its derivatives on Mcl-1 and Bak expression was evaluated by western blots.

#### Results and conclusions

Each member of miR-29 family



decreases the expression of Mcl-1 protein. Selective silencing of Mcl-1 by siRNA interference demonstrated that Mcl-1 plays an important role in modulation of etoposide toxicity. Our cell viability results shown that miR-29b is significantly more effective than miR-29a or miR-29c. Based on results with two mutated variants of miR-29b, one undergoes nuclear shuttling and the other stays in the cytosol, we conclude that miR-29b significantly increases toxicity of etoposide in HeLa cells due to the combination of Mcl-1 down-regulation and nuclear transport of the mature miR-29b.

This work was supported by grants LO1304 and IGA\_LF\_2016\_012.

## 5

### Surface antibodies-modification of apoferritin with encapsulated doxorubicin favorably influences the formation of protein corona.

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#### Introduction

Nano-sized carriers can encapsulate anticancer drugs and deliver them to the tumor tissue<sup>1</sup>. Upon entering the blood stream, many nanocarriers get covered by protein corona, hampering their entry to target cells<sup>1</sup>. Thus, we aimed to study the protein corona formation around bare and Abs-modified apoferritin bearing doxorubicin.

#### Materials/methods

Horse spleen bare and antibodies-modified apoferritin, was used as a nanocarrier for doxorubicin.

Protein coronas were formed using EDTA-treated plasma from healthy human donor. Unbound proteins were removed and samples were subjected to 2D electrophoresis with subsequent protein identification by mass spectrometry.

#### Results and conclusions

Bare horse spleen apoferritin showed only slight protein corona formed by the most plasma-abundant 66.5 kDa protein albumin. Very intensive protein corona was formed around bare apoferritin with encapsulated doxorubicin. This corona was formed in small percentage by albumin or 80 kDa transferrin, but mostly by high-molecular proteins, such as 340 kDa fibrinogen or >400 kDa mannan-binding lectin. The antibodies-modified apoferritin with encapsulated doxorubicin showed significant decrease in the amount of proteins bound onto its surface while maintaining their profile. It can therefore be concluded that the surface modification with antibodies favorably affects the formation of protein corona, and thus the entry of anticancer drug doxorubicin inside cancer cells.

The authors gratefully acknowledge financial support from the Grant Agency of the Czech Republic (NANO-CHEMO GA CR 14-18344S).

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## 6

### Size-related toxicological aspects of PVP-capped platinum nanoparticles.

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#### Introduction

Platinum drugs are used for the treatment of variety of solid tumors, including breast, ovarian, colorectal, neuroblastomas, etc.<sup>1</sup>. Due to undesired side effects, novel types of formulations are searched. Thus, we aimed on synthesis and testing of platinum nanoparticles of various sizes (PtNPs).

#### Materials/methods

PtNPs coated with polyvinylpyrrolidone were synthesized and characterized using TEM, DLS and FT-IR. Prostate (LNCaP), breast (MDA-MB-231), neuroblastoma (GI-ME-N) cell lines were used for testing of cytotoxicity. We also test the PtNPs-induced haemocompatibility, genotoxicity and free radicals formation.

#### Results and conclusions

Different sizes of PtNPs covered with polyvinylpyrrolidone were confirmed by TEM analysis and by DLS. The hydrodynamic diameters were as follows: Pt10-11.7 nm, Pt29-13.5 nm, Pt40-18.2 nm. Synthesized PtNPs possess exceptional haemocompatibility (no observed haemolysis) and genotoxicity (negligible amount of DNA fragments). Cytotoxicity dependence on different size of PtNPs was confirmed, where the highest toxicity was demonstrated for the smallest PtNPs (Pt10), and the lowest cytotoxicity was observed for the largest PtNPs (Pt40). Noteworthy, high resistance was observed in GI-ME-N cell line, where IC<sub>50</sub> was > 50 µg/ml. In the rest of tested cell lines the IC<sub>50</sub> was determined as approx. 6 µg/ml. It was found, that PtNPs cytotoxicity is due to formation of reactive oxygen species. Exceptional compatibility with blood environment



and no genotoxicity predispose PtNPs as an interesting potential therapeutic agent for breast or prostate anticancer therapy.

The authors gratefully acknowledge financial support from the AZV project 15-28334A.

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### Cross-Interactions between Trks Receptors and Neurotrophins: Insights from Molecular Docking and Molecular Dynamics.

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#### Introduction

One of the major challenges in targeting Tyrosine Receptor Kinases (Trks) is the side effects resulting from simultaneous targeting of several Trk kinases at the same time. Thus, the aim of this work is to study the cross-interactions between Trks and neurotrophins (NGF, NT3 and NT4/5) by comparative modeling.

#### Materials/methods

Structures were prepared by chain trimming and reconstruction of missing atoms/residues. Approximately 30 dockings were performed on GRAMM-X Protein-Protein Docking Web Server v.1.2.0. Molecular dynamics in Ascalaph MD were performed at 300K 10.0 ps with 2.5 fs steps.

#### Results and conclusions

The known interactions from structures of TrkA-NGF and TrkB-NT4/5 were used to superimpose the rest of the Trks with NGF and NT4/5, followed by analysis of intermolecular binding by molecular dynamics. Three rounds of molecular docking were used to predict possible interactions of Trks with NT3 in the presence and absence

of p75 protein. Our preliminary findings showed that Trks interact with NT3 in perpendicular fashion to the interaction by p75 and in the non-bound region of the NT3 dimer, thus allowing for the formation of the known Trk-NT3-p75. We believe the preference of some receptors to particular neurotrophins is attributed to non-conserved residues in a divergent loop in the Trks ligand binding domains. The loop that play role in selectivity spans 332-340 in TrkA, 334-340 in TrkB, and 352-357 in TrkC. Residues identified to affect binding to NGF include V336 in TrkB and E322, K367 and T369 in TrkC. Residues identified to affect binding to NT4/5 include Q350 in TrkA and E322 in TrkC. The understanding of the selectiveness and divergence of the Trks is important for development of therapeutics.

The authors gratefully acknowledge financial support from the AZV project 15-28334A.

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### Sarcosine up-regulates expression of genes involved in cell cycle progression of metastatic models of prostate cancer.

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#### Introduction

In Europe, prostate cancer (PCa) is the most common solid neoplasm. Sarcosine represents a potential urinary biomarker exploitable for early PCa diagnosis<sup>1,2</sup>. It also acts as potential oncometabolite. Thus, we investigated the effect of sarcosine treatment on behavior of PCa tumors *in vivo*.

