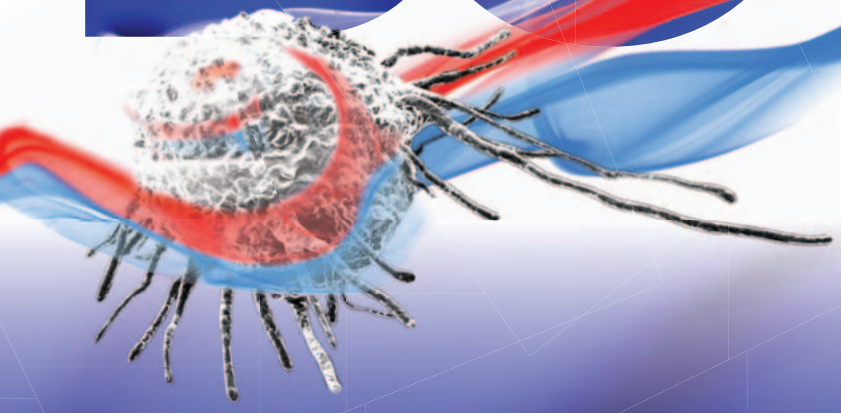
XV. DIAGNOSTIC, PREDICTIVE AND EXPERIMENTAL ONCOLOGY DAYS

ABSTRACT BOOK



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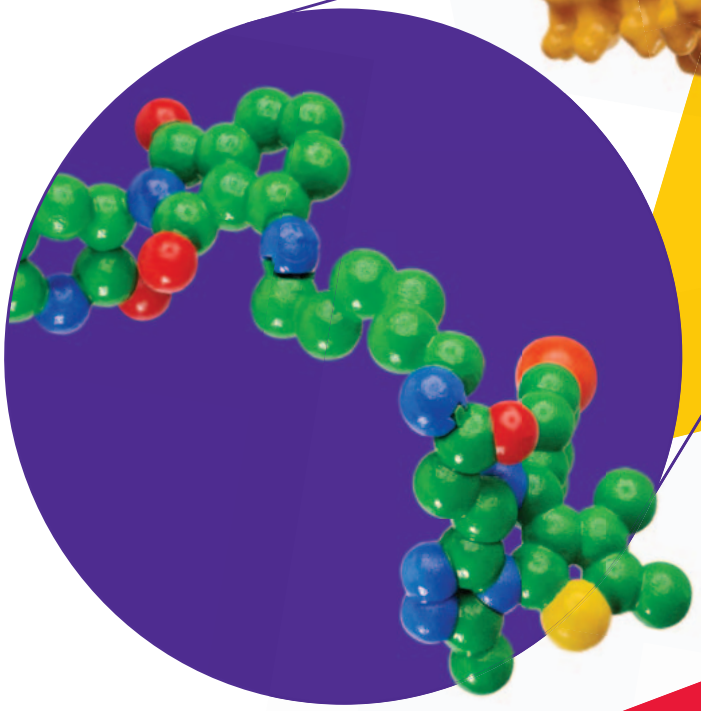

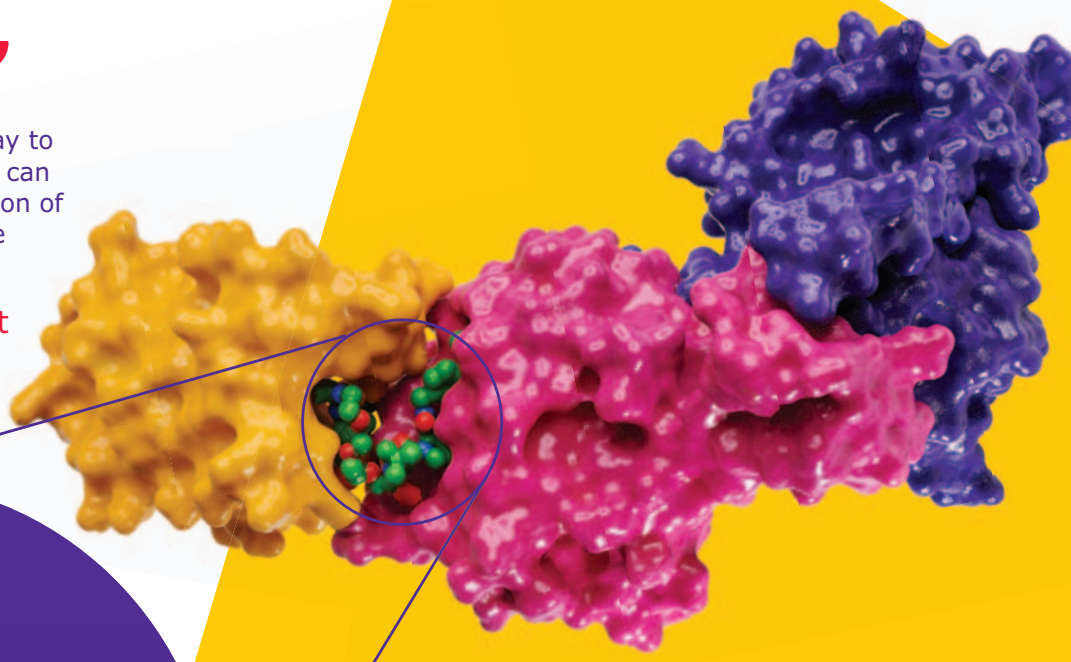

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PROGRAM

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

PONDĚLÍ / MONDAY - 25. listopadu 2019 / November 25th, 2019

13:00 13.15 CEREMONIAL OPENNING

PATHOPHYSIOLOGY OF CANCER AND MOLECULAR TARGETS I

Chairs: Maddalena Fratelli, Marian Hajduch

13:15 13:45 Retinoic-acid promotes viral mimicry and antigen presentation in immunologically-silent breast-cancer cells
Maddalena Fratelli

13:45 14:15 Low-sensitivity and drug-resistance to DNA-demethylating epi-drug decitabine in solid tumors: A challenge yet to overcome
Khushboo Agrawal

14:15 14:45 Role of CDK12 in the regulation of DNMT1 activity by altering expression of miR-152
Jiri Kohoutek

14:45 15:15 Pan-cancer analysis of single nucleotide variants affecting N-glycosylation sequence motifs
Miroslav Hruska

15:15 15.45 COFFEE BREAK

PATHOPHYSIOLOGY OF CANCER AND MOLECULAR TARGETS II

Chairs: Pavel Moudry, Josef Srovnal

15:45 16:15 Ribosomal biogenesis factors in p53-dependent stress response
Pavel Moudry

16:15 16:45 Bystin regulates c-myc on protein level
Zuzana Maceckova

16:45 17:05 Stroma-cancer interaction in PDAC
Pavol Szabo

17:05 17:25 Biological role of novel SNV of RPS7 in Diamond Blackfan anemia-model of ribosomal alterations in cance
Agata Kubickova

17:25 17:45 The microenvironment of melanoma cells
Barbora Dvorankova

17:45 18:00 Omega 3 fatty acid supplementation on NK cell cytotoxic activity and tumor reduction in rat Walker carcinoma model
Juan Bautista De Sanctis

ÚTERÝ / TUESDAY - 26. listopadu 2019 / November 26th, 2019

CANCER THERAPEUTICS I: NANOMEDICINE AND IMAGING

Chairs: Milos Petrik, Petr Cigler

9:00 9:30 PSMA-based cancer theranostics
Martina Benesova

9:30 10:00 Towards optimal nanobiointerface: molecular aspects of cellular targeting with nanoparticles
Petr Cigler

10:00 10:30 Bimodal immunoradiotherapy using thermoresponsive polysaccharide-based polymers
Martin Hruby

10:30 10:45 99mTc-labeled hydroxyapatite nanoparticles as potential tracers for solid tumors
Zbynek Novy

10:45 11:15 COFFEE BREAK

NEW TECHNOLOGIES IN CANCER RESEARCH I

Chairs: Khushboo Agrawal, Karel Koberna

11:15 11:30 New sensitive method for the detection of mycoplasmas using fluorescence microscopy
Karel Koberna

11:30 11:45 Hair follicles as a promising biological material and their use in cellular senescence and epithelial biomarkers
Natalie Kudlova

11:45 12:00 Can be human tears used as a source of brain proteins?
Tomas Ozdian

12:00 12:15 A high-throughput, whole cell-based assay for cytotoxicity profiling of new chemical compounds
Sona Gurska

12:15 12:30 Exhaled breath condensates proteomics as a tool to monitor condition of human lungs
Jana Vaclavkova

12:30 13:30 LUNCH

PATHOPHYSIOLOGY OF CANCER AND MOLECULAR TARGETS III

Chairs: Juan DeSanctis, Viswanath Das

13:30 14:00 MIDKINE as a novel tumor-secreted protein driving resistance to immune checkpoint blockade
Daniela Carolina Cerezo-Wallis

14.00 14:30 3D cell cultures are more predictive in vitro tumor models for cancer drug discovery and development
Viswanath Das

14:30 14:45 Influencing radiosensitizing properties in cancer cells
Miroslav Vetrik

14:45 15:15 COFFEE BREAK

NEW TECHNOLOGIES IN CANCER RESEARCH II

Chairs: Marian Hajduch, Martin Mistrik

15:15 15:45 Morphology-driven high-plex spatial analysis of tissue microenvironments with the GeoMx™ digital spatial profiling
Stephan McGoldrick

15:45 16:15 Microsatellite instability (MSI) – Biomarker for immuno - oncology research and Lynch Syndrome screening
Mónica Sevillano

16:15 16:45 Introduction to single molecule counting for ultrasensitive detection of protein biomarkers
Stanislav Kukla

16:45 17:15 Multimodal nuclear hybrid imaging in translational research
Sebastian Eigner

17:15 17:45 Making genius simpler: Orbitrap Exploris 480, a new quadrupole Orbitrap mass spectrometer
Maciej Bromirski

19:00 23:00 SOCIAL EVENING

STŘEDA / WEDNESDAY - 27. listopadu 2019 / November 27th, 2019

CANCER BIOMARKERS AND PERSONALIZED MEDICINE

Chairs: Jiri Drabek,Vladimira Koudelakova

9:00 9:20 Identification of circulating tumor cells (CTCs) in advanced breast cancer patients by EpCAM independent system CytoTrack
Małgorzata Szostakowska-Rodzoś

9:20 9:40 Determination of lung tumor mutation burden to predict the effects of immunotherapy with checkpoint inhibitors
Rastislav Slavkovsky

9:40 10:00 HPVPro study: Comparison of HPV detection in cervical and cervicovaginal swabs
Vladimira Koudelakova

10:00 10:20 Minimal Residual Disease Monitoring in Mantle Cell Lymphoma-from diagnosis to treatment
Anna Fabisiewicz

10:20 10:40 In search for potential biomarkers: deregulation of microRNAs in oropharyngeal carcinoma
Helena Kovarikova

10:40 11:00 Transcriptomic profiling in meningiomas for understanding of pathophysiology and biomerkers discovery
Hanus Slavik

11:00 11:15 COFFEE BREAK

CANCER THERAPEUTICS II: SMALL MOLECULES

Chairs: Petr Dzubak, Milan Urban

11:15 11:45 Disulfiram anti-cancer activity reflects targeting NPL4, not inhibition of aldehyde dehydrogenase
Zdenek Skrott

11:45 12:00 The impact of disulfiram on DNA replication dynamics in cancer cells
Dusana Majera

12:00 12:15 Transformations of lupane triterpenes in the positon C-30: synthesis, cytotoxic activity, SAR
Milan Urban

12:15 12:30 Triterpenoid thiazoles: from design and synthesis to in vitro biological evaluation
Lucie Borkova

12:30 12:45 Cytotoxic triterpenoid thiazoles induce apoptosis in CCRF-CEM cells via disruption of mitochondria
Ivo Frydrych

12:45 13:00 Functional screening of adenosine receptors: from model validation to active compound identification
Jana Kotulova

13:00 13:15 CLOSING REMARKS

13:15 14:15 LUNCH

Postery / Posters

1 *Epigenetic age estimation of the healthy Czech population by AgePlex*
Jiří Drábek

2 *A novel long non-coding RNA MIAT is associated with NMYC amplification in neuroblastoma*
Barbara Feriančiková

3 *Body Mass Index prediction through DNA methylation*
Lucie Kotková

4 *Prevalence and genotype-specific distribution of human papillomavirus in Czech nonvaccinated heterosexual couples*
Hana Jaworek

5 *Virtual screening using pharmacophore models retrieved from molecular dynamic simulations*
Pavel Polishchuk

6 *Novel antimicrobial activity of anticancer compounds*
Ermin Schadich

7 *CRISPR/Cas9-mediated tagging of proteins involved in ribosomal biogenesis*
Martin Ondra

8 *Subtyping of systemic amyloidosis in subcutaneous fat aspirates by mass spectrometry-based proteomics*
Dušan Holub

9 *In silico models of firefly luciferase inhibitors*
Mariia Matveieva

10 *Comparison of hgDNA quality control methods*
Patrícia Žižkovičová

11 *The effect of perioperative analgesia on CTCs occurrence in colorectal cancer patients*
Alona Rehulkova

12 *The quality and performance of HTS assays*
Pawel Znojek

13 *Identification of a four-gene methylation biomarker panel in high-grade serous ovarian carcinoma*
Ivana Baranova

14 *Applications of betulinic acid conjugates to explore its mode of action*
Jiri Rehulka

15 *KDM5D is associated with neuroblastoma chemoresistance to ellipticine*
Natália Podhorská

16 *Fluorescent Nanodiamonds Modified with Biocompatible Polymers*
Klaudia Kvaková

17 *Importance of V-ATPase for tumor cell chemoresistance and potentiation of cytostatic effects by V-ATPase inhibitors*
Marie Belhajova

18 *Epigenetically conditioned chemoresistance of cancer cell*
Marie Belhajova

19 *Preclinical absorption, distribution, metabolism, excretion, and thermodynamic properties of complexes with central atom of copper (II) and gold (I)*
Martina Medvedíková

20 *Development of a multi-target screening tool based on 3D pharmacophore models*
Alina Kutlushina

21 *Development of resistant cell lines to nucleosides based cytostatics*
Lenka Rehackova

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Pathophysiology of cancer and molecular targets I

Chairs: Maddalena Fratelli, Marian Hajduch

pondělí / 25. listopadu 2019 / Monday / November 25th, 2019 / 13:15 - 15:15

Retinoic-acid promotes viral mimicry ad antigen presentation in immunologically-silent breast-cancer cells

Maddalena Fratelli[1], Marco Bolis[1], Gabriela Paroni[1], Arianna Vallerga[1], Adriana Zanetti[1], Mami Kurosaki[1], Silvio Ken Garattini[2], Maurizio Gianni[1], Monica Lupi[1], Linda Pattini[3], Maria Monica Barzago[1], Mineko Terao[1], Enrico Garattini[1]

[1] *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.*
[2] *Azienda Ospedaliera di Udine, Udine, Italy.*
[3] *Politecnico di Milano, Milan, Italy*

Introduction

Breast cancer is a heterogeneous disease, which is traditionally classified in three groups for clinical purposes, i.e. estrogen receptor positive, HER2 positive and triple negative. Breast cancer heterogeneity is a major therapeutic problem and it requires the development of novel therapeutic strategies to be used in the context of the personalized treatment of the disease. This calls for the identification of the tumor groups and individual cases sensitive to the proposed therapeutic strategies. All-trans retinoic acid (ATRA) is used in the treatment of acute promyelocytic leukemia with remarkable results and it is a non conventional anti-tumor drug, being the first example of clinically effective cyto-differentiating agent. Pre-clinical data indicate that ATRA has significant potential in the personalized treatment of breast cancer. Indeed, we demonstrated that approximately 70% of ER + mammary tumors are sensitive to the anti-proliferative effects of ATRA. In contrast only 10-20% of the HER2 + and TNBC counterparts respond to the retinoid. This is at the basis of a clinical trial which we are conducting in post-menopausal women suffering from ER + breast cancer.

Materials/Methods

By means of total stranded RNA sequencing followed by network and pathway analysis, we define the perturbations afforded by ATRA on the gene expression profiles of 16 breast cancer cell lines characterized for their retinoid sensitivity and recapitulating the disease heterogeneity. We also extend some results to short-term tissue cultures and in vivo mice xenotransplant models

Results and Conclusions

The RNA-sequencing studies performed in cell lines indicate that the retinoid activates the interferon pathways and up-regulates interferon-responsive genes. The results are confirmed in short-term tissue cultures and in vivo models. In sensitive breast cancer, ATRA up-regulates gene networks involved in immune modulation and antigen presentation. “Immunologically cold” breast cancer cell lines and tumours are particularly sensitive to the action of ATRA. We provide evidence as to the cellular and molecular mechanisms underlying the anti-tumor action of ATRA. In particular, we demonstrate that the retinoid triggers a “viral mimicry” response, involving activation of endogenous retroviruses. “Viral mimicry” causes the induction of IRF1 and DTX3L, which exert functionally opposite effects on ATRA-dependent growth inhibition of breast cancer cells.

Besides its significance from a mechanistic point of view, the study is of relevance from a clinical and therapeutic perspective. In fact, ATRA stimulates antigen presentation, interferon-dependent responses and immune responses, three processes controlling the sensitivity of tumors to immuno-modulatory drugs, such as check-point inhibitors. This suggests that ATRA and immunotherapeutic agents represent novel and rational combinations to be tested for the personalized treatment of breast cancer. With respect to this, it is remarkable that ATRA sensitivity seems to be higher in “immunologically cold” mammary tumors, which are generally resistant to immunotherapy.

Low-sensitivity and drug-resistance to DNA-demethylating epi-drug decitabine in solid tumors: A challenge yet to overcome

Khushboo Agrawal, Petr Vojta, Rastislav Slavkosky, Ivo Frydrych, Petr Dzubak, Marian Hajduch

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

Based on the current understanding of the epigenetic landscape, cancer methylome is highly disrupted, which makes DNA methylation an excellent target for anti-cancer cures. To date, azacytidine and its congener, decitabine (DAC) are the most successful DNA demethylating epigenetic drugs. However, scientists are yet to find the reversal of clinical resistance to these drugs. Yet another challenge remains to be the decreased efficacy of these promising therapies in the cure of solid tumors. We used a soild tumor, HCT116 colorectal cancer cell line and developed resistance against DAC. DAC-resistant HCT116 cells were used to study the epigenetic cross-talk between DNA methylation and chromatin modifications. The screening of parental and DAC-resistant HCT116 cells against inhibitors of epigenetic

writers-erasers-readers unveiled increased sensitivity of resistant cells to BET inhibitor (inhibitor of reader enzymes), (+)-JQ1. BET inhibitor further mediated augmented response on cell cycle phases of resistant cells, and showed increased anti-proliferative effects in xenograft models of resistant cells. We then sequenced the transcriptome of DAC-sensitive and -resistant HCT116 cells using RNA-seq. The RNA-seq data revealed the overexpression of critical oncogenes, and their binding inactivation of key tumor suppressor genes (TSGs) in resistant cells. The most significant and biologically relevant transcriptional changes were further validated by qRT-PCR, and in addition their methylation status was determined by bisulphite sequencing. We discovered that the expressions of down-regulated TSGs were driven by promoter methylation, exposing these tumor-suppressive signatures as biomarkers which might differentiate between DAC-resistance and sensitivity, whereas, overexpression of oncogenes was independent of promoter methylation. Interestingly, the expressions of up-regulated oncogenes which define cell identity, mainly those involved in signaling of inflammatory pathways were reversed on treatment with (+)-JQ1. Further, siRNA-mediated genetic inhibition of bromodomains in resistant cells phenocopied therapeutic inhibition by (+)-JQ1. These data unveil the chromatin "reader proteins", as regulators of dysregulated oncogenic expressions in DAC-resistant cells. The present study provides novel insights into the epigenomic landscape of DAC-resistant colorectal cancer cells, and put forward, the alternative therapeutic regimen for DAC-resistant patients.

