IMTM

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PhD. conference
July 14 - 16, Pastviny

ABSTRACT BOOK 2017

PROGRAM OF THE CONFERENCE:

Wednesday 14/6/2017

Section from 13:00 to 15:30 chaired by Tomáš Oždian, Jiří Drábek

13:00 13:50 *Michal Kroča* **Overview of the Czech Army Centre for Biological Defence role and activities**

13:50 14:10 *Ermin Schadich* **Identification of constitutive expression of Glutathione S transferase P1 in human preneoplastic keratinocytes**

14:10 14:30 *Hana Jaworek* **Prevalence of HPV Infection in Sperm Donors and Men Treated for Infertility: a Prospective Study**

14:30 14:50 *Rastislav Slavkovský* **Novel technologies for predictive RAS genotyping in metastatic carcinoma**

14:50 15:10 S*oňa Gurská* **Cytotoxicity profiling of new chemical compounds in HTS facility**

15:10 15:30 *Pawel Znojek* **HCS based identification of subcellular staining by library of fluorescent dyes.**

Cofee 15:30 16:00

Section from 16:00 to 18:00 chaired by Radek Trojanec, Soňa Gurská

16:00 16:20 *Zuzana Macečková* **Bysl as DBA modifier**

16:20 16:40 *Martina Jakoubková* **Diagnostic Biomarkers of GI tract cancers**

16:40 17:00 *Kateřina Sikorová* **Evaluation of sarcoidosis genetic risk based on 18 susceptibility markers in a west-slavonic population**

17:00 17:20 *Karolína Světlíková* **Association between nod2/card15 polymorphisms and acute myocardial infarction**

17:20 17:40 *Hanuš Slavík* **Detection of ionizing radiation biomarkers in hair follicles**

17:40 18:00 *Alona Řehulková* **CTCs/DTCs as independent prognostic criteria in non-small cell lung cancer**

Thursday 15/6/2017

Section from 9:00 to 10:30 chaired by Marián Hajdúch

9:00 9:20 *Natálie Táborská* **The impact of antibiotics on Diamond Blackfan anemia cell models**

9:20 9:40 *Miloš Petřík* **Radiolabelled siderophores for specific imaging of infections**

9:40 10:00 *Petr Vojta* **MOLDIMED - analytical pipeline solution for DNA massive parallel sequencing**

Cofee 10:00 10:30

Section from 10:30 to 12:00 chaired by Martin Mistrík, Pawel Znojek

10:30 10:50 *Agáta Kubíčková* **Development of human c-Myc reporter cell line**

10:50 11:10 *Magdalena Houdova Megova* **Molecular Characteristics of Recurrent Glioblastomas**

11:10 11:30 *Jana Kotulová* **Aequorin-based Functional Assay for High-throughput Screening: focus on Adenosine receptors**

11:30 11:50 *Jana Potočková* **Detection of HPV in non-small cell lung cancer patients**

Lunch 12:00 13:00

Section from 13:00 to 15:30 chaired by Lakshman Varanasi, Dusan Holub

13:00 13:20 *Dušan Holub* **Mouse model of Bordetella pertussis infection for identification of the disease biomarkers**

13:20 13:40 *Ivo Vrobel* **Identification and structure elucidation of novel sulphur-containing imatinib metabolites**

13:40 14:00 *Jana Václavková* **Identification of proteomic molecular targets of potential anticancer drugs by affinity purification**

14:00 14:20 *Tomáš Oždian* **The comparison of mass spectrometry approaches in proteomic profiling of drug responses.**

14:20 14:40 *Lenka Lachnitová* **Difficult start of measuring molecular weight of compounds in Chemical library**

14:40 15:00 *Miroslav Hruska* **Peptide identification capabilities at IMTM**

15:00 15:20 *Lakshman Varanasi* **Circulating glycoprotein biomarkers of pancreatic cancer**

15:20 15:40 *Jan Václavík* **Structural elucidation of novel biomarkers of known metabolic disorders based on multistage fragmentation mass spectra**

Friday 16/6/2017

Section from 9:00 to 10:00 chaired by Milos Petrik

9:00 9:20 *Zbyněk Nový* **New cholin analogs as potentional diagnostics/therapeutics for prostate cancer**

9:20 9:40 *Narendran Annadurai* **Identification of potential inhibitors of tau aggregation for alzheimer's disease therapy**

9:40 10:00 *Lenka Řeháčková* **Analyses of potential inhibitors of P-glycoprotein**

Cofee 10:00 10:30

Section from 10:30 to 12:00 chaired by Josef Srovnal, Barbora Lišková

10:30 10:50 *Josef Srovnal* **Identification of three distinct molecular subtypes in meningioma samples using microarrays for copy-number variants**

10:50 11:10 *Barbora Lišková* **Preclinical absorption, distribution, metabolism, excretion, and pharmacokinetics of a novel potent nucleoside cytostatic PNH 173**

11:10 11:30 *Mariia Matveieva* **Mining molecular patterns important for toxicity**

11:30 11:50 *Martina Michalová* **In vitro inhibition of human hepatic cytochrome P450 enzymes by copper (II) and gold (I) complexes**

11:50 12:10 *Jarmila Stanková* **The Covalently bonded compounds and their proteins target detection**

12:10 12:20 Tomáš Oždian **Conference closing**

Lunch 12:30 13:30

Overview of the Czech Army Centre for Biological Defence role and activities

Michal Kroča

Centre for Biological Defence, Military Institute of Health, Army of the Czech Republic

In presentation, history of Czech Army bio-defence system will be mentioned, with clarification of position and role of Centre for Biological Defence (CBD) within this system. Main parts of CBD and their function will be described and major areas of activity explained. Special focus will be given to hospitalization and treatment of patients with highly contagious diseases, education and training of specialists and research and development in bio-defence. Cooperation on national level with IRS, institutions and organisations will be referred, as well as international cooperation and participation of CBD and its members in foreign missions. Last but not least, plans for further development and expansion of this facility will be presented.