#### Materials/methods

Metastatic PCa cell lines (PC-3 and LNCaP) were stimulated with sarcosine, as well as mice used for xenograft studies. cDNA electrochemical microarray and semi-quantitative PCR were used to identify sarcosine effects on expression of genes involved in a cell cycle and apoptosis.

#### Results and conclusions

Supplementation of metastatic PCa cells with sarcosine stimulates their proliferation *in vitro*. In PCa murine xenografts, sarcosine treatment induced a tumor growth and significantly reduced weight of treated mice compared to non-treated individuals. In tumor tissue, we found significantly increased glycine, serine and sarcosine concentrations post-treatment, as well as increased levels of sarcosine dehydrogenase. Two other enzymes involved in sarcosine pathway, dimethylglycine and glycine-N-methyltransferase were affected only slightly. cDNA electrochemical microarray followed by validation using the semi-quantitative PCR revealed 25 differentially expressed genes in PC-3, 32 in LNCaP tumors and 18 overlapping genes, which are involved in the negative regulation of apoptosis and cell cycle. Results indicate that direct, repeated administration to sarcosine has significant stimulatory effects on the growth of ectopic prostate tumors.

The authors gratefully acknowledge financial support from the GACR project 16-18917S.

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### A role of V-ATPase in cancer cells chemoresistance.

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#### Introduction

The cytostatic drugs have become very efficient in cancer therapy. However, they are known to induce resistance. A proton pump vacuolar-ATPase (V-ATPase) plays an important role in development of drug resistance, by upregulation of its gene. This resistance is associated with V-ATPase-mediated vacuolar trapping of drugs and it can influence its anticancer action. This supports that V-ATPase should be an effective target of anticancer strategy.

#### Materials/methods

Western blot and RT-PCR were used to detect expression of V-ATPase in sensitive (UKF-NB-4) and resistant neuroblastoma cells (UKF-NB-4DOXO, UKF-NB-4ELLI, UKF-NB-4CDDP). Confocal microscopy and staining of the cells with a lysosomal marker Lyso Tracker Red were used to detect ellipticine and doxorubicine in lysosomes. The cytotoxic effect of cytostatics to neuroblastoma cells in the presence or absence of bafilomycin A (V-ATPase inhibitor) was evaluated by detection of apoptosis (Annexin V/DAPI labeling).

#### Results and conclusions

An increase in expression of V-ATPase was significant in neuroblastoma cells resistant to doxorubicin and ellipticine. Cell lines resistant to cisplatin, however, showed downregulation of V-ATPase. Pretreatment of neuroblastoma

cells with a V-ATPase inhibitor bafilomycin A enhanced markedly the anticancer effect of ellipticine and cisplatin against neuroblastoma cells, both the sensitive and resistant to ellipticine or cisplatin. Resistance to doxorubicin and ellipticine in the tested cells is associated with V-ATPase-mediated vacuolar trapping of these drugs, which may be decreased by bafilomycin A. Moreover, we endorsed V-ATPase as one of the survival mechanisms of neuroblastoma UKF-NB-4 cells and as a promising selective therapeutic target to be considered for future trials.

10

### Effect of mutations in the post-translation modifications sites of carbonic anhydrase IX and study of their functional properties.

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#### Introduction

Carbonic anhydrase IX (CA IX) is a tumor-associated transmembrane protein, which is regulated by hypoxia. CA IX is implicated especially in pH regulation, but also in cell proliferation, cell adhesion and tumorigenic processes. CA IX exists in an oligomeric form. Oligomers are stabilized by intermolecular disulfide bonds between cysteines. CA IX has also the ability to internalize into the cells after binding some specific ligands or antibodies. The aim of this study was to prepare mutations in the sites responsible for oligomerization and internalization and to investigate their effect to post-translation modifications of CA IX and its biological properties.

#### Materials/methods

We used C33 cell line (human cervical carcinoma cells) for our experiments. Point mutations of CA IX were

prepared in sites responsible for its dimerization and internalization. Cells expressing CA IX with these point mutations were analyzed by western-blotting, immunofluorescence, proliferation and migration assays, wound-healing and ELISA.

#### Results and conclusions

There are 4 important cysteines in one CA IX molecule, namely Cys156, Cys174, Cys336 and Cys409. Our investigation showed that presence of two intermolecular disulfidic bonds is necessary for dimerization of CA IX. These bonds are formed between cysteines on positions 174 and cysteines on positions 409 on two nearby molecules. When these cysteines were replaced with serines the double mutant was not capable to form a dimer. CA IX with this double mutation is present only as a monomer. We also detected that di-leucine and cysteine motif comprising of leucines on position 416 and 418 and cysteine on position 409 are essential for the internalization of CA IX triggered by binding of the specific antibody. These leucines were replaced with alanines and cysteine with serine. These mutated forms of CA IX are not able to internalize into the cell but they remain localized on the cell membrane. Another interesting observation is that cells expressing CA IX with the double mutation of Leu416, Leu418 exhibit an increased motility in comparison to natural CA IX.

**Acknowledgement:** This work was supported by the grant from Slovak Scientific Grant Agency (VEGA 2/0122/16, 2/0108/16 and 2/0081/14).

11

### Detection of ionizing radiation biomarkers in mouse hair follicles.

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### Introduction

Hair follicles are very easy obtainable biological material, which allow repeatable and noninvasive sampling. It can be used as a rich source of biomarkers of intrinsic and extrinsic influences. Utility of such material is promising in experiments with mouse models, where repeatable, long-term and harmless sampling is hardly feasible. Ionizing irradiation is a suitable model for the study of senescence, apoptotic pathways or neoplastic transformation.

### Materials/methods

Hair follicles were collected from one healthy BALB/c mouse into the RNA later for RT-qPCR and into the 4% formaldehyde for immunofluorescence with special vacuum collector. Material was collected before 2.6 Gy X-ray irradiation and 30 min, 3 h, 24 h after irradiation. Then the next dose of 2.6 Gy were applied and the last samples were collected 24 h after that. RNA was extracted using miRNeasy mini kit (Qiagen) and two-tube RT-qPCR was performed for SESN1, p21, MDM2 and HPRT gene expression analysis. Immunofluorescence was done for  $\gamma$ -H2AX and DAPI was used for the nucleuses visualization.

