ABSTRACT BOOK 2019

2019 IMTM Reactor is a conference held in 16. – 18. Semptember 2019 at Bystřice pod Pernštejnem. 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

Monday 16/9/2019

9:00 **Departure from IMTM**

12:00 13:30 **Lunch**

Section from 13:30 to 16:10 chaired by Milan Urban

13:30 13:45 *Tomáš Ožd*ian **Opening of conference**

13:45 14:00 *Jiří Voller* **Modulation of pre-mRNA splicing by cytokinins**

14:00 14:15 *Lucie Borková* **Triterpenoid Thiazoles: Their Synthesis, Cytotoxicity and Pharmaceutical Properties**

14:15 14:30 *Jiří Hodoň* **Cytotoxic Triterpenes and Study of their Mechanism of Action**

14:30 14:45 *Barbora Lišková* **Cytotoxic activity and ADME properties of novel modified ribonucleoside derivatives**

14:45 15:00 *Martina Medvedíková* **Interaction of copper(II) and gold(I) complexes with human liver microsomal cytochrome P450**

15:00 15:15 *Miloš Petřík* **68Ga labelled siderophores for specific infection imaging**

15:15 15:30 *Zbyněk Nový* **99mTc-labeled hydroxyapatite nanoparticles as potential tracers for solid tumors**

Cofee 15:30 16:00

Section from 16:00 to 19:00 chaired by Vlastimil Mašek

16:00 16:15 *Miroslava Popper* **Animal facility**

16:15 16:30 *Ivo Frydrych* **Possible contribution of RAGE in pyrimidine derivatives and fenretinide induced signalling response**

16:30 16:45 *Ermin Schadich* **Antibiotic and antiparasitic activity of novel compounds**

16:45 17:00 *Jiří Řehulka* **Applications of betulinic acid conjugates in drug target research**

17:00 17:15 *Vishwanath Das* **The antagonistic functional duality of p21 WAF1/CIP1 in cells derived from multicellular tumor spheroids**

17:15 17:30 *Narendran Annadurai* **Mechanisms of intracellular tau aggregation and seeding**

17:30 17:45 *Juan de Sanctis* **CuEt enhances NK and T cell cytotoxic response against tumor cells**

17:45 18:00 *Tereza Buchtová* **Cannabinoid signaling and its interference with CuET uptake**

18:00 18:15 *Hanuš Slavík* **Transcriptomic profiling in meningiomas for biomarkers and molecular features discovery**

18:15 18:30 *Alona Řehulková* **Validation of CTC detection method using CytoTrack instrument**

Grill 19:00

Tuesday 17/9/2019

Breakfast 8:00 9:00

Section from 9:00 to 10:00 chaired by Tomáš Oždian

9:00 9:15 *Jiří Dostál* **Biomarkers of endometrial receptivity. A prospective multicenter study on proteomic biomarkers of endometrial receptivity in cervical mucus**

9:15 9:30 *Jana Václavková* **Proteomic Analysis of Exhaled Breath Condensates**

9:30 9:45 *Dušan Holub* **Mouse model of Bordetella pertussis infection – proteomic profile**

9:45 10:00 *Jarmila Stanková* **Human elongation factor eEF1A1 and its interactive partners**

Cofee 10:00 10:30

Section from 10:30 to 12:15 chaired by Petr Džubák

10:30 10:45 *Mariia Matveieva* **In silico models for luciferase inhibitors**

10:45 11:00 *Jana Kotulová* **Adenosine Receptors: from Model Validation to Active Compounds Identification**

11:00 11:15 *Soňa Gurská* **Biochemical and cell-based assays in HTS facility**

11:15 11:30 *Lenka Řeháčková* **Enzymatic reactions and their application in screening of MARK4 inhibitors**

11:30 11:45 *Natálie Kudlová* **Non-invasive approach of hair follicles sampling, their use and applications in the aging experiments**

11:45 12:00 *Vaishali Uniyal* **Metal chelators as novel atnicancer molecules**

12:00 12:15 *Martin Ondra* **CRISPR/Cas9-mediated tagging of endogenous proteins with HiBiT peptide**

Lunch 12:15 13:30

Dinner 19:00

Concert of Masožravá Berta 20:00

Wednesday 18/9/2019

Breakfast 8:00 9:00

Section from 9:00 to 10:00 chaired by Pavlo Polishchuk

9:00 9:15 *Zuzana Macečková* **Bysl drives Diamond Blackfan pathology through c-myc regulation**

9:15 9:30 *Agáta Kubíčková* **SNV in RPS7 causes Diamond Blackfan anemia**

9:30 9:45 *Olena Mokshyna* **MD Study of Wild-Type and in silico Mutated Bystin Complexes**

9:45 10:00 *Dušan Holub* **Application of proteomics for amyloid subtypization**

Cofee 10:00 10:30

Section from 10:30 to 12:15 chaired by Vladimíra Koudeláková

10:30 10:45 *Jiří Drábek* **Epigenetic age estimation of the healthy Czech population by AgePlex**

10:45 11:00 *Hana Jaworek* **HPVPRO STUDY: COMPARISON OF HPV DETECTION IN CERVICAL AND CERVICOVAGINAL SWABS**

11:00 11:15 *Katarína Chromá* **E3 ligase RNF168 as a new potential diagnostic and prognostic biomarker for Multiple Myeloma**

11:15 11:30 *Rastislav Slavkovský* **Determination of lung tumor mutation burden to predict the effects of immunotherapy with checkpoint inhibitors (pilot study).**

11:30 11:45 *Rastislav Slavkovský* **Determination of lung tumor mutation burden to predict the effects of immunotherapy with checkpoint inhibitors (pilot study). Study of clonal variants in hematopoietic system in relation to age and stroke.**

11:45 12:00 *Agáta Kubíčková* **Modulation of transcription by CRISPR/dCas9 technology**

12:00 12:15 *Lucie Kotková* **BMI prediction through detection of DNA methylation**

Lunch 12:15 13:30

14:00 **Departure**

Modulation of pre-mRNA splicing by plant hormones

Jiří Voller[1], Barbara Maková[2], Václav Mik[2], Barbora Lišková[1], Lenka Zahájská[3], Martina Medvedíková[1], Marek Zatloukal[2], Marián Hajdúch[1], Miroslav Strnad[2]

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Familial dysautonomia is debilitating congenital neurodegeneration with no causative therapy. In >99 % of the cases, the disease is caused by a homozygous mutation in the donor splice site of intron 20 of ELP1 gene. The decreased engagement of the splicing machinery leads to the production of the transcript without the exon 20 and subsequently to the expression of the truncated and dysfuntional protein. The compounds studied as potential treatments include clinical candidate N6-furfuryl adenine, a plant hormone from the cytokinin family. In the presentation, we summarize our exploration of structure-activity relationships in a library phytohormones. A panel of in vitro pharmacokinetics assays was used for an initial estimation of the potential bioavailability of selected compounds.