Identification of constitutive expression of Glutathione S transferase P1 in human preneoplastic keratinocytes

Ermin Schadich

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Glutathione S-transferase P1 (GSTP1), a member of the GST enzyme superfamily, is associated with pathogenesis of different cancers including two important skin cancers, skin squamous cell carcinoma and basal cell carcinoma. In normal skin cells, the expression of GSTP1 is regulated by transcription factor Nuclear factor erythroid related factor 2 (Nrf2) and associated Nrf2 ARE signaling pathway. Our study aimed to analyse the expression of GSTP1 of human precancerous keratinocytes (HaCaT cells) during activation of Nrf2 HaCaT cells and their corresponding Nrf2-ARE luciferase reporter cells were treated by the known Nrf2 activators, ginger phenylpropanoids and quercetin, and the level of Nrf2 activity was subsequently determined. Western blot analyses were used to determine the level of GSTP1 While both ginger phenylpropanoids and quercetin significantly increased. he level of Nrf2 activity in HaCat cells, the level GSTP-1 was not changed. Such phenomenon of unresponsive downstream target expression in HaCaT cells is consistent with a constitutive expression of GSTP1. As the constitutive expression of GSTP-1 is typical to different types of cancer, its role in initial phases of neoplastic and malignant transformation of skin keratinocytes is indicated.

Acknowledgment:
The research was supported by POST UPGrant CZ.1.07/2.3.00/30.0004.

Prevalence of HPV Infection in Sperm Donors and Men Treated for Infertility: a Prospective Study

Hana Jaworek[1], Blažena Zbořilová[2,3], Ivana Oborná[4,5], Jana Březinová[6,7], Vladimíra Koudeláková[1], Jana Vrbková[1], Marián Hajdúch[1]

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[2] *Fertimed, Ltd.*
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[5] *Dept. of Obstetrics and Gynaecology, University Hospital Olomouc*
[6] *Sperm Bank International, Ltd.*
[7] *Arleta IVF, Ltd.*

Human papillomavirus (HPV) infection is a cause of many cancers but may also affect human fertility. HPV infection estimated to be associated with poor semen quality including sperm DNA fragmentation and sperm morphology. HPV examination is not included into the obligatory panel of infection for both, donors and infertile couples because the association between infertility and HPV is still uncertain. Semen samples and penile swabs were analysed using cobas® 4800 system (Roche) and PapilloCheck® HPV- Screening system (Greiner bio-one).In a group of 429 asymptomatic men 42% were HPV positive. Men from infertile couples were more frequently HPV positive than potential sperm donors (43.5% vs. 37%). HPV infection was more frequently detected in penile swab then in semen sample (40.1% vs.12.8%).

Acknowledgment:
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Novel technologies for predictive RAS genotyping in metastatic carcinoma

Rastislav Slavkovský[1], Jiří Drábek[1], Lucie Kotková[1], Marián Hajdúch[1]

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

Treatment of metastatic colorectal carcinoma by anti-EGFR biological drugs requires the genotyping of NRAS and KRAS genes (RAS genotyping). Standard Next generation sequencing (NGS) allows high throughput RAS genotyping with a high sensitivity; however, with it has unacceptable failure rate and tedious manual steps.
To improve the robustness and user-friendliness of NGS based RAS genotyping, we developed a novel approach. Our approach is able analyse numerous number of codons of

highly degraded DNA. The technology is based on the single step polymerase chain reaction amplification of tumor DNA from formalin fixed paraffin embedded samples with parallel incorporation of sequence tags required for reliable ultra-deep sequencing using MiSeq® (Illumina) platform. Using this technology, only about 1% of samples remains unanalyzable what superceeds 5 to 20% failure rate of other Next generation sequencing methods. Moreover, our approach applied to RAS testing enables to get genotyping result within 16 hours from extracting DNA while hands-on time is only 30-60 min. The presented technology is fully validated and stable in form of a diagnostic kit at -20°C.
The principle of technology allows to be used for genotyping of other clinically relevant genes and also allows the implementation of unique molecular barcodes allowing sensitivity improvement if required for liquid based biopsy samples.

Cytotoxicity profiling of new chemical compounds in HTS facility

Soňa Gurská[1], Pawel Znojek[1], Petr Džubák[1], Marián Hajdúch[1]

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

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 very short time. This system requires automation, data processing and control software, precise liquid handling devices, and sensitive detectors.
In vitro cytotoxicity testing has become an essential aspect of drug discovery. In our HTS laboratory the MTS assay as test cytotoxicity was validated. Cytotoxic effects of unique chemical compounds are tested on 10 cell lines. Firstly, all compounds are tested at 1 concentration and the PI (percentage of inhibition) value is calculated. Consequently, compounds which can kill more than 50% of cell population at tested 50 μM concentration (PI > 50%) are selected for determining the IC50 values. To quantify the suitability of cytotoxic assay in a HTS the Z-factor is determined for each plate and cell line.
Results obtained during last three months cytotoxicity testing will be presented.

Acknowledgment:
This study was supported by grants LF_2017_030 and by the National Sustainability Program (LO1304).

HCS based identification of subcellular staining by library of fluorescent dyes.

Pawel Znojek[1], Filip Teply [2], Petr Dzubak[1], Marian Hajduch[1]

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[2] UOCHB

Using high contend screening microscopy a library of 1700 small molecule compounds with unknown fluorescent properties was screened. The goal of the screening was to reveal approximate emission spectrum of each dyes and to find their sub-cellular targets. Whole library was tested on live cells as well as on dead cells fixed with various fixing condition. Results analysis was carried on with several approaches. First, staining pattern of cellular substructures was classified by investigator. In second approach image analysis and clustering technique was used for detection of staining pattern. From screened library were able to identify several hundred dyes emitting fluorescence. The majority of dyes had broad emission spectrum and stained mostly perinuclear region of cells. However, we were able to identified dyes which are able specifically stain nucleus, nucleoli, cellular membrane or mitotic spindle. Analysis approach and staining pattern of hits will be presented.

Bysl as DBA modifier

Zuzana Macečková

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

Diamond Blackfan anemia (DBA) is rare congenital red cell aplasia. DBA is usually diagnosed early after the birth as severe macrocytic or normocytic anemia with reticulocytopenia, normal platelet and neuthrophil counts and deficiency of red blood cells precursors in bone marrow. In approximately 35-50% of cases are these symptoms accompanied with physical malformation, mostly of craniofacial region, upper limps and growth retardation. Other clinical manifestation is increased level of ADA. In 50-75 % of DBA cases can be cause accaanouted for mutation in ribosomal protein, in other cases cause is still unknown. DBA displays broad range of phenotype manifestation and phenotype can vary even in siblings with same mutation . It has been hypothesised that phenotype variability can be caused by disease modifing genes. In one of our patient we detected big deletion of RPL11 gene. In this patient we also diagnosted mutation in BYSL gene. Mother and brother of patient mutation carry same mutation, although neither of them translate mutated form. We assume that translation of mutant bysl in patient can modifie disease severity.