### Results and conclusions

Total RNA extracted from one pluck is usually less than 35 ng but it has good quality (RIN  $\geq$  7.5) even after the storage at room temperature. We detected a good response of ATM/CHEK2/p53 pathway genes after 2.6 Gy of ionizing irradiation. We confirmed higher levels of suppressor genes p21 and SESN1 expression and lower level of oncogenic MDM2 expression. The strongest response was detected 0.5 and 3 h after irradiation (FC  $\geq$  1.5). We didn't find any uniform cumulative irradiation effects after the second

dose. Our data were confirmed by immunofluorescence for direct marker of ionizing radiation  $\gamma$ -H2AX. We found strong signal within 0.5 and 3 hours after irradiation and weak signals after 24 and 48 hours. There were no significant differences between samples after 24 and 48 hours.

**Dedication:** IGA\_LF\_2016\_010 and TAČR TE02000058

### 12

#### DNA methylation changes in tumour invasivity associated genes in breast cancer.

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### Introduction

Breast cancer is one of the most common type of epithelial tumours in women and approximately 20% patients die as a consequence of metastatic disease. Before the epithelial cancer cells are able to release themselves from primary tumour, they need to undergo the epithelial mesenchymal transition (EMT). Recently, it was found that also EMT and addition to other processes of tumourigenesis are regulated by epigenetic mechanisms like DNA methylation. Therefore we hypothesize that specific methylation changes in genes associated with EMT and degradation of extracellular matrix could contribute to metastatic processes.

### Materials/methods

In our study we quantified DNA methylation levels in promoters of three EMT specific genes (TWIST1, SNAI1, and SNAI2) and one gene for degradation of extracellular matrix (uPA). We evaluated DNA methylation in blood samples and tumour tissues from 29 breast

cancer patients using quantitative pyrosequencing method. Thirty three blood cells samples and 10 normal breast tissues from healthy women were used as controls.

### Results and conclusions

We revealed significantly increased DNA methylation levels in TWIST1 and SNAI2 genes ( $P=$  0.001 for both) in blood cells of patients in comparing with control blood samples. Significantly increased methylation levels in TWIST1, SNAI2 and uPA genes ( $P=$  0.002,  $P=$  0.003 and  $P=$  0.001) we found in tumour tissues compared to normal breast tissues. Our preliminary results indicated that patients with invasive breast carcinoma had increasing levels of gene specific methylation what could have an impact on development of metastases. The new knowledge in epigenetic regulation of EMT and other invasive mechanisms can bring the novel epigenetic biomarkers for monitoring of metastatic potential.

### 13

#### X-irradiation-induced senescence model: detection of senolytic drugs in high-throughput system.

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### Introduction

Cellular senescence is an irreversible state of a proliferation arrest, cells are still metabolically active but functionally inactive in the tissue. This condition can be induced by different stimuli: DNA damage and telomere shortening, stress impulses, chronic mitogenic signaling, oncogene activation or tumor suppressor inactivation. Senescence can serve as a beneficial protection against



hyperplastic pathologies (most lethal is tumorigenesis), on the other hand can lead to the degeneration of tissues. Senescent cells accumulate in mammalian body with physiological aging and is associated with age-related disorders. Senolytic drug discovery can help to the highly selective destruction of senescent cells in the ageing tissue and lead to the tissue function renewal.

#### Materials/methods

X-ray senescence model was obtained by irradiation (10 Gy) of 3 human cell lines (MRC5, U2OS, HCT116). Cells were plated in 384-well plates, irradiated and subsequently cultivated for 5 weeks. Unirradiated cells served as the control. Cells were treated with 16 compounds, each compound in 7 concentrations, for 5 days. Actinomycin-D, mitomycin-C and DMSO were used as the system function control. Quercetin and dasatinib were tested as well, they were previously published as the senolytic active drugs. Then MTT cell viability and proliferation assay was performed and absorbances were measured, finally  $IC_{50}$  values were calculated. Senescence-associated  $\beta$ -galactosidase activity assay and the final analysis were optimized.

#### Results and conclusions

$IC_{50}$  values for each compound from senescent cells plate and control (unirradiated) plate were compared. Drugs with positive senolytic effect were selected. In total we obtained 11/16 positively acting drugs that were active at least in one cell line. 2 compounds were active in all 3 tested cell lines, 5 compounds were active in 2 cell lines and 4 compounds were active in only 1 cell line, the senolytic activity of the rest 5 compounds was not observed. Quercetin and dasatinib were positively active in 2 from all 3 cell lines, their combination worked as well. Senescence-associated  $\beta$ -galactosidase activity assay will be used as an additional method for validating of the senolytic effect. Drugs that will be confirmed as the active ones in both tests will be used in *in vivo* testing.

Supported by IGA LF UP 2016\_010.

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### Metformin and melatonin administration in a rat model of breast cancer improves liver antioxidant status.

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#### Introduction

Reactive oxygen species are involved in pathogenesis of various diseases including breast cancer which is the most frequent malignancy in women worldwide. Increased levels of oxidative stress biomarkers (e.g. lipid peroxidation products, protein oxidation products and DNA adducts) are often found in breast cancer patients. Oxidative stress may also induce structural modifications of estrogen and progesterone receptors and influence disease progression in hormone-responsive breast cancer. Therefore, attenuation of oxidative stress by various substances is one of the hypothesized mechanisms how chemopreventive substances inhibit cancer cell proliferation.

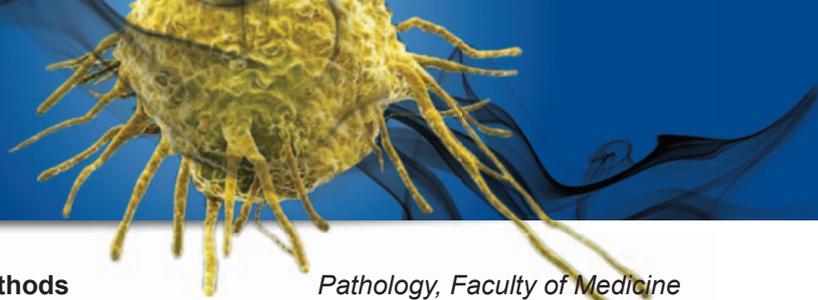
#### Materials/methods

Female Sprague-Dawley rats (Velaz, Prague, Czech Republic) aged 30 days were used in the experiment. The animals were fed the high-fat diet (10% total fat) and drank tap water or melatonin solution, respectively, *ad libitum*. Mammary carcinogenesis was induced by NMU (50 mg/kg i.p.) administered on the 42<sup>nd</sup> postnatal day. Chemoprevention with metformin and melatonin was initiated 12 days before the carcinogen administration and lasted until the termination of the experiment. Animals were assigned randomly to one of five experimental groups: NMU, control

group without chemoprevention; MF, chemoprevention with metformin; MEL, chemoprevention with melatonin; MF+MEL, chemoprevention with combination of metformin and melatonin; and INT, intact group. Metformin was administered in a diet (2000 ppm), melatonin was administered in tap water (20 mg/L, between 3 p.m. to 8 a.m., only water from 8 a.m. to 3 p.m.). The experiment was terminated 16 weeks after carcinogen administration. Liver samples were obtained for evaluation of oxidative status (thiobarbituric acid reactive substances concentration, oxidatively modified protein content, total antioxidant capacity, activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase), hepatotoxicity (levels of alanine aminotransferase and aspartate aminotransferase) and aerobic status (activity of lactate dehydrogenase and succinate dehydrogenase). Data were evaluated using one-way analysis of variance or the Kruskal-Wallis test, respectively.