Acknowledgment:

This study was partially supported by the Ministry of Education, Youth and Sports of the Czech Republic (GACR grant No. 17-14007S, NPU I LO1304, and OP VVV project ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868).

Triterpenoid Thiazoles: Their Synthesis, Cytotoxicity and Pharmaceutical Properties

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

[1] *Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc, CZ*
[2] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, CZ*

Pentacyclic triterpenes are secondary metabolites from plants and other living organisms with many interesting biological activities, such as antiviral, antibacterial, anti-inflammatory. Many researchers try to prepare their semi-synthetic derivatives with higher activity, better selectivity and pharmacological properties. Modification of the A-ring of natural triterpenes is currently a hot topic in the development of new antitumor triterpenoids.[1] More specifically, hundreds of the most active derivatives contain a heterocycle.[2] Recently, we

have developed and optimized synthetic approach for the synthesis of a large library of heterocyclic triterpenoids and evaluated their biological properties.

Starting from ketones of seven different triterpenic families, an efficient and straightforward synthesis of both substituted and unsubstituted aminothiazoles fused to the A-ring was developed. As a result, 70 new compounds were prepared.[3,4] A number of them had IC50 < 5 μM with high selectivity against cancer cells. Influence of the best compounds on the cell cycle and on the DNA/RNA synthesis was studied. Their pharmacological parameters were measured, such as chemical, plasma, and metabolic stability, membrane permeability, and binding to plasma proteins. Synthesis, cytotoxicity, structure-activity relationships, and pharmacological parameters of this set of compounds will be discussed.

Acknowledgment:

This work was supported by the Czech Science Foundation (15-05620S) and Palacký University Olomouc (IGA_PrF_2017_009, IGA_PrF_2018_029, and IGA_LF_2018_032). The infrastructural part was supported by the National Sustainability Programme (LO1304).

Citation:

1 Borkova, L.; Hodon, J.; Urban, M. Asian J. Org. Chem. 2018, 7, 1542.

2 Kvasnica, M.; Urban, M.; Dickinson N. J. et al. Nat. Prod. Rep. 2015, 32, 1303.

3 Borkova, L.; Adamek, R.; Kalina, P. et al. ChemMedChem, 2017, 12, 390.

4 Borkova, L.; Frydrych, I.; Jakubcova, N. et al. Eur. J. Med. Chem. Submitted.

Cytotoxic Triterpenes and Study of their Mechanism of Action

Jiří Hodoň[1], Milan Urban[1,2]

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Triterpenes are natural compounds that may be found in almost all living organisms, most often in plants, fungi and marine animals. Thousands of triterpenes have been isolated from natural sources and many of them are biologically active. Even though many researchers are studying terpenes quite extensively due to its antitumor and antiviral activities, their mechanisms of action often remain elusive.

The aim of this study is investigation of mechanism of action of highly cytotoxic triterpenoid pyrazines and pyridines.

Acknowledgment:

IGA_LF_2019_019

Citation:

1. Hill, R. A.; Connolly, J. D. Nat. Prod. Rep. 2015, 32, 273.
2. Dzubak, P.; Hajduch, M.;Vydra, D.; Hustova, A.; Kvasnica, M.; Biedermann, D.; Markova, L.; Urban, M.; Sarek, J. Nat. Prod.

Cytotoxic activity and ADME properties of novel modified ribonucleoside derivatives

Barbora Lišková[1], Anna Tokarenko[2], Soňa Gurská[1], Sabina Smoleń[2], Natálie Kudlová[1], Michal Tichý[1], Pavla Perlíková[2], Ivo Frydrych[1], Pawel Znojek[1], Petr Džubák[1], Marián Hajdúch[1], Michal Hocek[2]

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Three series of isomeric pyrrolo- and furo-fused 7-deazapurine ribonucleosides were synthesized and tested for cytostatic activity. The series of new ribonucleosides were divided to active or inactive cytostatics, where the most active were the methyl, methoxy, and methylsulfanyl derivatives exerting submicromolar cytostatic effects and good selectivity toward cancer cells.

The mechanism of action of the most active modified purine nucleosides and their analogs showed that the nucleoside is selectively phosphorylated only in proliferating cancer cells but not in nonmalignant cells, and the modified ribonucleotide is then incorporated into RNA (where it inhibits proteosynthesis) and to DNA (where it causes double-strand breaks). We have shown that some new synthesized nucleosides have the above properties and that the nucleosides are chemical, plasma or microsomal stable [1].

It was concluded that these compounds remain a promising prototype of compounds for the generation of novel class of potent cytostatics.

Acknowledgment:

Supported by IGA UPOL_LF_2019_011 and IGA_LF_2019_018

Citation:

[1] Tokarenko A, Lišková B, Smoleń S, Táborská N, Tichý M, Gurská S, Perlíková P, Frydrych I, Tloušťová E, Znojek P, Mertlíková-Kaiserová H, Poštová Slavětínská L, Pohl R, Klepetářová B, Khalid NU, Wenren Y, Laposa RR, Džubák P, Hajdúch M, Hocek M. Synthesis and cytotoxic and antiviral profiling of pyrrolo- and furo-fused 7-deazapurine ribonucleosides. J Med Chem. 2018 Oct 25;61(20):9347-9359.