Acknowledgment:
IGA LF UP 2017_13

Citation:
Danilova N, Gazda HT. Ribosomopathies: how a common root can cause a tree of pathologies. Dis Model Mech. 2015 Sep;8(9):1013-26.
Carron C et al. Analysis of two human pre-ribosomal factors, bystin and hTsr1, highlights differences in evolution of ribosome biogenesis between yeast and mammals. Nucleic Acids Res. 2011 Jan;39(1):280-91.

Diagnostic Biomarkers of GI tract cancers

Martina Jakoubkova

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Cancers of the GI tract are among the types of cancer in the world with the highest mortality. Early diagnosis is essential for treatment and patient survival. There is, therefore, a need for a method capable of detecting early stage disease. Because glycosylation is frequently a indicator of aberrant cellular physiology, glycoproteins may be useful as diagnostic biomarkers. Many glycosylated proteins are located on the outer surface of the cell or intended for export into the extracellular matrix and constitute a large proportion of tumor proteins in the bloodstream. Sensitive methods like mass spectrometry or immunoassays can detect some of these tumor proteins.
Sera from mice grafted with various GI cancer cell lines was used for identification of candidate biomarkers. A total of 147 unique human N-glycopeptides were identified in the screen. Individual candidates biomarkers from discovery phase will be validated in plasma samples from patients suffering from the appropriate type of cancer using a targeted proteomic technique.
Candidate biomarkers were analyzed by a gene ontology software to evaluate function of the target proteins. PANTHER statistical overrepresentation test was performed with data annotations for Biological process, Molecular function, Protein class and the Reactome pathway. Results show distribution of annotations for particular data sets.

Acknowledgment:
This project was supported by an internal grant of Placky University (IGA_LF_2016_019) and by the Czech Ministry of Education, Youth and Sports (LO1304).

Citation:
Tian et al, 2007, Solid-phase extraction of N-linked glycopeptides , Nature Protocols 2: 334-339
Sethi, Fanayan, 2015, Mass Spectrometry-Based N-Glycomics of Colorectal Cancer. Molecular Science 6:29278-304

Evaluation of sarcoidosis genetic risk based on 18 susceptibility markers in a west-slavonic population

Kateřina Sikorová[1], Amit Kishore[1], Martina Doubková[2], KRZYSZTOF Rębała[3], Anna Dubaniewicz[4], Vítězslav Kolek[5], Martin Petřek[1]

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Sarcoidosis is a multi-systemic, polygenic, inflammatory disease, affecting mostly lungs. Its sub-phenotype 'Löfgren's syndrome (LS)' is characteristic by acute onset and better prognosis. Several immune involved genes have been reported with the disease sub-phenotypes.
We analysed 18 SNPs among 564 sarcoidosis patients, its sub-phenotypes LS (n=94) and Non-LS (n=414), with compared to 301 healthy controls from the West-Slavonic population. Genotyping was performed using Sequenom MassARRAY iPLEX platform. Association analysis using allelic model and odds ratio (OR) were estimated using Fischer's exact test with Bonferroni correction.
Analysis of sarcoidosis cases revealed the association of total 7 variants significant with Bonferroni's correction. Among cases with sub-phenotypes LS were associated 6 variants located on chromosome 6 and involved in immune functions, 4 of them especially associated only with LS: HLA-DQA rs2187668 (OR=3.14, p=1.09x10-6); HLA-DRA rs3135394 (5.23, 8.25 x10-13); TNFαrs1800629 (2.66, 5.94x10-7); LRRC16A rs9295661 (2.97, 4.29x10-4) With Non-LS were associated 3 variants, specifically only with Non-LS it was ANXA11 rs1049550 (0.66, 2.71x10-4).
The results replicated the findings from a large cohort study in western Europeans and extended them especially of the Polish population in which hasn´t been yet investigated. Were found some genes variants for sarcoidosis which could be helpful in the diagnosis of early acute onset of the disease.

Acknowledgment:
Grant support: IGA_PU_LF_2016_009/2017_014 and LO1304.

Citation:
Costabel U., Hunninghake G.W., on behalf of the Sarcoidosis Statement Committee: Sarcoidosis Vasc Diffuse Lung Dis. 16 (2), 149 – 173 (1999)
Kishore A., Petrek M.: International Trends In Immunity 1 (4), 43-53 (2013)
Rivera N.V. et al.: Am. J. Respir. Crit. Care Med. 193(9), 1008-22 (2016)

Association between nod2/card15 polymorphisms and acute myocardial infarction

Karolína Světlíková[1], Veronika Žižková[1], Martin Petřek[1,2], Jana Petřková[1,3]

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Acute myocardial infarction (AMI) is a complex disease of polygenic and multifactorial nature. Inflammatory component plays a role in its pathogenesis (de Couto et al., 2015). Of possible polymorphic genes, NOD2/CARD15 may be of relevance. In this study we, therefore, analysed the association between AMI and NOD2/CARD15 gene variants (rs2066844, rs2066845, rs2066847). 250 AMI patients of Czech origin were enrolled and variant distribution compared with that in 220 healthy control subjects of West Slavonic, i.e. Czech (n=121) and Slovak (n=99) origin; the genetically close Slovak population was used to achieve adequate size of the control group. MassArray iPLEX methodology (AgenaBioscience, San Diego, CA) was used for SNP genotyping. Genotype and allele frequencies were determined; the distribution of genotypes complied to Hardy-Weinberg equilibrium. Significant difference in C allele frequencies was observed between the patient group (C: 0.04) and the control subjects (0.07) for rs2066847; no difference was detected for the other two SNPs tested. This finding, together with predominance of carriers of NOD2/CARD15 rs2066847 variant among healthy controls, suggests its possible protective character against AMI (Odds Ratio, OR 0.52; p=0.03). The plausible protective effect of the C allele of rs2066847 must be replicated in other centres/other ethnicities, otherwise it is considered preliminary and pertinent for Czech (West Slavonic) population only.