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1.01/0.0/0.0/16_019/0000868 and LM2015063, LM2015064 and foundation Cancer Research Czech Republic.

Role of CDK12 in the regulation of DNMT1 activity by altering expression of miR-152

Marta Dzimková[1], Marek Šupák[1], Monika Nováková[1], Jaroslav Klát[2], Jiří Kohoutek[1]

[1] *Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, Czech Republic.*
[2] *Department of Obstetrics and Gynecology, Faculty Hospital of Ostrava, Ostrava, Czech Republic*

Introduction

The DNA-damage-response (DDR) pathway is a cellular mechanism, which has evolved to protect cellular integrity by detection and repair of DNA lesions. At the moment the role of cyclin-dependent kinase 12 (CDK12) in the maintenance of genome integrity via the regulation of transcription of DDR genes, *BRCA1*, *RAD51* and others, is broadly established. Since various microRNA (miRNA) are situated within coding genes, such as DDR, we hypothesized that expression of some of them might be also affected by CDK12 depletion. Above all RNA polymerase II responsible for the transcription of miRNAs is regulated by kinase activity of CDK12.

Material/methods

Therefore, we performed the microarray analysis in order to identify potential miRNAs, which participate in the adverse effect of CDK12 on cell proliferation. Candidate miRNAs were validated by qPCR and chosen candidates were further evaluated in functional cellular assays.

Results and conclusions

Indeed, downregulation of CDK12 protein level led to aberrant expression of several miRNAs, among them, the level of miR-152 was significantly elevated. By using predictive algorithm, several proteins were indicated to be targeted by miR-152. We confirmed that upregulated expression of miR-152 leads to decreased expression of DNA methyltransferase 1 (DNMT1). At the moment, the impact of downregulated activity of DNMT1 on DNA methylation and epigenetics marks have been investigated at several downstream genes. We speculate that CDK12 regulates transcription of downstream genes either by phosphorylation of RNA polymerase II, DDR genes, or by altering DNA methylation status of certain genes by downregulating protein level of DNMT1.

The project is supported by the grant of the Ministry of Health AZV16-34152A.

Pan-cancer analysis of single nucleotide variants affecting N-glycosylation sequence motifs

Miroslav Hruska[1], Sharon Pitteri[2], Marian Hajduch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*
[2] *Canary Center at Stanford for Cancer Early Detection, Palo Alto, USA*

Introduction

N-glycosylation is a post-translational modification of proteins, which affects their fundamental properties such as stability, signaling, or intracellular trafficking. Glycans are attached to asparagines in N-X-S/T sequence motifs (N-motifs), and the recent availability of large genomic datasets enables studying alterations of such N-motifs by genetic variants.

Materials/methods

We have performed a pan-cancer analysis of single nucleotide variants (SNVs) affecting N-motifs in clinical data from The Cancer Genome Atlas (TCGA) program. In particular, we studied the differences between the behavior of somatic and germline variants, relations to known mutagenetic processes, implications for patients' survival, and the impact on the secondary structure of N-glycoproteins. Finally, we investigated the detection of novel N-glycosites using mass spectrometry of intact glycoproteomes.

Results and conclusions

SNVs affecting N-glycosites were less likely to be incorporated into the population, evidencing their potentially harmful effects. Removal of N-glycosites was most detrimental to tumor suppressor N-glycoproteins, as suggested by the shortened survival of patients. From a mutagenetic perspective, APOBEC enzymes, exposure to UV light, and deficient DNA polymerase epsilon had the highest tendency to create novel N-motifs, although their absolute strength varied significantly. Building on our recent work on the detection of variant peptides, we identified novel N-glycosite in fibrillin-1 protein. SNVs affecting N-glycosites have thus effect on patients' survival, their presence varies for individual mutagens, and we plan to further investigate these phenomena in conjunction with other clinical parameters.

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1.01/0.0/0.0/16_019/0000868 and LM2015064.

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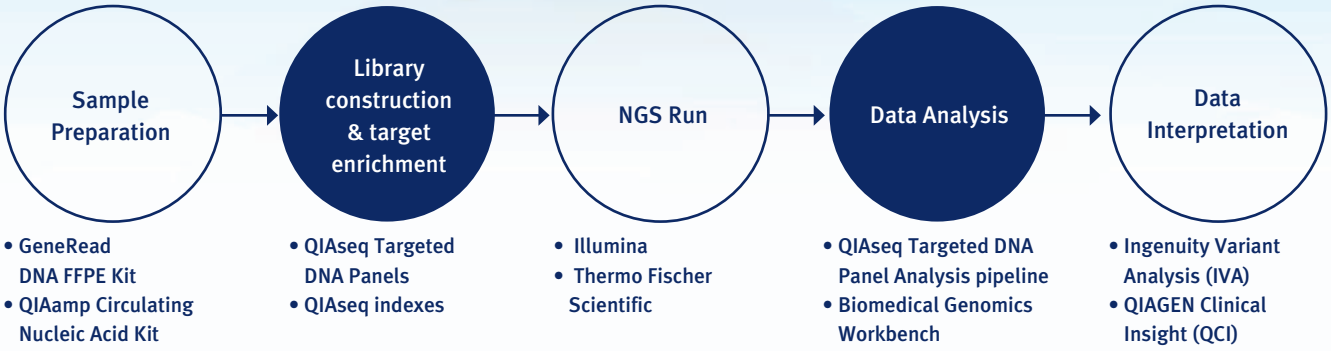

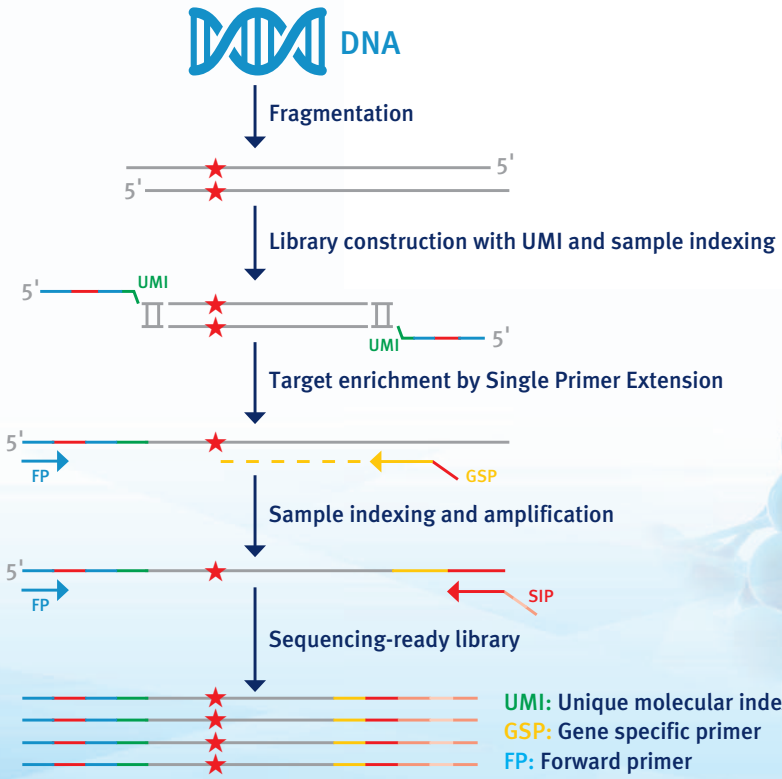

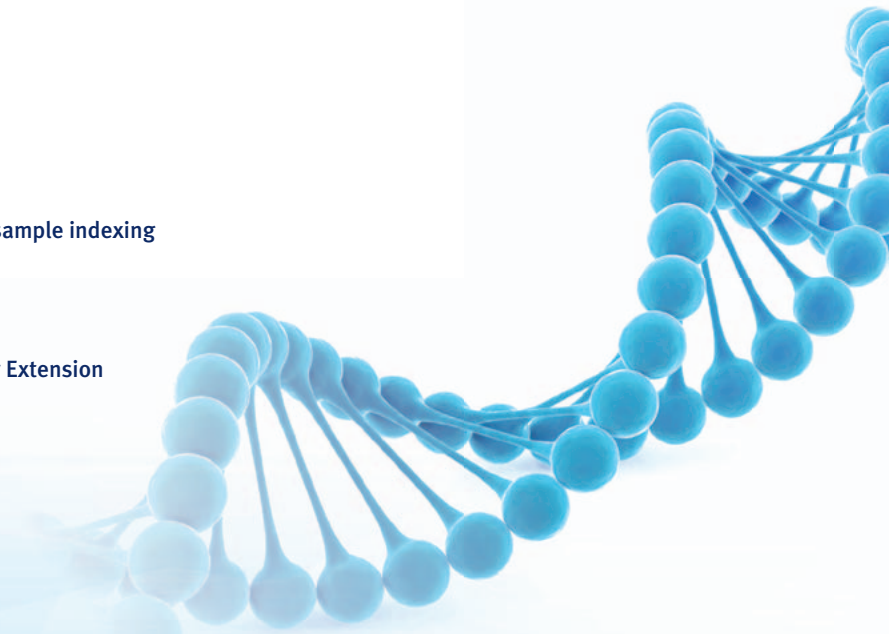

UMI: Unique molecular index
GSP: Gene specific primer
FP: Forward primer
UP: Universal primer
SIP: Universal primer

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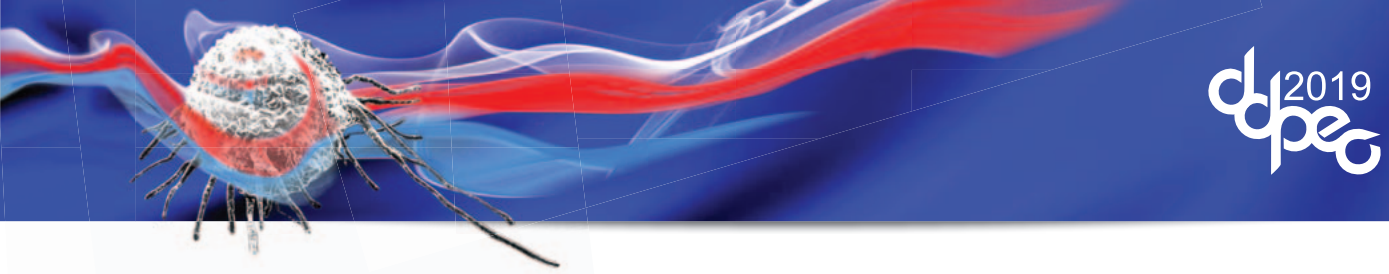

Pathophysiology of cancer and molecular targets II

Chairs: Pavel Moudry, Josef Srovnal

pondělí / 25. listopadu 2019 / Monday / November 25th, 2019 / 15:45 - 18:00

Ribosomal biogenesis factors in p53-dependent stress response

Pavel Moudry[1], Sinisa Volarevic[2], Jiri Bartek[1,3]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*
[2] *University of Rijeka, Rijeka, Croatia.*
[3] *Dansih Cancer Society Research Center, Copenhagen, Denmark*

Ribosomes are produced within the most prominent nuclear structure, the nucleolus, by a tightly controlled multistep process called ribosomal biogenesis. Disturbance of any single step in the process of ribosome biogenesis by extracellular or intracellular stimuli leads to ribosomal stress. Ribosomal stress activates p53 tumor suppressor that prevents cell-cycle progression and depending on the cellular context may induce cell cycle arrest, apoptosis or senescence. Studies over the last decade have identified mechanism of the p53 checkpoint activation upon ribosomal biogenesis stress. This is executed by release of ribosomal proteins RPL5 and RPL11 from the nucleolus to nucleoplasm, where they bind and inactivate main p53 negative regulator MDM2.

Using siRNA-based high content microscopy screening we aim to systematically assess how depletion of likely all ribosomal biogenesis factors affects steady-state level of p53. Results from the screen will be presented at the meeting.

Bystin regulates c-myc on protein level

Zuzana Macečková[1], Elena Mokshyna[1], Agata Kubíčková[1], Martin Ondra[1], Dagmar Pospíšilová[2], Petr Vojta[1], Marián Hajdúch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*
[2] *University Hospital, Olomouc, Czech Republic*

Introduction

Diamond Blackfan anemia (DBA) is rare congenital red cell aplasia. In 50-75 % of DBA cases can be cause accounted for mutation in ribosomal protein. In one patient from this cohort we detected small deletion in 1p36.11-1p36.12 area. This area includes RPL11 gene. Mutations in this gene are known cause of DBA phenotype and deletion of this gene was previously reported. Surprisingly, it was shown, that deletion of just one allele of RPL11 is not compatible with successful embryogenesis. Because affected patient was alive, we hypothesize, that disease modulating genes played role in his survival. We discovered mutation in BYSL gene which resulted in c-myc protein downregulation, which is necessary for successful erythropoiesis. Furthermore, we clarified mechanism by which bystin modulates c-myc protein levels. We also detected, that same mechanism is behind successful treatment of DBA with corticosteroids.

Material/methods

U2OS cell line was used for all experiments. Cells were transfected with WT or R343X bystin by x-tremegene 9 transfection reagent or by bystin siRNA by jet prime reagent or were treated by doxometasone. Treated cells were then harvested and lysed by RIPA buffer, part was used directly for western blotting and part was subjected to immunoprecipitation and following western blot.

Results and conclusions

We discover that bystin protein plays important role in c-myc turn-over. Under bysl down-regulation or mutation, c-myc stops bind to it and binds to nucleophosmin instead. When c-myc is bind to nucleophosmine, it gets translocated to nucleolus, where it binds to OAZ2, which leads to c-myc ubiquitin-independent degradation. Furthermore, we discover, that similar mechanism probably lays behind successful treatment of DBA by corticosteroids. We can say, that we discovered new c-myc regulatory gene and that this gene is targeted by corticosteroids which can have massive application in DBA and also cancer therapy.

Stroma-cancer interaction in PDAC

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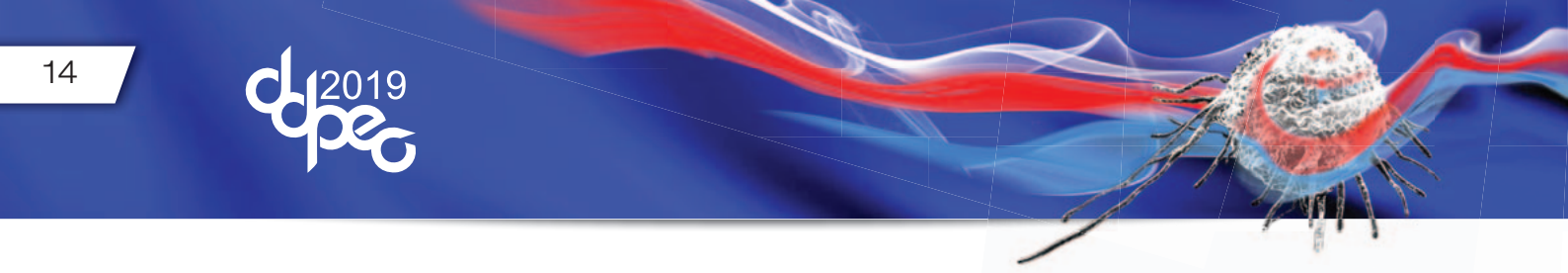

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Introduction:

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal tumours. Likewise, to another type of malignancy bad civilization habits including tobacco smoking, alcohol excess, obesity, diabetes, aging, immunodeficiency can lead to promote formation of PDAC. Unfortunately, there still no exist any successful screening methods to detect early stage of cancer spreading moreover, PDAC progression is extremely quick and often come across very late. Cancer cells colonized surrounding tissues and organs like lymph nodes and liver which finally lead to the death. Therefore, minimum success of current applied therapy leads experimental teams to find new therapeutic methods. PDAC belongs in the solid type of tumours, where mutual interaction between malignant cells and cancer microenvironment is crucial. The composition of cancer stroma is form by cellular part (infiltrating leukocytes and macrophages, blood wessels, cancer associated fibroblasts) and non-cellular fibrous extracellular matrix together with signals molecules. Cancer associated fibroblasts (CAF) produce wide spectrum of bioactive molecules and components of extracellular matrix, which support pancreatic cancer cells proliferation and migration.

Material and methods:

In our study we focus our interest on CAF – pancreatic cancer cell interaction. CAF were isolated like primoculture from patients suffering with PDAC. Their expressing profile were measured by using ILLUMINA system and compared with another type of CAF obtained from different tumors. Biological impact of CAF was tested on established cell lines from this type of cancer. Furthermore, phenotype changes on cancer cell lines were matched against primoculture cells isolated from ascitic fluid of PDAC patients.

Results:

Four molecules and their receptors are important signals couriers in interactions between cancer cells with other members of cancer microenvironment.

This study was supported from Centre for Tumour Ecology—Research of the Cancer Microenvironment Supporting Cancer Growth and Spread (reg. no. CZ.02.1.01/0.0/0.0/16_019/0000785), Charles University project PROGRES Q28

Biological role of novel SNV of *RPS7* in Diamond Blackfan anemia-model of ribosomal alterations in cancer

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Introduction:

Diamond Blackfan anemia is an infrequent innate red blood cell aplasia caused particularly by large genetic alterations like chromosomal translocations or mutations in ribosomal genes. Patients with this disease suffer not only with a substantially lower level of red blood cells but also with an increased incidence of osteogenic sarcoma or colon cancer. In our study, we had focused on an SNV in *RPS7* which has been found in a family suffering from this disease. This case is interesting because only one member of this family has symptoms of DBA and the others have just elevated marker of this disease - adenosine deaminase. According to recent literature, only 1% of DBA cases are caused by a mutation in the *RPS7* gene.

Methods and materials: Due to the lack of patient´s samples, we had designed and prepared a cellular model with this concrete variant in the *RPS7* gene by CRISPR/Cas9 technology. This model was used for studying p53-mediated ribosomal stress. Alterations in the processing of ribosomal RNA were determined by Northern blotting and rRNA FISH, changes in nucleolar morphology by immunofluorescence, cell cycle progression and protein synthesis rate were measured by flow cytometry, changes in protein expression and stability were determined by Western blotting, cell proliferation rate was measured by MTS viability assay after 72 hours.

Results and conclusions:

Our results suggest that this SNV in the *RPS7* gene causes higher extraribosomal accumulation of this ribosomal protein and subsequent activation of ribosomal stress pathways, namely p53 along with its downstream target p21. We have evidence that this SNV causes decreased ability of rRNA processing in its initial steps. This leads to the accumulation of rRNA precursors detected by the ITS1 probe. Activation of the p53 pathway is coupled with slower proliferation with small changes

in cell cycle progression. Interestingly protein synthesis is only slightly altered by this SNV.

The microenvironment of melanoma cells

Barbora Dvořánková[1,2], Karel Smetana[1,2], Karolína Strnadová[1], Lukáš Lacina[1,3], Petr Přikryl[1]

[1] *Charles University, 1st Faculty of Medicine, Prague, Czech Republic.*
[2] *Biocev, Vestec, Czech Republic.*
[3] *General Faculty Hospital, Prague, Czech Republic*

Introduction:

The number of people suffering from tumour generally as well as melanoma is increasing worldwide. Thanks to the relationship of melanoma cells with neural crest stem cells, melanoma can generalize almost everywhere in body. Therefore, the mortality of melanoma, despite the very new therapeutic options, remains quite high. Melanoma is highly heterogeneous and its metastasis differ in the gene profile from the original tumour. Detailed study of melanoma microenvironment and the mechanisms that facilitate the spreading of melanoma cells could cure or prolong the survival of the patients. **Materials and methods:** To study the crosstalk (tumour cells-stroma) in melanoma, we used the commercial lines of malignant and healthy melanocytes; we isolated several lines of melanoma cells ourselves as well as the melanoma-associated and normal dermal fibroblasts. Using the immunohistochemical, proteomic as well as genomic approach, we studied single cell types and their mutual communication. To correlate the in vitro experiments with in vivo practice, we made the proteomic analysis of the serum of 12 melanoma patients in different phases of the treatment**Results and conclusions:** Melanoma cells (similarly to other tumour cells) influence their ecosystem to be helpful to stimulate melanoma cell growth and metastasization. Melanoma associated fibroblasts strongly differ from healthy dermal fibroblasts on RNA as well as on protein level. They produce wide spectrum of chemokines, cytokines and growth factors, stimulating mainly migration of melanoma cells but not their growth. IL-6, IL-8 and TGF-beta are the leading molecules and they are transported via vessels. They prepare premetastatic niche and support formation of lymph node/ distant metastases. Serum proteomic analysis of melanoma patients verified these findings.