Interaction of copper(II) and gold(I) complexes with human liver microsomal cytochrome P450

Martina Medvedikova[1,2], Vlastimil Masek[1], Zuzana Racova[2], Jan Vanco[3], Zdenek Travnicek[3], Pavel Anzenbacher[2]

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Cytochromes P450 are the most important enzymes of the first phase of their metabolism. The copper (II) mixed–ligand complexes of the type [Cu(qui)(mphen)]Y·H2O, where Y=NO3 (complex 1) or BF4 (complex 2) and the gold (I) mixed–ligand complexes of the type [Au(L)(PPh3)], where L=6-isopropyloxy or 6–benzyloxy–9–deazapurin (complex 3 and 4) were tested with human liver microsomal cytochrome P450. An interaction of both compounds with CYP was detected using difference spectroscopy. The compounds were shown to bind to CYP enzymes with spectral binding constants 7,59±1,8 µM and 8,56±1,0 µM (complex 1 and 2, respectively) and 4,49±0,7 µM (complex 3) and 4,81±0,6 µM (complex 4). The study of their potencial to inhibit the activities of CYP is based on the biotransformation of marker substrates, which were quantified by HPLC. The results have shown no prominent inhibition of individual CYP activities with either compounds except in the case 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). 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. These results were supported by analysis of studied complexes with CYP1A2 and CYP3A4 using isothermal titration calorimetry.

Acknowledgment:

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Citation:

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

68Ga labelled siderophores for specific infection imaging

Milos Petrik[1], Eva Umlaufova[1], Vladislav Raclavsky[2], Andrea Palyzova[3], Vladimir Havlicek[3], Hubertus Haas[4], Zbynek Novy[1], Clemens Decristoforo[5], Marian Hajduch[1]

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Despite the success of antimycotics and antibiotics, microbial infections in hospitals are on the rise, particularly with the emergence of resistant pathogenic fungi and bacteria (1). Current diagnostics based on positive cultures, serology and morphologic imaging (CT, MR) have major limitations in specificity, sensitivity and/or time to diagnosis. Molecular imaging, in particular positron emission tomography (PET) has the potential for specific and sensitive detection of infections. The siderophore-based iron acquisition system, upregulated during infection (2), represents one of few fundamental differences between microbial and mammalian cells. Siderophores are low-molecular mass, iron-specific chelators secreted by bacteria or fungi (3). Almost all microorganisms possess dedicated transporters for uptake of siderophores produced by the pathogen itself or other microorganisms (so-called xenosiderophores). We have recently demonstrated that several siderophores can be radiolabelled with 68Ga, a short lived radionuclide, replacing iron without loss of bioactivity and allowing molecular imaging of microbial infections by PET (4). Here we report on the specific imaging and monitoring of Aspergillus fumigatus and Pseudomonas aeruginosa infection in vivo with 68Ga-siderophores using PET/CT.

Acknowledgment:

We gratefully acknowledge the financial support of Technology Agency of the Czech Republic (Project No. TE01020028) and the Czech Science Foundation (Project No. 19-10907S).

1. Mascellino MT. (2014) Bacterial and mycotic infections in immunocompromised hosts: clinical and microbiological aspects. OMICS Group eBooks.

2. Schrettl M et al. (2004) Siderophore biosynthesis but not reductive iron assimilation is essential for Aspergillus fumigates virulence. J Exp Med 200:1213-1219.

3. Drechsel H et al. (1998) Peptide siderophores. J Pept Sci 4:147-181.

4. Petrik M et al. (2010) 68Ga-siderophores for PET imaging of invasive pulmonary aspergillosis: proof of principle. J Nucl Med 51:639-645.

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

Zbynek Novy[1], Milos Petrik[1], Marian Hajduch[1], Jan Kozempel[2], Martin Hruby[3]

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The aim of this work was to label newly prepared hydroxyapatite nanoparticles with 99mTc using clinical radiotracer 99mTc-HDP, further verification of radiochemical stability of labelled nanoparticles in vitro and to describe their biodistribution employing SPECT/CT and ex vivo biodistribution approach. Radiolabeling of nanoparticles was done under mild conditions, followed by quality control step represented by radiochemical purity check by iTLC to reveal labeling efficiency and in vitro stability of the tracer. Ex vivo studies were performed on normal and tumor mice. The biodistribution of labeled nanoparticles was also monitored by μSPECT/CT system for two different application approaches. We have studied influence of antiangiogenic therapy with bevacizumab to HAP-NP biodistribution in tumor-bearing mice too. The nanoparticles were labeled with high radiochemical purity and stability. Ex vivo biodistribution studies revealed dominant accumulation in the liver and spleen. The favorable tumor/blood ratio was determinated from tumor study. In vivo imaging showed mainly the same organs as ex vivo study. Ex vivo data from bevacizumab-treated tumor animals reported significant differences in nanoparticle accumulation. Tested nanoparticles could be labeled with 99mTc-HDP. Their in vitro stability is on satisfactory level. Biodistribution studies revealed high accumulation in liver and spleen. These findings were confirmed by SPECT/CT imaging.

Acknowledgment:

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Animal facility

Miroslav Popper

IMTM

The facility provides preclinical in vivo efficacy models for numerous diseases, including neurology, oncology, pharmacokinetic, toxicology and metabolic studies. Facility is also poised to customize preclinical pharmacology to speed up a development of molecules and substances, eventually potential drugs.

Preclinical studies at Animal facility have utilized species of laboratory animal models (including immunodeficient and GMO animal models) in SPF conditions. The employees of the Animal facility can administer test agents into rodent via many routes and perform surgical techniques or procedures with special imaging and analysis capabilities for specialized and/or custom programmes and have experience, expertise and equipment for Microsurgery and Stereotaxy.

In vivo toxicology studies can encompass dosing regimens from acute (single dose) to chronic (multiple doses). Several routes of exposure (e.g. oral, intravenous, intramuscular, topical, etc.) can be accommodated and multiple species of rodents are available. The full complement of toxicology evaluations includes Biochemistry, Hematology, urinanalysis, histopathology, bioanalysis and toxicokinetics.

The preclinical oncology studies are conducted from traditional xenografts to advanced humanized mouse models of cancer.

As a part of the animal facility is the irradiation department.

The animal facility is also equipped for behavioral neuroscience tests (e. g. locomotor activity, anxiety, and habituation, cognition).