Acknowledgment:
Grant support: LO1304, IGA_PU_LF 2016_009 / 2017_014

Citation:
de Couto, G et al. (2015) Macrophages mediate cardioprotective cellular postconditioning in acute myocardial infarction. J Clin Invest. 125:3147–3162

Detection of ionizing radiation biomarkers in hair follicles

Hanuš Slavík[1], Petra Švarcová[1], Pavlína Dušková[1], Martin Mistrík[1], Josef Srovnal[1], Marián Hajdúch[1]

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Hair follicles are very easy obtainable biological material, which allow repeatable and noninvasive sampling. It can be used as a rich source of biomarkers of intrinsic and extrinsic influences. Ionizing irradiation is a suitable model for the study of senescence, apoptotic pathways or neoplastic transformation. We use special vacuum pistol for collection of the material. Expression of the markers SESN1, p21 and MDM2 was investigated by RT-qPCR and presence of γ-H2AX was investigated by immunofluorescence. Experiments were performed on human and murine fibroblast cell lines, human hair follicles (ex vivo irradiation) and murine hair follicles (in vivo irradiation). Comparison of the results from different biological material will be presented as a fold change to non-irradiated samples.

Citation:
Kabacik S., Ortega-Molina A., Efeyan A., Finnon P., Bouffler S., Serrano M., Badie C. (2011) A minimally invasive assay for individual assessment of the ATM/CHEK2/p53 pathway aktivity. Cell Cycle, 10: 1152-1161

CTCs/DTCs as independent prognostic criteria in non-small cell lung cancer

Alona Rehulkova[1], Andrea Prokopova[1], Josef Srovnal[1], Monika Vidlarova1, Josef Chudacek[2], Jana Vrbkova[1], Josef Skarda[3],Tomas Bohanes[4], Jiri Klein[5,6,] Marian Hajduch[1,5]

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Purpose: CTCs have been widely introduced as research object in recent years. However, a small number of CTCs in early stages of lung cancer complicates the analysis. It was reported better results from blood drawing closer to primary tumor - pulmonary vein, as

well collection from their accumulation reservoir – bone marrow. In this regard, we have hypothesized that simultaneous analysis of peripheral blood (PB), tumor-draining blood (TDB) and bone marrow (BM) would be more productively.
Materials and methods: 119 IA-IIIA stages of NSCLC patients were enrolled. The CTCs/DTCs presence has been detected by qRT-PCR of CEA, EGFR, LunX, c-met, EpCAM mRNA expression in PB, TDB and BM before/during surgery. Statistical analysis was carried out using software R.
Results: We determined that CTCs/DTCs are prognostic criteria independent of clinical-morphological parameters. Correlation with tobacco-smoking status was established using EGFR and EpCAM as CTCs biomarker. The presence of CEA-positive CTCs/DTCs in TDB samples indicated significantly shorter CSS and in BM indicated significantly shorter OS and CSS. The EpCAM-positive CTCs presence in TDB has affected the CSS ($p=0.026$) and DFS ($p=0.041$).
Conclusion: Detection of CTCs/DTCs is a "liquid biopsy" embodiment, allowing predict the course of the disease in early time. Identifying CTCs-positive patients will allow to more effectively navigate in the systemic treatment.

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The impact of antibiotics on Diamond Blackfan anemia cell models

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Diamond Blackfan anemia is a rare inherited bone marrow failure syndrome characterized by a red blood cells production failure, this state leads to the lack of oxygen in tissues. DBA is caused mainly by mutations in genes encoding ribosomal proteins and that is why it is highly connected with ribosomal stress with the subsequent influence on the protein synthesis. Here we present experiments related to the protein synthesis level measurement on DBA cell models after the treatment of selected antibiotics which have their mechanism of action based on the protein synthesis inhibition in bacteria. We verified the hypothesis: Do antibiotics worsen or reduce protein synthesis level in DBA cell models?.
DBA models were prepared by transient transfection of U2OS cells with ptdTomato-C1 vector carrying one of the target genes: RPS19, RPS26, RPL5 and RPL11 (wild-type and mutant form). Cells were treated with for 24 hours. Then newly synthesized proteins were detected by Click-IT system and fluorescence signals from this assay were scanned in Cell Voyager 7000S. Data were analyzed in Columbus Image Analysis System.
We found out that there are effects of antibiotics on DBA cell models. When comparing wild-type versus mutant form of the target gene in cells, we can conclude that the best results

(dose-dependent decrease of protein synthesis level) occurred after Clarithromycin treatment as well as in the case of Erythromycin. Study funding: IGA_LF_2016_010.

Acknowledgment:
Study fuding: IGA_LF_2016_010.

Citation:
Ito E. et al.: Molecular pathogenesis in Diamond-Blackfan anemia. Int JHematol. 2010 Oct;92(3):413-8.
Lipton JM, et al.:Diamond-Blackfan anemia: diagnosis, treatment, and molecular pathogenesis.Hematol Oncol Clin North Am. 2009 Apr;23(2):261-82.
Narla A, et al.: Ribosome defects in disorders of erythropoiesis. Int J Hematol. 2011Feb;93(2):144-9.

Radiolabelled siderophores for specific imaging of infections

Miloš Petřík[1], Eva Umlaufová[1], Vladislav Raclavský[2], Vladimír Havlíček[3], Andrea Palyzová[3], Zbyněk Nový[1], Clemens Decristoforo[4], Marián Hajdúch[1]

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Introduction: Invasive infections remain a major cause of morbidity and mortality in immunocompromised patients. Early and accurate diagnosis is a key factor for the successful treatment. Siderophores are chelators produced by microorganisms to scavenge iron, which is an important nutrient for them. Replacing iron in siderophores by suitable radiometal opens approaches for targeted imaging of infection.
Methods: Radiolabelling of siderophores with 68Ga was performed using acetate. Stability in different media, protein binding and log P values were determined. In vitro uptake was tested under various conditions using various fungal and bacterial cultures. Biodistribution was studied in normal and infected mice or rats using PET/CT imaging.
Results: Studied siderophores (TAFC and PYOA) were labelled with 68Ga. The resulting complexes showed hydrophilic properties, low protein binding and high stability for 68Ga-TAFC, while 68Ga-PYOA showed certain instability. In vitro uptake of 68Ga-siderophores was highly dependent on type of microbial culture. In normal mice 68Ga-siderophores showed rapid renal excretion and low blood values. PET/CT imaging in infected animals showed accumulation of 68Ga-siderophores in infected tissues.
Conclusions: Siderophores can be easily labelled with 68Ga. The high and specific in vitro uptake in appropriate microbial cultures as well as excellent pharmacokinetics makes them promising agents for imaging both fungal and bacterial infections.

