ABSTRACT BOOK 2018

2018 IMTM Reactor is a conference held in 18. – 21. Semptember 2018 at Pastviny recreation areal. It is focused to recent develompent in IMTM and related laboratories and should serve to connecting and strenghtening of inter-laboratory collaboration within the Institute

Organizer

The Institute of Molecular and Translational Medicine (IMTM), Faculty of Medicine and Dentistry, Palacký University in Olomouc is a leading Czech translational medicine institute established in 2010. IMTM was established within the infrastructural project initiated by the Palacký University, in close partnership with the University Hospital in Olomouc, the Institute of Chemical Technology and the Institute of Organic Chemistry and Biochemistry, AS CR, v.v.i. in Prague. Our research at IMTM is focused on better understanding of human diseases and development of future medicines and diagnostics.

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Programme

Tuesday 18/9/2018

Travel and Těchotín visit

7:00 **Departure from IMTM**

9:00 12:00 **Visit in Centrum of biological defence Těchonín**

13:00 **Arrival to Pastviny, Lunch**

Section from 14:30 to 16:10 chaired by Tomáš Oždian

14:30 14:40 *Tomáš Oždian* **Opening of conference**

14:40 15:30 *Lucie Tučková* **WHO classification of the CNS tumours. IDH status in diffuse gliomas and its importance. Testing of IDH mutations in diffuse gliomas: retrospective immunohistochemical and mutation analysis**

15:30 15:50 *Alena Mičková* **3D culture as a better model for personalised medicine**

15:50 16:10 *Lenka Řeháčková* **Screening of potential inhibitors of MARK4 kinase**

Cofee 16:10 16:40

Sporting and touristic activities

Dinner 18:30 19:00

Wednesday 19/9/2018

Breakfast 8:00 8:30

Section from 9:00 to 10:00 chaired by Marián Hajdúch

9:00 9:40 *Juan de Sanctis* **Role of fenretidine in lipid metabolism in cystic fibrosis mouse model and human patients**

9:40 10:00 *Hanuš Slavík* **MicroRNA profiling in cystic fibrosis model cell lines after zinc/fenretinide treatment**

Cofee 10:00 10:30

Section from 10:30 to 12:30 chaired by Josef Srovnal

10:30 10:50 *Alona Řehulková* **MiRNA profiling as a method of identifying a meningioma with the prospect of recurrence**

10:50 11:10 *Juan de Sanctis* **Importance of 45 genetic polymorphisms in Venezuelan patients with Asthma or COPD**

11:10 11:30 *Karolína Bartáková* **Genetic biomarkers of clinical response to bevacizumab in colorectal cancer patients**

11:30 11:50 *Rastislav Slavkovský* **Search for new predictive epigenetic biomarkers and advanced diagnostics of tumor response to anticancer drugs**

11:50 12:10 *Hana Jaworek* **Pitfalls in HPV68a detection**

12:10 12:30 *Zuzana Šporiková* **Mapping of molecular landscape underlying drug resistance and recurrence in glioblastoma paired primary and recurrent tumours and glioblastoma resistant cell lines**

Lunch 12:30 13:30

Section from 13:30 to 15:10 chaired by Jiří Drábek

13:30 13:50 *Lenka Lachnitová* **Measuring the molecular weight of compounds in Chemical library**

13:50 14:10 *Ivo Frydrich* **Betulinic acid derivatives inhibit GLI-mediated transcription in human glioblastoma cell line**

14:10 14:30 *Natálie Táborská* **Fluoroquinolones and their new role in the alternative splicing**

14:30 14:50 *Zuzana Macečková* **Fluorescent dye labeling nucleolus through covalent interaction with BYSL**

14:50 15:10 *Martin Ondra* **Implementation of a novel HiBiT Protein Tagging System for monitoring proteins expression**

Cofee 15:10 15:40

Section from 15:40 to 16:40 chaired by Jan Bouchal

15:40 16:00 *Barbora Lišková* **Pharmaceutical formulation and stability testing of metabolite of disulfiram (CuET)**

16:00 16:20 *Martina Medvedíková* **Spectral analysis of lymphatic tissue**

16:20 16:40 *Agáta Kubíčková* **Lentiviral CRISPR libraries for identification and validation of molecular mechanisms of action**

Dinner 18:30 19:00

Concert 19:30 22:30 Masožravá Berta

Thursday 20/9/2018

Breakfast 8:00 8:30

Section from 9:00 to 10:00 chaired by Gabriela Kořínková

9:00 9:20 *Alejandro Carazo* **Hepatocyte-based studies in preclinical stages of drug development**

9:20 9:40 *Vishvanath Das* **The effect of anti-cancer drugs on prion-like propagation of tau inclusions**

9:40 10:00 *Narendran Annadurai* **Anti-cancer drug inhibit the aggregation of aggregation-prone tau peptides**

Cofee 10:00 10:30

Section from 10:30 to 12:30 chaired by Milan Urban

10:30 10:50 *Juan de Sanctis* **Antineoplastic activity of derivatives of 7-substituted-4-chloro-quinoline**

10:50 11:10 *Jiří Hodoň* **Synthesis of modified triterpenoids and study of their mechanism of action**

11:10 11:30 *Mariia Matveieva* **Understanding properties making compounds a hit in primary screening**

11:30 11:50 *Jiří Řehulka* **siRNA transfection for high-throughput screening of small-molecules**

11:50 12:10 *Vaishali Uniyal* **Effect of the compound in the histidine phosphorylation in cancer**

12:10 12:30 *Khushboo Agrawal* **Chromatin reader machinery as target for overcoming resistance to DNA-demethylating epi-drugs**

Lunch 12:30 13:30

Section from 13:30 to 15:30 chaired by Vlastimil Mašek

13:30 13:50 *Ermin Schadich* **Antibiotic and antiparasitic activity of novel compounds**

13:50 14:10 *Jarmila Stanková* **What can HPLC methods offer to Drug Design and Development?**

14:10 14:30 *Dušan Holub* **Quantitative proteomic evaluation of normal tissue and tissue with amyloid mass**

14:30 14:50 *Martina Jakoubková* **Diagnostic biomarkers of pancreatic cancer**

14:50 15:10 *Lakshman Varanasi* **Anatomy of a chromatography problem**

15:10 15:30 *Jana Václavková* **Proteomic analysis of exhaled breath condensates as a non-invasive diagnostics of pediatric asthma**

Cofee 15:30 16:00

Section from 16:00 to 17:00 chaired by Martin Mistrík

16:00 16:20 *Jana Kotulová* **High-Throughput Screening of GPCRs Using Aequorin-Based Functional Assay**

16:20 16:40 *Soňa Gurská* **Cytotoxicity profiling of new chemical compounds in HTS facility**

16:40 17:00 *Robert Szabó* **Development and Optimization of High-Throughput Screening Assay for Antiviral Therapeutics**