Acknowledgment:

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Possible contribution of RAGE in pyrimidine derivatives and fenretinide induced signalling response

Ivo Frydrych[1], Pavlo Polishchuk[1], Martina Medvedíková[1], Marián Hajdúch[1]

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The receptor for advanced glycation end-products (RAGE) is a single transmembrane receptor of the immunoglobulin superfamily that plays an important role in many pathological processes and thus represents potential for research and development of diagnostic and therapeutic strategies. Pyrimidine appears attractive as a template for the design of novel small-molecule RAGE inhibitors in terms of the structural simplicity and the chemical stability. Inspired by argpyrimidine having a common structure with known RAGE inhibitors, we synthesised and screened small library of 45 pyrimidine derivatives on cell-based model for monitoring NFκB activation, which represents key transcription factor of RAGE signalling. We also included synthetic retinoid derivative Fenretinide, extensively studied because of its anti-inflammatory and anti-tumor properties. We have identified

6 compounds, Fenretinide including, with strong inhibitory effect on RAGE mediated NFκB activation. To confirm possible interaction with RAGE, isothermal titration calorimetry study has been done. Additionally, we made molecular docking for predicting how the molecules interact with protein receptor and elucidating the most probable binding site. Finally, the effort has been put to find correlation between docking scores and biological activities.

Acknowledgment

This research was financially supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and by the Technology Agency of the Czech Republic (TE01020028).

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 BigLopac, 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 two strains of 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. The 22 compounds were active against intracellular bacteria in primary screen. The ongoing dose-response analyses showed 10 active compounds while the activity of others is to be tested. It is also worth to mention that the assay for analyses of antiviral compounds is also calibrated as reported in previous report.

Acknowledgment:

Studies are supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Applications of betulinic acid conjugates in drug target research

Jiri Rehulka[1], Milan Urban[1], Sona Krajcovicova[2], Miroslav Soural[2], Petr Dzubak[1], Marian Hajduch[1]

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Bi-functional molecules have numerous applications in basic research as well as in drug development. The hybrid molecule usually consists of two protein-binding moieties connected with a suitable linker. Another modality is conjugation with fluorescent label or an anchor that enables immobilization on a resin or magnetic beads. Such conjugates serve as probes for small-molecule-protein interaction studies using microscopic techniques or mass spectrometry. Currently, a new emerging class of bi-functional drugs PROTAC is being evaluated in clinical trials and preclinical studies. The proteolysis targeting chimeras are hybrid molecules that enable specific knockdown of a targeted proteins by a proteasomal degradation. The hybrid molecule consists of two active moieties, one binds a protein target and the another is recognized by E3 ubiquitin ligase. In addition, PROTAC technology can be used for identification of drug targets. The talk will be focused on development and experimental use of hybrid molecules related to betulinic acid and results will be discussed to sum up the research activities on drug targets of bioactive triterpenes.

Acknowledgment:

This work was supported by Technology Agency of the Czech Republic TE01020028, Ministry of Health of the Czech Republic (15-31984A), internal grant of Palacky University (IGA_LF_2019_018) and by the Czech Ministry of Education, Youth and Sports (LO1304).

Citation:

Preparation of Conjugates of Cytotoxic Lupane Triterpenes with Biotin. Bioconjugate Chemistry 2015 26 (12), 2563-2570

A Synthetic Approach for the Rapid Preparation of BODIPY Conjugates and their use in Imaging of Cellular Drug Uptake and Distribution. Chem. Eur. J. 2018;24:4957-4966.

Design and applications of bifunctional small molecules: Why

two heads are better than one. ACS Chem Biol. 2008 November 21; 3(11): 677-692.

The antagonistic functional duality of p21 WAF1/CIP1 in cells derived from multicellular tumor spheroids

Viswanath Das

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The cyclin-dependent kinase inhibitor p21 is a key mediator of p53-dependent cell cycle arrest after DNA damage, in addition to p53-independent mechanisms. Being one of the major transcriptional targets of p53 and due to its anti-cell proliferative activity, p21 was considered initially as a potent tumor suppressor. However, emerging studies now show the anti-apoptotic and pro-cell proliferative effects of p21, highlighting the oncogenic role of p21 in cancer. In particular, p21 results in genomic instability and the development of aggressive and chemo-resistant traits in a subset of highly proliferating tumor cells through p53-independent pathways. The cellular localization of p21 has been proposed to be critical either in promoting cell survival or in inhibiting cell growth. Herein, using a combination of gene knockout, cytotoxicity assay, immunofluorescence, immunoprecipitation, and mass spectrometry techniques, we study the consequence of nuclear and cytoplasmic localization of p21 on cell survival and drug response in cells derived from multicellular spheroids of multiple human cancer cell lines.

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Mechanisms of intracellular tau aggregation and seeding

Narendran Annadurai[1], Marian Hajdúch[1], Viswanath Das[1]

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Alzheimer's disease (AD) is characterized by the accumulation of neurofibrillary tangles (NFTs) and aggregated tau seeds may underlie the progression of NFTs induced tau pathology. The relationship of tau seeding activity and phospho-tau pathology remains to be understood. In AD, tau found mostly in the hyperphosphorylated and aggregated form. It's not clear yet that the hyperphosphorylation drives the tau aggregation and its seeding. Recent findings show that under in vitro conditions, tau phosphorylation at Ser202/Thr205/Ser208 induces fibril formation confirmed using Thioflavin T (ThT) assay.

We wanted to study the effect of tau peptide aggregated fibrils (R1, R2, R3 and R4) on tau hyperphosphorylation and the correlation between tau hyperphosphorylation and seeding using cell line models. By using tau P301S biosensor cell line, we found that R2 and R3 fibrillar aggregates induced the phosphorylation at Ser262 in the triton-insoluble fractions. Both R2 and R3 aggregates are able to reseed the tau P301S biosensor cell line. Further studying the effect of phosphorylation on intracellular tau aggregation under low and transient tau expression conditions will help in understanding the mechanisms of tau aggregate seeding and progression of tau pathology.