Acknowledgment:
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MOLDIMED - analytical pipeline solution for DNA massive parallel sequencing

Petr Vojta

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Nowadays, massive parallel sequencing (MPS) have started to play a key role in DNA diagnostics. MPS methods generate enormous amount of data and require command line based analytical tools linked to Linux, what is often limiting factor for potential users. Here we demonstrate straight-forward and user-friendly solution for MPS variant analysis. Designed pipeline is separated into two main parts. The first part contains processing of raw sequencing reads including quality check, read trimming, alignment to reference genome and variant calling. Default settings is based on general recommendations of good practice, neverthless atributes might be configurable by a user. The computational steps are carried out by integration of several GNU licensed tools. The second part provides annotation of obtained DNA variants, their localization on gene level and effect on translation. Variants are characterized by frequency in population, pathogenicity, damaging protein predictions in silico and linked to gene ontologies and human phenotype ontologies terms. The compact and intuitive platform enabling analysis from raw data to variants annotation. Variant filtering options are allowed by frequency, patogenicity, diseases phenotype or selected molecular pathways criteria. Moreover, variant characterization could be extended by uploading of user's custom files in standard formats (VCF/GTF/GFF).

Acknowledgment:
MOLDIMED – TACR

Development of human c-Myc reporter cell line

Agáta Kubíčková

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

Here we report a process of generating a novel reporter cell line for determining the expression level of c-Myc. c-Myc is known as a master regulatory factor of cell proliferation, metabolism, differentiation and apoptosis1. Nearly 20 % of human cancers are associated with dysregulation of c-Myc2. This human reporter cell line will serve as a high throughput compound screening and testing tool and could also aid basic research in the study of

ribosomal biogenesis. There is an evidence of several mechanisms how to lower the level of c-Myc. We will focus on testing small molecule ligands, that can specifically induce the formation of and stabilize the G-quadruplex structure at NHE III 1, a G-rich sequence of c-Myc promoter3.
Methods: transduction, stable cell line generation, single cell sorting, induction of c-Myc with IL-6 and EGF, luciferase assay, generation of c-Myc overexpressing lentivirus using molecular biology tools.
Results: The level of c-Myc after induction by interleukin 6 and epidermal growth factor is not sufficient enough for validation of novel reporter cell line. This problem could be probably solved by co-transduction with lentivirus designed for c-Myc overexpression.

Acknowledgment:
This work is supported by the National Programme of Sustainability II LO1304.

Citation:
1. Chen, B. J. et al. Small molecules targeting c-Myc oncogene. Int. J. Biol. Sci. 10, 1084-1096 (2014).
2. Albihn, A. et al. MYC in oncogenesis and as a target for cancer therapies. Advances in cancer research 107, 163-224 (2010).
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Molecular Characteristics of Recurrent Glioblastomas

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Background:
Glioblastoma (GBM) is the most frequent and the most malignant brain tumor of adults. Currently, there is no standard treatment and lack in knowledge of molecular biology of recurrent GBM. Molecular and genetic aberrations occurring after recurrence of GBM are able to elucidate biological changes developed due to the selection pressure of the therapy.
Materials and methods
24 patients with the recurrent GBM were analyzed by OncoScan assay, fluorescent in situ hybridization for determination of EGFR, p53, RB1, MDM2, CDKN2A genes and 1p, 19q and 10p chromosomal regions statuses, methylation-specific PCR for MGMT promoter methylation status and CADMA PCR for IDH1/2. OncoScan data were processed by GISTIC 2

software.
Results:
The most significant aberrations were EGFR amplification (19/24 primary tumors, 16/24 recurrent tumors), CDKN2A loss (18/24 primary tumors, 16/24 recurrent tumors), MDM2 gain (4/24 primary tumors), PDGFRA gain (3/24 recurrent tumors) and GSST1 loss in primary tumors (7/24) and GSTM1 gain in recurrent tumors (7/24). Status of GSST1 and GSTM1 gene were verified in control glioblastoma group.
Conclusion
Molecular genetic profiling of 24 recurrent GBM showed association between recurrence and status of glutathione S-transferases genes.

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Aequorin-based Functional Assay for High-throughput Screening: Focus on Adenosine Receptors

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Background: High-throughput screening (HTS) of active compounds is a dominant strategy in drug discovery since the 1990s. Cell-based HTS can provide a deeper understanding of G protein-coupled receptor interactions with active molecules. Here we present a highly sensitive functional assay based on utilizing coelenterazine-reconstituted aequorin as a probe for intracellular calcium level that was used for identifying active molecules in HTS of adenosine receptors (ARs).
Methods and findings:
4,560 small molecules were used in primary screen of potential AR agonists and antagonists. Active candidates were examined in secondary screen by using 8-point dose-response curve and IC50 value calculation. Our findings suggest that confirmation rate of primary hits is affected by (i) the HTS assay quality, (ii) hit limit/threshold selection, and (iii) primary hit profile. Therefore, we also focused on data processing.
Conclusions:
HTS is a critical step in early drug discovery. Here we established robust HTS method of ARs screening that could provide a guidance on hit identification of other GPCRs in luminescence-based assay step by step.

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Detection of HPV in non-small cell lung cancer patients

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Introduction:Worldwide,lung cancer is the most common cause of death associated with cancer.Cigarette smoking remains the main risk factor which contributes to the development of lung cancer.However,we know that not everyone who smokes develops lung cancer and it is likely smoking works in synchrony with other risk factors (genetic, environmental or stochastic) to cause cancer [1].Could human papillomavirus (HPV) be one of those factors?The objective of this study is to determine the HPV prevalence in Czech patients and its potential clinical significance.Material and methods:A cohort of 80 patients was enrolled to this study.The DNA from FFPE and frozen tissue in RNA later was isolated using Cobas kit (Roche, Germany).The method, which was used for the detection of presence/absence of HPV, is based on a quantitative multiplex real-time PCR using specific TaqMan® probes.The detection limit was set to 4 copies of the virus/1 reaction.As an internal control, a gene glyceraldehyde 3-Phosphate Dehydrogenase was used.Results:All 80 samples of both available biological materials were negative similarly to [2], [4], [5]. In contrast,a majority of performed studies detected HPV positivity in lung cancer [3].These data vary in several factors-human race (the highest HPV positivity detected in Asia), gender (HPV more frequently detected in women) or histotype.Our findings did not confirm any ethiologic correlation between HPV16, 18, 31, and 56 and NSCLC in Czech population.