Omega 3 fatty acid supplementation on NK cell cytotoxic activity and tumor reduction in rat Walker carcinoma model

Juan Bautista De Sanctis[1,2]

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[2] *Institute of Immunology. Universidad Central de Venezuela, Caracas, Venezuela, Bolivarian Republic of*

Introduction:

Omega 3 fatty acids have been shown to reduce Walker carcinoma growth is the rat *in vivo* model (1); however, the exact mechanism is still unknown. The omega 3 fatty acid diet was shown to affect the tumor and modify the cachexia observed in the animals after 14 days. It has been postulated that the increase in omega 3 fatty acids decreases the inflammation at the site of the tumor and decrease the metabolic changes induced by exacerbated tumor growth and inflammatory cytokines. However, the possibility that omega 3 fatty acids would enhance the immune response against the tumor has not been assessed in this model.

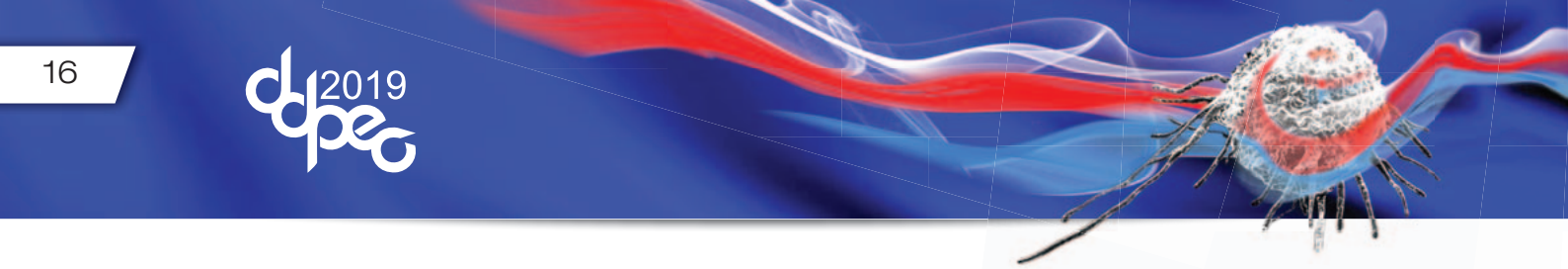
Material and Methods:

Thirty-two adult Sprague Dawley rats weight 225± 25 g were divided into four equal groups. Group one was control: diet-fed with standard rat chow without tumor, the second group was control diet but treated with the tumor, group three was supplemented with omega 3 fatty acids, 300 mg per kg per day, by gastric gavage and the fourth group was fed with saturated fatty acids from palm oil, 300 mg per kg per day, by gavage as in group three. Walker carcinoma, 5 million cells in 1 ml of saline solution were inoculated in the right flank. The animals were killed after 3 weeks (tumor was visible in the controls). Animals fed with omega-three had significantly less amount of tumor mass and metastasis ($p<0.01$ as compared to other groups). NK was obtained from the spleen and was challenged against the same tumor at a ratio effector target of 20:1, 10:1 and 5:1.

Results and discussion:

A significant reduction ($p<0.001$) in NK activity was observed in animals fed with a standard diet with the tumor as compared to the healthy controls (55±17 vs. 155±42 lytic units). Moreover, animals fed with omega 3 fatty acids showed the highest NK activity as compared to palm oil-treated animals and controls (238±29 vs. 108±29 vs. 155±42 lytic units, p,0.01 by ANOVA). Omega 3 fatty acids enhance NK cytotoxic response as compared to standard chow and palm oil. Future experiments should assess the effect of both the tumor cells and immune response.

1.- Iagher F, et al. Nutr Cancer. 2011 Nov;63(8):1307-15.



Cancer therapeutics I: Nanomedicine and imaging

Chairs: Milos Petrik, Petr Cigler

úterý / 26. listopadu 2019 / Tuesday / November 26th, 2019 / 9:00 - 10:45

PSMA-based cancer theranostics

Martina Benešová

German Cancer Research Center (DKFZ), Research Program Imaging and Radiooncology, Research Group Molecular Biology of Systemic Radiotherapy, Heidelberg, Germany

Prostate cancer (PCa) belongs to the deadliest and most commonly diagnosed tumor disease in men worldwide. The main cause of death is connected with the progression of androgen-independent disease-stages. The prostate-specific membrane antigen (PSMA) is a well-recognized biological target for addressing PCa, whether for diagnosis or therapy.

The broader utilization of PSMA was initiated by the by the development of ^{111}In-labeled monoclonal mouse antibody 7E11-C5, the first FDA-approved imaging agent for PCa known as Prostascint®. In an effort to further improve both the diagnosis and therapy of PCa, diverse chemical constructs have been explored as capable PSMA vectors including several peptidomimetic Glu-ureido-based PSMA inhibitors. First in-human studies with such PSMA radioligands showed highly sensitive and specific imaging of PSMA-positive PCa by means of positron emission tomography (PET) and single photon emission computed tomography (SPECT) as well as significant response after PSMA radioligand therapy (PSMA-RLT).

This presentation will summarize the impact of clinically-relevant low-molecular weight PSMA inhibitors (e.g., MIP-1404/-1072/-1095, PSMA-11, PSMA-1007, DCFBC, DCFPyL, PSMA I&T) on prostate cancer management. The main focus will be dedicated to design, pre-clinical evaluation and clinical application of theranostic PSMA radioligand PSMA-617 (^{44}Sc-, ^{68}Ga-, ^{90}Y-, ^{152}Tb-,^{177}Lu-, ^{213}Bi- and ^{225}Ac-PSMA-617).

Based on the current evidence, combination of PSMA-PET and PSMA-RLT is implemented in a growing number of centers worldwide as it represents an important approach stepping towards the concept of theranostics and personalized nuclear medicine.

Towards optimal nanobiointerface: molecular aspects of cellular targeting with nanoparticles

Petr Cígler

Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic

Nanoparticles used for biological applications provide range of imaging modalities and possibility of polyvalent display of molecules. Attachment of ligands to nanoparticles leads for selective and highly effective targeting of biological receptors. However, the interface of nanoparticles has to be optimized in terms of non-specific binding, which can strongly reduce the advantages of multivalent binding and interaction selectivity. In the talk, I will exemplify strategies for colloidal stabilization of nanoparticles under physiological conditions, coatings of nanoparticles with biocompatible polymers and influence of the coating strategies and ligand attachment on targeting of various cancer cells. The results obtained using diamond nanoparticles and chemically reprogrammed virus-like particles will be discussed.

Bimodal immunoradiotherapy using thermoresponsive polysaccharide-based polymers

Lenka Loukotová[1], Jan Kučka[1], Mariia Rabyk[1], Kristýna Venclíková[1], Daniela Machová[1], Olga Janoušková[1], Věra Kolářová[2], Petr Páral[2], Luděk Šefc[2], Petr Štěpánek[1], Martin Hrubý[1]

[1] *Institute of Macromolecular Chemistry AS CR, Prague, Czech Republic.*

[2] *Charles University in Prague, First Faculty of Medicine, CAPI, Prague, Czech Republic*

Introduction:

We designed a new bimodal therapeutic agent based on synergic combination of radio- and immunotherapy [1]: Radiation kills the tumour cells as well as the cells protecting tumour from immune response such as the regulatory T cells, while after the decay of the radionuclide, immunomodulatory enhances the following immune response against the remaining tumour cells.

Materials/methods:

The selected polysaccharides β-glucan, κ-carrageenan and lentinan were grafted with poly(2-isopropyl-2-oxazoline-*co*-2-butyl-2-oxazoline) s (POXs) (different graft lengths and grafting densities) that induced a cloud point temperature (CPT) of the final polymers to keep them in the solid tumor after intratumoral administration. The polymers were modified to bear the complexes of DOTA chelator with 90-Y. The antitumor efficiency of the immunoradiotherapy was tested *in vivo* on mice with EL4 lymphoma using the chosen synthesized polymer.

Results and conclusions:

We observed oxidative burst response of the leukocytes established the immunostimulatory properties of the polymer, which were also studied *in vivo* after the injection into the thigh muscles of healthy mice showing extensive immune activation reactions at the site of injection. The biodistribution study *in vivo* indicated the formation of the polymer depot, which was gradually

degraded and excluded from the body. The radiolabelled polymer was used during *in vivo* antitumor efficiency experiments on mice with EL4 lymphoma, while 7 of the 15 were completely cured and the others exhibited significantly prolonged survival time compared to the control group. The *in vivo* experiments indicated the considerable synergistic effect of using this compared to separately using immunotherapy or radiotherapy.

Financial support of the Ministry of Education, Youth and Sports of the CR (grants # LM2015064 ERIC and 8J18FR038) is gratefully appreciated.

Reference:[1] *J. Controlled Release*, **2017**, *268*, 78-91.

99mTc-labeled hydroxyapatite nanoparticles as potential tracers for solid tumors

Zbynek Novy, Milos Petrik, Marian Hajduch
Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic

Introduction:

The aim of this work was to label newly prepared hydroxyapatite nanoparticles with 99mTc using clinical radiotracer 99mTc-HDP, further verification of radiochemical stability of labelled nanoparticles in vitro and to describe their biodistribution employing SPECT/CT and ex vivo biodistribution approach.

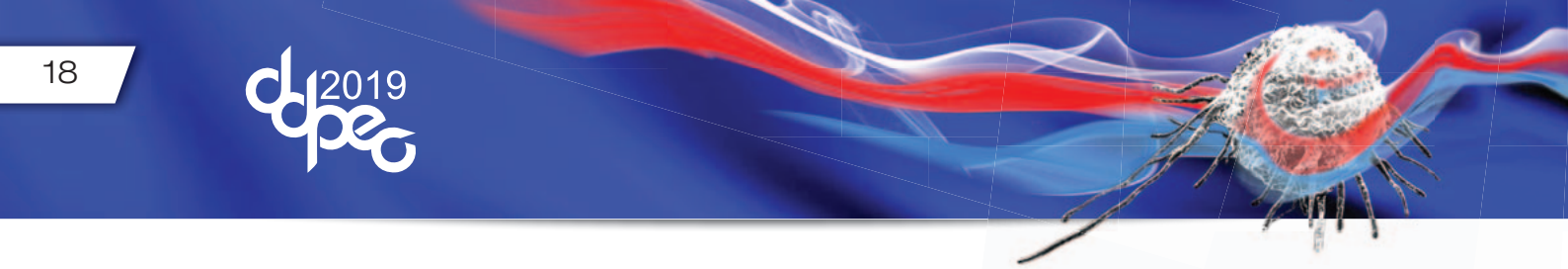
Materials/methods:

Radiolabeling of nanoparticles was done under mild conditions, followed by quality control step represented by radiochemical purity check by iTLC to reveal labeling efficiency and in vitro stability of the tracer. Ex vivo studies were performed on normal and tumor mice. The biodistribution of labeled nanoparticles was also monitored by μSPECT/CT system for two different application approaches. We have studied influence of antiangiogenic therapy with bevacizumab to HAP-NP biodistribution in tumor-bearing mice too.

Results and conclusions:

The nanoparticles were labeled with high radiochemical purity and stability. Ex vivo biodistribution studies revealed dominant accumulation in the liver and spleen. The favorable tumor/blood ratio was determinated from tumor study. In vivo imaging showed mainly the same organs as ex vivo study. Ex vivo data from bevacizumab-treated tumor animals reported significant differences in nanoparticle accumulation. Tested nanoparticles could be labeled with 99mTc-HDP. Their in vitro stability is on satisfactory level. Biodistribution studies revealed high accumulation in liver and spleen. These findings were confirmed by SPECT/CT imaging.

This project was supported by the Ministry of Health of the Czech Republic (grant No. 16-30544A).



New technologies in cancer research I

Chairs: Khushboo Agrawal, Karel Koberna

úterý / 26. listopadu 2019 / Tuesday / November 26th, 2019 / 11:15 - 12:30

New sensitive method for the detection of mycoplasmas using fluorescence microscopy

Anna Ligasová[1], Markéta Vydržalová[2], Lenka Brůčková[2], Anna Janošťáková[1], Renata Buriánová[1], Renata Večeřová[3], Karel Koberna[1]

[1] *Palacký University Olomouc, Faculty of Medicine and Dentistry, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*

[2] *University of Pardubice, Faculty of Chemical Technology, Pardubice, Czech Republic.*

[3] *Palacký University Olomouc, Faculty of Medicine and Dentistry, Department of Microbiology, Olomouc, Czech Republic*

Introduction

Contamination of cell cultures by mycoplasmas is a very common phenomenon. As they can substantially alter cell metabolism and potentially spread to all cell cultures in laboratory, their early detection is necessary. One of the fastest and cheapest methods of mycoplasma detection relies on the staining of mycoplasmas' DNA by DAPI or Hoechst dyes. Although this method is easy and fast to perform, it suffers from the low signal provided by these dyes comparing to the nuclear DNA. Therefore, the indirect staining of the reporter cell lines using DNA dyes is usually used.

Materials/methods

Human A549 and Lep cells and mycoplasma strains *M. hominis*, *M. fermentans* and *M. arginini* were cultured at 37 °C in an atmosphere containing 5 % CO_2. The mycoplasma detection was performed by the developed approach, by direct DNA staining, RT-PCR or MycoAlert PLUS Mycoplasma Detection Kit.

Results and conclusions

In the study presented, we have developed and tested a new immunofluorescence assay for the detection of mycoplasmas. The method is based on the enzymatic labelling by modified nucleotides utilizing nicks in the mycoplasmas' DNA. Modified nucleotides are incorporated into mycoplasmas' DNA and subsequently visualised by immunofluorescence microscopy. The developed approach is independent of the mycoplasma strain, does not intensely stain nuclear DNA, does not stain other bacteria and provides higher sensitivity than the approach based on the direct labelling using DAPI or Hoechst dyes.

Funding:

This research was supported by the Technology Agency of the Czech Republic, grant number TE02000058, the Ministry of Education, Youth and Sports of the Czech Republic -- Project EATRIS-CZ, grant number LM2015064 and the European Regional Development Fund - Project ENOCH, grant number CZ.0 2.1.01/0.0/0.0/16_019/0000868.

Hair follicles as a promising biological material and their use in cellular senescence and epithelial biomarkers

Natalie Kudlova, Hanus Slavik, Pavlina Duskova, Marian Hajduch, Martin Mistrik

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

Introduction

Regarding experiments performed on laboratory animals, we naturally need to sample them to obtain biological material as a source of information hidden in DNA, RNA, proteins etc. Nowadays it is quite common to get samples invasively from soft tissues especially tails and ears from mice by biopsies. But if we consider the rules set by law - so called *The principles of 3Rs* (Replacement, Reduction, Refinement), we will find out that, for some approaches and biological methods in at least basic research, it is possible to use non-invasively collected material that is sufficient for our purposes. Herein, we present scientific evidence that mouse hair follicles (mouse hair bunches) sampled by our plucking device can serve as a beneficial material for genotyping from DNA, gene expression level measurements from RNA and protein labeling in nucleus and cytoplasm as well.

Materials/methods

For genotyping purpose, we isolated DNA from 151 animals (151 mouse hair bunches sampled by our new pistol-like device and 151 commonly obtained tail biopsies) using cobas® DNA Sample Preparation Kit, then we performed PCR amplification of two target transgenes identifying GMO animals of Alzheimer disease model and compared both result lists. For RNA isolation and gene expression measurement we used mouse hair samples of naturally aged mice (age between 6 months - 2,5 years) and compared p16 and p21 (senescent markers) expression by real-time PCR. Expression of both mentioned markers were also measured in artificially-induced senescence mouse model (3 x 8 Gy of ionizing radiation induction in right legs) vs left legs serving as a control. We also performed an immunofluorescence assay to visualize target proteins, e.g. senescent markers in aging experiments and other nuclear and cytoplasmic proteins just to verify if it is possible in such a biological

material without huge changes in standard protocols.

Results and conclusions

DNA and RNA isolation yields from all samples were sufficient for subsequent procedures. Mean DNA yield from hair follicles was 16,356,91 ng/µl in contrary to 30,6613,18 ng/µl from tail biopsies. Average RNA yield was 10 ng/µl. 100% match between genotyping results from tail biopsies and hair follicles was observed. This fact leads us to the idea of the soft tissue samples´ replacement by less invasively obtained hair samples. We got statistically significant differences of p16 and p21 expression levels among various age groups of naturally aged animals - the older the animal, the higher the expression. We also confirmed higher p16 protein level by IF assay. Then we designed, optimized and developed the irradiation-induced senescence mouse model as a model of accelerated aging *in vivo* that can be used for potential senolytic drugs identification and validation. Generation of such a model takes 3 - 4 weeks (after IR). Hair follicles from the irradiated tissue significantly reveals increased expression of senescence markers. Thus, we confirm the feasible use of hair follicles as an input biological material for a wide variety of experiments instead of soft tissue samples.

Study funding: IGA_LF_2018_005.

Can be human tears used as a source of brain proteins?

Tomáš Oždian[1], Pavel Hok[2], Jan Mareš[2], Petr Kaňovský[2], Petr Džubák[1], Marián Hajdúch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*

[2] *Department of Neurology, Faculty hospital in Olomouc, Olomouc, Czech Republic*

Introduction

In the current proteomics, there is an increasing interest in the analysis of body fluids in biomarker discovery. They offer several advantages. They are often low to non-invasive, close to diseased organs and easily accessible. However, this is not the case for the brain. The only body fluid available for brain disease biomarker studies is cerebrospinal fluid. The acquisition of this fluid is, however, far from non-invasiveness. In this study, we evaluate proteome of the tears as a potential source for brain protein biomarkers.

Materials/methods

In our preliminary study, 5 healthy volunteers and 11 patients with retrobulbar neuritis were involved in study. The tears were acquired using standard Schirmer test. Acquired tears were dissolved and processed for LC-MS proteomic analysis. The mass spectra were searched using Thermo ProteomeDiscoverer v2.3. The proteins have been further annotated for localization using proteinatlas.org.

Results and conclusions

In the healthy controls, there has been identified on average about 700 proteins per sample, whereas in patients it was only about 500 proteins per sample. In total, there are more than 1500 unique proteins identified and quantified in the pilot study. Manual annotation of protein localization showed that about 50% of all identified proteins are expressed in brain, where 20% of proteins from this group are brain-specific. Other relevant protein groups present in tears were proteins of the immune system (25%), plasma proteins (10%) and proteins expressed in saliva (3%). Those numbers suggest potential usefulness of tears for brain biomarker search.

Supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and Internal Grant Agency of Palacky University (IGA_LF_2019_018).

A high-throughput, whole cell-based assay for cytotoxicity profiling of new chemical compounds

Soňa Gurská, Lenka Lachnitová, Petr Džubák, Marián Hajdúch

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

Introduction

High-throughput screening (HTS) is widely used in the field of pharmaceuticals and academic institutes as a primary tool for early-stage drug discovery. This technique was developed to evaluate the biological activity of thousands of individual small molecules and to identify potential drug candidates in a short time.

Materials/methods

One of the methods routinely used in our HTS laboratory is *in vitro* cytotoxicity screening. It is a convenient, phenotypic and predictive mean of characterizing the toxic potential of new chemical entities. The MTS assay as a cytotoxicity test was validated on 10 cell lines (8 cancer cell lines and 2 non-cancer cell lines) in 384 and 1536 well plate format.