Acknowledgment:

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CuEt enhances NK and T cell cytotoxic response against tumor cells

Juan De Sanctis

IMTM

CuEt is have been currently used to treat cancer cells; however, early reports on diethyldithiocarbamate, mainly in the mouse model, suggest that it may enhance lymphocyte proliferative response and cytotoxic response (Int. J. Immunopharmac., Vol. 13, No. 8, pp. 1073-1084, 1991, and Renoix et al.). The aim of the project was to treat mononuclear lymphocytes (PMN) with CuEt and then challenge them with Jurkat cells. For NK spontaneous cytotoxic response, the PMN cells were treated with CuEt 1 hr (10 pM to 100 nM) prior to the assay, then washed and challenged with the Jurkat cells at effector target ratios of 1:1, 2:1, 5:1, 10:1 for 4 hr at 37 C. Cell death was measured by 7Add fluorescence. T cell cytotoxicity

was assessed by challenging PMN for 7 days with fixed Jurkat cells in the presence of CuEt. After the incubation, cells were again challenged with alive Jurkat cells at the same ratio as described above. CuEt treatment significantly increased in both assays the cytotoxic response against Jurkat cells, from basal spontaneous cytotoxicity lytic units 150+/-25 to 475 +/- 45 with 10 nM CuEt. In similar fashion specific T cell, cytotoxic response was 75 +/- 12 lytic units to 308+/- 65 lytic units $p<0.001$ $n=5$ for both assays. It is suggested that this enhancement of cytotoxic response could be due to the secretion of IL-2 and/or IFN gamma by the treated cells.

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Cannabinoid signaling and its interference with CuET uptake

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Cannabis sp. and its extracts have been studied for many years for their almost miraculous effect on human health. Most of the pharmaceutical effects originate from a chemical group called cannabinoids highly presented in the whole plant of cannabis sp. and were primarily studied due to their interaction with cannabinoid signaling system which is affecting multiple aspects of cellular and organismal physiology. From the pharmacological point of view, cannabidiol (CBD) belongs to the most studied as it is a major non-psychoactive constituent and displays many biological effects within different tissues. Importantly, CBD and marijuana's extracts are often used in combination with standard chemotherapeutics for reduction of treatment's side-effects.

Recently described antitumor drug and inhibitor of NPL4/p97 protein degradation system CuET was tested in combination with CBD and other cannabinoids. The rationale for combining these compounds with CuET originates in a high probability of their interaction in current and future oncological treatments. Data which will be presented are showing that cancer cells pretreated by CBD and some other selected cannabinoids become relatively resistant to CuET. This effect seems to be related to the CuET cellular uptake, a mechanism of which is still unknown. These findings may change the view of CBD and marijuana as a substance generally improving the anticancer treatments and shed new light on CuET uptake mechanism.

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Transcriptomic profiling in meningiomas for biomarkers and molecular features discovery

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Meningioma growth rates are highly variable, even within benign subgroups, causing some cases to remain stable while others grow rapidly despite radiotherapy. Biomarkers that differentiate meningiomas by aggression and enable prediction of their biological behavior would therefore be very clinically beneficial. We revealed the miRNAs as very promising independent markers of recurrence with strong prognostic value. Multivariate miR-331-3p based regression model can successfully predict recurrence status in 83% cases (n = 191). This model contains also clinical characteristics such as WHO grade, gender, tumor localization etc. Motivated by these results, we decided to investigate meningioma's transcriptomes more deeply in context of clinical features and manifestations. Thus, we also performed longRNA profiling by NGS and analyzed mRNA and lncRNA across the used samples (n = 70) of various clinical parameters. Differential expression between the certain groups of samples can reveal more detailed insight in meningioma pathogenesis and can help to improve prediction of prognosis and suitable therapy for every individual patient.

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Validation of CTC detection method using CytoTrack instrument

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Background: CytoTrack is an instrument for rare cells detection, for example circulating tumor cells in peripheral blood. The method is based on the detection of target cells fluorescent signal. CytoTrack scans 2 million cells/sec and fixes the position of each target cell on the disc. In this study, we validated the preservation and staining conditions of blood samples spiked with A549 cell line for further scaling on the instrument.

Material and methods: Selection of tubes for the preservation of blood samples was the first phase of validation. We selected different commercial tubs (Cell-Free DNA BCT® Streck, CellSave and EDTA test-tubes) for comparison. Samples from 5 donors were stored at room temperature for 0, 24, and 72 hours. The cell lysis degree, DNA and RNA degradation were determined in tubes. In-vitro detection of CTCs was performed in the second validation phase. A549 cell line was added to the whole blood samples. Staining was performed with commercial and home-made CK20, DAPI, EpCAM, CD-45 stain mix.

Results: We determined the minimum of cell lysis and DNA degradation even after 72 hours of storage in commercial tubes. However, almost complete RNA degradation was observed in commercial tubes. There is less strong RNA degradation using standard EDTA tube (RIN 6.4). The cell line with high CK expression was selected. The tubes were tested for the preservation of A549 cells with peripheral blood cells. Subsequently, the cells were detected on the instrument.

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Biomarkers of endometrial receptivity. A prospective multicenter study on proteomic biomarkers of endometrial receptivity in cervical mucus

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Endometrial receptivity (ER) is the sensitivity of the uterine cavity mucosa for implantation of the embryo. ER occurs over a relatively short period of time and cannot currently be determined otherwise than by invasive diagnostic methods. ER is currently investigated directly in the endometrium morphologically, by genomics and proteomics and in the secretions obtained from the uterine cavity by genomics, proteomics and lipidomics.

The uterine cervix is filled with cervical mucus, which reflects the hormonal changes during the menstrual cycle. For this reason, a correlation between the development of the endometrium and the molecular-biological profile of the cervical mucus can be expected.

Treatment of infertility by in vitro fertilization and embryo transfer, with or without making intracytoplasmic sperm injection (IVF/ICSI/ET) is a good model for study of ER.

The primary goal of our proposed project is to reduce the difficulty, unpleasantness and pain of sampling biomarkers of ER for the patient. The second and equally important objective, is to identify biomarkers of ER by proteomic examination of the cervical mucus.

If in the project there will be found new biomarkers of ER in the cervical mucus, their asessment could significantly reduce severity of infertility diagnostics for the woman and generaly increase the efficacy of infertility treatment.

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Proteomic Analysis of Exhaled Breath Condensates.

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Collecting exhaled breath condensates (EBC) is a cheap and non-invasive method to obtain human samples. Finding non-invasive methods for early detection of lung diseases would be highly beneficial. EBC represents a source of biomarkers which can provide valuable information about respiratory and systemic diseases. Proteomic analysis of EBC is a prospective method to detect early changes in the status of the respiratory system and possibly other organs. It could also replace or complement some invasive sampling methods in future and provide non-invasive lung diseases screening technique.