Dinner 18:30 19:00

Friday 21/9/2018

Breakfast 8:00 8:30

Section from 9:00 to 10:20 chaired by Petr Džubák

9:00 9:20 *Petr Pavliš* **Clindata**

9:20 9:40 *Petr Vojta* **Moldimed pipeline**

9:40 10:00 *Jan Lošťák* **Pre-clin data: Modul for management of in-vivo experiment results**

10:00 10:20 *Martin Szotkowski* **The MEDCHEMBIO portal**

Cofee 10:20 10:50

10:50 12:30 *Marián Hajdúch* **Panel discussion: LIMS and cybernetic security**

Launch 12:30 13:30

14:00 **Departure**

WHO classification of the CNS tumours. IDH status in diffuse gliomas and its importance. Testing of IDH mutations in diffuse gliomas: retrospective immunohistochemical and mutation analysis

Lucie Tučková

Dpt. of clinical and molecular pathology University Hospital Olomouc

Short overview of the classification of the CNS tumours. IDH status in diffuse gliomas and its importance. Testing of IDH mutations in diffuse gliomas: retrospective immunohistochemical and mutation analysis.

3D culture as a better model for personalized medicine

Alena Mičková

Laboratory of molecular pathology

Preclinical testing is an important part of a drug development process. One of the key reliability determinants is, what testing subjects are used during the tests and how closely are these subjects modeling the real-life conditions. There are currently 2 methods of in vitro cultivation - 2D, which produces an adherent cell culture and 3D, which produces a 3D culture - known as spheroid culture if cell lines were used, or organoids if patient samples were used.

We believe that 3D cell structure is the most suitable model for purpose of preclinical drug testing because it is very well matching microenvironment and tumor metabolism of real patient in comparison with a classical adherent cell culture. In fact, the characteristics are very close to mice xenografts, however the time costs are similar to 2D cultivation.

So far, we have been able to cultivate 30 cell samples from patients with advanced prostate cancer who had undergone radical prostatectomy and lymphadectomy. In these cultured patient samples, the growth of epithelial cells was confirmed by IHC (immunohistochemistry), but we were not able to confirm presence of tumor cells yet, as the IHC is not sufficient for this test. Our main goal, once we are able to confirm presence of tumor cells in organoids, will be to focus our research on patient groups where standard therapy was not successful, and personalize the therapy more specifically for them.

Acknowledgment:

3D cultivation, organoids, prostate cancer

Screening of potential inhibitors of MARK4 kinase

Lenka Řeháčková

Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry Palacky University

MARK4 (microtubule affinity regulated kinase 4) is an enzyme encoded by gene MARK4. This enzyme phosphorylates proteins associated with microtubules and affects cell cycle and mitosis. Expression of MARK4 gene has been identified as a cancer marker and MARK4 enzyme has also a possibly role in Alzheimer´s disease. One of the substrates of MARK4 is TAU protein, which forms neurofibrillary tangles in brain tissue after its hyperphosphorylation. Regulation of TAU protein phosphorylation can be a potential target in Alzheimer´s disease therapy.
We developed enzymatic assay for detecting activity of MARK4 kinase which is used for screening of new compounds which can be used as a selective inhibitors of MARK4 and potential drugs in treatment of neurodegenerative diseases.

Acknowledgment:

IGA-LF-2018-031

Role of fenretidine in lipid metabolism in cystic fibrosis mouse model and human patients

Juan De Sanctis[1], Danuta Radzioch[2], Marian Hajduch[1]

[1] *IMTM*
[2] *McGill University*

Fenretidine (FEN) is a synthetic modified molecule of retinoid acid which has been shown to induce changes in lipid metabolism. It has been used in for treatment of several cancers; however, due to its low effectiveness it is only used in few cases. FEN was found to enhance immune response in mice infected with Pseudomonas aeruginosa, primer pathogen in cystic fibrosis. Cystic fibrosis is a genetic disease in which the chloride channel is mutated. Since in CFTR KO mouse model and in humans there is a low production of long chain ceramides, treatment with FEN increased long chain and decreased low chain ceramide a mechanism involving ceramide synthase modulation. FEN also decreases inflammatory cytokine production, decreases abnormal cell death decreasing peroxide formation. Currently there is a phase II clinical trial ascertain the clinical use of FEN in cystic fibrosis patients. Future therapy in these patients may involve a mixture of therapeutic options to enhance chloride

transport and enhance immune response decreasing morbidity and mortality of these patients

Acknowledgment:

American and Canadian Cystic fibrosis foundations. Institute of Immunology, Venezuelan Central University at Caracas. Venezuelan Institute of Scientific Research Laurent Pharmaceuticals.

MicroRNA profiling in cystic fibrosis model cell lines after zinc/fenretinide treatment

Hanuš Slavík[1], Jana Vrbková[1], Josef Srovnal[1], Marián Hajdúch[1], Danuta Radzioch[2]

[1] IMTM, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc
[2] McGill University in Montreal

Cystic fibrosis is the most common autosomal recessive disorder with 3 – 5% carriers in Caucasian population. It is usually caused by mutation in CFTR protein, which is responsible for balance of chloride anions on the surface of mucous membranes. Mutation in this protein causes more viscous mucus, which favors bacterial growth and damage the vital organs. In these days, life expectancy with cystic fibrosis is much better during last few decades because of novel medications. Fenretinide is one of the newly tested drugs, which are able to compensate the aftermaths of cystic fibrosis. This substance influences the levels of inflammatory proteins and stabilizes the concentrations of the specific lipid molecules. Typically, there is lower incidence of Pseudomonas aeruginosa infections after the treatment of fenretinide. There are clear benefits of fenretinide, but the certain mechanism of action is still unknown. MiRNA markers can stead to decipher the task because of its regulatory features. Understanding of the mechanism can help to make the treatment more suitable and miRNA markers can be also used for monitoring of the treatment and disease. Two model cell lines with different treatments and time points have been used for microarray analyses in this study. Differentially expressed miRNA profiles have been studied for setting up the best conditions of the treatment and the most promising molecules will be used for GO and pathway analyses.

Acknowledgment:

IGA_LF_2018_005

MiRNA profiling as a method of identifying a meningioma with the prospect of recurrence

Alona Řehulková[1], Hanuš Slavík[1], Josef Srovnal[1], Tereza Lausová[1], Jana Vrbková[1], Vladimír Balik[1], Marián Hajdúch[1]

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

Meningioma are usually benign tumors, but they can have clinically malignant characteristics, such as local recurrence, progression (relapse with a higher histopathological class), or an aggressive clinical picture. Therefore, the identification of patients with a high probability of recurrence and progression is the aim of our study. We chose miRNA profiling for this purpose as one of the directions of our project. The analysis included 65 FFPE tissue samples (30 samples from patients without recurrence, 30 paired samples from 15 patients relapsing negative controls and 5 samples of histologically normal tissue membranes of the brain). After total RNA extraction with miRNeasy Mini kit (QIAGEN), Affymetrix miRNA 4.0 Array and FlashTagTM Biotin HSR RNA Labeling Kit were used for potential biomarkers identification. 54 differentially expressed miRNAs have been found in the samples from recurrent patient before relapse. 16 candidate miRNAs have been chosen for further validation using RT-qPCR and 5 potential molecules for data normalization with the most stable expression in whole data set. Weak differences have been found between first and subsequent tumors in recurrent patients.