Results and conclusions

In the primary screen, all compounds were tested at one concentration (50 µM) and the PI (percentage of inhibition) value was calculated. To calculate IC50 values for selected active compounds (PI > 50%), a secondary (dose-response) screen was performed. Data were analysed by Dotmatics software. To quantify the suitability of cytotoxic assay in HTS, the Z-factor was determined for each plate and cell line. Some results obtained in the cytotoxicity testing will be presented and discussed.

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1.01/0.0/0.0/16_019/0000868 and LM2015063, LM2015064 and

foundation Cancer Research Czech Republic.

Exhaled breath condensates proteomics as a tool to monitor condition of human lungs

Jana Václavková[1], Jana Vrbková[1], Petr Džubák[1,2], Dušan Holub[1], Tatiana Gvozdiaková[2], František Kopřiva[2], Vendula Látalová[2], Juraj Kultan[3], Vítězslav Kolek[3], Petr Jakubec[3], Ondřej Fischer[3], Marián Hajdúch[1]

[1] *Laboratory of Experimental Medicine, Institute of Molecular a Translational Medicine, Faculty of Medicine and Dentistry Palacky University, Olomouc, Czech Republic.*

[2] *Department of Pediatrics, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*

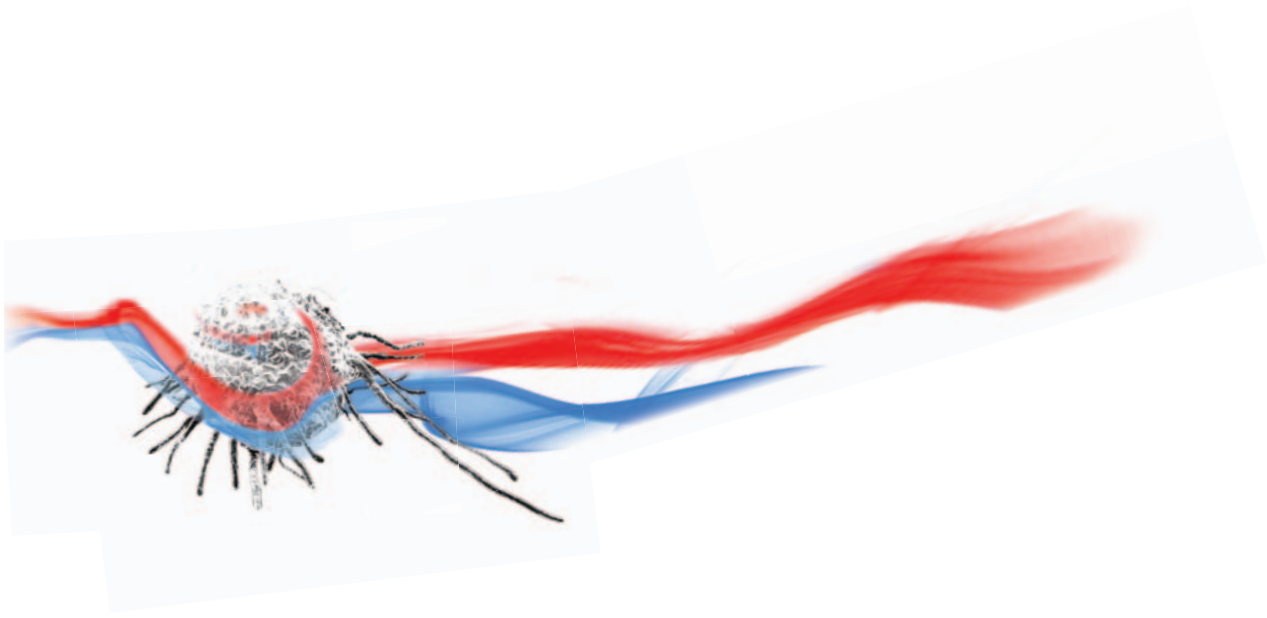
[3] *Department of Respiratory Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*

Exhaled breath condensates (EBC) collection is a cheap and non-invasive method to obtain human samples. EBC represents a rich source of biomarkers which can provide valuable information about respiratory and systemic diseases. Finding new non-invasive methods for early detection of lung diseases (such as lung cancer, asthma COPD, cystic fibrosis etc.) would be highly beneficial. Proteomic analysis of EBC is a prospective method to detect early changes in the status of the respiratory system and possibly other organs. It could also replace or complement some invasive sampling methods in future and provide non-invasive lung diseases screening technique. Our studies are focused on lung cancer, COPD, children's asthma, cystic fibrosis and biomarkers of premature aging of lungs. Together, our studies will advance the development and validation of EBC as a novel tool for the proper diagnosis of asthma, choosing a proper treatment and monitoring treatment efficacy.

Exhaled breath condensate proteins in the sample are solubilized, denatured, reduced, digested and concentration of peptides is measured. Purified samples are diluted for HPLC-MS/MS (LTQ Orbitrap Elite) analysis which is performed in 3 technical replicates. Measured spectra are analyzed by Proteome Discoverer software (Thermo Scientific). Data are further statistically evaluated by Statistica and Bioconductor R – package.

Currently we have analyzed more than 1.000 EBCs from healthy controls and disease groups. We typically detect and quantify low hundreds of proteins per sample. Preliminary statistics indicates combined protein biomarker signatures can detect specific lung disease conditions with both high specificity and sensitivity (typically > 95 %). We were able to develop the biomarkers for multiple disease compared to healthy controls and we have developed a model to identify the biomarkers of premature aging of lungs. Discovered biomarkers will be validated by targeted mass spectrometry analysis, eventually immunochemical methods and Western blot.

This work is supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), by the grants of AZV 16-32302A, AZV 16-32318A and by the Internal Grant of Palacky University IGA_LF_2019_018.



Pathophysiology of cancer and molecular targets III

Chairs: Juan DeSanctis, Viswanath Das

úterý / 26. listopadu 2019 / Tuesday / November 26th, 2019 / 13:30 - 14:45

MIDKINE as a novel tumor-secreted protein driving resistance to immune checkpoint blockade

Daniela Carolina Cerezo-Wallis, María Soledad Soengas, David Olmeda Casadomé

CNIO, Madrid, Spain

Immunotherapy using anti-PD1/PDL1 blocking antibodies offers new options for cancer treatment, but clinical responses remain limited and/or transient in most cases. Primary resistance to immunotherapy is often observed in patients with either low immunogenic tumors (cold tumors), or with lesions infiltrated with immune suppressive cells, such as tumor-associated macrophages (TAMs), T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Yet, the mechanisms that define the immunogenicity of tumors, and more important, biomarkers to predict clinical responses to immunotherapy, are still pending needs in the field. We have previously identified a novel melanoma-secreted protein, called MIDKINE (MDK), with critical roles in lymphangiogenesis and metastasis. Now we have defined a MDK-associated gene expression profile (MAGEP) that is highly predictive of survival in melanoma and other tumor types. Bioinformatics analysis show that MAGEP expression in melanoma correlates with increased immune cell infiltrates, in particular, of MDSCs, TAMs and Tregs. In vivo and In vitro experiments showed that MDK activates NF-κB in melanoma cells via an ALK-dependent manner, leading to an inflammatory secretory program that increases intratumoral recruitment of TAMs and Tregs. Additionally, paracrine signaling of MDK induces STAT3 phosphorylation in myeloid cells, promoting their immune suppressive polarization. This proved to have clinical implication as we found that MDK secretion prevents immunotherapy responses to anti-PD1/PDL1 blocking antibodies *in vivo*. Moreover, we have discovered a potential correlation between MDK expression and poor response to immunotherapy in melanoma patients, highlighting the relevance of our results. As an therapeutic alternative, we have observed that dsRNA-based nanoparticles can reverse MDK-driven immunosuppression by a combined action in melanoma cells and macrophages. Thus in conclusion, we have characterized new mechanisms of resistance to immunotherapy mediated by MDK; yet, we also found that dsRNA-based therapies are able to potentiate anti-PD1/PDL1 blocking antibodies responses, even in refractory tumors.

3D cell cultures are more predictive *in vitro* tumor models for cancer drug discovery and development

Viswanath Das, Narendran Annadurai, Marian Hajduch

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

Three-dimensional (3D) cell cultures are increasingly being recognized as physiologically relevant *in vitro* tumor models in drug discovery and development. As more predictive models that closely reproduce *in vivo* tumor characteristics, 3D cultures are expected to augment translational cancer research. While monolayer two-dimensional (2D) cultures are still a predominant choice for drug screening and target validation studies, they may not be suitable for certain drug-target studies when the target of interest is expressed under certain *in vivo* conditions, such as hypoxia. Herein, taking two examples, we show how 3D environment-induced cellular alterations affect drug-target interaction and how 3D cell cultures can serve as an important bridge to understand spatial dimension-mediated cellular alterations.

In the first example, we show the reliability of spheroid cultures as a better model for studying the cellular effects of carbonic anhydrase IX (CAIX) inhibitors. The human CAIX is a transmembrane enzyme that regulates pH in hypoxic tumors, and its expression is associated with tumor metastases and poor prognosis. CAIX overexpression alters intra- and extra-cellular pH and results in tumor metastasis and resistance to weakly basic anticancer drugs. Human colorectal cancer HCT116 and HT-29 cells express CAIX when simulated with hypoxia or hypoxic mimetics in 2D cultures but abundantly express CAIX under 3D culture conditions without any exogenous stimuli. The selective CAIX inhibitors show low-to-moderate toxicity in 2D cultures but significant toxicity in spheroids. A time-dependent effect of compounds on spheroid growth is proportional to a time-dependent increase in CAIX expression, suggesting that the inhibitors specifically target CAIX-expressing spheroids. Due to the anti-CAIX effects, the compounds facilitate better penetration of doxorubicin in co-treated spheroids, suggesting the potential for future synergistic combinations to combat hypoxic tumors.

Protein $p21^{Cip1/Waf1}$ (p21) is a cyclin-dependent kinase inhibitor and plays an important role in cell cycle arrest, senescence, and apoptosis. The upregulation of p21 and its frequent cytoplasmic relocation correlate positively with poor prognosis, tumor grade, invasiveness and/

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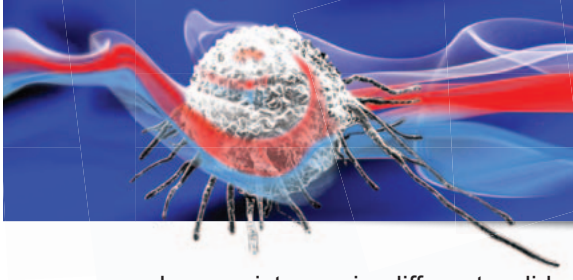


or drug resistance in different solid tumors. In the second example, we present the excellent reproducibility of p21 upregulation and cytoplasmic relocation of p21 in spheroids of colorectal cancer cells and the subsequent development of resistance to anti-cancer drugs. This increased resistance potentially results due to two factors. First, spheroids with increased cytoplasmic show an increased expression of intracellular junctional proteins that affect drug absorption and penetration. Second, a subset of spheroid cells with cytoplasmic p21 appears to form an '*anti-apoptosome*'-like complex by sequestering released cytochrome C or prevent the release of cytochrome C from mitochondria that play a key role in the initiation of apoptosis.

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1. 01/0.0/0.0/16_019/0000868 and LM2015063 and foundation Cancer Research Czech Republic.

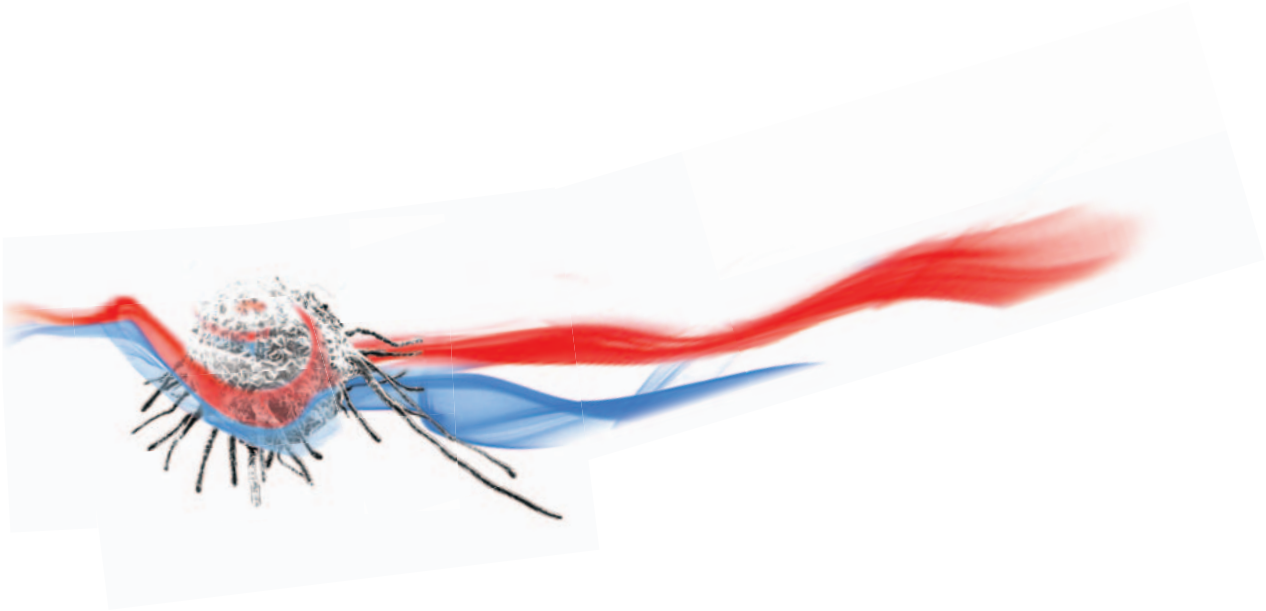
Influencing radiosensitizing properties in cancer cells

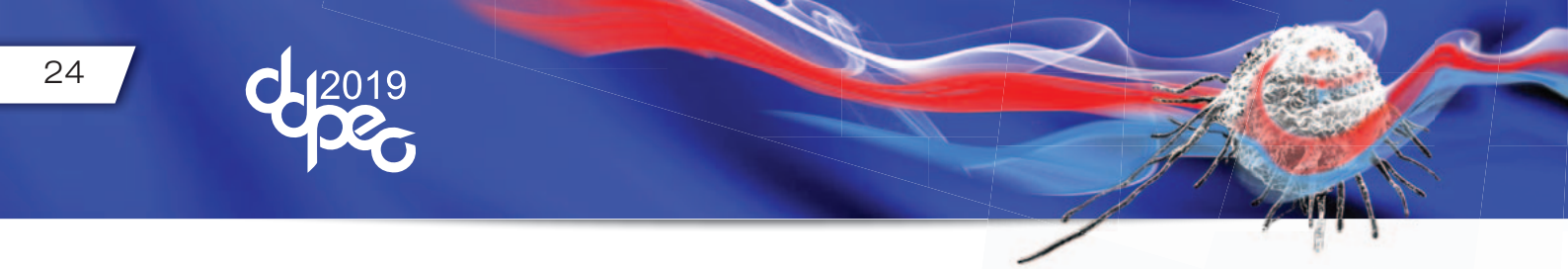
Miroslav Vetrik[1], Libor Kobera[1], Rafał Konefał[1], Volodymyr Lobaz[1], Martin Hruby[1], Guillem Pratx[2]

[1] *Institute of Macromolecular Chemistry, Prague, Czech Republic.*

[2] *Stanford School of Medicine, Stanford, USA*

Radiation treatment (RT) together with surgery intervention and chemotherapy is one of the most applied cancer therapies nowadays. We developed nanoparticles that are able to alter radiation sensitization of cancer cells. Radiation outcome can be boosted or lowered using self-assembled fluorinated dialkyl diselenide nanoparticles. Highly hydrophobic nature of fluorinated chains give molecules its self-assembly properties and are able to prolong oxygen release. Amphiphilic character of the nanoparticles is achieved by incorporation of selenocystine linker that can be cleaved during the radiation treatment. Radiation initialize the cleavage of diselenic bond that act as potent redox-active system. Intracellular generation of reactive oxygen species (ROS) during X-ray application was significantly lowered when 2 Gy and 4 Gy was applied. Nanoparticles interact with RT and surrounding radicals (produced by the irradiation) and the decomposition occurs followed by oxygen burst release. Presented fluorinated nanoparticles with cleavable selenocystine bond can be utilized during RT as radioprotectants potentially protecting healthy tissues from radiation while allowing delivering higher radiation doses to cancer cells. When saturated with oxygen they can act as radiosensitizers. Financial support of the Ministry of Education, Youth and Sports of the CR (grant # LM2015064 ERIC) and of the Czech Academy of Sciences (grant # DAAD-19-09) and Czech Academy of Science (# MSMJ200501801) is gratefully appreciated.





New technologies in cancer research II

Chairs: Marian Hajduch, Martin Mistrik

úterý / 26. listopadu 2019 / Tuesday / November 26th, 2019 / 15:15 - 17:45

Morphology-driven high-plex spatial analysis of tissue microenvironments with the GeoMx™ digital spatial profiling

Stephan McGoldrick

BIOMEDICA, Vienna, Austria

Historically, immunohistochemistry and immunofluorescence have been used to assess spatial heterogeneity of proteins and nucleic acids in tissue specimens. However, these techniques are of limited utility due to restricted dynamic range, difficult quantification and limited multiplexing capability. NanoString's GeoMx™ Digital Spatial Profiling (DSP) is a novel, non-destructive, highly multiplexed assay for the digital characterization of protein and RNA expression from spatially discrete regions of interest (ROI) in FFPE tissue sections. GeoMx™ DSP can simultaneously quantify 96 protein targets and 1,000s of transcripts in multiple ROI and for up to 20 slides per day. Current applications include biomarker discovery, mechanism of action studies, and rare cell characterization.

Microsatellite instability (MSI) – Biomarker for immuno - oncology research and Lynch Syndrome screening

Mónica Sevillano

Promega, GMBH, Mannheim, Germany

Microsatellite Instability has become an increasingly relevant tool in genetic and immuno- oncology research. Deficiencies in DNA mismatch repair (dMMR) can be caused by hereditary, germline mutations or hypermethylation. Either mechanism disrupts expression of functional MMR proteins, allowing replication errors to accumulate across the genome. This can lead to unchecked growth and cancers, but also produces novel proteins. These "foreign" proteins can be immunogenic, recruiting immune effector cells to that tissue. Mononucleotide repeat microsatellite sequences are particularly sensitive to replication errors (mutation) and can be the first evidence of an MMR deficiency. Microsatellite instability (MSI) analysis provides an assessment of DNA mismatch repair (MMR) status, and is clinically useful both for Lynch Syndrome (LS) screening and stratifying patients for checkpoint-inhibitor immunotherapy. Based on recent studies FDA approved the use of MSI tests as an indicator of response to immunotherapeutic drug. Traditionally the FDA approves drugs based on the organ where the cancer originated. It is the first time the approval was based on a molecular signature in cancers (MSI-H/dMMR) which can been found in different parts of the body. This talk explains the clinical workflow of MSI testing from DNA extraction to data interpretation, with focus on the molecular details and advantages of STR analysis.

Introduction to single molecule counting for ultrasensitive detection of protein biomarkers

Stanislav Kukla

Merck spol., s.r.o., Prague, Czech Republic

Protein profiling technologies have become indispensable tools that are being used in all stages of drug discovery research, including basic biomedical research dealing with established cell lines, emerging translational research and pre-clinical screening using various animal models and, finally, also in clinical diagnostics where patient samples such as liquid biopsies or tissues are used. One of the hallmarks of current protein biomarker research is achieving higher sensitivity which can lead to identification of new potential biomarkers and elucidation of their role not only on the system, but also on the molecular level. In short, detecting the previously undetectable.