#### Results and conclusions

Carcinogen administration enhanced oxidative stress as the level of lipid peroxidation (measured by thiobarbituric acid reactive substances concentration) and oxidatively modified protein content increased in the NMU group compared with the INT group. Induction of antioxidant enzymes activity by chemopreventive agents was particularly noticeable in the MEL group. Chemoprevention-induced increase in antioxidant defence attenuated lipid peroxidation, decreased oxidatively modified protein content and, in case of combined treatment, increased total antioxidant capacity. In conclusion, treatment with metformin and melatonin enhanced liver antioxidant defence system and attenuated oxidative stress in a rat model of breast cancer. This effect was more pronounced in combined treatment in comparison with single use of substances. Overall improved



antioxidant status may have an impact on cancer progression.

## 15

### Importance of promoter methylation of GATA5 and THSB1 genes in malignant tumors of the sinonasal area.

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#### Introduction

Epigenetic changes are considered to be frequent events during tumor development. Hypermethylation of promoter CpG islands represents an alternative mechanism for inactivation of tumor suppressor genes, DNA repair genes, cell cycle regulators and transcription factors. The aim of this study was to investigate promoter methylation of specific genes in sinonasal carcinoma by comparison with normal sinonasal tissue.

#### Materials/methods

To search for epigenetic events we used MS-MLPA (Methylation-specific Multiplex ligation-dependent probe amplification) to compare methylation status of 57 formalin fixed, paraffin embedded tissue samples of sinonasal carcinomas with 18 control samples. Detected changes in methylation were compared with clinicopathological characteristics.

#### Results and conclusions

Using a 20% cut-off for methylation, we observed significantly higher methylation in *GATA5* ( $p=0.0005$ ), *THSB1* ( $p=0.0002$ ) and *PAX5* ( $p=0.03$ ) genes in the sinonasal cancer group compared to the control group. Methylation in *THSB1* gene was significantly higher in samples of high grade carcinoma compared to low grade carcinoma ( $p=0.028$ ). These observations provide evidence that changes in methylation of these genes may be one of the major mechanisms in sinonasal carcinogenesis. In addition, changes in methylation could be potentially used in screening of sinonasal cancer and may have implications for future individualised therapy based on epigenetic changes.

## 16

### The prognostic aspect of the perioperative circulating tumor cells detection in non-small cell lung cancer patients.

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#### Introduction

About 10 years ago, circulating tumor cells has been widely introduced as a clinical research tool. Elevated levels of circulating tumor cells (CTCs) predict a poor prognosis in patients with epithelial cancer. However, CTCs number paucity, which detected in early lung cancer stage, complicates the analysis. It was reported better results from blood drawing closer to primary tumor (namely from the pulmonary vein) as well collection from their accumulation reservoir (bone marrow). To verify this hypothesis we have conducted detection of the circulating/disseminated tumor cells (CTCs/DTCs), by comparing blood samples from peripheral blood, tumor-draining blood and bone marrow.

#### Materials/methods

The CTCs/DTCs presence has been detected by quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) in peripheral blood, tumor-draining blood and bone marrow before/ during surgery in 119 IA-IIIa stages of non-small cell lung cancer (NSCLC) patients. Cut-off gene expression values were determined for CEA, EGFR, LunX, c-met and EpCAM mRNA copies, for showing of the CTCs/DTCs presence/absence. Statistical analysis was carried out using software R, ver. 3.2.3 and additional R packages. The Kruskal-Wallis/ANOVA tests, Wilcoxon exact rank/Student's t-tests, Bonferroni, Kaplan-Meier method, log-rank tests and Cox regression univariate analysis were used.

#### Results and conclusions

We found no correlations between



the presence of CTCs/DTCs and conventional clinical prognostic characteristics, such as the tumor size, local lymph node metastases, stage of the disease, tumor histological type and the malignancy level. Tobacco-smoking status was correlated with EGFR mRNA positive cells in peripheral blood samples ( $p=0.021$ ) and for EpCAM mRNA positive cells in bone marrow samples ( $p=0.032$ ). During follow-up period cancer cause of death was determined in 32 (28,3%) patients from 54 (45,4%) patients that died. The presence of CEA mRNA-positive CTCs/DTCs in tumor-draining blood samples indicated significantly shorter CSS and in bone marrow indicated significantly shorter OS and CSS, compared to patients without CEA mRNA-positive CTCs. The EpCAM mRNA-positive CTCs/DTCs presence in tumor-draining blood has affected the CSS ( $p=0.026$ ) and DFS ( $p=0.041$ ). Detection of CTCs/DTCs in the blood/bone marrow of patients is a "liquid biopsy" embodiment, allowing predict the course of the disease in early time. Evaluation of the CTCs/DTCs may be an important step to optimize the adjuvant and palliative treatment in the future.

**Acknowledgement:** The work was supported by grants IGA LF UP 2016\_010, TACR TE02000058 and NPU LO1304.

**17**

### NGS technology as a way for monitoring of IgVH clones in ALL patients - pilot study.

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#### Introduction

Acute lymphoblastic leukaemia (ALL) is the most common form of cancer

in children but it also affects adults. The clinical course of ALL is highly variable. Nowadays, determination of the mutational status of rearranged immunoglobulin heavy chain variable (*IGHV*) genes in large series of patients ALL has shown powerful and independent prognostic value. Thus, *IGHV* mutational status is being considered as a one of the most important prognostic factors to stratify patients in clinical prognosis. Newly introduced massively parallel sequencing technology enables through deep sequencing of rearranged IgVH CDR3 regions analysis of a previously inaccessible level of BCR repertoire. The CDR3 diversity reflects clonal composition, the potential antigenic recognition spectrum, and quantity of available B cell responses.

In the present study, we have used NGS profiling to follow up minimal residual disease (MRD) in samples from our ALL patients.