Acknowledgment:

The work was supported by grants IGA LF UP 2018_005, TACR TE02000058, NCMG LM201591 and NPU LO1304

Importance of 45 genetic polymorphisms in Venezuelan patients with Asthma or COPD

Juan De Sanctis[1,2], Jenny Garmendia[1], Dolores Moreno[3]

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[2] *IMTM, Palacky University*
[3] *University Hospital of Caracas. UCV*

Asthma and chronic obstructive pulmonary disease (COPD) are common inflammatory diseases of the airways. The main difference is the type of inflammation, its appearance (asthma childhood,

COPD above 60 years of age) and the reversibility upon bronchodilator drugs, asthma bronchoconstriction is reversible and COPD partially reversible. In order to ascertain possible genetic markers, 45 different single nucleotide polymorphisms were assessed in 100 adult patients with asthma, 100 patients with COPD, 100 non atopic controls aged matched for asthma, 100 non atopic controls aged for COPD, 100 atopic controls with no airway diseases. The screening was based upon spirometry, 6 minute walk test, and response to bronchodilators, Prick test for different allergens and laboratory tests assessing IgE, C reactive protein, hematology, stool analysis for parasitic diseases. The 45 polymorphisms analyzed were divided as follows: inflammatory cytokines, non inflammatory cytokines and proteins, expression markers, beta adrenergic receptor, oxidative enzymes, anti-radical production, proteases, anti-proteases, signal transduction, prostaglandin and leukotriene pathways, C-reactive protein, mucin, serotonin transporters. Results: the most important markers were beta adrenergic receptor 16, for asthma, predicts therapeutic response, ADAM 33/V4, and serotonin transporter 2 could be used to differentiate pathology. These three polymorphisms may have significant clinical value.

Acknowledgment

Project financed by FONACIT and Central University of Venezuela

Genetic biomarkers of clinical response to bevacizumab in colorectal cancer patients

Karolína Bartáková[1], Barbora Blumová[1], Helena Štefanová[1], Veronika Holinková[1], Jana Vrbková[1], Rastislav Slavkovský[1], Jiří Drábek[1]

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

The incidence of CRC in the Czech Republic has stabilized around 80 new cases per year per 100 thousand inhabitants in the last 10 years (Dušek et al. 2005). Although bevacizumab is widely used in the treatment of metastatic CRC (according to the clinical registry CORECT (IBA) there is a total of 6,759 patients treated with bevacizumab in the Czech Republic complex oncology centres from 2005 to 2015 only), the validated predictive biomarker of the reaction to bevacizumab treatment is still unknown. In this project, we try to identify molecular genetics predictive biomarker of bevacizumab.
Formalin-fixed paraffin-embedded tumour tissues were obtained from 30 patients of University Hospital, Olomouc with diagnosed metastatic colorectal carcinoma. Copy number analysis (CNV) was performed using the OncoScan FFPE Assay Kit 1.0 (Thermo Fisher Scientific). The data were analyzed using software the R (RCore Team). The standardized segmentation file analyzed by GISTIC 2. The DAVID version 6.8 database was used for gene annotation.
In the group of responders region 18p11.32 was significantly amplified with 14 genes found in this region. Significantly deleted regions were 1p36.33, 8p11.22, 10q11.23, 14q32.33,

16p13.3 and 20p12.1. In the group of non-responders regions 8q24.21, 14q12 and 19q13.2 were significantly amplified, but no significantly deleted region was found.

Acknowledgment:

This study was supported by the Ministry of Health of the Czech Republic, grant number NV16-32198.

Citation:

Dušek L., Mužík J., Kubásek M., Koptíková J., Žaloudík J., Vyzula R. Epidemiologie zhoubných nádorů v České republice [online]. Masarykova univerzita, [2005], [cit. 2018-5-31]. Available on: http://www.svod.cz. Version 7.0 [2007], ISSN 1802 – 8861.

Search for new predictive epigenetic biomarkers and advanced diagnostics of tumor response to anticancer drugs

Rastislav Slavkovsky[1], Lucia Kotková[1], Karolína Bartáková[1], Marián Hajdúch[1], Jiří Drábek[1]

[1] *IMTM, LF, UPOL*

Our aim is to find epigenetic biomarkers of the response to the bevacizumab used in the treatment of metastatic colorectal carcinoma (mCRC). Bevacizumab is an antibody targeted to VEGF and thus it contributes to the suppression of angiogenesis, on which the tumor is often dependent. We assume that methylation change in the tumor may lead to a gene expression change that cause resistance. Since no reliable predictive marker of response to bevacizumab has been found so far, the methods of our study are genome-wide. From the tumor DNA isolated from the group of responsive and group of non-responsive patients, we enriched the methylated DNA and sequenced on NGS platform. Differential methylation was determined based on the number of reads in the area using MACS2, diffreps and bedtools. We identified dozens of statistically significant candidate areas, including those in vicinity of promoter. Selected markers of resistance to bevacizumab will be validated using alternative methods and may be the subject of detailed functional studies, used potentially in the future as part of the diagnosis of mCRC.

Our another activity is advanced molecular diagnostics of tumors where standard treatment is not appropriate or possible. By identifying genetic alterations, it is possible to characterize the tumor, to target the drug more accurately and to increase the likelihood of the response. We used the Trusight Tumor 170 kit for tumor genotyping of DNA and RNA of 170 genes. However, interpretation of the findings requires complex aggregation of informations from different sources, and may end unclear or inconclusive. We have verified this approach on

several occasions and, after extensive validation, may be used for advanced diagnostics of selected cases.

Acknowledgment:

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Pitfalls in HPV68a detection

Hana Jaworek[1], Katerina Kubanova[1], Vladimira Koudelakova[1], Jiri Drabek[1], Marian Hajduch[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, UP Olomouc, CR*

Despite proved oncogenic potential, HPV68 genotype may be excluded from HPV screening tests and from newly developed vaccines due to its rarity in cervical cancer. HPV68 may exist in two subtypes (a and b), differing in 6% E6, 5% E7 and 7% L1 ORF sequence, and the HPV68a subtype is usually not detectable by primers targeting L1 gene. The aim of the study was to evaluate the efficacy of routinely used cobas® 4800 HPV Test (targeting L1 gene) in HPV68a detection.
Cervical swabs (n=2198) obtained by physicians and self-sampled cervicovaginal swabs (n=217) were analysed for the presence of HPV by cobas® 4800 HPV Test (cobas, Roche) and PapilloCheck® HPV-Screening test (PapilloCheck, Greiner Bio-One). Real-time PCR followed by high resolution melting (HRM) curve analysis was used for HPV68a/b subtyping.
HPV68 was detected in 39 of 2198 (1.77%) cervical swabs and 4 of 217 (1.84%) cervicovaginal swabs using PapilloCheck, with 33 single-type positive cases altogether. Cobas gave false negative result in 20 of 33 (60.6%) HPV68+ cases. HPV68a subtype was detected in all (20/20) false negative cases by HRM analysis. HPV68a subtype was detected in 5 of 13 (38.5%) and HPV68b in 8 of 13 (61.5%) of true positive cases, respectively.
Though cobas is routinely used HPV screening test, the false negative result was detected in 60.6% of HPV68 single-type infection cases due to its lower sensitivity for HPV68a.