This talk will be an introduction to a new immunoassay technique [1] that combines a traditional and familiar immunoassay workflow and assay anatomy (such as for ELISA) with patented single molecule counting (SMC™) read-out technology and that enables the detection of low-abundance protein biomarkers with unparalleled sensitivity and accuracy, capturing concentrations down to the femtogram/mL levels. You will learn about the principles how ultra sensitivity in this emerging immunoassay technique has been achieved by combining improved sample preparation steps together with cutting-edge laser technology and special algorithms for signal analysis. Digital counting of individual fluorophore-labeled antibody detection molecules that are released from the sandwich immunocomplex improves signal-to-noise ratios and can lower the limits of detection by two or three orders of magnitude when compared e.g. to classical ELISA, opening completely new horizons in protein biomarker research not only in cancer, but also in immunology and neuroscience. Several typical case studies (ultrasensitive detection of VEGF in breast cancer samples or detection of anti-drug antibodies as part of immunogenicity testing of new cancer therapies) will be also included in the talk.

[1] Joseph Hwang, Munmun Banerjee, Adam S. Venable, Zara Walden, John Jolly,

Cathleen Zimmerman, Elizabeth Adkisson, Qiang Xiao. Quantitation of low abundant soluble biomarkers using high sensitivity Single Molecule Counting technology, Methods,

Volume 158, 1 April 2019, Pages 69-76

Multimodal nuclear hybrid imaging in translational research

Sebastian Eigner, Jens Waldeck, Claudia Oerther, Ali Asgar Attarwalla, Michael Heidenreich

Bruker BioSpin, Ettlingen, Germany

Introduction:

Multimodality imaging with PET/CT and SPECT/CT have become common place in clinical, preclinical and basic medical research. The pivotal question however remains: Do other combinations of imaging modalities had and will have a similar impact in medical science and clinical medicine?

Clearly Multimodal Nuclear hybrid imaging becomes more and more applicable due to latest developments in PET, MRI and CT instrumentation as well as applications.

Methods and Materials

Low dose micro-CT in combination with dedicated full-body PET was used to obtain fast and absolutely quantitative in vivo data. In addition, MRI from translational up to high-field field-strength was applied either sequential or in a simultaneous approach and the patented self-gating IntraGate as well as PolyGate sequences (Bruker BioSpin MRI, Ettlingen, Germany). Analysis had been performed via ParaVision 360 (Bruker) and/or pmod (Zurich, Switzerland)

Results and conclusion:

Clearly, multimodal imaging is thriving and still evolving rapidly. Although software approaches for image fusion will continue to be widely used, the role of hybrid imaging system becomes an more relevant set-up, both in research and clinical practice. Inspired by the success of PET/CT and SPECT/CT implementations like CT-based motion correction, low dose filtering as well as simultaneous multi-mouse gated kinetic PET/MR can be considered the new frontline in (pre)clinical imaging. In addition, Cost saveings , minimized radiation exposure for mice and man as well as drastically increases information gain go hand-in-hand with these development. The integration of structural, functional and molecular imaging with therapeutic intervention represents in the ultimate multimodality platform for biomedical research and eventual clinical application.

Hence multimodal nuclear hybrid imaging is the method of choice for peer-reviewed translational research.

Making genius simpler: Orbitrap Exploris 480, a new quadrupole Orbitrap mass spectrometer

Maciej Bromirski

Thermo Fisher Scientific, Warszawa, Poland

Introduction

Since its introduction, the Orbitrap-based-MS have and are still playing a pivotal role in many different research areas such as proteomics, metabolomics, biopharma, and applied markets including sport doping, forensic toxicology, clinical research, food safety and environmental analysis. Each of these applications comes with different challenges to mass spectrometry. To address some of these challenges, new technological developments, as well as improvements on existing mass spectrometers is a necessity.

Material/methods

Here we evaluated the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer for proteomics applications with data dependent and data independent acquisitions. We demonstrate the benefit of Thermo Scientific™ FAIMS Pro interface for deeper proteome coverage.

Results and conclusions

The FAIMS Pro interface coupled to the Orbitrap Exploris 480 MS provides more unique peptides & proteins identification and moreover better accuracy in TMT-11 plex quantitation. Improvements from PRM in targeted quantitation is illustrated with novel SureQuant methodology, which is integrated into a predefined template ready for use. Furthermore, we determined the high precision and accuracy for LFQ using yeast spiked into a constant HeLa background analyzed in data-dependent acquisition mode on the Orbitrap Exploris480 equipped with a FAIMS Pro interface.

Finally, we focus on instrument robustness, data reproducibility and ease of use with easy maintenance of the new Orbitrap Exploris 480 mass spectrometer.

keywords: Orbitrap, FAIMS Pro, DDA, DIA, TMT Quantiation, Sure-Quant Quantitation, High-Resolution & High-Mass Accuracy

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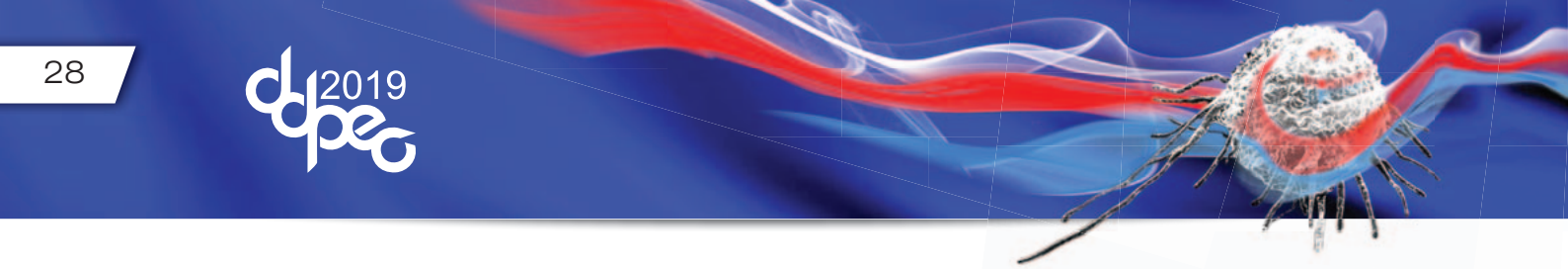

Cancer biomarkers and personalized medicine

Chairs: Jiri Drabek,Vladimira Koudelakova

středa / 27. listopadu 2019 / Wednesday / November 27th, 2019 / 9:00 - 11:00

Identification of circulating tumor cells (CTCs) in advanced breast cancer patients by EpCAM independent system CytoTrack

Małgorzata Szostakowska-Rodzoś, Anna Fabisiewicz, Katarzyna Pogoda, Agnieszka Jagiełło-Gruszfeld, Zbigniew Nowecki, Urszula Śmietanka, Ewa Grzybowska

The Maria Skłodowska-Curie Cancer Center and Institute of Oncology, Warsaw, Poland

Introduction:

Current adjuvant treatment strategies are guided by characterization of the primary tumor, despite the fact that metastases can differ in terms of genetic characteristics. Thus, there is a need for molecular characterization of the metastatic lesion. However, the inaccessibility of the metastatic sites and invasiveness of biopsy are challenging. Therefore, analyzing circulating tumor cells (CTCs) as a surrogate for the metastatic lesion would serve as non-invasive method for determination of heterogeneity among primary tumor and metastases. Identification of resistance associated mutations in CTCs may improve treatment decisions and patient outcome.

Aim:

Identification and enumeration of CTCs in peripheral blood of advanced breast cancer patients. The second aim of the project is to identify mutational changes in single CTCs in metastatic breast cancer associated with resistance to anti-estrogen treatment and cell migration. Comparison of molecular profile in primary tumor and metastases.

Materials and Methods: Primary tumors and peripheral blood (PB) samples were collected from patients with advanced breast malignancy during treatment with anti-estrogen therapy, in The Maria Skłodowska-Curie Institute of Oncology, in Warsaw. Nuclear cells were isolated and prepared for further analysis. Identification, enumeration and isolation of CTCs form PB samples was performed using novel instrument CytoTrack (Denmark), that combines flow cytometry and fluorescent microscopy. Single CTCs isolated via micromanipulation (CytoPicker) were lysed and single cell whole genome amplification (WGA) was performed using MALBAC system. Mutations in genes associated with anti-estrogen resistance and migration (ESR1, ESR2, AKT1, AKT2, PIK3CA) are going to be identified via NGS and compared with mutation status from primary tumor samples.

Results:

Our preliminary data show utility of CytoTrack for identification, enumeration and isolation of single CTCs from breast cancer patients. We identified 1332 CTCs in 25% of 88 patients included in the project. The studies of CTCs heterogeneity are ongoing project. The single cell NGS studies are in progress.

Conclusion:

Circulating tumor cells are rare and their number in blood is dynamically changing during therapy. CytoTrack enables to isolate CTCs independently of their EpCAM status.

Determination of lung tumor mutation burden to predict the effects of immunotherapy with checkpoint inhibitors

Rastislav Slavkovský[1], Barbora Koblihová[1], Lucie Kotková[1], Ondřej Fischer[2], Jiří Drábek[1], Marián Hajdúch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic*

[2] *Department of Tuberculosis and Respiratory Diseases, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital in Olomouc, Olomouc, Czech Republic*

Introduction:

High tumor mutation burden (TMB) is an emerging biomarker of sensitivity to immune checkpoint inhibitors (ICI) and has been shown to be significantly associated with response to PD-1 and PD-L1 blockade immunotherapy.

Materials/methods :

In this pilot study we established method of TMB assessment by massive parallel sequencing (MPS) using QIAseq Targeted DNA panel - Human Tumor Mutational Burden Panel (QIAGEN) and HISEQ 2500 (Illumina). Two groups of lung adenocarcinoma samples acquired from patients treated with nivolumab were compared. First group included responding or stable disease patients and second group included patients with progressive disease.

Results and conclusions:

The method of library preparation and sequencing was optimized. It was shown that DNA re-purification using Monarch DNA clean up kit is required for proper library preparation. Out of total 25 sequenced samples, data from 18 tumor samples and 2 negative controls passed high quality filter. Out of these high confidence samples 3 samples showed high TMB values (>15 mut/Mb) and all the patients showed good overall clinical response (partial remission). 13 samples showed low TMB values (<10 mut/Mb) and majority of the patients showed bad overall clinical response (progressive disease).1 samples showed intermediary TMB value, 1 sample need to be removed due to the confusing clinical data and 2 negative samples were correctly negative for

somatic mutations. Althought with limited statistical power due to the small sample size, our pilot study in overal showed good correspondence of TMB value and clinical response to ICIs support the role of TMB for prediction of nivolumab response.

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grant NCLG CZ.02.1.01/0.0/0.0/16_013 /0001634) and Palacky university (grant IGA_LF_2019_003).

HPVPro study: Comparison of HPV detection in cervical and cervicovaginal swabs

Vladimíra Koudeláková[1], Hana Jaworek[1], Magdalena Uvírová[2], Marián Hajdúch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.*

[2] *CGB Laboratyory, Inc, Olomouc, Czech Republic*

Introduction

The cervical screening program in the Czech Republic is based on cytology with HPV triage. The *implementation* of primary *HPV screening and increasing cervical screening attendance* are a major challenge. The objective of the HPVPro study was to find out the HPV prevalence in the screening population of Czech women since there are no data for the Czech Republic. The second objective was to compare HPV DNA detection in paired self-sampled cervicovaginal swabs and physician-obtained cervical swabs.

Methods

Cervical swabs were taken by a gynaecologist from 1044 Czech women (age 30-64 years) during the regular screening examination. Cervicovaginal swabs were obtained by self-sampling using digene HC2 collection device, Qiagen (HPVPro1, 544 women) and EvalynBrush, Rovers Medical Devices (HPVPro2, 500 women). All samples were analysed using Hybrid Capture 2 (HC2, Qiagen), 500 paired samples from the HPVPro2 study were analysed also using Qiascreen HPV PCR Test (Qiagen).

Results and conclusions

Hybrid Capture 2 detected hrHPV positivity in 11.2% (117/1044) of cervical swabs and 14.0% (76/544) of swabs sampled by HC2 collection device and 10.4% (52/500) of swabs sampled by EvalynBrush. HC2 detected hrHPV positivity in at least one sample in 15.2% (159/1044). Qiascreen detected hrHPV in 9.7% (47/486) of cervical swabs and 10.5% (51/486) of cervicovaginal swabs. Qiascreen detected hrHPV positivity in at least one sample in 11.5% (56/486). Concordance of cervical and cervicovaginal hrHPV positivity was 93% for HC2 and 97.1% for Qiascreen.

HPV prevalence in the screening population of Czech women is between 11% and 15% depending on the used HPV detection method. HPV detection in cervical and cervicovaginal swabs was highly concordant. The offering of self-sampling could significantly increase the attendance of Czech women in the cervical screening program.

This work was supported by grants: IGA LF UP 2019_003, CZ.02.1.01/0 .0/0.0/16_019/0000868, LM2015064 and foundation Cancer Research Czech Republic.

Minimal Residual Disease Monitoring in Mantle Cell Lymphoma-from diagnosis to treatment

Anna Fabisiewicz[1], Michał Szymczyk[2], Małgorzata Szostakowska- Rodzoś[1], Zbigniew Bystydzieński[3], Grzegorz Rymkiewicz[3], Monika Świerkowska-Czeneszew[2], Joanna Romejko-Jarosińska[2], Ewa Paszkiewicz- Kozik[2], Krystyna Domańska-Czyż[2], Beata Ostrowska[2], Włodzimierz Osiadacz[2], Renata Zub[1], Janusz Siedlecki[1], Jan Walewski[2]

[1] *Department of Molecular and Translational Oncology,Maria Sklodowska-Curie Institute-Oncology Centre, Warsaw, Poland.*

[2] *Department of Lymphoproliferative Diseases,Maria Sklodowska-Curie Institute- Oncology Centre, Warsaw, Poland.*

[3] *Laboratory of Cytometry Maria Sklodowska-Curie Institute-Oncology Centre, Warsaw, Poland*

Mantle cell lymphoma (MCL) is a rare and incurable disease of lymphoid system. MCL is characterized by an aggressive course and multiple relapsing. No therapeutic standard is available neither in the first nor in the subsequent lines of treatment of this disease. The main cause of death in MCL patients is relapse due to undetermined minimal residual disease (MRD) and therefore monitoring MRD is crucial for making the best treatment decisions. The gold standard method for MRD analysis is the quantitative polymerase chain reaction. The most commonly used molecular markers for measuring MRD in MCL are: t(11;14)(q13;p32) translocation or *CCND1* expression and IGH rearrangement.

In this lecture we present data from European MCL Network and Nordic MCL2 and MCL3 studies as well as our own research on monitoring of MRD in MCL patients who were treated in the Department of Lymphoproliferative Diseases in the Maria Sklodowska-Curie Institute- Oncology Centre in Poland. We also studied *SOX11* expression as molecular marker for MRD in MCL and compared it with previously used markers: t(11;14) and IGH rearrangement. The predictive value of *SOX11* expression was confirmed, when high *SOX11* expression is correlated with a poor prognosis. *SOX11* expression is highly specific for MCL and independent of the presence of t(11;14) and thus it can be used as a molecular marker even for the *CCND1(-)* negative MCL subtype.

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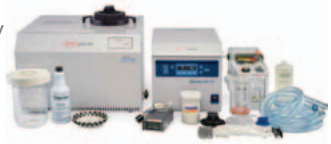

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laboratorní myčky
velkokapacitní myčky
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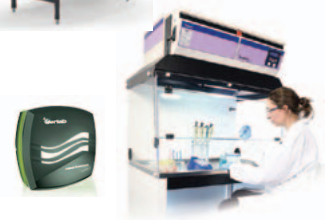
PŘÍPRAVA ČISTÉ VODY
reverzní osmóza
deionizační systémy
kompaktní laboratorní systémy
poloprovozní systémy

PIPETY LABORATORNÍ PLASTIK
pipety Finnpipette a ClipTip
kompletní sortiment špiček
dávkovače a pipetovací nástavce
spotřební plastik Nalgene, Nunc a Matrix
plastik pro environmentální výzkum

BIOHAZARDY, IZOLÁTORY LAMINÁRNÍ BOXY
biohazard boxy tř. II
laminární boxy
biohazardy tř. III a izolátory
lamimární moduly
monitoring prostředí

BEZODTAHOVÉ DIGESTOŘE
digestoře a vážící boxy
filtrační systémy pro místnosti
monitoring laboratorního prostředí



DEKONTAMINAČNÍ SYSTÉMY H_2O_2
VHP generátory pro dekontaminaci
aerosolové generátory
dekontaminace prostor a zařízení

INKUBÁTORY, TERMOSTATY
CO_2, CO_2/O_2 inkubátory
termostaty do +100°C, +300 °C
anaerobní a hypoxické boxy
sušárny do +500°C
klimatické a růstové boxy
systémy pro monitoring teploty

MRAZICÍ A CHLADICÍ BOXY, KRYO BOXY
mrazicí boxy do -86 °C až -180 °C
řízené zamrazování LN_2 -180 °C
mrazicí boxy -5 °C až -40 °C
chladicí boxy 0 °C až +15 °C

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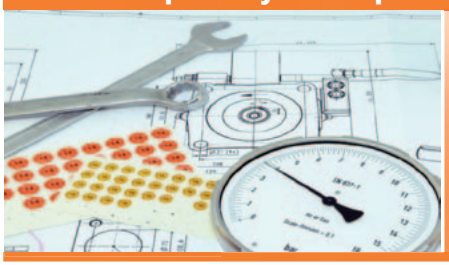

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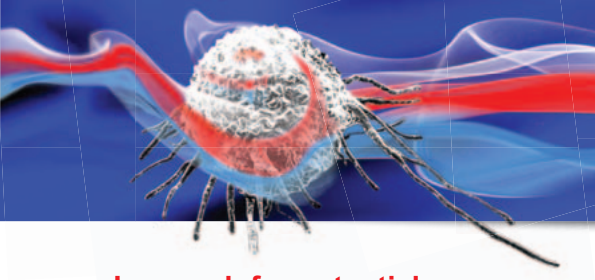

In search for potential biomarkers: deregulation of microRNAs in oropharyngeal carcinoma

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Introduction

Head and neck squamous cell carcinomas are a group of heterogenic tumors arising from epithelial tissue of aerodigestive tract characterized by difficult diagnosis, treatment and prognosis. Alcohol consumption, smoking and highrisk human papillomavirus infection are very well described risk factors of head and neck cancer development. Oropharyngeal carcinomas are malignancies developing in the throat area downstream to oral cavity.

microRNAs (miRNAs) are short (~23 nt) non-coding RNA molecules participating in regulation of gene expression. Primary function of miRNAs is negative translation regulation as part of RISC (RNA-induced silencing complex) by translational repression and mRNA degradation. miRNAs are involved in various disease pathologies such as neurodegenerative diseases, metabolic disorders and others. Moreover, microRNAs have been recognized as key molecules in cancer development and progression in various types of tumors.

In the present study we studied relative expression of miRNA in unique set of formalin-fixed, paraffin-embedded (FFPE) squamous cell oropharyngeal cancer samples and related metastases. In selected samples we analyzed the miRNome using Agilent miRNA microarray platform and small RNA sequencing in Illumina platform. Preselected miRNA deregulations were further confirmed by qPCR analysis with TaqMan Advanced Assays. The data were further analyzed in context of recorded clinicopathological and follow-up data.

Materials and Methods

As the primary aim of the study was the comparison of miRNA expression profiles between primary tumors, corresponding lymph node metastasis and control samples, only surgically treated oropharyngeal squamous cell carcinoma patients with positive cervical lymph node metastases were enrolled in the study. FFPE samples from 73 primary oropharyngeal tumors and corresponding metastases were analyzed. As controls we used 44 samples of palatine tonsils resected due to chronic tonsillitis. Thus, a total of 190 formalin fixed, paraffin-embedded samples of oropharyngeal tissue were analyzed.