Our study is focused on children's asthma. Together, our studies will advance the development and validation of EBC as a novel tool for the proper diagnosis of asthma, choosing a proper treatment and monitoring treatment efficacy.

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

We collected and analyzed from 62 pediatric asthma patients. We have successfully analyzed the EBC of diagnosed and treated asthma and compared them with the samples of 62 healthy controls. We have found proteomic biomarkers of asthma.

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Mouse model of Bordetella pertussis infection – proteomic profile

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Bordetella pertussis is 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 fluid samples (BALF: supernatant and pellet) were collected from infected and control mice. Liquid chromatography and mass spectrometry were used to collect information about all proteins that are made in the samples. We found some significantly up- and 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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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/

Human elongation factor eEF1A1 and its interactive partners

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Human translational elongation is driven by two families of elongation factors, eukaryotic elongation

factor 1 A 1 (eEF1A1) is a member of eEF1A family with moonlighting functions. Besides ribosomal

synthesis, proliferation and cell cycle progression, eEF1A family has other several functions in cell

organization. Moreover, eEF1A1 has been reported in development and progression of various cancers,

and thus it has been proposed as a target for anticancer therapy. Different active compounds were

identified as targets of eEF1A1, among them flavonoid derivatives such as 2-phenyl-3-hydroxy-4(1H)-

quinolinones (3-HQs) exhibit significant anticancer activities. The interaction site of contraceptive drug

gamendazole was using computational modeling identified, the same site was found to be potential

target for 3-HQs.

A purified protein 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 by ITC (Isothermal

Calorimetry) and SEC-HPLC (size-exclusion HPLC). Different variants of recombinant protein were used

for analysis and their influence to analysis was discovered. To conclude our study, the interaction

between eEF1A1 and HP-23c was confirmed.

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In silico models for luciferase inhibitors

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The inhibition of luciferase in high-throughput screening (HTS) assays could lead to a false positive result. This issue has been known for a long time, and there have been significant efforts to identify luciferase inhibitors in order to enhance recognition of false positives in screening assays. However, although a large amount of publicly available luciferase counterscreen data is available, to date little effort has been devoted to building a chemoinformatics model that can identify such molecules in a given data set. One recent study provided models with high accuracy, but these models cannot be used otherwise than via web service *. In this study we developed models for luciferase inhibitors using machine learning methods and they showed high accuracy. They are freely accessible and their usage doesn't require the user to share proprietary compounds with a third party.

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Grant

Citation:

* Luciferase Advisor: High-Accuracy Model To Flag False Positive Hits in Lyciferase HTS Alssays. Gosh et al. J. Chem. Inf. Model. 2018, May 29 58(5): 933-942.

Adenosine Receptors: from Model Validation to Active Compounds Identification

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Adenosine receptors (ARs) are involved in regulation of a plethora of biological processes, including pathophysiological conditions such as cancer. Anti-cancer properties of ARs were confirmed both, in vitro and in vivo. Given the ubiquitous expression of ARs and the lack of

specificity of compounds targeting them, there is a prevailing need for highly selective and potent agonists and antagonists of ARs.

Each AR reporter cell line performance in high-throughput screening was evaluated. Thereafter, small molecule proprietary chemical library of IMTM (over 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 dose-response secondary screen. We identified several potent modulators of ARs. Most promising compounds were subjected to another series of verification. Data analysis includes assay robustness parameters and quantification of the biological response of compounds.

In summary, building a G protein-coupled receptor (GPCR) screening platform has provided us with a comprehensive tool for the characterization of small molecules as GPCR modulators. We developed a pipeline comprised of (1) series of validation, (2) primary screen for selection of preliminary actives, (3) counter-screen for unambiguous hit identification, (4) secondary screen for active confirmation, and (5) orthogonal assay for further verification.

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Biochemical and cell-based assays in HTS facility

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High-throughput screening (HTS) was developed to evaluate the biological activity of thousands of individual small molecules and to identify potential drug candidates in a short time.

I. One of the methods routinely used in our HTS laboratory is in vitro cytotoxicity screening. It is a convenient, phenotypic and predictive mean of characterizing the toxic potential of new chemical entities. Initially, the MTS assay as a cytotoxicity test was validated on 10 cell lines in 384.

In the primary screen, all compounds were tested at one concentration (50 μM) and the PI (percentage of inhibition) value was calculated. To calculate IC50 values for selected active compounds (PI > 50%), a secondary (dose-response) screen was performed. Data were analyzed by Dotmatics software. To quantify the suitability of cytotoxic assay in HTS, the Z-

factor was determined for each plate and cell line. Some results obtained in the cytotoxicity testing will be presented.

II. New fluorescence-based assay for identification of small molecule inhibitors of carbonic anhydrases was introduced into our HTS platform. It is a simple, homogeneous mix-and-read biochemical test. The assay was validated by screening the LOPAC library of 1280 compounds. Obtained results as well as identified hits will be presented and discussed.

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Enzymatic reactions and their application in screening of MARK 4 inhibitors

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MARK 4 is one of the enzymes which phosphorylates TAU protein. In its physiological state, TAU protein stabilizes microtubules, but when it is hyperphosphorylated, it leads to oposite state. Microtubules are depolymerized which causes degradation of neuronal cells and this pathalogical state is typical for example for Alzheimer's disease. In our project, we are focused on development of enzymatic assays for detecting enzyme activity. We have already developed method for MARK4 enzymes and identified promising inhibitors of its activity, which currently undergo cellular experiments.