Acknowledgment:

IGA LF 2018_005

Mapping of molecular landscape underlying drug resistance and recurrence in glioblastoma paired primary and recurrent tumours and glioblastoma resistant cell lines

Zuzana Šporiková[1], Magdalena Megová Houdová[1], Radek Trojanec[1], Ondřej Kalita[2], Jana Vrbková[1], Jiří Drábek[1], Marián Hajdúch[1]

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[2] Department of Neurosurgery, Faculty Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University in Olomouc

Glioblastoma (GBM) is the most common primary brain tumour in adults with nearly 100% mortality. Despite aggressive therapy, overall survival of GBM patients remains poor due to intrinsic or acquired resistance to maximal treatment modality based on combination of radiation and chemotherapy. The biological background behind GBM progression after first line therapy is mostly unknown and thus, there are only limited targeted therapeutic options for the second line treatment. The main goal of this study was to elucidate genetic landscape in paired samples of primary versus recurrent GBM and to validate prognostic and predictive value of the most significant differences in independent cohort of GBMs.
Patients and methods: The cohort consists of 43 paired samples obtained during first surgery followed by radiochemotherapy (at least 54 Gy plus 3 cycles of temozolomide) and during the second palliative surgery. The analysis comprises genome copy number variations and FISH determined gene and chromosomal region statuses for EGFR, p53, RB1, MDM2, CDKN2A and 1p, 19q, 10p. Methylation-specific PCR was used for analysis of MGMT promoter methylation and competitive amplification of differentially melting amplicons PCR for IDH1/2 mutations detection. The validation of the most relevant alterations was performed on independent cohort of 104 non-paired tumors.
Results: Full data with statistical analysis will be presented at oral presentation.

Acknowledgment:

IGA_LF_2018_005, TACR TE02000058

Measuring the molecular weight of compounds in Chemical library

Lenka Lachnitová[1], Sandra Benická[1], Pawel Znojek[1]

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Actually, in the Chemical library of IMTM there are about 9.300 compounds. It would be beneficial to check the purity of compounds and find out older compounds already decomposed.
In cooperation with chemist Sandra Benická, we have already measured 3000 compounds from Chemical library. We used 96-well plates for testing and measured molecular weight by the mass spectrometer. Problem with a poor solubility of many compounds was partly resolved by replacing methanol with acetonitrile.
Compounds were distributed according to the log P value to five groups from strongly polar to strongly non-polar. At first, we have measured rather polar compounds with a log P value from 1.5 to 3. After that, we have selected non-polar compounds up to log P value 4. Strongly non-polar compounds will be measured by HCTultra Ion Trap. The fifth measured group was polar and strongly polar compounds with a log P value from 1.5. to zero. Unfortunately, the problems with precipitation of the compounds in the mobile phase persisted. We think that log P is not such a convenient value for distribution of compounds according to their polarity. In addition, about a quarter of the compounds have to be measured again by another method or at a higher concentration. Also, it is difficult to distinguish whether the compound was already degraded or if it disintegrated during the measurement in the spectrometer.

Acknowledgment:

This study was supported by grants LF_2018_031 and by the National Sustainability Program (LO1304).

Betulinic acid derivatives inhibit GLI-mediated transcription in human glioblastoma cell line

Ivo Frydrych[1], Milan Urban[1], Marián Hajdúch[1]

[1] *Institute of Molecular and Tranlation Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc*

The evolutionary important Hedgehog (Hh) signaling pathway has recently been implicated in several forms of solid tumors, such as basal cell carcinoma of the skin, cerebellar meduloblastoma, rhabdomyosarcoma, or cancers of the pancreas, stomach, lung, and prostate. Constitutive activation of Hh pathway has also been reported in a subset of human gliomas. The Gli family of proteins represent key mediators of the Hh pathway. The naturally occurring pentacyclic triterpenoid betulinic acid (BA) has been shown to induce apoptosis and inhibit Hh signalling in rhabdomyosarcoma. Inspired by this finding, we screened library of BA derivatives as potential inhibitors of the transcriptional activator Gli1 by using a U87-MG derived cell-based assay. We identified group of potent Gli1 inhibitors with the effect even better than Hh pathway inhibitor cyclopamine. These compounds also remarkably decreased U87-MG cells proliferation and induced apoptosis in a dose-dependent manner. To examine the molecular mechanism of the phenotypic changes, we performed real-time quantitative (RT-PCR) analysis to monitor the expression changes of critical component of Hh signaling pathway and corelated the results with protein level expression.

Acknowledgment:

This work is supported by the National Programme of Sustainability II (LO1304) and Technology Agency of the Czech Republic (TE01020028).

Fluoroquinolones and their new role in the alternative splicing

Natálie Táborská[1], Gabriela Rylová[1], Jan Hlaváč[1], Marián Hajdúch[1], Petr Džubák[1]

[1] *IMTM*

Fluoroquinolones are antibiotics approved for bacterial infection treatments. They share the similar chemical structure with quinolones and contain extra fluorine atom. Recent publications indicate these compounds have another role in the alternative splicing, it is supported by experiments showing MDMX is alternatively spliced after the treatment by at least 2 fluoroquinolones. Based on this information we focused on another attractive target that is highly cited - Pyruvate Kinase, Muscle. PKM2 is typically highly upregulated in the most cancer cells and plays very important role in the glycolysis of tumor cells. U2OS cell

line was used (less CCRF-CEM), cells were treated with selected fluoroquinolones (ciprofloxacin, enoxacin, ofloxacin) and one new drug 05-0777. RNA from this biological material and subsequent cDNA was used for MDMX alternative splicing confirmation in PCR, then for PKM isoforms´ expression measurement in RT-PCR. Isolated proteins were used for western blots using antibodies against PKM isoforms and 2 spicing factors. Now we are preparing samples for transcriptome sequencing. We confirmed exon 6 skipping in MDMX gene after ciprofloxacin and enoxacin treatment. Ofloxacin and 05-0777 did not show such results. PKM1 and PKM2 isoforms´ expression is slightly changed, those findings will be verified and confirmed by transcriptome sequencing in the nearest future. We can also conclude that there are changes in U2AF35 and EFTUD2 proteins after 05-0777 treatment.

Acknowledgment:

Supported by IGA_LF_2017_013.