Total RNA including miRNAs was isolated from FFPE tissue samples using AllPrep DNA/RNA FFPE kit (Qiagen). Concentration and purity of isolated RNA was first assessed by NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific), Qubit Fluorimeter (Thermo Fisher Scientific) or Agilent Bioanalyzer (Agilent). G3 Human miRNA Microarray, Release 21, 8×60K (Agilent) was used for high throughput miRNome expression analysis of 40 selected samples of OPSCC (tumors, related metastases and controls) in cooperation with Central European Biosystems, s. r. o. For sRNA NGS library preparation NEBNext Small RNA Library Prep Kit for Illumina (New England Biolabs) was used (40 samples) and sequenced on Illumina MiSeq using MiSeq Reagent Kit v2 (50 cycles). The FASTAQ files were analyzed using Oasis 2.0 detection software. The synthesis of cDNA was performed using TaqMan™ Advanced miRNA cDNA Synthesis Kit with universal reverse transcription primers (Applied Biosystems). Real-time PCR was done with specific TaqMan™ Advanced miRNA Assays (Applied Biosystems) on Rotor-Gene Q (Qiagen). Relative expression of each miRNA was determined using the $2^{-\Delta\Delta Ct}$ method. All statistical tests (Student's t-test, ANOVA, Logrank test were two tailed and $P < 0.05$ results were considered statistically significant.

Results and Discussion

The first phase of the study was preselection of interestingly deregulated miRNAs using high-throughput miRNA expression methods. The second phase of the study was designed to verify the changes in expression of pre-selected miRNAs by real-time PCR.

From all the results of miRNA microarray analysis miR3656 and miR206 were selected for further validation second phase of the study. After evaluation of all small RNAs NGS data two miRNAs were selected for further validation by realtime PCR: miR3753p and miR1505p.

The real-time PCR validation confirmed that miR3656 was significantly upregulated in tumor samples (FC = 6.56 and P = 0.0006) and less but significantly upregulated in metastasis samples (FC = 3.04 and P = 0.012) compared to control samples. Moreover, deregulation between the three sets of samples was significant according to ANOVA (P = 0.0012). Correspondently, miR1505p was significantly downregulated in tumor samples (FC = 14.75 and P < 0.0001) and significantly downregulated in metastasis samples compared to control samples (FC = 8.94 and P < 0.0001). Moreover, ANOVA analysis confirmed statistical significance in

expression between all three sets of samples (P < 0.0001) and there was difference in expression between metastases and tumors (FC = 1.30 and P = 0.049).

On the other hand, miR206 was significantly downregulated in metastasis samples compared to tumor samples (FC = 39.99 and P < 0.0001). It was significantly upregulated in tumor samples compared to controls (FC = 10.93 and P < 0.0001). In contrast, there was a significant downregulation in metastasis samples compared to control samples (FC = 3.93 and P = 0.0061). Finally, there was a trend of downregulation of miR375 in metastasis samples compared to tumor samples (FC = 2.45 and P = 0.146). Even though, the deregulation was statistically significant according to small RNA NGS data, we were unable to confirm the statistically significant results using realtime PCR analysis.

Patients with lower expression of miR-150-5p in primary tumors and metastases (based on median value) had impaired survival in comparison to higher expression group (P = 0.0124 and P = 0.0192).

All the miRNAs selected for second phase of the study correlated with at least one of the tracked clinicopathological characteristics. miR-150-5p correlated with several recorded parameters. The trend of higher downregulation of miR-150-5p in HPV positive samples was observed with P = 0.071. Keratinizing tumors had lower expression of miR-150-5p than non-keratinizing tumors (P = 0.010). Size and extension of the tumor also correlated with miR-150-5p expression, when lower expression of the miRNA was present T3 – T4 stage tumors (P = 0.0016). Moreover, downregulation of miR-150-5p was less prominent in tumors with higher regional lymph nodes metastases involvement (N2 – N3) (P = 0.028). miR-150-5p expression also correlated with recurrence (three types of recurrence were recorded: local, regional and distant) of the disease and the downregulation was more significant in patients with recorded follow-up recurrence (P = 0.053).

We have concluded that miR-150-5p (among others) may be the best potential biomarker for oropharyngeal squamous cell carcinoma. miR-150-5p was significantly in both tumor and metastasis samples, its low expression in tumor and metastasis samples was associated to impaired survival of the patients and it was less expressed in advanced stages of the disease. Moreover, we have confirmed that sRNA sequencing is a suitable method for miRNome analysis.

Funding

The study was supported by the program MH CZ – DRO (UHHK, 00179906), SVV grant 260398, by the program PROGRES Q40/11 and by European Regional Development FundProject BBMRICZ.: Biobank network – a versatile platform for the research of the etiopathogenesis of diseases No. EF16 013/0001674.

Transcriptomic profiling in meningiomas for understanding of pathophysiology and biomerkers discovery

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Introduction

Meningiomas represent about 20 % of all intracranial tumors and their growth rates are highly variable. Even within benign subgroups, causing some cases to remain stable while others grow rapidly despite radiotherapy. Biomarkers that differentiate meningiomas by aggression and enable prediction of their biological behavior would therefore be very clinically beneficial.

Material, methods and patients

RNA was obtained from 70 FFPE meningioma samples with various clinical characteristics. The cDNA libraries for NGS were prepared using TruSeq Stranded Total RNA Library Prep Kit with RiboZero Gold - Set A (Illumina) and sequenced on HiSeq 2500 (Illumina). Differentially expressed mRNA and lncRNA were analyzed at q ≤ 0.050 and logFC ≥ 2 or ≤ -2 in studied subgroups (gender, recurrence status, WHO grade and tumor localization). Selected markers were chosen for further validation using independent patient cohort.

Results and conclusions

In total, 15 mRNA and 59 lncRNA were differentially expressed in males and females. These results showed the most accurate hierarchical clustering. However, the WHO grades expressed the highest transcripts deregulation. Also, primary recurrent and non-recurrent tumors showed a high level of deregulation (69 mRNA and 108 lncRNA). The differentially expressed genes overlapped strongly in both, recurrence status and WHO grades subgroups.

Gene expression levels differ in meningioma subgroups and can reveal more detailed insight in its pathogenesis improving prediction, prognosis and personalized therapy.

The project was financially supported by grants IGA_UP_LF_2019_003 and European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Cancer therapeutics II: Small molecules

Chairs: Petr Dzubak, Milan Urban

středa / 27. listopadu 2019 / Wednesday / November 27th, 2019 / 11:15 - 13:00

Disulfiram anti-cancer activity reflects targeting NPL4, not inhibition of aldehyde dehydrogenase

Zdenek Skrott[1], Dusana Majera[1], Jan Gursky[1], Tereza Buchtova[1], Marian Hajduch[1], Martin Mistrik[1], Jiri Bartek[1,2,3]

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Introduction:

Aldehyde dehydrogenase (ALDH) is a proposed biomarker and possible target to eradicate cancer stem cells. ALDH inhibition as a treatment approach is supported by anti-cancer effects of the alcohol-abuse drug disulfiram (DSF, Antabuse). Given that metabolic products of DSF, rather than DSF itself inhibit ALDH *in vivo,* and that DSF's anti-cancer activity is potentiated by copper led us to investigate the relevance of ALDH as the suggested molecular cancer-relevant target of DSF.

Materials/methods:

Cell viability was analysed by XTT test or by calculation of growth area. The integrity of cell membrane was analysed by propidium staining and flow-cytometry. The levels of proteins and their subcellular localisation was detected by western blot or immunofluorescence coupled with quantitative microscopy. Formation of CuET in media was analysed by HPLC-MS. The activity of ALDH was measured by ALDEFLUOR assay and flow-cytometry.

Results and conclusion:

In this study we show that DSF does not directly inhibit ALDH activity in diverse human cell types, while DSF's *in vivo* metabolite, S-methyl-N,N-diethylthiocarbamate-sulfoxide inhibits ALDH activity yet does not impair cancer cell viability. Our data indicate that the anti-cancer activity of DSF does not involve ALDH inhibition, and rather reflects the impact of DSF's copper-containing metabolite (CuET), that forms spontaneously *in vivo* and in cell culture media, and kills cells through aggregation of NPL4, a subunit of the p97/VCP segregase. We also show that the CuET-mediated, rather than any ALDH-inhibitory activity of DSF underlies the preferential cytotoxicity of DSF towards BRCA1- and BRCA2-deficient cells. These findings provide evidence clarifying the confusing literature about the anti-cancer mechanism of DSF, a drug currently tested in clinical trials for repositioning in oncology.

The impact of disulfiram on DNA replication dynamics in cancer cells

Dusana Majera[1], Zdenek Skrott[1], Martin Mistrik[1], Jiri Bartek[1,2,3]

[1] *Institut of Molecular and Translational Medicine, Olomouc, Czech Republic.*

[2] *Danish Cancer Society Research Center, Copenhagen, Denmark.*

[3] *Karolinska Institute, Stockholm, Sweden*

Introduction:

Recently reported preferential cytotoxicity of disulfiram to cancer cells lacking BRCA1 and BRCA2 is opening new possibility for targeted treatment of cancers with mentioned genetic backgrounds, such as breast and ovarian cancer. In this study we investigate the unknown effect of disulfiram on DNA replication dynamics and DNA damage response pathways, since BRCA1 and BRCA2 are both involved in this processes.

Materials/methods:

For efficient knockdown of BRCA1 and BRCA2 genes, we used doxycycline-inducible shBRCA1 and shBRCA2 H1299 cells. Effect of disulfiram on DNA replication was evaluated using DNA combing assay, which allows determination of DNA replication fork velocity. DNA damage responses were analysed by immunofluorescence staining of various DNA damage proteins using quantitative microscopy. Replication stress responses were assessed by immunoblotting and immunofluorescence.

Results and conclusions:

We concluded that disulfiram is causing DNA damage, as documented by γH2AX foci formation, preferentially in S/G2 cells. Further it reduces DNA replication and DNA replication fork velocity, while increasing origins of replication. Induction of replication stress was confirmed by RPA foci formation and accumulation of ssDNA detected as BrdU foci in non-denaturated cells. Homologous recombination repair pathway is also activated after disulfiram treatment and this was evaluated by Rad51 foci formation. Despite exhibiting strong replication stress as a consequence of disulfiram treatment, cells fail to activate ATR-Chk1 signalling cascade. Exact mechanism behind compromised ATR-Chk1 signalling pathway after disulfiram treatment is not known and needs to be study further.

Transformations of lupane triterpenes in the positon C-30: synthesis, cytotoxic activity, SAR

Milan Urban[1], Jiří Hodoň[1], Lucie Borková[1], Jan Pokorný[1], Anna Kazakova[1,2], Ivo Frydrych[1], Soňa Gurská[1], Jiří Řehulka[1], Marián Hajdúch[1]

[1] *IMTM, LF-UP, Olomouc, Czech Republic.*

[2] *Dept. of Organic Chemistry, Faculty of Science, UP, Olomouc, Czech Republic*

Introduction:

Triterpenoids are natural compounds from plants, fungi, marine invertebrates or other organisms. They have often a variety of biological activities.1 Betulinic acid is one of the lupane triterpenes with moderate cytotoxicity and its derivatives are proven to inhibit maturation of HIV.2

Results:

In our earlier work, betulinic acid was oxidized in the position C-30 in order to improve its cytotoxic activity.3 Resulting 30-oxobetulinic acid had IC50 in low micromolar concentrations in multiple cancer cell lines but the compound suffered from its high toxicity against normal human fibroblasts which is caused by the presence of a Michael acceptor. Therefore the molecule was further chemically transformed to either weaken the Michael acceptor by the introduction of azine moiety,4 or by the complete removal of this part using Wittig reaction followed by various cyclocondensations. As a result, triterpenes substituted with various polycyclic aromatic and heterocyclic compounds were obtained that had low cytotoxicity in micro to nanomolar scale and high selectivity index.

Conclusion:

New synthetic approach was applied to betulinic acid that yielded novel compounds with selective cytotoxicity in nanomolar range. Currently, the mechanism of action and basic assumptions about SAR are being evaluated and preliminary results will be discussed as well as the possible pharmacophores.

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1. 01/0.0/0.0/16_019/0000868 and LM2015063.

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Triterpenoid thiazoles: from design and synthesis to in vitro biological evaluation

Lucie Borková[1,2], Soňa Gurská[2], Barbora Lišková[2], Marián Hajdúch[2], Milan Urban[2]

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[2] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic*

Introduction

Pentacyclic triterpenes are secondary metabolites from plants and other living organisms with many interesting biological activities, such as antiviral, antibacterial, anti-inflammatory. The aim of many research groups is to prepare their semi-synthetic derivatives with higher activity, better selectivity and better pharmacological properties. Modification of the A-ring of natural triterpenes is currently a hot topic in the development of new antitumor triterpenoids.[1] More specifically, hundreds of the most active derivatives contain a heterocycle.[2] Recently, we have developed and optimized synthetic approach for the synthesis of a large library of heterocyclic triterpenoids and evaluated their biological properties.

Materials/methods

Starting from ketones of seven different triterpenic families, an efficient and straightforward synthesis of both substituted and unsubstituted aminothiazoles fused to the A-ring was developed. All prepared compounds were tested for their cytotoxic activity on 8 cancer and 2 non-cancer cell lines by MTS assay. The most potent derivatives were further tested for their impact on cell cycle analyzed by flow cytometry. Pharmacological parameters, such as chemical, plasma, and metabolic stability, membrane permeability, and binding to plasma proteins were evaluated.

Results and conclusions

As a result, 70 new compounds were prepared and a number of them had IC_{50} < 5 μM with high selectivity against cancer cells. The study of the influence of the best compounds on cell cycle revealed an accumulation of cells in G2/M phase, which corresponds with decreased DNA/RNA synthesis. Moreover, four compounds have appropriate pharmacological parameters and are selected for future *in vivo* ADME-Tox evaluation on mice.[3,4]

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1. 01/0.0/0.0/16_019/0000868 and LM2015063.

4) References

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Cytotoxic triterpenoid thiazoles induce apoptosis in CCRF-CEM cells via disruption of mitochondria

Ivo Frydrych[1], *Lucie Borková*[1,2], *Soňa Gurská*[1], *Marián Hajdúch*[1], *Milan Urban*[1,2]

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Introduction

Semisynthetic triterpenes containing a heterocycle in their molecule are often selectively cytotoxic to cancer cells, making them promising candidates for the treatment of various cancers. We have focused on the synthesis of aminothiazoles that all contain free carboxylic acid (C-28) and in addition, we have also prepared analogues with unsubstituted amino-group that were not part of the previous studies. As a result, a set of triterpenoid thiazoles derived from free betulonic acid, dihydrobetulonic acid, and ursonic acid was synthesized. Among them, we identified four highly cytotoxic compounds (4m, 5m, 6m, 7b) with good pharmacological profile and studied them more deeply.

Materials/methods

Cytotoxicity against CCRF-CEM cells has been determined by MTS assay. For the investigation of apoptosis induction, we have used AnnexinV/propidium iodide double labelling with subsequent flow cytometry analysis. Caspase 3/7 enzymatic activity was detected and measured in cell extracts using artificial fluorogenic substrate acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin (Ac-DEVD-AMC). The effect of the compounds on the expression of apoptosis-related proteins was studied by western blot. Mitochondrial membrane potential was analysed by flow cytometry using fluorescent sensitive probe JC-1. For the cell cycle evaluation, propidium iodide staining with subsequent flow cytometry analysis has been used.

Results and conclusions

Based on Annexin V/PI double labelling, we found that all selected derivatives induce apoptosis. Apoptotic signalling was further confirmed by caspase-3/7 enzymatic activity increase induced by all derivatives. To further examine the mechanism of apoptosis, the effect of the compounds on the expression of apoptosis-related proteins was studied. Protein expression analysis revealed caspase-3 and -7 processing, as well as PARP cleavage in cells treated by 5m and 7b. On the other hand, compounds exhibited no effect on caspase-8 activation, supporting the evidence of mitochondrial (intrinsic) pathway of apoptosis. This assumption was further supported by observed modulation of Bcl-2 as well as BAX protein expression. Moreover, all the compounds induced mitochondrial depolarization. Among the compounds studied, 5m and 7b showed the most promising results and they are the best candidates to become potentially new anticancer drugs.

This work was supported by grants: IGA LF UP 2019_018, CZ.02.1.01/0.0/0.0/16_019/0000868 and LM2015063, LM2015064 and foundation Cancer Research Czech Republic.

Functional screening of adenosine receptors: from model validation to active compound identification

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Introduction

G-protein coupled receptor (GPCR) protein family of adenosine receptors (AdRs) is involved in the regulation of a plethora of biological processes, including pathophysiological conditions such as cancer. The anti-cancer properties of ARs were confirmed both *in vitro* and *in vivo*. Given the ubiquitous expression of ARs and the lack of specificity of compounds targeting them, there is a current need for highly selective and potent agonists and antagonists of AdRs.

Materials/methods

We developed a pipeline comprised of (1) a series of validation, (2) primary screen for selection of preliminary actives, (3) counter-screen for unambiguous hit identification, (4) secondary screen for active confirmation, and (5) orthogonal assay for further verification.

Results and conclusions

Offering high sensitivity and specificity with minimal interference (such as that from autofluorescent compounds), our principal cell-based functional assay utilizes aequorin luminescence induced by activation of GPCR of interest. Moreover, cell-based functional screening provides more complex insight into interactions of compounds and AdRs in comparison to biochemical assays.

Although several AdRs ligands are currently in clinical trials for various conditions, including cancer, AdR potential is yet to be unravelled and fully understood. AdR ligands are often reproached for their low selectivity. Hence, targeting only a single AdR could substantially contribute to the elimination of any potential off-targets. We can thus precisely determine which receptors are being activated. Also, we can specify the mechanism of action in following profiling studies. Building a GPCR screening platform has provided us with a comprehensive tool for the characterization of small molecules as modulators of GPCRs, including AdRs.

This study was supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and internal grant of Palacky University IGA_LF_2019_018.

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Posterová sekce / Poster section

1

Epigenetic age estimation of the healthy Czech population by AgePlex

Helena Štefanová[1], Barbora Blumová[1], Veronika Holinková[1], Jana Stránská[1,2], Lucie Kotková[1], Rastislav Slavkovský[1], Karolína Bartáková[1], Jiří Drábek[1,2]

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Introduction

Epigenetic analysis of DNA gains momentum in forensic genetics because it can be used for authentication of natural DNA, tissue of origin typing, time of sample deposition estimation, and/or revealing behavioural/ physiological pattern of perpetrator (smoking, alcoholism, drug addiction, socioeconomical status, physical activity, body mass index, vegetarianism, and age).

Estimation of age has the direct application in investigative mode of forensic genetics: e.g. if applied during investigation of Barbora Němečková rape and murder in Kmetiněves, the Czech Republic, the 15 years old perpetrator Robin would have been found earlier than as a number 632 out of 700 tested in DNA dragnet.

Also, epigenetic age estimation would help to distinguish true children among refugees that claim preferential treatment for underaged.

In this project, we applied for the first time QiaGen (BioVectis) system to the Czech population sample to see the method applicability.

Materials/ methods

We extracted DNA from peripheral blood in EDTA of blood donors (n=250, with informed consent and ethical committee approved), performed bisulfitation reaction using EpiTect Fast Bisulfite kit and PCR followed by pyrosequencing with 5 markers (ELOVL2 C7, C1orf132 C1, TRIM59 C7, KLF14 C1, FHL2 C2) using AgePlex kit (QIAGEN) on PyroMark Q48 pyrosequencing machine, as described in the manual. Age was calculated online at http://biovectis.com/forensic1/age-calculator webpage.

Results and conclusions

We did not find any significant difference between the first and second epigenotyping run of 47 samples (p=0.076) or between sexes

AgePlex performs unsatisfactorily in our hands with the original statistical algorithm and laboratory method as described in manual. Though we were able to improve the model to fit our data better (with exception of age category 18-25 yrs in batch 1), we were still not satisfied with the found difference between chronological and epigenetic age. There can be two main explanations for our findings.