#### Materials/methods

Peripheral blood mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation. For *IGHV* analysis we used both complementary DNA (cDNA) and genomic DNA (gDNA). First step was fragment analysis with Biomed2 primers for clonality testing. Samples for NGS we amplified with Biomed2 primers (FR1 and FR2) with adapters for Multiplicom MIDs. Prepared libraries were analyzed using paired-end Illumina MiSeq sequencing. Raw data were processed by our own pipeline and on-line Vidjil software (<http://www.vidjil.org/>).

#### Results and conclusions

We analyzed samples gained from 8 patients with ALL in time. We were able to set up the method's sensitivity up to  $1 \times 10^{-5}$ . Some discrepancies in IgVH CDR3 sequences repertoire between DNA and cDNA were observed. NGS showed the potential to be a very sensitive method for MRD monitoring. Furthermore, within this monitoring we can detect a new potential pathologic clone.

Supported by MH CZ - DRO (UJHK,

00179906)

**18**

### Oncogenic action of S100P in cancer includes sequestration of p53 and stimulation of therapy-induced senescence.

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#### Introduction

S100P overexpression in different types of cancer has been reported to correlate with the aggressivity of tumors and response to therapy. The role of S100P in drug resistance remains controversial. We focused our experimental studies on demonstration of S100P-p53 interaction and its effects on the response to chemotherapeutic treatment.

#### Materials/methods

S100P-p53 interaction was demonstrated in GST pull-down experiments, Far Western blotting, co-immunoprecipitation and proximity ligation assay. Biological effects of S100P - cell proliferation and cytotoxic responses during chemotherapeutic agent treatment - were evaluated in the real-time xCELLigence system, confocal microscopic analysis, colony outgrowth, SA- $\beta$ -gal senescence assay. All experiments were done in two models: overexpressing S100P-transfected cells, as well as cells with silenced endogenous S100P.

#### Results and conclusions

We confirmed that S100P binds p53 and through interfering the complex p53-HDM2 increases the p53 level. Paradoxically, the S100P-induced p53 is unable to activate its transcriptional targets *hdm2*, *p21*,



and *bax* following the DNA damage. This appears to be related to reduced phosphorylation of serine residues in both N-terminal and C-terminal regions of the p53 molecule. Furthermore, the S100P expression results in lower levels of pro-apoptotic proteins, reduced cell death in response to cytotoxic treatments, followed by stimulation of therapy-induced senescence and increased clonogenic survival. Thereby, the S100P protein may contribute to the outgrowth of aggressive tumor cells resistant to cytotoxic therapy and promote cancer progression.

**Financial support:** ERDF Biomed park (ITMS 26240220087).

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### The effect of metallothionein - doxorubicin combination approach in the targeted anticancer therapy.

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### Introduction

Metallothionein (MT), a low molecular weight family of metal binding proteins, plays an important role in the organism defense against various threats such as oxygen radicals (ROS), heavy metals, chemotherapeutics and UV and ionizing radiation. The unique property of MT is the regulation and control of redox-homeostasis, thiol-disulphide equilibrium in the cell in synergism with GSH. The structure of MT includes the two domains, alpha and beta. The metal ions in the MT structure stabilize its molecule. It was proved; the MT is able to bind zinc, copper, cadmium and so mercury, lead, arsenic, nickel, silver and many others. However, the MT gene promoter has response elements to metals (MRE), to glucocorticoids (GRE) and to oxidative agents, electrophilic compounds and xenobiotics (ARE). Expression and synthesis of MT are induced not only for heavy metals, but also for stress-hormones and cytokines; free radicals, peroxides, cancerogens and antitumor drugs. The MT could play protective role in anthracycline cardiotoxicity caused by doxorubicin during the anticancer treatment. Nowadays, the involvement of MT to cardiac cell protection in oncologic treatment efforts the nanomedicine.

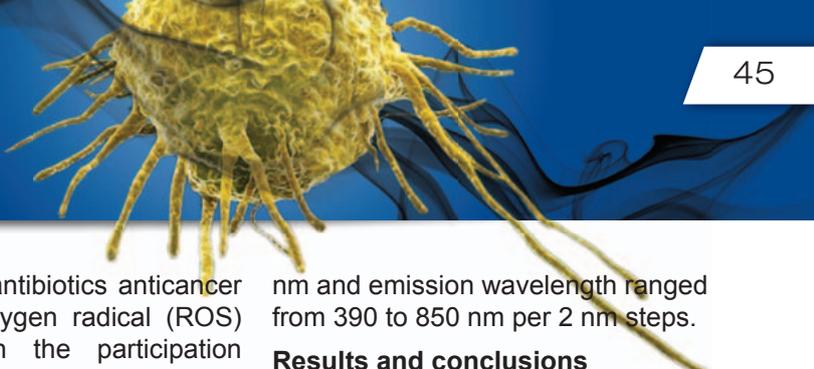
### Materials/methods

Doxorubicin and all other chemicals were purchased from the Sigma-Aldrich, unless stated otherwise. Spectrophotometric measurements of total protein content and oxidative stress (ABTS, DPPH) were carried using an automated chemical analyser BS-200 (Mindray, Shenzhen, China). Reagents and samples were placed on cooled sample holder ( $4 \pm 1$  °C) and automatically pipetted directly into plastic cuvettes. Incubation proceeded at  $37.0 \pm 0.1$  °C. Mixture was consequently stirred. The washing steps of pipetting needle with distilled water (18 mΩ) were

done in the midst of the pipetting. For detection itself, the following range of wave lengths can be used -340, 380, 412, 450, 505, 546, 570, 605, 660, 700, 740 and 800 nm. The instrument was operated using the BS-400 software (Mindray). The fluorescence of doxorubicin was measured by multifunctional microplate reader Tecan Infinite 200 PRO 132 (TECAN, Switzerland). Excitation wavelength was 480 nm and emission wavelength ranged from 550 to 850 nm per 2 nm steps.

### Results and conclusions

Nanomedicine is perspective field of the nanotechnology. The main idea is to apply an unique properties of „Nano-word” such as better bioavailability, surface properties, enhance therapeutic effect and possibility of targeting or in the case of nanocages - release of therapeutic in the damaged tissues. The construction of nanocarriers could be done using polymeric, biodegradable materials such as chitosan or PEG. Preferably, it could prove a reduced toxicity and immune responses. Moreover, the decoration of nanocarriers by specific peptides or antibodies could provide targeted transport of the therapeutic directly to the affected tissue. In this experiment, the antioxidant properties of doxorubicin and MT were studied using spectroscopic based methods such as DPPH, FRAP, ABTS and ORAC. The effect of various MT concentrations was compared with the therapeutic dose of doxorubicin. The results were statistically evaluated and the correlation between mentioned methods was proved. From the obtained data it is clearly follows the MT has antioxidant and protective effect against oxidative stress produced by doxorubicin. The application of metallothionein in such nanotechnologic modification is perspective approach to design a nanocarrier due to the fact of the synergic and protective effect of the metal binding protein metallothionein. The further experiments should be focused on the clarifying the molecular mechanism of the MT action.