Citation:

1) Valianatos, G. et al. (2017) A small molecule drug promoting miRNA processing induces alternative splicing of MdmX transcript and rescues p53 activity in human cancer cells overexpressing MdmX protein. PLOS ONE 12(10).
2) Dong, G. et al (2016) PKM2 and cancer: The function of PKM2 beyond glycolysis. Oncology letters 11(3).
3) Cieply, B. et al (2015) Functional roles of alternative splicing factors in human disease. Wiley Interdisciplinary Reviews, RNA 6(3).

Fluorescent dye labeling nucleolus through covalent interaction with BYSL

Zuzana Macecková[1], Pawel Znojek[1], Tomas Ozdian[1], Petr Dzubak[1], Marian Hajduch[1]

[1] Faculty of Medicine and Dentistry, Institute of Molecular and Translational Medicine, Palacky University Olomouc, Hněvotínská 1

Our institute has been given a library of fluorescence dyes. After high throughput screening we selected eleven dyes specifically labeling nucleolus. After the following tests, we selected one dye that labeled nucleolus only in green channel and showed covalent biding towards unknown protein. This compound co-localized with ribosomal proteins and with nucleolar protein BYSL. Using three BYSL antibodies we performed an immunoprecipitation detected fluorescent signal. Immunoprecipitated was subjected to MS analysis. Out of this analysis we selected three proteins as the possible target of compound. We suspect that the compound binds specifically to BYSL and we want to prove it in following experiments.

Acknowledgment: IGA_LF_2018_005

Implementation of a novel HiBiT Protein Tagging System for monitoring proteins expression

Martin Ondra

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

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 assay. The detection reagent contains an inactive rest of luciferase, Large Bit (LgBiT), which rapidly binds to HiBiT to produce a highly active luciferase enzyme. CRISPR-mediated insertion was used for HiBiT tagging into the specific genomic locus of genes under the control of their native promoters. We tried to tagged 2 proteins on C-terminus with HiBiT by lipofection and electroporation.
HiBiT Protein Tagging System was performed with U-2OS and K562 cell lines. We have succeeded with CRISPR-mediated insertion of HiBiT by electroporation in K562 cell line. The luciferase activity was detected by Nano-Glo® HiBiT Lytic Detection System in whole cell lysate and by Nano-Glo® HiBiT Blotting System after SDS-PAGE and transfer onto a nitrocellulose membrane.

Acknowledgment:

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Citation:

Oh-Hashi, K. et al. (2017): Application of a novel HiBiT peptide tag for monitoring ATF4 protein expression in Neuro2a cells. Biochem Biophys Rep. 12:40-45

Pharmaceutical formulation and stability testing of metabolite of disulfiram (CuET)

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Pharmaceutical formulation is the process of combining various chemical substances with the active drug to form a final medicinal product, which is called a drug mixture or drug formulation. A drug formulation can be given to the patient in various forms like liquids,

suspensions, extended and controlled release polymers, emulsions, self-emulsifying systems and a host of others. The type of the formulation given depends upon the patient's age, sex, and health condition and is specific for particular routes of administration and biological activity and site of effect of the drug.
During the development of a new drug, it is necessary to select as soon as possible the formulation with the best stability characteristics. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under storage conditions and under the influence of a variety of stress factors such as hydrolytic, oxidation, photolytic and thermal conditions.
The study of aforementioned terms the pharmaceutical formulation and stress stability are shown on metabolite of disulfiram (CuET) that is responsible for its anti-cancer effects [1].

Acknowledgment:

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Citation:

[1] Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4. Skrott Z, Mistrik M, Andersen KK, Friis S, Majera D, Gursky J, Ozdian T, Bartkova J, Turi Z, Moudry P, Kraus M, Michalova M, Vaclavkova J, Dzubak P, Vrobel I, Pouckova P, Sedlacek J, Miklovicova A, Kutt A, Li J, Mattova J, Driessen C, Dou QP, Olsen J, Hajduch M, Cvek B, Deshaies RJ, Bartek J; Nature. 2017 Dec 14;552(7684):194-199. doi: 10.1038/nature25016. Epub 2017 Dec 6

Spectral analysis of lymphatic tissue

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Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma. DLBCL is an aggressive (fast-growing) lymphoma which can arise in lymph nodes or outside of the lymphatic system. There are several subtypes of DLBCL that may affect a patient's prognosis and treatment options. The most significant are named according to their cell of origin and include germinal centre B-cell-like (GCB) and activated B-cell-like (ABC). These groups of patients may have different prognosis with treatment and information about what subtype („GCB" or „ABC") is involved will be key to choosing the appropriate therapy.

According to our hypothesis, differences in the expression profile of ABC and GCB subtypes also result in a different chemical composition of tumour tissue, and hence a different fingerprint in infrared spectra. The study was done on a thin sections of a paraffin-embedded tissue samples, sections were than analysed using Fourier transform infrared (FTIR) spectrometer coupled with a focal plane array (FPA) and Quantum cascade lasers (QCL). The thin section is "scanned" in a suitable raster, where each point on the map represents one spectrum. The maps contained up to hundreds of thousands of spectra and carry information about the chemical composition of the sample. Principal component analysis will be performed to reveal basic patterns in data. And then we should get the answer if we are able to distinguish cancer or non-cancer tissue.

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Lentiviral CRISPR libraries for identification and validation of molecular mechanisms of action

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In this study, we focus on elucidation of the mechanism of action of a novel, potent 7-deazaadenine analogues (AB61, PNH173, PNH192) using CRISPR libraries. Pooled lentiviral CRISPR libraries are a heterogeneous population of lentiviral transfer vectors, each containing 10 gRNAs per gene to ensure modification of every target gene in a human

genome. We will conduct a primary genome-wide screen using an ultra-complex gRNA library. Fraction of infected cell population will be subjected to a selection in the presence of a particular drug. The frequencies of gRNA-encoding cassettes in the selected population and an unselected control population will be determined by deep sequencing. From these data, hit genes and gRNAs that effectively target them will be identified. Finally, we are going to test identified gRNAs individually to ensure that the genetic modification reproduces the phenotype we had screened for in the first place.

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Citation:

Jost M, Weissman JS. (2018) CRISPR Approaches to Small Molecule Target Identification. ACS Chem Biol, 13: 366-375.
Jost M, Chen Y, Gilbert LA, Horlbeck MA, Krenning L, Menchon G, Rai A, Cho MY, Stern JJ, Prota AE, Kampmann M, Akhmanova A, Steinmetz MO, Tanenbaum ME, Weissman JS. (2017) Combined CRISPRi/a-Based Chemical Genetic Screens Reveal that Rigosertib Is a Microtubule-Destabilizing Agent. Mol Cell, 68: 210-223.

Hepatocyte-based studies in preclinical stages of drug development

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The liver is one of the most complexes organs in the body and its function is critical for the correct functioning of the organism. This organ is involved in multiple key metabolic functions, and thus, the implementation of hepatocyte-based in vitro experiments are a valuable tool for the study of liver-related molecular mechanisms and physiological conditions.