Acknowledgment:
This work was supported by grants LF_2016_010, Ministry of School, Education and Youth (LO1304 and LM2015064) and Technological Agency of the Czech Republic (TE02000058).

Citation:
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Mouse model of Bordetella pertussis infection for identification of the disease biomarkers

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Bordetella pertussis and Bordetella parapertussis are bacteria that cause pertussis, also known as whooping cough. In adults, because of the atypical course of the disease without characteristic coughing spells, is often not correctly recognised and therefore not treated. The disease can be deadly for newborns and infants, because they do not have any immunity to pertussis until they are vaccinated. They are therefore particularly at risk of suffering serious complication. Therefore, the early and reliable diagnosis of whooping cough is extremely important in order to promptly begin antibiotic treatment.
Therefore, we proposed to perform preclinical study using B. pertussis infected mice for identification of potential host and/or pathogen protein biomarkers. The bronchoalveolar lavage (BAL) samples were collected from infected and control mice. Mass spectrometry quantified 721 proteins from total BAL samples. We found 133-up and 294-down regulated proteins. As expected, some of these proteins play important roles in immune response and immune system process. We identified one specific chaperonin protein of B. pertussis, which could be potential pathogen biomarker.

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Citation:
Finger H, von Koenig CHW. Bordetella. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 31.Available from: https://www.ncbi.nlm.nih.gov/books/NBK7813/

Identification and structure elucidation of novel sulphur-containing imatinib metabolites

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Untargeted metabolite profiling using high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS), followed by data analysis with the Compound Discoverer 2.0TM software, was used to study the metabolism of imatinib in humans with chronic myeloid leukemia. Plasma samples from control (drug-free) and patient (treated with imatinib) groups were analyzed and the unknown ions occurring only in the patient group were then, as potential imatinib metabolites, subjected to multi-stage fragmentation. The application of an untargeted approach enabled detection of 24 novel structurally unexpected metabolites (1). Several sulphur-containing compounds, probably originating after reaction of reactive intermediates of imatinib with endogenous glutathione, were found and annotated as cysteine and cystine adducts. In the proposed mechanism, the cysteine adducts were formed after the rearrangement of piperazine moiety to imidazoline. In vivo S-N exchange occurred in the case of the cystine adducts. N-O exchange was observed in the collision cell in the course of the fragmentation of the cystine adducts. The presence of sulphur in cysteine and cystine conjugates was proved by means of ultra-high resolution measurements using Orbitrap Elite. The detection of metabolites derived from glutathione might improve knowledge about the disposition of imatinib towards bioactivation and help to improve understanding of the mechanism of its hepatotoxicity or nephrotoxicity in humans.

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Citation:
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Identification of proteomic molecular targets of potential anticancer drugs by affinity purification

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Affinity purification is one of the methods which can be used in drug discovery to identify a molecular target of a compound of interest. In this work affinity purification was performed using streptavidin coated magnetic beads, which bind biotinylated analogues of tested compounds, and SILAC labeled cell lysates. Potential proteomic molecular targets were

identified by UHPLC/MS-ESI/LTQ Orbitrap.
A new pyrazine compound was synthetized and tested for its molecular target. The experiments were performed with CCRF-CEM cell line and we have identified some candidate proteins that are considered to be a molecular target of our compound. The identified proteins include oncogenes, parts of mitochondrial electron transport chain, fatty acid metabolism and some transport proteins. These proteins could have important biological significance and will be further validated to confirm our findings.

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Citation:
Ong SE, Li X, Schenone M, Schreiber SL, Carr SA (2012) Identifying cellular targets of small-molecule probes and drugs with biochemical enrichment and SILAC. Methods Mol Biol. 803: 129-40.

The comparison of mass spectrometry approaches in proteomic profiling of drug responses.

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There are two main mass spectrometry approaches in proteome analysis: electrospray (ESI) and matrix assisted laser desorption/ionization (MALDI) based. For purposes of their comparison, the SILAC labeled CCRF-CEM cell line was treated by three platinum drugs and whole cell lysate was analyzed. Samples were measured in parallel by three mass spectrometers – ion trap (ESI-IT), time-of-flight (MALDI-TOF) and orbital ion trap (nESI-Orbitrap). Each spectrometer was coupled with high performance liquid chromatography and independently optimized for best performance. Data were analyzed independently and average count of identified proteins was 660 ± 124 for ESI-IT with 66% coverage in all three replicates, MALDI-TOF with 355 ± 68 proteins and 41% coverage, nESI-Orbitrap with 3430 ± 306 proteins and 76% coverage. Quantification accuracy of ESI-IT determined as R2 was 0.454 ± 0.047, MALDI-TOF 0.524 ± 0.134 and nESI-Orbitrap 0.692 ± 0.063. Proteins identified by ESI-IT and MALDI-TOF in at least one replicate was nearly the same and all of them were identified in nESI-Orbitrap as well.

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Difficult start of measuring molecular weight of compounds in Chemical library

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Since the beginning of this year 400 of compounds was added. Actually there is 5.600 compounds. Actually the biggest challenge is a measurement of molecular weight of all compounds in IMTM chemical library. The purpose is to find out compounds already decomposed, to check the purity of compounds. There is a danger of confusion of some compounds too. In cooperation with chemist Tereza Volná we have already measured three 96-well plates for testing. Unfortunately several issues appeared. The first is a different solubility of compounds. I tried to split the compounds into 5 groups from strongly polar to non-polar according to their chemical structure. But it took too much of time. Thus Pavlo Polischuk split the compounds according to log P value. Different solubility of compounds should be resolved by different ratio of methanol and water for each group. There is also a problem with drying out of methanol before a measurement of Mw was completed. We also have to solve a risk of mass spectrometer clogging. Last but not least in cooperation with Pawel Znojek I try to find any solution to avoid manual processing and evaluation of measured results.