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**A new type of carbon nanotransporter for doxorubicin targeted delivery in breast cancer: the effect of metallothionein.**

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**Introduction**

Anthracyclines antibiotics have a wide spectrum of the potential use in anticancer treatment of malignant and solid haematological tumours. The group of daunorubicin, doxorubicin, idarubicin, epirubicin (the structures are shown in the Figure 1) evinced a higher limitation of their use, appreciable cardiotoxicity (cumulative dose is 300-550 mg/m<sup>2</sup>). The main mechanism of action of

anthracyclines antibiotics anticancer effect is the oxygen radical (ROS) generation with the participation of the creation Fe<sup>2+</sup> ions complex with anthracyclines antibiotics (commonly used doxorubicin). The lipid peroxidation is induced by the complex penetration through lipid membrane of cardiomyocyte. Moreover, in the case that the DOXO is already in the cell the complex DOXO- Fe<sup>2+</sup> or DOXO-semiquinon is formed. These molecules are able to produce ROS and lead to lipid peroxidation of the membranes, mitochondrial damage, changes of energetic metabolism of the cell and apoptosis induction. In addition, the molecules of DOXO affects to the energetic metabolic pathway. Except this fact, the strong affinity of anthracycline antibiotic to the DNA is not inconsiderable due the fact that compounds intercalate into the double helix structure DNA very fast. The formation of DNA adducts causes the disruption of the replication process, and also the transcription damage. In our previous experiments, the formation of DNA-DOXO adduct have been detected and described by change of biophysical signal of DNA. Our designed procedure could be used for the monitoring of the effectiveness of the applied cytostatic therapeutics.

**Materials/methods**

Doxorubicin and all other chemicals were purchased from the Sigma-Aldrich, unless noted otherwise. Voltammetric measurements were carried out using μ-AutolabIII (Eco Chemie, Amsterdam, The Netherlands) potentiostat/galvanostat controlled by Autolab GPES software. The cyclic voltammetric parameters were as follows: initial potential 0 V; vertex potential 1.5 V; end potential 0 V; step potential 5 mV. Absorption spectra of doxorubicin were measured with WWR Germany spectrophotometer. The fluorescence of doxorubicin was measured by multifunctional microplate reader Tecan Infinite 200 PRO 132 (TECAN, Switzerland). Excitation wavelength was set to 360

nm and emission wavelength ranged from 390 to 850 nm per 2 nm steps.

**Results and conclusions**

Nanotechnology is the field of science and industry with the aim of targeted manipulation with individual atoms lead to the formation of compound and materials with the unique properties or objects composed of single atoms. Furthermore, these objects could be employed as an miniaturized tools, robots or integrated circuits which are more smaller than manufactured by common technology. The perspective branch of nanotechnology is nanomedicine. Decrease of the toxicity of anthracycline antibiotics could provide a wide range of therapeutic approaches and also, the nanotechnology surface modifications of the drug delivery systems are one of the promising ways in the cancer treatment. In our experiment we focused on several nanotechnology surface modification of the doxorubicin for its therapeutic concentration increase in the place of the malignant tumour. The interaction of doxorubicin and metallothionein has been studied in our work by various biophysical approaches. The redox properties of the mentioned molecules and their mutual influence were monitored. It was shown, the MT is possible to use as a protective molecule against DOX-mediated cardiotoxicity. However, the molecular and biochemical mechanisms of these effects are still unclear. It seems the mitochondria play a significant role in this mechanism. The cardiac cells contain substantial number of mitochondria and MT is perspective molecule to have a protective effect on those mitochondria. Obtained results could help the understanding of a new and potential utilization of MT in the targeted defence of selected tissues during the anticancer therapy.



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### Bid-dependent activation of mitochondrial pathway is essential for cisplatin- or LA-12-mediated enhancement of TRAIL-induced apoptosis in human prostate cancer cells.

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#### Introduction

In order to enhance the killing effects of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) in human prostate cancer cells, we employed a recently introduced platinum(IV) complex LA-12 and compared its sensitizing properties with commonly used anticancer drug cisplatin.

#### Materials/methods

Using the state-of-the-art methods of the cell and molecular biology, biochemistry and analytical cytometry, molecular mechanisms responsible for the cooperative killing effects of the drugs were examined, especially at the level of mitochondria and caspases.

#### Results and conclusions

We showed that exposure of human prostate cancer cells to sub-lethal doses of cisplatin or LA-12 resulted in significant potentiation of TRAIL-induced apoptosis via engagement of mitochondrial pathway, associated

with enhanced cleavage/activation/level of several Bcl-2 family proteins, loss of mitochondrial membrane potential and caspase activation. RNAi-mediated silencing of Bid or Bak abrogated drug combination-induced cytotoxicity, underscoring the critical involvement of these proteins and mitochondria. The more detailed molecular mechanisms involved in stimulation of Bid protein activation induced by platinum drugs and TRAIL in prostate cancer cells will be presented within our contribution.

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### Regulation of NEU4 sialidase expression in tumor hypoxia.

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#### Introduction

Alterations in sialic acids found on most glycomolecules have been associated with the malignant phenotype of tumors. Since downregulation of sialidase NEU4, which cleaves sialic acid residues from glycomolecules, has been associated with invasive properties, we decided to investigate its expression in respect to hypoxia implicated in such cases.

#### Materials/methods

Colorectal, breast, renal, and pancreatic cancer cell lines were incubated in normoxia (21% O<sub>2</sub>) and hypoxia (2% O<sub>2</sub>) for 24-48 hours. Expression of NEU4 along with other genes of interest was assessed by real-time PCR and Western Blot.

#### Results and conclusions

In all the cancer cell lines tested, NEU4 transcription was significantly induced in hypoxia, indicating that NEU4 is a hypoxia-responsive gene induced by the hypoxia-inducible transcription factor HIF. In HIF1-silenced cells, NEU4 transcription was restored to its normoxic level.