In the decade of the 60´s, Berry and Friend developed a hepatocyte isolation method and reported successful rat hepatocyte isolations (Berry and Friend, 1969). A few years later, Seglen further developed and improved this technique, modifying it into a two-step perfusion process (Seglen P.O., 1976). This method, with several minor modifications, is the one used to date. In research, the most frequently used hepatocytes are from human, mouse and rat origin. Gene expression, liver diseases, hepatic clearance and toxicity are some of the experiments that have been developed for these cultures. In addition, the enzymatic modifications of xenobiotic and new chemical entities by hepatocytes cultures offer

significant information regarding metabolic, biochemical and molecular activities on early stages of drug development.

Hepatocytes cultures offer a good in vivo/ in vitro correlation. However, novel approaches to hepatocyte-based methods are the focus of some recent efforts which try to improve the drawbacks that these methods present, especially regarding quick enzymatic activity loss (Shulman M., 2013).

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The effect of anti-cancer drugs on prion-like propagation of tau inclusions

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For several years, tau aggregation in Alzheimer's disease was believed to be an autonomous cell disorder, where the events leading up to the formation of toxic tau protein aggregates were thought to be confined within the affected cells. However, the development and spread of tau aggregates from one region of the brain to other in Alzheimer's disease patients gave the notion of prion-like spreading of tau aggregates within the brain. These findings were first corroborated in transgenic mice where intracerebral injection of brain extract of mice expressing mutant P301S tau resulted in the assembly of tau in mice expressing wild-type. Recent studies using *postmortem* brain samples provide an important evidence that seed-competent soluble tau inclusions exist in brains of Alzheimer's disease patients prior to the development of tau pathology.

There is an intriguing correlation that indicates cancer survivors have a lower risk of Alzheimer's disease development. There are no studies that have shown a therapeutic link between cancer survivors and Alzheimer's disease; however, there is strong evidence in the literature that indicates a positive effect of anti-cancer drugs for Alzheimer's disease. Although it is not impossible to know the presence of seed-competent tau inclusions in

brains of cancer survivors who do not eventually develop Alzheimer's disease, we speculated that chemotherapy in cancer patients inhibits the prion-like spreading of tau and Alzheimer's disease development. Our results from cell and nematode models of Alzheimer's disease suggest that anti-cancer drugs inhibit seed-competency toxic tau aggregates, prevent the effect of tau aggregates on native tau protein.

Anti-cancer drug inhibit the aggregation of aggregation-prone tau peptides

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Introduction

Tau aggregates are associated with over 20 neurological disorders.Hyperphosphorylated neurofibrillary tangles are pathological hallmarks of Alzheimer's disease and correlates with the clinical progression of the disease.A growing body of evidence shows that prefibrillar tau aggregates that precede the appearance of tangles contribute to the neuronal loss.Tau R3 reproduces much of the aggregation behaviour of tau in cells and animal models.Taking this advantage, we examined the effect of 20 clinically-used anti-cancer drugs and four compounds with anti-cancer properties on the in vitro aggregation of R3.

Methods

The effect of drugs on R2 and R3 aggregation was monitored by thioflavin T binding assay and R3 aggregation inhibition was visualized by fluorescence imaging, atomic force microscopy, and non-reducing gel electrophoresis.In order to find out the effect of these compounds on disulfide bond cross-linked R3 dimer formation, we tested the aggregation of mutant R3 peptide in which the cysteine residue at position 322 was replaced with alanine (R3C322A).

Results

Our data show that not all anti-cancer compounds inhibit the aggregation of R3 peptide.Paclitaxel, cisplatin, doxorubicin, resveratrol and quercetin dose-dependently inhibited R3 and R2 aggregation in ThT assay, inhibited formation of fibrils.Interestingly, none of the most active compounds inhibited R3 dimer formation.Resveratrol and quercetin were less effective in inhibiting R3C322A aggregation.

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Antineoplastic activity of derivatives of 7-substituted-4-chloro-quinoline

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Derivatives of 7-substituted-4-chloro-quinoline were obtained by a nucleophilic aromatic substitution between 4,7-dichloroquinoline and the 3 and/or 4-amino acetophenone. Generating a key intermediate 1-(3 or 4-(7-chloroquinolin-4-ylamino) phenyl)ethanone and consequently 16 different compounds. The compounds were tested for cytotoxicity against cell lines lines Jurkat E6.1, HL60, MCF-7 and A549. Compounds named 4a, 4g, 4l, 4m and 6e show greater activity growth inhibitory HL60 leukemia cells after 24 h of treatment with IC50 values of 1.19 μM, 1.08 μM, 0.59 μM, 0.43 μM and 0.94μ M (to 3 and 100 times more active than doxorubicin and the chloroquine, respectively). Regarding the evaluation of pro-apoptotic activity on neoplastic cell lines Jurkat E6.1, HL60, MCF-7 and A549, was demonstrated that derivatives 4, 5 and 6, like the controls, an increase in generated percentage of cells positive for Annexin V/FITC dose dependent (early and late apoptosis). None of these derivatives induced necrosis process in these cells. These compounds may be useful tools for cancer therapy.

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Faculty of Pharmacy. Universidad Central de Venezuela

Synthesis of modified triterpenoids and study of their mechanism of action

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Synthesis of modified triterpenoids and study of their mechanism of action

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3. Biologically active terpenoids usable as prodrugs. Urban, M.; Kvasnica, M.; Dick

Understanding properties making compounds a hit in primary screening

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When creating HTS libraries it is critical to have good criteria for compound selection. Existing rules which describe properties of molecules, e.g. Lipinski rules, are designed to determine rather drug-like molecules than primary hits. We took an attempt to develop rules for library design. For this purpose we built a random forest model for prediction of "HTS-like" compounds which would be hits more probable across different assays. The model had high quality. But the better the model predicts, the more difficult it is to "understand" it. We aimed to interpret the model, simplify it to that extent, that it becomes understandable - which physicochemical properties molecules should have.
This was done via 2 steps: extracting decision rules from the forest and simplifying them. From each decision tree we extracted rules. Geometrically, these rules are rectangles in multidimensional space, each coordinate stands for one physicochemical property: molecular weight, logP, and number of rotatable bonds, H-bond donors and H-bond acceptors. The rectangle shows which minimum and maximum values of these properties molecules should have. The whole (classification) forest can be represented as the set of intersections between rectangles, where >=50 % of trees take part. For regression grid search was used to determine the number of trees whose intersections represent the forest better. The method was applied to 230326 train set ompounds (49 assays) and 72671 test componds (42 assays).