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Non-invasive approach of hair follicles sampling and possibilities of their use

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There is the increasing evidence of the 3R sentences (Replacement, Reduction, Refinement) importance, we present data confirming the possibility of mouse hair follicles´ usage in a variety of experiments based on different approaches and purposes. We show how to collect this promising biological material using a new pistol-like device in the least invasive manner in the contrary with stressful and painful tail biopsy or ear punch. We isolated DNA from 151 genotyping samples of 5XFAD mouse strain, performed PCR to identify GMO. Other samples were processed in RNA isolation step and reverse transcription to obtain the material for qPCR. Finally, the gene expression of senescence markers (p16, p21) in naturally aged animals and artificially aged tissues was measured. Mean DNA yield from hair follicles was 16,35±6,91 ng/µl in contrary to 30,66±13,18 ng/µl from tail biopsies. Average RNA yield was 10 ng/µl. In genotyping, we obtained 100% conformity of hair follicle and tail samples. Measurement of senescence markers expression in mouse groups of different age showed a statistically significant increase in older animals in comparison with younger. We also confirmed a higher p16 protein level in old animals in IF assay. We also developed a mouse model which mimics aged tissue after the artificial induction, which was used for senolytic drugs testing in vivo. Thus, we confirm the feasible use of hairs as an input material for a range of experiments instead of soft tissues.

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Metal chelators as novel atnicancer molecules

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With a continuous increase in the resistance of cancer cells against chemotherapy, it is needed to find alternative and more effective treatments. In collaboration with the chemists we designed and prepared cyclobut-3-ene-1,2-dione-3-hydrazones bearing a benzothiazole moiety with substituted 2-hydroxyaryl- or 2-N-heteroaryl group using the molecular hybridization approach(also called as hybrid pharmacophore approach). It allows to prepare compounds that can affect multiple targets and utilizing combination of several different mechanisms of action. We are trying to evaluate their cytotoxic activity and the role of their biologically important metallocomplexes.

CRISPR/Cas9-mediated tagging of endogenous proteins with HiBiT

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HiBiT is an 11 amino acid peptide tag, developed from C-terminal region o NanoLuc Luciferase, which can be attached to any protein and detected quickly and easily by using bioluminescent assays. The detection reagent contains an inactive rest of luciferase, Large Bit (LgBiT), which rapidly binds to HiBiT to produce a highly active luciferase enzyme. HiBiT was knocked-in the C-terminus of genes of interest by CRISPR/Cas9 system.

U2OS human cell lines were used for CRISPR/Cas9 knock-in of HiBiT into genomic regions targeted by specific gRNAs. Monoclonal cell lines were prepared by limiting dilution and functionally validated using siRNA treatment to prove specificity and accuracy of HiBiT knock-in. We performed luminescence assays (lytic detection, blotting system) and Western blotting. Time-dependent decay of luminescence signal for lytic detection of tagged proteins was checked. We were able to quantify changes in protein levels after specific siRNA treatment. Luminescence signal for lytic assay was measurable even after 6 hours. These reporters could be used as in vitro model and for high-throughput screening in future to find compounds from chemical libraries which will interact with gene expression and stability of proteins expressed at physiological levels.

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Bysl drives Diamond Blackfan pathology through c-myc regulation

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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 neutrophil 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. In 50-75 % of DBA cases can be cause accounted for mutation in ribosomal protein. In one patient from this cohort we detected small deletion in 1p36.11-1p36.12 area. This area includes RPL11 gene. Mutations in this gene are known cause of DBA phenotype and deletion of this gene was previously reported . Surprisingly, it was shown, that deletion of just one allele of RPL11 is not compatible with successful embryogenesis. Because affected patient was alive, we hypothesise, that disease modulating genes played role in his survival. We went through exome sequencing data to detect any significant variant in genes involved in ribosomogenesis and/or protein synthesis. We detected non-sense mutation R343X in gene for BYSL protein. Unexpectedly, when we perform protein analysis of family members, we detected shortened version of BYSL protein only in patient and not in her mother or brother. We performed protein stability assay, which confirmed, that knock down of RPL11 gene leads to stabilisation of mutated BYSL. Therefore, we theorised that mutation in

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SNV in RPS7 causes Diamond Blackfan anemia

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Diamond Blackfan anemia is rare congenital red blood cell aplasia caused mainly by large genetic alterations like chromosomal translocations or mutations in ribosomal genes. In our study we had focused on a SNV in RPS7 which has been found in a family suffering with DBA. This case is interesting because only one member of this family has symptoms of DBA and the others have just elevated marker of this disease adenosine deaminase. According to the recent literature only 1% of DBA cases are caused by mutation in RPS7 gene. Due to lack of patient´s samples we had designed and prepared cellular models with this particular variant in RPS7 gene. We had used cellular models for studying p53 mediated ribosomal stress, alterations in processing of ribosomal RNA, nucleolar morphology, cell cycle, protein expression and synthesis. Our results suggest that SNV in RPS7 gene causes higher extraribosomal accumulation of ribosomal protein S7 and subsequent activation of ribosomal stress pathways, accumulation of rRNA precursors, slower proliferation with small changes in cell cycle progression and size of nucleoli. Interestingly protein synthesis is not altered by this SNV.

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Citation:

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MD Study of Wild-Type and in silico Mutated Bystin Complexes

Olena Mokshyna

IMTM

The known data indicates that bystin is overexpressed in human cancers and promotes cell growth, which makes bystin a promising protein target for a potential anticancer agents.

The IMTM researchers obtained experimental data on a range of bystin inhibitors. To understand underlying binding mechanisms we employed state-of-art molecular dynamics (MD) techniques using GROMACS 2016 free software; in all simulations Amber 99SB force field was used. As previously no ligand-protein crystal structures were known, first we used simulated annealing MD and geometric site detection method to detect the putative binding site. Afterwards to explore modes of binding of different inhibitors several iterations of MD simulations up to 200 ns were launched both for wild type bystin and various in silico mutated forms. Both single residue mutations and several residues mutations were simulated. In this study we focus on summarizing the obtained results and further in-depth analysis of in silico mutated forms, changes in binding mode for different ligands, as well as specifics of behavior of important residues.