However, at the level of protein, NEU4 was significantly decreased under hypoxic conditions in all the cancer cell lines tested signifying its proteasomal degradation. SIAH2 is an ubiquitin-ligase induced in hypoxia targeting proteins for degradation in the proteasome under conditions of limited oxygen. It is possible that SIAH2 also ubiquitinates NEU4 in hypoxia and facilitates its degradation.

**Grant support:** APVV 14-0753,  
 VEGA 2/0122/16, VEGA 2/0155/16

23

### The role of the soluble form of tumor-associated CA IX protein in vivo.

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#### Introduction

The regulation of cell migration, adhesion and proliferation can be performed through soluble forms of transmembrane (TM) proteins produced by posttranslational cleavage - shedding. Carbonic anhydrase IX (CA IX) is a TM protein involved in the progression of tumor phenotype. Extracellular part of CA IX is shed from the cell surface and therefore its functions in tumor microenvironment and circulation are currently examined.

#### Materials/methods

We prepared stably transfected mouse melanoma cell lines B16-F0 FL CAIX (normal CAIX shedding) and B16-F0 NS CA IX (CA IX shedding malfunction) for *in vivo* experiments. In tumor xenograft experiments we used immunodeficient female mice NMRI-Foxn1<sup>nu</sup> nu/nu (8 mice per group; subcutaneous administration, 14 days) and in colonization assay C57 BL/6J female



mice (7 mice per group; intravenous administration, 21 days) were used.

### Results and conclusions

Previously, we showed that CA IX shedding can affect migration and invasion of tumor cells *in vitro* therefore we decided to perform *in vivo* experiments. We observed differences between cell lines with normal and malfunctioned CA IX shedding in size and number of formed xenografts/metastatic foci, in both experimental settings. After subcutaneous administration of tumor cells we measured 50% higher weight and 58% more volume of B16-F0 NS CA IX xenografts compared with B16-F0 FL CA IX xenografts. In colonization assay we observed 25% more lung foci in the B16-F0 NS CA IX group. Moreover, these foci covered 2.45 times larger area than B16-F0 FL CA IX foci. Nevertheless, in blood serum of mice with experimental metastases formed by B16-F0 FL CA IX, we detected increased levels of several protumorigenic cytokines. Based on these data we propose that soluble form of CA IX serves as signaling molecule influencing tumor progression. Grant support: VEGA 2/0134/12; VEGA 2/0152/12; VEGA 2/0081/14, APVV-16-0697

### 24

#### Plasma-based biomarkers of gastrointestinal cancers.

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#### Introduction

Cancers of the GI tract are among the most common types of cancer in the world with high mortality. Early diagnosis is essential for treatment and patient survival. Symptoms of

the disease are often not specific to a particular type of GI cancer. Additionally, the first symptoms appear in the later stages of the disease, when the chances of survival are significantly lower. There is a need for a method capable of detecting early stage disease. The composition of the proteome is dynamic and reflects changes in the cellular physiology. Specific proteins, whose qualitative and quantitative changes indicate such a change in physiology, may be used for development of a biomarker or biomarker panel for the early diagnosis of disease, for prognosis or for prediction of response to drug. Alterations in protein glycosylation patterns may be indicative of cancer. Glycoprotein changes are related to basic features of the cancer cell biology and can provide source of biomarkers. Glycosylated proteins are usually located on the outer surface of the cell or intended for export into the extracellular matrix. For this reason, these proteins are likely to be present in larger quantities than other non-glycosylated proteins in the bloodstream. Plasma is the most desirable kind of sample for clinical tests because it can be obtained non-invasively. It is assumed that some of the tumour proteins are secreted into the bloodstream in trace quantities. Sensitive methods like mass spectrometry or immunoassays can detect some of these tumour proteins.

#### Materials/methods

Mouse xenograft model was used for identification of candidate biomarkers. Cancer cell lines representing 7 types of cancers of gastrointestinal tract were implanted in immunocompromised mice. Mice were sacrificed after tumours grew to a specific size and blood and tumour tissue samples collected. The premise for the method is that human protein in mouse plasma originated in human tumour tissue. N-glycopeptides were isolated by solid phase extraction (Solid-phase extraction of N-linked glycopeptides, Tian et al, 2007 Nature Protocol)

and analysed by an Orbitrap Fusion (Thermo) mass spectrometer and Ultimate 3000 RSLC Nano liquid chromatograph (Thermo). Acquired data were analyzed by software developed in-house and unique human N-glycopeptides were identified for another phase of biomarker development.

### Results and conclusions

Murine xenograft 147 unique human N-glycopeptides were identified in murine xenograft serum. The list includes proteins that have been approved by the FDA for use in biochemical tests in clinical laboratories. Individual candidate biomarkers from discovery phase will be verified and validated in plasma samples from patients suffering from the appropriate type of cancer. These samples will be prepared in the same way as the samples from the discovery phase and analyzed by a targeted proteomic method. Verification and validation of candidate biomarkers for pancreatic cancer are in progress.

### 25

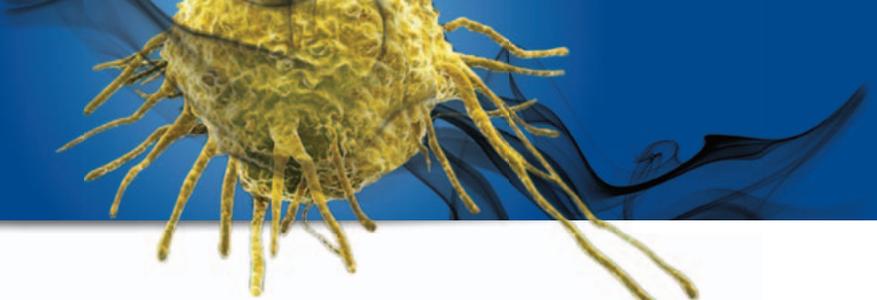
#### Detection of HPV in lung cancer patients.

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#### Introduction

Human papillomavirus, or HPV, is a commonly occurring virus. It is known approximately about 120 HPV types. Most of them are harmless, infection subsides without symptoms. Only 40 types of HPV are sexually transmitted. According to risk we are divided into low-risk (low risk HPV, lrHPV) and high risk (high risk HPV, hrHPV). Low-risk, non-carcinogenic HPV types can cause cervical changes of benign (abnormal, but not cancerous changes), and genital warts. HPV high risk induces changes in epithelial cells, e.g. the surface of the cervix, which may lead to cancer. HrHPV infection also causes carcinoma of the vulva



(external genitals) and the vagina, rectum carcinoma, penile carcinoma, and head and neck cancer. Also HPV affects male fertility. Increasingly serious issue, on which we focus in our work, is the detection of HPV in samples of lung tumors in patients with non small cell lung cancer (NSCLC). Looking at the causes of lung cancer is extremely important. Unfortunately, the stigma that lung cancer is a smoker's disease has in some ways slowed progress in evaluating other possible causes. We also know that not everyone who smokes develops lung cancer and that likely smoking works in synchrony with other risk factors to cause cancer. Is HPV one of those factors? Though HPV has been found in lung cancer cells, especially in squamous cell carcinoma of the lungs, the clinical significance of finding HPV in lung cancer cells still isn't known or understood.