First, improvement of statistical model for the Czech individuals based on the Czech data may be caused by a difference in recruitment of tested persons: Czech individuals were blood donors that are considered the most healthy (epigenetically young) both by medical examination and by themselves while information about original Polish tested population is not provided in QiaGen manual. We do not think that there are such large epigenetic differences in two Slavonic populations of Czechs and Poles in general.

Second, technological replicates starting from bisulphitation (n=8) and starting from pyrosequencing (n=47) show systemic problem for ELOVL2 marker and room for optimization for all markers tested, where bisulphitatiom seems to be the major bottleneck. It may be advantageous to use some internal control for efficiency of bisulphitation (e.g. qBiCo) that would provide stop/ go quality check before using the method in live settings.

Acknowledgements: Supported by NV16-32198A, CZ.02.1.01/0.0/0.0/16_013/0001674, TE02000058, IGA_LF_2019_003, and CZ.02.1.01/0.0/0.0/16_019/0000868.

2

A novel long non-coding RNA MIAT is associated with NMYC amplification in neuroblastoma

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Introduction:

Long non-coding RNAs (lncRNA) are a type of RNA with lengths exceeding 200 nucleotides that are not translated into protein. Neuroblastoma (NBL) is the most common solid, extracranial malignant tumor of children. Amplification of the oncogene NMYC is a well-established poor prognostic marker in NBL and it correlates with higher tumor aggressivity.

Methods

The expression level of MIAT was assessed using qRT-PCR. We transfected MIAT siRNA or control siRNA into NBL cells to knock-down MIAT. PrestoBlue detected cell viability. Apoptosis rate was analyzed using flow cytometry. Mitochondrial respiration and glycolysis were measured using the Agilent Seahorse XFp Cell Mito and Glycolysis Stress Tests.

Results and conclusion

We examined expression of lncRNA MIAT in panel of NBL cell lines with and without NMYC amplification. We found that MIAT expression strongly correlates with NMYC amplification. Knock-down of MIAT by siRNA resulted in a significant arrest of cells at G_0 phase and a decrease in S phase in both UKF-NB-4 (MYCN ampl.) and SH-SY5Y (MYCN nonampl.) cells. Moreover, MIAT downregulation decreases UKF-NB-4 and SH-SY5Y cells migration. Silencing MIAT in human neuroblastoma cell lines resulted in induction of apoptosis in lines with NMYC amplification compared to cell line without NMYC amplification where we found only growth inhibition. Mitochondrial oxidative phosphorylation, as measured by oxygen consumption rate, and also glycolysis function, as measured by extracellular acidification rate were reduced in the siMIAT group. Knock-down MIAT decreased NMYC protein expression in neuroblastoma cell lines with NMYC amplification.

These results demonstrate that MIAT levels are potentially associated with neuroblastoma and with NMYC amplication and seem to be important for NBL proliferation, metabolism and aggressivity, particularly for those with MYCN amplification.

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Body Mass Index prediction through DNA methylation

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Introduction

DNA methylation is the most widely used epigenetic modification in a forensic setting. Its changes may arise as a result of lifestyle and environmental factors. Thus, epigenetics can contribute not only to the estimation of the tissue origin in the biological sample but also help predict various phenotypical characteristics of the trace donor. The most important phenotypic characteristics tested by now is age, but markers for other characteristics like time of trace deposition, donor smoking, alcohol consumption, or diet are being searched for as well. These predictions can lead the investigation by narrowing down the population from which sample donor recruits.

In medical and obesity research, a correlation between methylation of specific gene loci and body mass index (BMI, a measure for indication nutritional status in adults) was found. The methylation status of these markers could predict body structure of an unknown sample donor.

Materials/methods

We decided to verify a correlation between BMI and methylation status in three previously validated CpGs in *HIF3A* gene and other previously published candidate markers using a group of healthy Czech blood donors (n>20) with BMI under 21 kg/m^2 (n>10) and over 36,5 kg/m^2 (n>10).

For methylation assessment, we choose next-generation amplicon bisulfite sequencing over the pyrosequencing method, mostly because of lower sample requirements (from 0,5 ng DNA for NGS vs 10 ng DNA using PyroMark method) which are in a lot of cases the bottleneck for the usage of the method in the forensic setting.

Results and conclusions

The statistically significant association between BMI status and methylation levels was found in two tested CpGs. Univariate and multivariate prediction models were constructed using this small sample subset in the pilot study.

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Prevalence and genotype-specific distribution of human papillomavirus in czech nonvaccinated heterosexual couples

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Introduction

Human papillomaviruses (HPVs) are agents of a common sexually transmitted disease (STD) that affects the majority of sexually active population during their lives. HPVs are important factors responsible for cancer development in ano-genital and aero-digestive region. The aim of this study was to evaluate the genotype-specific prevalence and genotype-specific distribution of HPV infection in semen samples/penile swabs and cervical swabs in non-vaccinated heterosexual partners. HPV genotyping becomes attractive for use in cervical cancer screening, as it offers a possibility to integrate HPV screening with HPV vaccination monitoring.

Methods

Semen samples and penile swabs were collected from male partner and cervical swabs were collected from female partner of heterosexual couples (n = 195). Presence of HPV DNA in semen samples and cervical swabs was analysed using the cobas® 4800 HPV Test (Roche) and followed by HPV

genotyping by PapilloCheck® HPV-Screening (Greiner Bio-One). Penile swabs were examined using PapilloCheck® HPV-Screening. The genotype-specific prevalence and concordance of vaccine-targeted HPV infection were evaluated using R statistical software.

Results and conclusions

HPV DNA was more frequently detected in penile swabs (41.0%) than in cervical swabs (22.1%) or semen samples (15.9%; $P < 0.001$). The most frequently detected genotypes in all analyzed sample types were vaccine non-targeted genotypes. Among 27 couples in which both partners were HPV positive, 55.6% (15/27) harbored at least one genotype in common.

Only in 4 couples, both partners were positive for bivalent vaccine targeted genotypes as well as for quadrivalent vaccine targeted genotypes. Nonavalent vaccine targeted HPV genotypes were detected in both partners of 8 couples.

The highest protective potential of bivalent, as well as the quadrivalent vaccine, was observed in penile infection (16%, and 20%). The highest protective potential of the nonavalent vaccine was observed in cervical infection (32.6%). Data presented here may be the last population-based Czech data on HPV prevalence in virtually unvaccinated women and men and as such form a valuable baseline for future assessment of vaccine impact

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Virtual screening using pharmacophore models retrieved from molecular dynamic simulations

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Introduction

Pharmacophore models are widely used for identification of promising primary hits in large libraries. They can be developed on the basis of a x-ray structure of a protein-ligand complex by identification of observed interactions between the partners. However, in x-ray complexes the number of observed interactions is greater due to experimental conditions that would be usually observed under normal conditions. Recently is was demonstrated that using an ensemble of pharmacophore models retrieved from molecular dynamic (MD) simulations of protein-ligand complexes may overcome this issue. However, two issues are arisen: 1) a large number of pharmacophore models which can make virtual screening inefficient and 2) a strategy for prioritization of compounds based on multiple pharmacophore models.

Materials and methods

Here, we developed new approach for identification of redundant pharmacophore models based on their 3D pharmacophore hashes. Pharmacophores having identical hashes are recognized as redundant and can be removed from an ensemble. We proposed new strategy for compound scoring based on the percentage of compound conformers matching at least one non-redundant pharmacophore model. Compounds whose conformers more frequently match pharmacophores might lose the less number of degrees of freedom upon binding event and thus entropy decrease would be smaller and binding may be more favorable.

Results and conclusion

To validate the developed approach we performed 50 ns MD simulations of four CDK2 complexes with their ligands. The retrieved list of non-redundant pharmacophores was used for virtual screening of compounds from DUD-E data set. Our scoring strategy demonstrated good ability to select active compounds with enrichment factors greater that those for previously published approaches. In the case if several protein-ligand complexes are available the consensus of compound scores for individual complexes may results in better performance. We also demonstrated that more complex models resulted in less number of hits but with higher enrichment factors. The developed approach was implemented as an open-source software.

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Novel antimicrobial activity of anticancer compounds

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Introduction

Infectious diseases such as tuberculosis and leishmaniases are associated with high morbidity and mortality in human populations globally. So far, therapeutic measures against these disease that are based on the use of classical drugs are limited by low efficiency and secondary effects. The novel drug candidates and commercial drugs with activity against different cancer cell lines can also have the significant antimicrobial activity. Our aim was to analyses their activity against two microbial pathogens associated, *Mycobacterium bovis* and *Leishmania major.*

Materials and Methods

In one study, 4, 800 compounds from proprietary chemical library were tested for activity against two

strains of bacterium *M.bovis* in high-throughput analyses. In another study, 1,280 compounds from BigLopac, a commercial chemical library of bioactive compounds were tested activity against axenic forms of *L. major*.

Results

Screening 4, 800 compounds from proprietary chemical library for activity against two *M. bovis* strains showed a set of 120 active compounds that belong to different classes of anticancer compounds. After filtering compounds with known activity and/or cytotoxicity to BJ fibroblasts and human macrophages, the 68 compounds were selected for further analyses. The 22 compounds were active against intracellular bacteria in primary screen. These compounds could be classified into two groups based on the mode of their action. The ongoing dose-response analyses showed 10 active compounds while the activity of others is to be tested. Screening of 1,280 compounds from BigLopac, a commercial chemical library of bioactive compounds, using high-throughput analyses of their activity against *L. major* showed a set of active compounds. After filtering out the compounds with known activity and/or with cytotoxicity to human THP-1 macrophages, one compound was selected for further analyses. This compound was also effective in elimination of parasites from infected macrophages.

Conclusion

These results showed that novel anticancer compounds have antimicrobial property indicating their potential for development of novel therapeutic measures.

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CRISPR/Cas9-mediated tagging of proteins involved in ribosomal biogenesis

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Ribosomal biogenesis is a process which is sensitive to any changes in protein level. In that case, overexpression of recombinant reporter genes is not appropriate way to study mechanisms and effects of compounds on ribosomal proteins and its regulators. To solve this problem, we created the reporter cell lines by CRISPR-Cas9 mediated knock-in of HiBiT (Marie KS et al. ACS Chem. Biol. 13, 2017) into the genomic locus of proteins which are involved in ribosomal biogenesis. HiBiT is an 11 amino acid peptide tag, developed from C-terminal region o NanoLuc Luciferase, which can be attached to any protein and detected quickly and easily by using bioluminescent assays. The detection reagent contains an inactive rest of luciferase, Large Bit (LgBiT), which rapidly binds to HiBiT to produce a highly active luciferase enzyme.

U2OS human cell line was used for CRISPR/Cas9 knock-in of HiBiT into genomic regions of RPL11 and c-Myc targeted by specific gRNAs. Monoclonal cell lines were prepared by limiting dilution and functionally validated using siRNA treatment to prove specificity and accuracy of HiBiT knock-in. We performed luminescence assays (lytic detection, blotting system) and Western blotting. Time-dependent decay of luminescence signal for lytic detection of tagged proteins was checked.

We were able to quantify similar changes in protein levels with HiBiT lytic assay and western blotting after specific siRNA treatment. Luminescence signal for lytic assay was measurable even after 6 hours. These reporters could be used as *in vitro* model and for high-throughput screening in future to find compounds from chemical libraries which will effect gene expression or stability of proteins expressed at physiological levels.

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Subtyping of systemic amyloidosis in subcutaneous fat aspirates by mass spectrometry-based proteomics

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Introduction

The systemic amyloidosis is a rare disorder characterized by the abnormal deposition of misfolded amyloid protein in various organs [1]. Over time, the accumulating amyloid damages the tissue microenvironment and causes organ failure. To date, there are 36

known fibril proteins in human that can cause amyloidosis [2]. Early diagnosis is critical for effective patient management. Preferred method for routine amyloid subtyping is IHC, an antibody-based method with numerous unspecificities [3]. We have introduced mass spectrometry-based proteomic analysis to screen of systemic amyloidosis from subcutaneous fat aspirate (SFA) samples.

Materials/methods

So far, we have obtained 67 SFA samples for subtyping of amyloid deposits. In the SFA samples, the proteins were solubilized and digested directly with trypsin. All peptide samples were subsequently separated by liquid chromatography, and individual peptides were acquired by tandem mass spectrometry. Acquired spectra were identified and quantified using a search engine - MaxQuant. The most abundant amyloid protein determined the amyloid subtype.

Results and conclusions

The mass spectrometry-based proteomic analysis enabled subtyping of different kinds of amyloid proteins (e.g. Ig kappa, Ig lambda) in SFA samples. In addition, proteins associated with the amyloid formation (e.g. SAP, ApoA-IV, ApoA-I, ApoE) were identified. Mass spectrometry-based proteomic analysis of SFA samples offers a sensitive and specific tool for subtyping of systemic amyloidosis.

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In silico models of firefly luciferase inhibitors

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Introduction.

One of the most common reporter enzymes employed for constructing HTS assays are luciferases, which generate a bioluminescent signal through oxidation of a luciferin substrate. Although luciferases are attractive as assay reporters due to the high signal to background ratio, the inhibition of luciferase in high-throughput screening (HTS) assays could lead to a false positive result. Studies of FLuc inhibitors among large compound libraries show that approximately 10% of the compounds inhibit FLuc at a screening concentration of 11μM [1]. To date little effort has been devoted to building a chemoinformatics model that can identify such molecules in a given data set. One recent study provided models with high accuracy, but these models can be used only via web service , which requires the user to share proprietary compounds with a third party [2].

Materials/methods.

In this study we developed models for luciferase (*p.pyralis*) inhibitors using machine learning methods : gradient boosting, random forest, deep neural networks. The source of data were two Pubchem counterscreen assays: AID1006 and AID588342, containing approximately 195K and 364K compounds respectively.

Results and conclusions.

Models showed high accuracy (maximum balanced accuracy 0.94). They are planned to become freely accessible and their usage will not require sharing proprietry data.

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Comparison of hgDNA quality control methods

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Introduction

Quality control (QC) of hgDNA (human genomic DNA) is a crucial step before every procedure in molecular-diagnostics laboratory. Especially when formalin-fixed paraffin-embedded (FFPE) samples are processed, the quality, quantity and integrity of hgDNA are critical in downstream analysis (e.g. NGS or qPCR) and can save a lot of money and effort. Aim of this project is a comparison of 5 different methods for FFPE hgDNA quality control.

Material and methods

The study comprises quality testing of 29 hgDNA samples isolated from FFPE tissues. Quality control was

performed on 21 hgDNA samples with 5 different methods. Due to insufficient amount of sample volume, remaining 8 samples were analysed with 3 methods only.

The QC methods used for quality, quantity, and integrity measurement were: NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific); Qubit® 2.0 Fluorometer using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific); 2100 Bioanalyzer using High Sensitivity DNA kit (Agilent Technologies); LightCycler® 480 Real-Time PCR System (Roche Diagnostics) using Quantifiler™ Human DNA Quantification Kit (Thermo Fisher Scientific) and LightCycler® 480 Real-Time PCR System using KAPA Human Genomic DNA Quantification and QC Kit (Roche Diagnostics).

Before Agilent measurement, all samples were diluted to 2 ng/µl if needed and before qPCR measurements, all samples were diluted to 0.5 ng/µl – same aliquots were used for both qPCR kits (Quantifiler™ Human DNA Quantification Kit and KAPA Human Genomic DNA Quantification and QC Kit).

Results and Conclusions

Spectrophotometric NanoDrop measurements enables quantity and purity evaluation of samples, but concentrations acquired are remarkably overestimated in an incalculable manner comparing to fluorometric Qubit measurement; however, there is a positive correlation (Graphs 1 and 2). Qubit provides more precise results and that is why those results were used for dilutions to further methods (capillary gel electrophoresis on Agilent 2100 Bioanalyzer and qPCRs on LightCycler® 480).

Due to FFPE sample´s origin, DNA integrity was estimated by capillary gel electrophoresis with High Sensitivity DNA kit on Agilent 2100 Bioanalyzer and qPCR with KAPA Human Genomic DNA Quantification and QC Kit (Roche Diagnostics) on LightCycler® 480 (Graph 3). Bioanalyzer electropherograms were empirically divided into 3 categories: very-fragmented (VF), fragmented (F) and lightly-fragmented (LF) (Figures 1 and 2). KAPA Human Genomic DNA Quantification and QC Kit (Roche Diagnostics) determines different DNA fragments of 41 bp, 129 bp, and 305 bp. Quality scores, defined as ratio of different fragment size´s concentrations for the same sample, were noticeably low for tested samples. Only one sample achieved recommended value for Q129/Q41 Ratio (0.5) (Table 1). The main advantage of the qPCR method is that it gives information not only about fragment sizes, but also about amplifiability of detected fragments (Graph 4).

In an effort to get the most accurate results, we compared concentration values between 41 bp fragments from KAPA Human Genomic DNA Quantification and QC Kit (Roche Diagnostics) and fragments from Quantifiler™ Human DNA Quantification Kit (Thermo Fisher Scientific) that has fragments with length 62 bp only. In graphs, we acquired quite similar line between concentration values of those 2 methods (Graphs 5 and 6).

In case when only informative quantity measurement is needed, it is possible to use NanoDrop, though it measures both double-stranded (dsDNA) and single-stranded DNA (ssDNA) or more precise method Qubit that specifically measures only dsDNA. Besides concentration measurements, qPCR KAPA Human Genomic DNA Quantification and QC Kit enables acquiring information about PCR-amplifiable fragment distribution sizes of tested samples. On the other side, Agilent gives information only about fragment lengths, not about amplifiability. Therefore KAPA qPCR method is recommended for precious samples to decide if downstream analysis can proceed with the chosen sample. Even if Quantifiler™ Human DNA Quantification Kit produced similar results compared to KAPA Human Genomic DNA Quantification and QC Kit, unfortunately it indicates quality and quantity information only about one fragment lenght (62 bp) and not about longer fragments that are used in downstream applications.

Acknowledgements

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The effect of perioperative analgesia on CTCs occurrence in colorectal cancer patients

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Background

Colorectal cancer (CRC) is one of the most common cancer diseases in the Czech Republic and is the second leading cause of malignancy-related deaths worldwide. It is known that circulating tumor cells are precursors to metastatic disease. However, the effect of perioperative analgesia on CTCs and on the tumor metastatic potential has not been investigated. The aim of the study was to analyze the effect of opioid analgesia on circulating tumor cell (CTC) levels in colorectal cancer patients after surgical treatment.

Material and methods

In total, sixty-five patients who underwent radical surgery treatment

were prospectively included into the study. Eight patients were excluded because of noncompliance or postsurgical complications. Patients were randomly enrolled into the 3 groups: the first received epidural analgesia (17 patients), the second received piritramide (19 patients), and the third one received morphine (21 patients). The blood samples were collected before surgery, immediately after surgery, 2nd postoperative day and one month after surgery. The types of analgesia were compared to the presence of CTCs detected by real-time PCR quantification of CEA and CK20 mRNA positive CTCs.

Conclusions

The analysis of samples from 2nd postoperative day revealed a higher level of the CEA and CK20 mRNA positive CTCs in blood of patients who received morphine analgesia in comparison with patients that received piritramide. The study will be conducted to assess disease-free survival of patients in different groups.

Acknowledgment

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The quality and performance of HTS assays.

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Over the past decade, High Content Screening (HCS) became a leading technology widely used in drug discovery. As a starting point in the drug development pipeline, HCS allows for rapid selection of most active compounds that are further evaluated in order to determine their mechanism of action, specificity or physiological properties. Screening campaigns come with significant costs and efforts which can be reduced providing proper quality control and increasing screening performance. This can be accomplished only by recognition and elimination of adverse procedural or environmental conditions in which the screening is conducted. In practice however, such level of control is often hard to achieve therefore considerable attention has been paid to develop data normalization procedures that can correct experimental bias. Screening performance strongly depends on the determination of the compound's activity threshold at which a decision can be made about the classification of the active compound for further testing. The activity limit is usually determined by the method used for data normalization. However, the nature of HCS measurements itself often makes a challenge for the use of such methods according to their theoretical assumptions. Although such an approach is not prohibited there is still little data comparing the performance of various methods in the normalization of real HCS measurement collected for a particular assay format. Presented results of several screening experiments will show how the application of appropriate methods is essential for improving the quality of screening data.