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Application of proteomics for amyloid subtypization

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The systemic amyloidosis is a rare disorder characterised by the abnormal deposition of misfolded amyloid protein in various organs [1]. Over time, the accumulating amyloid damages the tissue microenvironment and causes organ failure. To date, there are 36 known fibril proteins in human that can cause amyloidosis [2]. Early diagnosis is critical for effective patient management. IHC is the preferred method for routine amyloid subtyping. However, it is an antibody-based method with numerous unspecificities [3]. Therefore, we have introduced mass spectrometry-based proteomic analysis for subtyping of amyloid deposits in FFPE and SFA samples.
So far we have obtained 400 FFPE and 63 SFA samples for subtyping of amyloid deposits. In FFPE samples, Congo red positive-stained amyloid deposits were dissected using laser microdissection; the proteins were extracted from excised materials and digested using

trypsin. In the SFA samples, the proteins were solubilized and digested directly with trypsin. All samples were subsequently separated by liquid chromatography, and individual peptides were acquired by tandem mass spectrometry. Acquired spectra were identified and quantified using a search engine – MaxQuant. The most abundant amyloid protein determined the amyloid subtype.
The mass spectrometry-based proteomic analysis enables correctly subtyping of different kinds of amyloid proteins.

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Epigenetic age estimation of the healthy Czech population by AgePlex

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

In medical and obesity research, a correlation between methylation of specific gene loci and body mass index (BMI, a measure for indication nutritional status in adults) was found. The methylation

status of these markers could predict body structure of an unknown sample donor.

We decided to verify a correlation between BMI and methylation status in three previously validated

CpGs in HIF3A gene and other previously published candidate markers using a group of

healthy Czech blood donors (n>20) with BMI under 21 kg/m2 (n>10) and over 36,5 kg/m2 (n>10).

For methylation assessment, we choose next-generation amplicon bisulfite sequencing over

the pyrosequencing method, mostly because of lower sample requirements (0.5 ng DNA for

NGS vs 10 ng DNA using PyroMark method) which are in a lot of cases the bottleneck for the usage

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HPVPRO STUDY: COMPARISON OF HPV DETECTION IN CERVICAL AND CERVICOVAGINAL SWABS

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Introduction

The implementation of primary HPV screening and increasing cervical screening attendance are major challenges. The offering of self-sampling to cervical screening non-attenders could increase women's participation as it was shown in several European countries. The objective of the HPVPro study was to find out the HPV prevalence in the screening population of Czech women since there are no data for the Czech Republic. The second objective was to compare HPV DNA detection in paired self-sampled cervicovaginal swabs and physician-obtained cervical swabs.

Methods

Cervical swabs were taken by a gynecologist from 1044 Czech women (age 30–64 years) during the regular screening examination. Cervicovaginal swabs were obtained by self-sampling using two types of self-sampling device (HPVpro 1 and HPVpro2 arms). All samples were analyzed using digene Hybrid Capture 2 (HC2, Qiagen) HPV DNA detection method, 500 paired samples from the HPVPro2 study were analysed also using Qiascreen HPV PCR Test (Qiagen). HrHPV positive and discrepant samples were genotyped using PapilloCheck HPV-Screening (Greiner Bio-One).

Results and conclusion

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

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E3 ligase RNF168 as a new potential diagnostic and prognostic biomarker for Multiple Myeloma

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The ubiquitin proteasome system (UPS) is the major component that controls steady-state protein levels regulating multiple biological processes, such as cell cycle, cellular proliferation, apoptosis, and DNA damage response involved in cancerogenesis as well as drug resistance. Cancer cells display complex genetic alterations and excess of mutant proteins have increased demands on UPS resulting into cell status referred as proteotoxic stress. The best-established model for elevated cancer related proteotoxic stress is haematological malignancy Multiple myeloma (MM) standardly treated by proteasome

inhibitors (PIs). However, the majority of MM patients eventually relapse to this class of drug and become resistant. We have recently described phenomenon of increased E3 ligase RNF168 as an adaptation to heightened proteotoxic stress in cancer cell lines including multiple myeloma. In addition to this, we have shown the upregulation of RNF168 in response to intrinsic proteotoxic stress in multiple myeloma cells isolated from clinical tumour specimen. Identification of elevated RNF168 in patient´s multiple myeloma cancer cells may provide a new plausible diagnostic or even prognostic biomarker of response to existing or emerging therapies. The presentation will bring a brief overview of current knowledge of multiple myeloma disease and highlight novel promising treatment strategy based on recently described protein degradation inhibitor- disulfiram´s metabolite termed as CuET.

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Determination of lung tumor mutation burden to predict the effects of immunotherapy with checkpoint inhibitors (pilot study).

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

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

Results and conclusions will be presented.

Determination of lung tumor mutation burden to predict the effects of immunotherapy with checkpoint inhibitors (pilot study). Study of clonal variants in hematopoietic system in relation to age and stroke.

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

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It was recently discovered that one of the hallmark of aging is the accumulation of clonal variants within the cells of hematopoietic system without presence of malignant transformation. This phenomenon is also known as clonal hematopoiesis of indeterminate potential (CHIP). Interestingly, the presence of CHIP correlates with the risk of cardiovascular system diseases. We plan to study this phenomena using advanced deep sequencing in elderly patients with or without stroke using liquid blood biopsy and atherosclerotique plaques. Details of study plans will be presented.

Modulation of transcription by CRISPR/dCas9 technology

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These days CRISPR/Cas9 technology is widely used in the field of genomic engineering for generation of cellular and animal models by editing of the sequence of particular gene. Small modifications of this tool enabled us to regulate transcription of many genes in just one experiment. For this application Cas9 enzyme was modified in two aspects. Firstly, the active site of this nuclease was mutated in such a way to gain inactive enzyme. Secondly, an adaptor was linked to dCas9 for enhancing of transcription modulation activity. dCas9 is programmed with a guide RNA (gRNA) that directs it to a DNA locus of interest via sequence complementarity. This can be used for the elucidation of the mechanism of action of small molecules with therapeutic potential. 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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BMI prediction through detection of DNA methylation

Lucie Kotková[1], Rastislav Slavkovský[1], Veronika Holinková[1], Barbora Blumová[1], Helena Štefanová[1], Jana Stránská[1], Pavla Kouřilová[1], Jiří Drábek[1]

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

We decided to verify a correlation between BMI and methylation status in three previously validated CpGs in HIF3A gene and other previously published candidate markers using a group of healthy Czech blood donors (n>20) with BMI under 21 kg/m2 (n>10) and over 36,5 kg/m2 (n>10).

For methylation assessment, we choose next-generation amplicon bisulfite sequencing over the pyrosequencing method, mostly because of lower sample requirements which are in a lot of cases the bottleneck for the usage of the epigenetic methods in the forensic setting.

Here we present our preliminary data.

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