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siRNA transfection for high-throughput screening of small-molecules

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Gene silencing by small interfering RNAs is a widely used tool in biological research and drug discovery. Silencer Select siRNA Library enable specific knockdown of target gene transcripts and is designed for silencing human druggable genome, drug targets, kinases, phosphatases, G protein-coupled receptors, proteases and ion channels. Gene silencing will allow synthetic lethality screens with anti-cancer small molecule anticancer drugs. The implementation of the assays involves optimization of siRNA transfection conditions for selected cell lines and acoustic liquid handler for a rapid screen of siRNA libraries in high throughput.

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Effect of the compound in the histidine phosphorylation in cancer

Vaishali Uniyal

IMTM

Cells use phosphorylation to modulate intracellular signaling. Protein phosphorylation has been widely studied in the cancer research. The phosphoproteome study mainly focused on the serine, threonine and tyrosine. Histidine phosphorylation is less studied because of the heat and pH-sensitive nature of phosphoramidate bond. NME23, the mammalian histidine kinase was found upregulated in hepatocellular carcinoma and LHPP, histidine phosphatase was found to be a tumour suppressor. Histidine phosphorylation is also related with some divalent cation binding proteins. In our lab, we have found a potent cytotoxic compound that acts as a metal chelator. We are interested in finding the relation between the metal chelation by the compound and its effect if any, in the histidine phosphorylation. Targeting this new mechanism might help to solve the problem of the cancer drug resistance.

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Chromatin reader machinery as target for overcoming resistance to DNA-demethylating epi-drugs

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The clinical resistance and decreased efficacy of prototypal DNA-demethylating drug, 5-aza-2'-deoxycytidine (DAC) in solid tumors limits the successful epigenetic therapy. We developed in vitro resistance against DAC using a solid tumor, colorectal cancer cell line, and studied epigenetic cross-talk between DNA methylation and chromatin modifications. The study unveiled the increased sensitivity of resistant cells to bromodomains (BET) inhibitor, (+)-JQ1 in cytotoxicity assay, augmented response on cell-cycle phases of resistant cells, increased anti-proliferative effects in xenograft models of resistant cells, and synergystic effects in combination with DAC in parental cells, both in vitro and in vivo. Further, the transcriptomics and methylomics profiling revealed the methylation-independent overexpression of critical oncogenes, and methylation-dependent inactivation of key tumor suppressor genes in resistant cells. The study exposes the methylation-driven tumor-suppressive signatures as biomarkers which might differentiate between DAC-resistance and sensitivity. Interestingly, the expressions of up-regulated oncogenes were reversed on treatment with (+)-JQ1. Further, siRNA-mediated genetic inhibition of BET 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, and put forward, the alternative therapeutic regimen for DAC-resistant patients.

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Antibiotic and antiparasitic activity of novel compounds

Ermin Schadich

UMTM

Infectious diseases such as leishmaniases and tuberculosis are associated with high morbidity and mortality in human populations globally. Our research is focused on identification of novel drug candidates that could be used in the development of the

therapeutic measures against these two diseases. Screening of 1,280 compounds from Big Lopac, a commercial chemical library of bioactive compounds, using high-throughput analyses of their activity against axenic forms of Leishmania mexicana and Leishmania 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. Screening 4, 800 compounds from proprietary chemical library for activity against Mycobacterium bovis showed a set of 120 active compounds. After filtering compounds with known activity and/or cytotoxicity to BJ fibroblasts and human macrophages, the 68 compounds were selected for further analyses. Currently, their activity against intracellular bacteria the in infected macrophages is tested by using in vitro assays.

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What can HPLC methods offer to Drug Design and Development?

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Identification and validation of small molecule targets is the main interest for academic and pharmaceutical research. High pressure liquid chromatography (HPLC) technology provide a effective tool to separate and analyze biomolecules on a state-of-the-art level, especially when s chromatographic system is coupled with a very sensitive detector such as fluorescent detector or mass spectrometer. Therefore, various applications of HPLC methods are developed for target identification and validation.

A purified protein, Elongation factor 1-alpha 1 (EEF1A1), and its small molecule binding partner, 5-(3-hydroxy-4-oxo-1,4-dihydroquinolin-2-yl)-2-(morpholin-4-yl)benzamide (HP-23c), were analyzed with two different HPLC methods. Firstly, we used RP-HPLC (reverse phase HPLC) for analysis, followed by SEC-HPLC (Size-exclusion HPLC). Both of these analyses were performed on a UltiMate 3000 RS system (Thermo, USA) equipped with a UV and a FLD detectors. Further, we used confocal microscopy for the visualization of EEF1A1 interaction with its fluorescent ligand, HP-23c, in U2-OS a human osteosarcoma cell line.

Combining these orthogonal methods can give us wide information about relationship of EEF1A1 and HP 23c.

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Quantitative proteomic evaluation of normal tissue and tissue with amyloid mass

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The amyloidosis is a multiorgan disease with significant morbidity and mortality. The disease is characterized by the abnormal deposition of misfolded amyloid protein in various organs. Over time, the accumulating amyloid damages the tissue microenvironment and causes organ failure. IHC is the preferred method for routine amyloid subtyping. However, it is an antibody-based method with many unspecificities. Therefore we have introduced mass spectrometry-based proteomic analysis for subtyping amyloid deposits in FFPE tissues.
In this study, we report a proteomic analysis of microdissected material from FFPE testicular tissue. One nl of normal tissue with different quantities of amyloid mass (0%, 20%, 40%, 60%, 80% and 100%) were collected with the aim to test the sensitivity of the method and measure the ratio of the amyloidogenic and normal proteins. The proteins were extracted from microdissected materials and digested. All recovered peptides were separated and analyzed by LC-MS. Acquired spectra were identified and quantified using the MaxQuant software.
The proteomic analysis allowed the identification of more than 800 proteins per samples. In our tested tissue, the most abundant amyloidogenic protein Semenogelin 1 determines the amyloid subtype ASem1. In tissues with increasing amounts of amyloid mass, we observed increased levels of proteins related to amyloid fibril formation such as SAP, ApoE, ApoA-IV, ApoA-I, VIM, VTN and CLU.