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Peptide identification capabilities at IMTM

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During the last five years, considerable efforts were put to build a reliable system for identification of mutant peptides using mass spectrometry of proteome. While conceptually clear, identified peptides in preliminary studies almost did not correspond to alterations observed on RNA level. The first realization—absence of one-to-one correspondence between peptides and mass spectrometric measurements—was considered as a candidate for explanation of large proportion of (likely) false positives.. Subsequent series of intentionally designed validations further enabled understanding of deeper aspects of identification, associated problems and guided design of solution. Nowadays, the latest version of claire, went through dramatic change from viewpoint of computational

complexity. Specifically, the calculation of spectral match was reformulated to scale logarithmically with size of the database. This, in effect, enables extremely fast testing of large number of hypotheses and opens widely the door for deep interpretation of mass spectrometric measurements. Recently, the system was used to test around trillion hypotheses, evaluated in around 11 hours. The increase in computational capability could also possibly enable precise modelling of tail of distribution of random matches to spectrum—an essential step in deriving the probability of correct interpretation of measurement.

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Circulating glycoprotein biomarkers of pancreatic cancer

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Biomarkers are physiological indicators of diseased tissue and have value in diagnosis, prognosis and in the evaluation of a treatment regimen. The larger goal of this project is to develop serum-based N-glycoprotein markers that can warn of gastrointestinal cancers early enough for a treatment to be effected successfully. N-Glycosylation is frequently indicative of perturbations in cellular physiology and this makes the glycosylated proteins clinically valuable. Many of these proteins are secretory or cell-surface proteins and are more likely to be secreted or shed into serum than proteins in the cytoplasm. A comprehensive screen of sera from mice grafted with various GI cancer cell lines has yielded several N-glycoprotein markers, some of which are novel and some of which have been previously reported (1). Two of these N-glycoproteins have been reported in a recent diagnostic signature, suggesting that our workflow is sound (1,2). A few hundred sequence alterations were also identified in the data from the discovery screen using a software developed in house. The wildtype candidate markers are being assayed in murine xenograft sera and in a pilot cohort of pancreatic cancer patient sera, using a targeted proteomic method. Preliminary analyses have been completed and the analysis of samples from a larger patient cohort will shortly be underway.

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Citation:
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Structural elucidation of novel biomarkers of known metabolic disorders based on multistage fragmentation mass spektra

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Specific diagnostic markers are key to effective diagnosis of inherited metabolic disorders. Current mass spectrometry provides powerful tool for structural elucidation of unknown compounds even in complex biological matrices. This approach was used to determine molecular structure of uncharacterized compounds observed in plasma samples from phenylketonuria and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency patients. We used liquid chromatography coupled to a high-resolution mass spectrometer Orbitrap Elite. Multi-stage fragmentation mass spectra were acquired via two different fragmentation techniques, Collisional-induced Dissociation and High-energy Collisional Dissociation. In order to determine the molecular structure, we searched Metlin, ChemSpider, mzCloud and HMDB databases together with fragmentation predictive software MassFrontier. Exact masses of molecular ions as well as their fragments were determined with resolution 120 000 FWHM within mass accuracy Δppm < 1.0. The fragmentation experiments led to structural elucidation of 3-methylglutaconylcarnitine in 3-hydroxy-3-methylglutaryl-CoA lyase deficient patients. In samples from phenylketonuria patients two novel compounds were characterized: Phe-Glu-Glu conjugate and Phe-hexose conjugate. Our results proved that structural elucidation of unknown metabolites in human biofluids can be done by current exact mass multi-stage fragmentation techniques even at relatively low concentrations.

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Citation:
Sana TR, Roark JC, Li X, et al (2008) J Biomol Tech 19:258-266.
Wishart DS, Knox C, Guo AC, et al (2009) Nucleic Acids Res 37:603-610.

New cholin analogs as potentional diagnostics/therapeutics for prostate cancer

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As tumor cells are dividing rapidly their uptake of cholin is significantly increased. We are testing various cholin analogs as new potentional tumor tracers based on this knowledge. Our goal is to reveal suitable candidates according their in vitro IC50s for in vivo testing and than verify the suitable properties of these candidates under in vivo conditions. We have performed in vitro experiments to establish IC50 values for alll studied choline derivatives using PC-3 prostate cancer cell line. In this assay the compounds competed in uptake with tritium labelled choline. The best cadidates with IC50 comparable to choline where transfered into in vivo biodistribution study and to single photon emission computed tomography (SPECT) imaging of prostate cancer mouse model too. Fifty different choline analogs were tested for their IC50 values. Ten of them revealed suitable IC50s. This candidates were tested in vivo for their biodistribution in tumor model and finally ratio of accumulation tumor to blood was calculated. In case of compound PS152 this ratio was 2,09 and this compound was also used as SPECT tracer in tumor model. We can conclued that this ten compounds are promising candidates for further testing as prostate cancer tracers/therapeutics.

Identification of potential inhibitors of tau aggregation for alzheimer's disease therapy

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Accumulation of amyloid beta (Aβ) and tau aggregates are two characteristic pathological hallmarks of Alzheimer's disease (AD). In AD, dysfunctions in signaling pathways that regulate tau phosphorylation result in the hyperphosphorylation of tau and its subsequent aggregation into neurofibrillary tangles that contribute to neuronal loss in AD. Therefore, inhibition of abnormal aggregation of tau is a potent therapeutic strategy to prevent the extensive loss of neurons, and potentially to limit the progression of AD into advanced stages. With this goal in mind, we established a novel in vitro tau peptide repeats

aggregation assay for the screening and identification of potential anti-aggregation inhibitors.

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NARENDRAN ANNADURAI, ALFREDO CAGNOTTO, ANTONIO BASTONE, MARIO SALMONA, PETR DŽUBÁK, MARIÁN HAJDÚCH, VISWANATH DAS*. IDENTIFICATION OF POTENTIAL INHIBITORS OF TAU AGGREGATION FOR ALZHEIMER'S DISEASE THERAPY (abstract). In: IMTM REACTOR CONFERENCE; 2017 Jun 14-16; Czech Republic

Analyses of potential inhibitors of P-glycoprotein

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The mechanism of multidrug resistance (MDR) in the cancer therapy has been intensively investigated for many years and in connection with MDR several proteins with altered expression were identified. One of the first identified proteins was P-glycoprotein (Pgp). The level of this protein is increased in cells with the MDR phenotype allowing the decreasing of the intracellular concentration of drugs. In our study we analysed 30 compounds and we identified 10 potential inhibitors of Pgp which can be helpful for overcoming MDR in cancer treatment.