#### Materials/methods

As a biological material, 50 samples of patients with NSCLC has been used in two storage method - such as formalin-fixed paraffin embedded sections (FFPE) and frozen tissue in RNA later. In both cases the DNA were isolated using Cobas kit. The method which were used to the detection of presence/absence HPV, is based on a quantitative multiplex real-time polymerase chain reaction (PCR). We are able to detect E2 and E6 HPV genes using specific TaqMan® probes. We have tried to detect subtypes HPV 16, 18, 31 and 56. As an internal control, a gene Glycerolaldehyde 3-Phosphate Dehydrogenase (GAPDH) was used.

#### Results and conclusions

To this day, all 50 samples tested were HPV negative for all four sub-strains of HPV (16, 18, 31 and 56). We continue in further testing, as well as data in the literature vary both according to race (Asians have the highest percentage of positives), according to histological types (it is said that the largest percentage of positives often found in squamous type), as well as gender (women are in this direction vulnerable

group). In Europe, the data vary between 0-20% of HPV positivity with respect to lung tumors. It is necessary to continue with further testing to map the incidence of HPV positivity in the group of patients from University Hospital Olomouc. Although some publications show a higher presence of the virus in our group of patients, its presence still failed to prove in our cohort of patients. We will continue to further testing at least 50 patients.

Acknowledgement: This work was supported by internal grant IGA LF 2016\_10.

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#### Molecular diagnostics in glioma.

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#### Introduction

Gliomas are the most common, malignant, and highly infiltrating diffuse brain tumors and their prognosis still remains unfavorable despite aggressive therapy. Recently, new World Health Organization (WHO) classification of tumors of central nervous system established an important role of IDH mutations in glioma diagnostics. We investigated association of IDH mutations with other molecular genetic markers and clinical characteristics of glioma patients.

#### Materials/methods

A group of 153 glioma patients was retrospectively analyzed for the presence of IDH mutations by competitive amplification of differentially melting amplicons (CADMA) PCR. Copy number of EGFR, p53, RB1, MDM2, CDKN2A genes and 1p, 19q and 10p chromosomal regions were investigated by fluorescent in situ hybridization. MGMT promoter methylation status was determined by bisulfite conversion followed by methylation-specific PCR. Results of molecular genetic analysis were correlated with clinical characteristics.

#### Results and conclusions

Thirty six IDH1 R132H, one IDH1 R132C and no IDH2 R172K mutations were found. In the group of all gliomas, 50% harbored the MGMT methylation, 25% EGFR amplification, 30% CDKN2A loss, 30% 1p loss, 25% RB1 loss, 16% p53 loss, 20% 10p loss, 17% 19q loss, 9% 1p/19q co-loss, 24% MDM2 gain. IDH1 mutations were positively associated with MGMT methylation, 1p/19q co-loss and negatively associated with EGFR amplification and 10p loss. Overall survival of IDH1 mutated glioblastomas were almost 3 times longer than these without IDH1 mutations ( $P = 0.035$ ), unlike MGMT methylated vs. unmethylated glioblastomas ( $P = 0.166$ ). In conclusion, we retrospectively detected IDH mutations in glioma patients by CADMA PCR method and investigated association between IDH status and other analyzed molecular genetics and clinical characteristics. Our findings demonstrated that in glioblastomas, IDH1 mutations are probably stronger prognostic markers than MGMT methylation. We suggested IDH1/2 mutations analysis as a prime procedure in glioma sample genetic examination. This work was supported by grants from the Ministry of Health (NT 13581) and Technology Agency (TE02000058) of Czech Republic. The infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported by the National Program of Sustainability (NPU LO1304).





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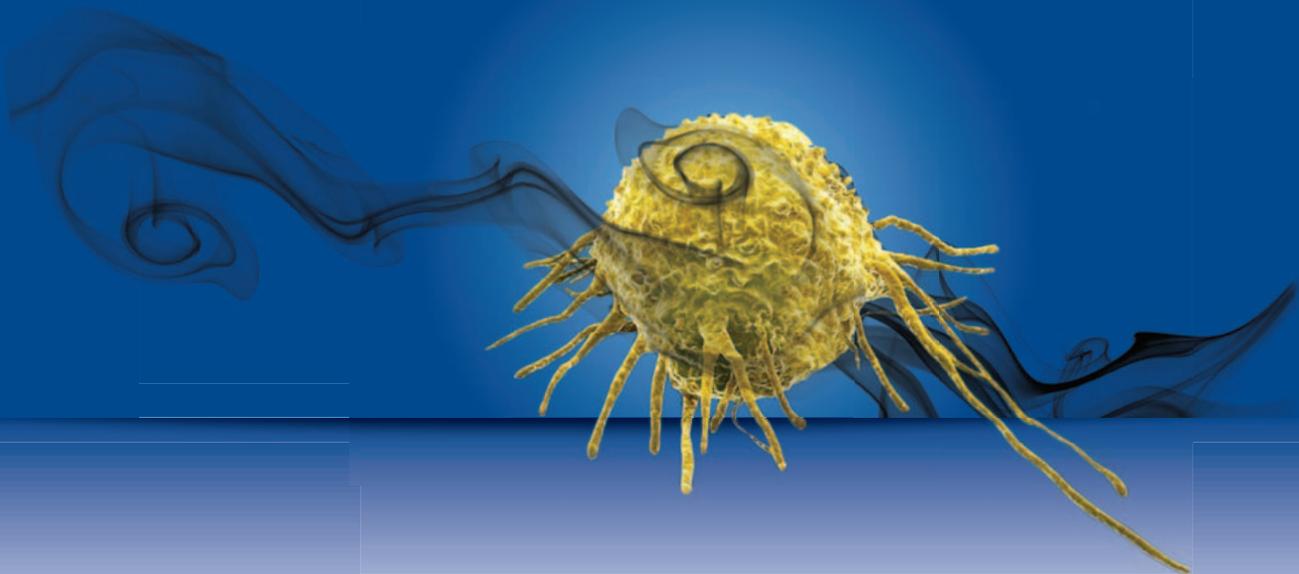
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