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Identification of a four-gene methylation biomarker panel in high-grade serous ovarian carcinoma

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Introduction

The lack of effective biomarkers for screening and early detection of ovarian cancer is currently considered to be one of the most pressing problems in oncogynecology. The most common histological subtype accounting for up to ~80 % deaths from all forms of ovarian cancer is highly invasive high-grade serous carcinoma (HGSOC). Due to vague early symptoms and aggressive nature it is mostly diagnosed after the disease has metastasized beyond the ovary. Just like any other malignancy, HGSOC is the consequence of progressive genetic and epigenetic alterations. Because the epigenetic alterations occur early in the cancer development, they provide great potential to serve as biomarkers. In our study, we investigated a possibility of four-gene methylation panel (including *CDH13*, *HNF1B*, *PCDH17* and *GATA4* genes) to be of clinical use in early detection of HGSOC.

Materials and methods

Study group consisted of 68 patients with HGSOC and 53 patients who had undergone surgery for non-malignant diagnosis. Control group was extended by samples of healthy ovaries collected from 13 women during autopsy. The set of analyzed samples contained 86 samples of formalin-fixed, paraffin-embedded tissue and 52 fresh frozen tissue samples.

For methylation detection we used next-generation sequencing as preliminary scan followed by methylation sensitive high-resolution melting analysis and real-time

methylation specific analysis. We also investigated the relation between gene hypermethylation and gene relative expression using $2^{\Delta\Delta Ct}$ method.

Results and conclusions

The sensitivity of the examined panel reached 88.5 %. We were able to detect methylation in 85.7 % (12/14) of early stage tumors and in 89.4 % (42/47) of late stage tumors. The total efficiency of the panel was 94.4 % and since the analyzed CpGs were selected in the regions without any methylation present in the control samples, the specificity achieved 100% rate. Our results also showed significantly lower gene expression in the tumor samples in comparison to control samples. The more pronounced downregulation was measured in the group of samples with detected methylation.

To conclude, in our study we designed the four-gene panel for HGSOC detection in ovarian tissue with 100% specificity and sufficiently high sensitivity. The next challenge is translation of the findings to the less invasive source for biomarker examination, such as plasma. Our results indicate that combination of examined genes deserve consideration for further testing in clinical molecular diagnosis of HGSOC.

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Applications of betulinic acid conjugates to explore its mode of action

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Introduction

Hybrid molecules are used in various applications in drug development and basic research. The bi-functional molecule typically consists of two protein-binding moieties connected with a suitable linker. For small-molecule-protein interaction studies can be designed conjugates with fluorescent label or an anchor that enable microscopic imaging or protein enrichment and subsequent mass spectrometry analysis. In addition, PROTAC technology can be used for validation of drug-protein interaction.

Materials/methods

The PROTAC chimeras are hybrid molecules that enable specific knockdown of targeted proteins via proteasome degradation. Several conjugates of betulinic acid with a moiety recognized by E3 ubiquitin ligase were synthesized. The degradation of a potential protein target was monitored using western blot.

Results and conclusions

The assumed interacting partner of betulinic acid was validated using proteolysis targeting chimeras. The challenges in chemistry and biological part of the study will be discussed. This work was supported by Technology Agency of the Czech Republic TE01020028, Ministry of Health of the Czech Republic (15-31984A), internal grant of Palacky University (IGA_LF_2019_018) and by the Czech Ministry of Education, Youth and Sports (LO1304).

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KDM5D is associated with neuroblastoma chemoresistance to ellipticine

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Introduction

Chemoresistance is a major problem in cancer therapy. Rising number of evidence refers an importance to epigenetically based acquired drug resistance. Our results show, that cells surviving single exposure to high dose of cytostatics form about 1-5 % of cell population and do not differ from the major sensitive cell population, but they exhibit epigenetic changes. Histone methylation is at the heart of transcription regulation, genes expression and the maintenance of genome integrity. Its dysregulation has been observed in many cancers. Histone lysine-demethylases KDM5s are enzymes, that are capable of removing tri- and di- methyl marks from lysine 4 on histone H3 (H3K4) and depending on the methylation site, their effect on transcription can be activating or repressing. That means, they could play a crucial role in downregulation of tumor suppressors or *vice versa* upregulation of oncogenes and in appearance of drug tolerance. There has been reported, that in a number of tumor cell types hypoxia induced resistance to different cytostatics. Our hypothesis, based on published studies and our previous observations, is that tolerance to cytostatics is related to the ability of transcription regulation by KDM5s enzymes. Therefore, we focused on role of KDM5s demethylases in neuroblastoma (NBL) sensitive cells, chemoresistant sublines

and persisting cells after cytostatic exposure both in normoxia and hypoxia.

Materials/methods

Sensitive NBL cell line (UKF-NB4) and its derived chemoresistant cell lines to vincristine (VCR), ellipticine (ELLI), doxorubicin (DOXO) and cisplatin (CDDP), which has been created after long-term cultivation with rising concentration of these cytostatics were treated with 20 nM VCR, 5μM ELLI, 2 μM DOXO and 20 μM CDDP in normoxic (21% O_2) and hypoxic (1% O_2) conditions. Detection of KDM5A-D mRNA expression levels in cells were analyzed by RT-PCR and Droplet Digital PCR. Detection of apoptosis was analyzed by flow cytometry annexin V/FITC assay after KDM5s inhibition, especially KDM5D, by a multi-KDM inhibitor JIB-04 in UKF-NB4^{ELLI} (80 nM), where the change of KDM5D expression was highest and control UKF-NB4 (40 nM) cell line.

Results and conclusions

We found that expression of KDM5A-D were significantly increased in all chemoresistant NBL cell lines compared to sensitive NBL cell line. Expression of KDM5A-C in normoxia in sensitive NBL cells after treatment with cytostatics, were reduced or remain unchanged, however in hypoxia were their expressions increased. KDM5D wasn't expressed both in normoxia and hypoxia in sensitive NBL cell line. Also, KDM5D wasn't expressed in VCR resistant cells in normoxia while in all other chemoresistant cells was KDM5D highly overexpressed, the highest expression was in NBL cells resistant to ELLI.

We observed that inhibition of KDM5D in ELLI resistant NBL cell lines lead to inhibition of cell growth followed by induction of apoptosis compared with sensitive NBL cell lines, where we found only inhibition of cell growth. These results demonstrate that KDM5D expression levels are potentially associated with NBL chemoresistance to ELLI.

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Fluorescent Nanodiamonds Modified with Biocompatible Polymers

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Introduction

In the past decade, sentinel node(s) mapping became standard procedure used in cancer diagnostics. However, agents commonly used in these applications, mostly blue dyes and radiotracers, still have several disadvantages. Fluorescence probes currently show the most promising results as potential alternatives. Fluorescent nanodiamond (FND) is a biocompatible material which exhibits unique optical properties. The origin of the nanodiamond fluorescence is based on artificially created nitrogen-vacancy (NV) centers. Emission maximum of NV centres is in near-infrared region (approximately 700 nm) which belongs to the tissue imaging window. NV centers are extremely resistant towards photobleaching. These properties make FND an ideal candidate for bioimaging applications.

Materials and methods

This work is focused on preparation FNDs coated with D-mannosylated polyglycerol for sentinel node(s) visualisation. Polyglycerol coating overcomes limited colloidal stability of FNDs in the biological environment and enables surface modification possibilities. Dmannose targets macrophages, which are abundantly present in the sentinel nodes. The functionalization with D-mannose was achieved using click chemistry (azide-alkyne cycloaddition). First, treatment with glycidyl propargyl ether provided alkyne-modified polyglycerol which was connected with azidated D-mannose via click reaction. The optimal polymerization and click reaction conditions were extensively studied.

Results and conclusions

Resulting particles (both mannosylated and non-mannosylated) were highly stable in the high-salt condition (1M NaCl) and non-specific protein binding in FBS was completely eliminated. The mannosylated particles interacted specifically with macrophages and showed enhanced retention in mice lymphatic nodes, providing a clear imaging contrast.

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Importance of V-ATPase for tumor cell chemoresistance and potentiation of cytostatic effects by V-ATPase inhibitors

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Introduction

Neuroblastoma (NB) is a malignant embryonic tumor of undifferentiated sympathetic cells and is the most

common solid extracranial tumor in children. Cytostatic drugs such as cisplatin, ellipticine and doxorubicin have become very important in the treatment of cancer. . However, they are known to induce resistance in cancer cells including NB. Many mechanisms are involved in this resistance, one of which is vacuolar ATPase (V-ATPase) -mediated vacuolar trapping of hydrophobic weak base chemotherapeutic drugs via a mechanism known as a lysosomal sequestration and it can influence its anticancer action.

Materials/methods

The aim of study is to investigate whether the V-ATPase action is the possible mechanism for surviving of NB cell lines and how to suppress this type of chemoresistance.We examined expression of V-ATPase in resistant and sensitive cell lines after their exposure to cytostatics, lysosomal capacity (volume) and cellular localization of cytostatics. We also studied possibilities of inhibition of this enzyme pump.

Results and conclusions

In resistant NB cells, we observed a higher expression of V-ATPase, which was further increased by the action of appropriate cytostatics. Doxorubicin and ellipticin resistance in the tested NB cells is associated with vacuolar capture of these drugs, mediated by V-ATPase in lysosomes, and we observed lysosomal compartments increase in sensitive cell lines compared to resistant and lower nuclear fluorescence. By acting with a specific V-ATPase inhibitor, bafilomycin A, the number of lysosomes is reduced and the cytostatics are localized predominantly in the nuclei. Fluorescence intensity measurements indicate that lysosome specific day LTR accumulates depending on the concentration of ellipticin but not cisplatin. V-ATPase is associated with resistance to some cystotatics and seems to be a promising selective therapeutic target to be considered for future trials.

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Epigenetically conditioned chemoresistance of cancer cell

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Introduction

Chemoresistance is one of the main reasons for the failure of cancer therapy. We believe, based on the literature and our observations that the “tolerance” to cytostatics is related to the ability of transcription regulation. It has been suggested that the KDM5 family of demethylases (KDM5A/B/C/D) plays a role in the appearance of drug tolerance. Also the felxibility in using different energy pathways may indicate a survival adaptation to achieve a higher cellular fitness that might be associated with chemoresistance.

Materials/methods

The aim of the project is to find out what mechanisms are responsible for the early development of chemoresistance and characterize the persistent cell subpopulation, to identify markers of persistent cell subpopulation and to verify whether they could serve as possible therapeutic and predictive targets. We examined expression of KDM5A/B/C/D in resistant and sensitive cell lines. We also tested mitochondrial and glycolytic function of sensitive and persistant cell lines using Seahorse Fxp analyzer.

Results and conclusions

Preliminary results show the difference in expression of KDM5 demethylases in the sensitive and resistant NB cell lines. We also observed lower glycolysis in sensitive cells compared to rezistatnt and survivors. The results of the bioenergetic organization indicate that platinum resistant cells prefer oxidative phosphorylation (OCR rate). The results of the cytotoxicity analysis correlated with the rate of the Mitostress Test in the cell lines. The higher the OCR, the more cells are resistant to the cytostatics tested.

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Preclinical absorption, distribution, metabolism, excretion, and thermodynamic properties of complexes with central atom of copper (II) and gold (I).

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Introduction

The discovery of cisplatin and other platinum-based drugs started a research of new metal–based coordination compounds with the aim to achieve compounds with better antitumor activity and the less side effects. Since that time, a variety of analogous compounds with another central atom has been synthesized. For this study, the two copper (II) complexes were selected due to high cytotoxic effects against a wide spectrum of human cancer cell lines. In selecting the other compounds, the two gold (I) complexes were chosen for their anti-inflammatory and anti-cancer properties. Pharmacokinetics is the way the body acts on the drug once it is administered and

includes absorption, distribution, metabolism, and excretion (ADME). Complexes were studied for their pharmacokinetics and thermodynamic parameters.

Materials/methods

ADME parameters, including chemical, plasma and microsomal stability, protein binding, permeability of compounds across a Caco-2 and MDCK-MDR1 cells monolayer. Analysis of samples is performed using a quadrupole mass spectrometer (QTrap 5500, AB Sciex) that was connected to the RapidFire 300 (Agilent). The thermodynamic parameters for binding tested complexes with the difference forms of cytochrome P450 (human bactosomes CYP1A2, CYP3A4 and recombinant human CYP3A4) were further determined using isothermal titration calorimetry (ITC, TA Instruments, USA). It's a technique that allows determination of binding constants, change of enthalpy and stoichiometry with high accuracy.

Results and conclusions

The ADME properties of all tested compounds were in favorable values. All studies compounds were found to be stable in PBS and human plasma after incubation for 2 h. Metabolic stability in human liver microsomes were measured up to 60 min and *in vitro* intrinsic clearance was determined as medium. These complexes showed high protein binding and low permeability through artificial membrane. The compounds with central atom of copper (II) showed high permeability in MDCK-MDR1 cells, indicating a high potential for penetration through the blood-brain barrier. Titration of all compounds into bactosomes CYP1A2 showed no interaction. The strong interactions have seen between the complexes and form of cytochrome P450 3A4. The thermodynamic parameters for these interactions reported exothermic reactions.

Acknowledgment

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Development of a multi-target screening tool based on 3D pharmacophore models

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Introduction.

Pharmacophore modeling is one of the major drug design approaches in the absence of structural data about the target receptor. A pharmacophore is an description of molecular features that are necessary for molecular recognition of a ligand by a biological macromolecule. A pharmacophore model explains how structurally diverse ligands can bind to a common receptor site. In modern computational chemistry, pharmacophores are used to find hits. If compound has pharmacophore pattern, then this structure is potentially active against a specific target protein. As a rule, structures are not selective they bind to several types of proteins. Therefore, it is logical to assume that pharmacophore models obtained from ligands of different target proteins can match the same ligands. Thus, it becomes possible to identify several targets with which the compound could potentially bind. This makes it possible to predict side effects of the compounds.

Materials and methods.

We have developed a tool that generates and validates 3D ligand-based pharmacophore models. We generated 3D pharmacophore models for 109 data sets using this tool. Further, these models were screened on ChEMBL databases. Compounds that were matched by different types of models were assembled.

Results and conclusions.

It has been assumed that compounds which were map by several models bind different targets. These results were verified by published data. Does it has been shown the tool can be used as a multi-target screening.

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Development of resistant cell lines to nucleosides based cytostatics.

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Cancer is a widespread group of diseases. Statistics show that there are 96 500 new cases of cancer every year in the Czech republic and 27 261 patients die (2016 statistic) because of this disease. The fundamental problem of cancer therapy is the ability of cancer cells to resist treatment. There can be primary resistance (to the first administration of the drug), secondary resistance (occurs during treatment), cross-resistance (resistance to drugs with similar chemical structure) and also multidrug resistance (resistance to structurally different drugs, MDR).

A common cause of drug resistance in cancer cells is the intra-tumor heterogeneity. During treatment, resistant cancer cells are selected from which a new resistant tumor may grow. For better understanding of the mechanisms of drug resistance in cancer treatment, we have created cell models of resistant cancer cells. Currently, we have been able to prepare **leukemia cell lines**(CCRF-CEM, K562) resistant to **fludarabine**, **cytarabine**, **6-mercaptopurine,**

and **6-thioguanine**, which are nucleosides based cytostatics.

Fludarabine is used in the treatment of chronic lymphocytic leukemia (CLL) and as salvage therapy for acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL). In some cases, combination therapy of fludarabine with other cytostatics is used (for example sequential infusion of fludarabine and cytarabine). Cytarabine, 6-mercaptopurine and 6-thioguanine are mainly used in treatment of ALL and AML. All these drugs are classified as anti-metabolites, which can incorporate into cancer cells metabolism and stop their division. Cytarabine is a pyrimidine antagonist, 6-mercaptopurine and 6-thioguanine are purine antagonists and fludarabine is an adenosine deaminase inhibitor.

In our resistant cell line models, we were able to detect not only primary and secondary resistance to cytarabine, fludarabine, 6-mercaptopurine or 6-thioguanine but also cross-resistance and MDR. Resistant cell lines characterization and testing of new drugs capable of overcoming their resistance are currently underway.

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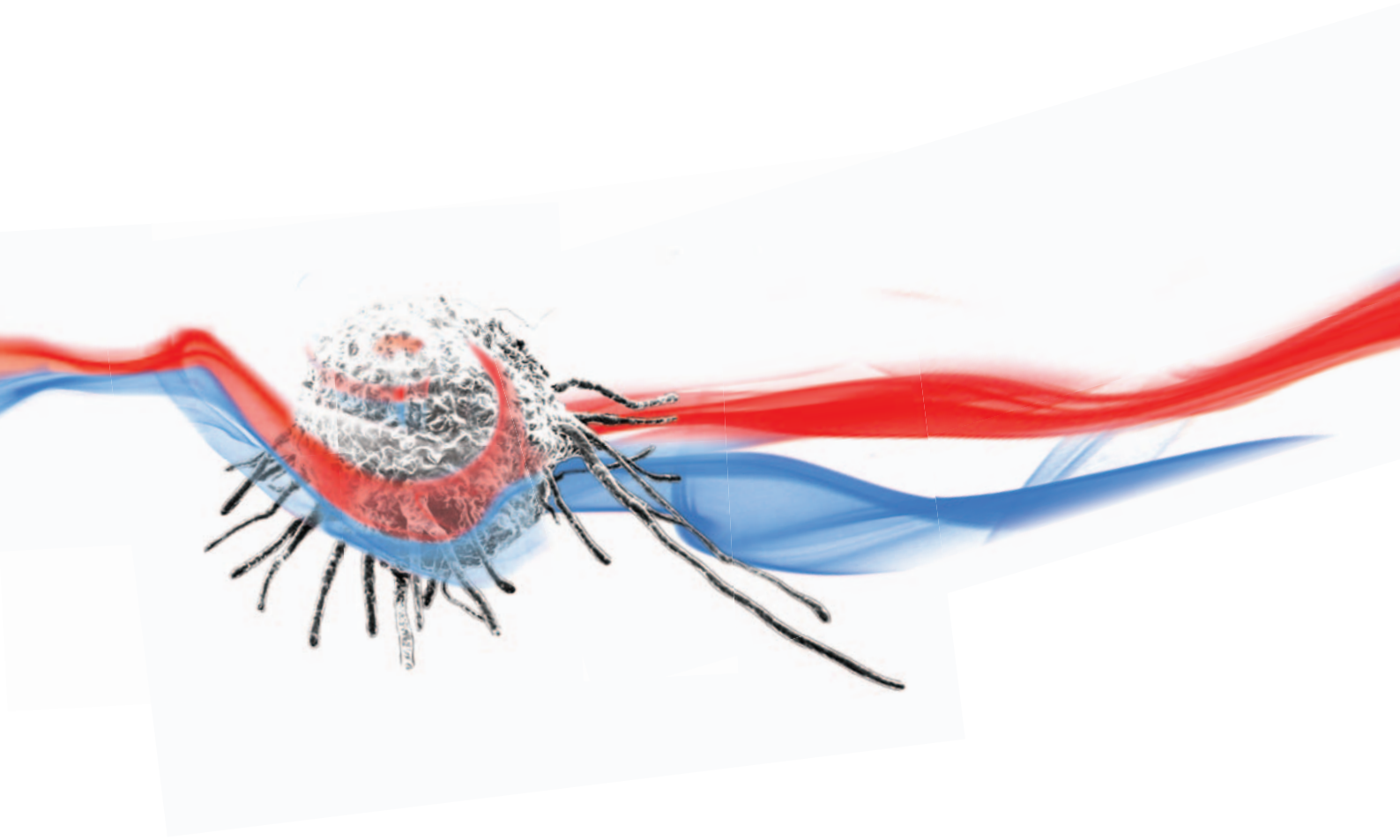

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