Identification of three distinct molecular subtypes in meningioma samples using microarrays for copy-number variants

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We have identified three distinct meningioma molecular patterns – normal-like profile, deletion profile and complex profile. Chromosome 22 deletion, del(1p) and dup(3q) are the most common CNV (55%, 47%, resp. 31% of cases) in meningioma. Potential CNV changes in recurrent meningioma were identified. However, it will require further validation using FISH.

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Preclinical absorption, distribution, metabolism, excretion, and pharmacokinetics of a novel potent nucleoside cytostatic PNH 173

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Pharmacokinetics is the way the body acts on the drug once it is administered and includes absorption, distribution, metabolism, and excretion (ADME); and relationship between time and plasma drug concentration. Compound PNH 173 was studied for pharmacokinetics parameters. This compound is structurally similar to a AB61 that is potent nucleoside cytostatic. [1] First, it was determined intraperitoneal pharmacokinetics test of PNH 173 in murine serum and then in vitro and in vivo models generate many 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 liquid chromatography system Dionex UltiMate 3000 or the RapidFire 300 (Agilent). Investigated compound PNH 173 was tested by pharmacokinetics assay with two differently doses (maximum tolerated dose (MTD) and ½ MTD). The highest concentrations of MTD and ½ MTD were observed in 1h, where were observed cmax = 18 and 10 μM, t1/2elim = 3.2 and 4.7 h, respectively. PNH 173 was studied for ADME basic methods and for example it has been found that PNH 173 was stable in PBS, plasma and hepatic microsomes; 67% of PNH 173 was bound to plasma protein. The pharmacokinetics of PNH 173 is known, the subject of further study will be determined cytotoxic activities and mechanisms in cancerous and non-cancerous cell lines.

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Citation:
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Mining molecular patterns important for toxicity

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Interpretation of QSAR models can assist in practical aspects of drug development. One interpretation approach calculates the contribution of any fragment to the property modelled [1, 2] . Such analysis reveals SAR trends captured by the model but it does not take into account the molecular environment of fragments. We aimed to enhance this method by means of identifying the structural context for each fragment. To enable "tracking" of molecular surroundings we used explicit context encoding. The contexts of radii 1 to 3 bonds were subsequently added to fragments. Thus fragments connected to different atoms (moieties) became distinguishable. We compared this technique to Gaussian mixture modelling (GMM) proposed by us previously for context analysis.
The approach was applied to analyse the toxicity of 1984 compounds to T. pyriformis. The results revealed some fragments exhibiting substantially different contributions when connected to aromatic systems compared to aliphatic moieties, while others become distinguishable at a deeper level of context details. Results revealed the superiority of the explicit encoding of local environments compared to unsupervised detection of context by means of Gaussian mixture model. Known toxicophoric patterns were captured, e.g. electrophiles, uncouplers of oxidative phosphorylation, thus showing that the method is able to discover reasonable patterns important for biological activities and understanding mechanisms of action.
Acknowledgment:
A new feature was proposed to improve the earlier developed QSAR model interpretation approach. We proposed to encode the local environment (of radius up to 3 bonds) for each fragment to capture context dependence of fragment contributions. This was applied for analysis of the toxicity compounds to T. pyriformis.

Citation:
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In vitro inhibition of human hepatic cytochrome P450 enzymes by copper (II) and gold (I) complexes

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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. The copper (II) mixed-ligand complexes of the type [Cu(qui)(L)]Y · H2O, where Hqui=2-phenyl-3-hydroxy-4(1H)-quinolinone, Y=NO3 (complex 1) or BF4 (complex 2) and L=5-methyl-1,10-phenanthroline, and the gold (I) mixed-ligand complexes of the type [Au(L)(PPh3)], involving triphenylphosphine, where L=6-isopropyloxy-9-deazapurin and 6-benzyloxy-9-deazapurin (complex 3 and 4) were studied. Herein, we examined the interactions of these complexes with cytochromes P450. The study is based on the biotransformation of marker substrates of nine forms of CYP450, which were quantified by HPLC. All the complexes showed strong inhibition of CYP3A4 and CYP2C9 (to 5–13 % of control activity at 10 μM and to 5–14 % of control at 100 μM complex concentration, respectively). Only compounds with copper as the central metal inhibited activity of CYP2C19 (to 24–36 % of control activity). CYP2A6 form was inhibited to approximately 60 % of control activity by all complexes and activities of other forms were almost unchanged. Experimental data obtained with the complexes were analysed by Dixon plots to evaluate the possible mechanisms of enzyme inhibition and to determine inhibition constants Ki. The results document that the complexes should be more thoroughly tested for possible interactions.

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Buchtík R, et al.(2011) Synthesis, characterization, DNA interaction and cleavage, and in vitro cytotoxicity of copper(II) mixed-ligand complexes with 2-phenyl-3-hydroxy-4(1H)-quinolinone. Dalton Trans., 40, 9404
Vančo J, et al.(2014) Gold(I) Complexes of 9-Deazahypoxanthine as Selective Antitumor and Anti-Inflammatory Agents. PLoS ONE 9(10)e109901

The Covalently bonded compounds and their proteins target detection

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The targeted covalent inhibitors specifically covalently bind to a molecular target and suppress its biological functions. A „protein silencing“ is achieved by the creation of protein-drug adduct, usually over a cysteinyl -SH group. The benefits of this covalent drug approach are numerous, including high potency, using a low dose, extended duration of action and applicability for all classes of proteins. The proteins targets can be identified mainly due to methods based on proteomics mass spectrometry (fragmentation of small molecules) and variable LC techniques. Other techniques can use a click chemistry and different tagging of small molecules and then their detection by LC-MS approach or visualisation by a confocal microscopy.
Materials/Methods: Cancer cell lines U2OS and HCT116 were treated by experimentally prepared compounds with a fluorescent tag. For visualization were used the confocal microscopy, SDS-PAGE.
Results and conclusions
We detected the protein-drug complexes localised mainly in cytoplasm with possible ER and mitochondrial targets. We have also observed differences between used fluorescent tags with different size and the protein-drug complex creations. We confirmed this set of compounds as covalent inhibitors and based on these pre-screening data further target investigation will be continued.

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