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Diagnostic biomarkers of pancreatic cancer

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IMTM

Based on the GLOBOCAN 2012 project, Pancreatic cancer (PC) ranks7th in cancer-related mortality worldwide. The Czech Republic had the highest incidence of PC in the world in 2012. It is one of the most aggressive types of cancer. Patients are usually diagnosed at the advanced stage due to the asymptomatic course of the disease and most of them die from metastatic dissemination within 5 years. Resection remains the most effective treatment of PC. Because only 15-20% patients are diagnosed with resectable tumor, early diagnosis is essential to patient survival. Development of LC-MS techniques enables the identification of new protein biomarkers for diagnosis of various types of diseases. The changes in the levels of glycosylated protein in plasma may reflect a pathological condition in certain tissues/organs. Majority of glycoproteins are located in the cytoplasmic membrane or secreted from the cell. This increase the chances of their presence in the bloodstream and is the reason why they can potentially serve as biomarkers.
Candidate biomarkers were identified in serum of mice grafted with human cell lines representing 17 types of cancers of the gastrointestinal tract (GI). N-glycopeptides were isolated by solid phase extraction and analyzed by an Orbitrap Fusion (Thermo) mass spectrometer and Ultimate 3000 RSLC Nano liquid chromatography (Thermo) Acquired data were analyzed by software developed in-house. 128 human N-glycopeptides in murine serum originated in GI tumor tissu

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Anatomy of a chromatography problem

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Faulty chromatography is frequently the reason for poor mass spectrometry. We present here a persistent problem in a Dionex Ultimate 3000 RSLC Nano liquid chromatograph. The problem manifested as inconsistent and poor chromatography, variable pump pressure, faulty analytical and trap columns, and faulty column-switching and injection valves. It was eventually traced to a piston seal that was in use past its certified lifetime. The problem was diagnosed using BSA peptide standards, ChromeleonTM, Skyline and MSStatsQC software. Debris from the degrading piston seal had damaged components “downstream” in the flow path and caused all the observed problems. The problem was solved by the replacement of the piston seals and corresponding pistons. We discuss the problem, its eventual diagnosis and subsequently the appropriate repairs. The exercise revealed several issues that could recur in the other chromatographs of the same make in the laboratory. It points to a serious, and costly, lapse in maintenance. Measures are being taken to prevent similar lapses in instrument maintenance. These include, among others, a schedule for the (periodic and) timely replacement of certain parts, and the use of the statistical process control software, MSStatsQC, for the continuous monitoring of chromatography performance.

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Longitudinal system suitability monitoring and quality control for targeted proteomic experiments. Dogu et al, 2017, Molecular and Cellular Proteomics

Proteomic analysis of exhaled breath condensates as a non-invasive diagnostics of pediatric asthma.

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Collection of exhaled breath condensate is a non-invasive, cheap and simple method to obtain samples from the lungs. Exhaled breath condensate (EBC) represents a source of biomarkers including volatile organic molecules and macromolecules such as proteins, lipids, oxidants, and nucleotides. These analytes could be the biomarkers used for diagnosis of lung cancer; respiratory, inflammatory, metabolic, cardiac and other systemic diseases. The aim of this project was to find the proteomic biomarkers in exhaled breath condensates of asthma children patients and cystic fibrosis children patients and to compare them with exhaled breath condensates proteomes of healthy children.

Exhaled breath condensate proteins are digested by trypsin overnight, reduced by DTT and concentration of peptides is measured. Samples are purified and prepared for HPLC/MS analysis which is performed in three technical replicates using high-resolution LTQ Orbitrap Elite mass spectrometer (Thermo Scientific). Measured spectra are analyzed by Proteome Discoverer™ 2.1 software (Thermo Scientific). Data are further statistically evaluated by Statistica and Bioconductor R – package.

We have successfully collected and analyzed samples from 68 pediatric asthma patients and 73 healthy controls. Across all the patient's samples, we have identified almost 3400 proteins. We performed an analysis of protein and peptide level and selected the candidate biomarkers. We have identified candidate predictors of asthma treatment and treatment response. Suggested biomarkers will be further validated using targeted proteomics.

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High-Throughput Screening of GPCRs Using Aequorin-Based Functional Assay

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G protein-coupled receptors (GPCRs) are among the most studied pharmacological targets for ~34 % of all Food and Drug Administration-approved drugs. New avenues in GPCR pharmacology (e.g. biased agonism, allosteric modulation) are emerging and high-throughput screening (HTS) is a tool to facilitate GPCR drug discovery in its early phase. This study aims to contribute to the characterization of complex interactions between a receptor and an active molecule using a highly sensitive functional assay based on utilizing coelenterazine reconstituted aequorin as a probe for intracellular calcium level flux (AequoScreen platform). Here we focus on identification of active compounds in HTS of GPCR family of adenosine receptors (ARs).
Each cell line performance in HTS was evaluated according to various parameters. Thereafter, a small molecule proprietary chemical library of IMTM (up to 5,000 compounds) was analysed in primary screen of potential AR agonists and antagonists. Compounds were retested in counter-screen for unspecific calcium release possibly interfering with the assay followed by the secondary screen.
GPCRs are important medicinal chemistry targets and the finding of new active molecules could be speeded up by using HTS platform. Here we established a robust functional HTS method for identification of GPCRs interacting compounds, including ARs. Our method could provide guidance on hit identification of other GPCRs in luminescence-based calcium flux assay step-by-step.

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Cytotoxicity profiling of new chemical compounds in HTS facility

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The HTS (high throughput screening) is usually one of the first steps in the drug discovery process. This technique was developed to evaluate the biological activity of thousands of chemicals to identify potential drug candidates in a very short time. The system requires automation, data processing and control software, precise liquid handling devices, and sensitive detectors.

One of the methods routinely used in our HTS laboratory is cytotoxicity screening as in vitro cytotoxicity testing has become an essential aspect of drug discovery. At the beginning, the MTS assay as test cytotoxicity was validated. Cytotoxic effects of unique chemical compounds are tested on 10 cell lines (8 cancer cell lines and 2 non-cancer cell lines). Firstly, all compounds are tested at 1 concentration (50 μM) and the PI (percentage of inhibition) value is calculated. Consequently, compounds which can kill more than 50% of the cell population at tested 50 μM concentration (PI > 50%) are selected for determining the IC50 values. For analysis and calculations the software Dotmatics is used. To quantify the suitability of cytotoxic assay in an HTS the Z-factor is determined for each plate and cell line.

Our proprietary chemical library contains nearly 8.000 compounds and is continuously growing. Results obtained during last three months cytotoxicity testing will be presented.

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Development and Optimization of High-Throughput Screening Assay for Antiviral Therapeutics

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Coxsackie B virus is a worldwide distributed enterovirus infecting humans resulting in gastrointestinal distress, and/or pericarditis and myocarditis [1]. A plaque reduction assay represents a reliable simple tool for the antiviral therapeutics screening against different DNA and RNA viruses, Coxsackie B virus including [2, 3, 4]. We developed a high-throughput screening method based on the similar principles as the plaque reduction assay with which is possible to screen thousands of different chemical compounds in a relatively short time. This assay requires a distinctive cytopathic effect resulting in death of the infected cells as the reading is performed by MTT or MTS [5]. A plaque reduction assay mentioned before is commonly used for other viruses such as Herpes simplex virus-1, Vaccinia virus and Vesicular stomatitis virus. All the mentioned viruses infect VERO 76 cell line as well as Coxsackie B virus does [2, 3, 4]. However, the adjusted high-throughput screening assay as we designed for Coxsackie B virus could be utilized with a slight changes for Vesicular stomatitis virus, but not for Herpes simplex virus-1 and Vaccinia virus because of the insufficient death rate of the cells even though the cell morphology changes caused by infection are noticeable early after infection. In results, we found 25 out of 5114 compounds inhibiting the replication of Coxsackie B5 virus. Some of them were previously described as anti-cancer inhibitors [6].

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