REACTOR

IMTM Reactor Conference

Semptember 7–9, 2020
Bystřice nad Pernštejnem

INSTITUTE OF MOLECULAR AND
TRANSLATIONAL MEDICINE

PROGRAM

Monday 7th

Chair: Karel Koberna

10:30 10:40 **Welcome speak**
Tomáš Oždian

10:40 11:00 **Inhibition of α-synuclein peptide aggregation in vitro**
Narendran Annadurai

11:00 11:20 **Inhibition of α-synuclein peptide aggregation in vitro part II**
Viswanath Das

11:20 11:40 **MARK4 inhibitors**
Lenka Řeháčková

11:40 12:00 **Targeting the Sonic Hedgehog Signaling Pathway with Betulinic Acid Derivatives**
Ivo Frydrich

12:00 13:00 **LUNCH**

Chair: Tomáš Oždian

13:00 13:20 **Bystin regulates c-myc on protein level**
Zuzana Maceckova

13:20 13:40 **CRISPR/Cas9 technology - not only a genome editing tool**
Agáta Kubíčková

13:40 14:00 **Profiling of novel anti-cancer agents from the perspective of adenosine receptor**
Jana Kotulová

14:00 14:20 **High-throughput screening of chemical compound libraries in Nematodes**
Dominik Vítek

14:20 14:40 **Development of a novel high throughput screening tool for CFTR modifiers discovery**
Martin Ondra

14:40 15:00 **The MicroScale Thermophoresis: Principles and advantages of technology**
Jarmila Stanková

15:00 15:20 **COFFEE BREAK**

Chair: Milan Urban

15:20 15:40 **Fatty acids enhance expression of Killing receptors in human NK cells of young and old volunteers**
Juan De Sanctis

15:40 16:00 **Antibiotic, antiparasitic and antiviral activity of novel compounds**
Ermin Schadich

16:00 16:20 **Cytotoxic activity of triterpenoid pyrazines and their bioisosteric pyridines**
Jiří Hodoň

16:20 16:40 **Study of the basic pharmacokinetic properties of a new carboranes**
Barbora Lišková

16:40 17:00 **Identification of compounds with CYP2W1-specific cytotoxic activity**
Soňa Gurská

19:30 **GRILL**

Tuesday 8th

Chair: Miloš Petřík

9:00 9:20 **Proteomic signature in exhaled breath condensates for a non-invasive diagnostics of lung cancer and COPD**
Jana Václavková

9:20 9:40 **Identification of biomarkers in bronchoalveolar lavage of Bordetella pertussis infected mice**
Dušan Holub

PROGRAM

9:40 10:00 **Behavioral research laboratory: IMTM and ICRC cooperation**
Aleksandra Bartelik

10:20 10:40 **Preclinical tumor imaging with 89Zr-labeled monoclonal antibody ramucirumab**
Zbyněk Nový

10:40 11:00 **COFFEE BREAK**

Chair: Josef Srovnal

11:00 11:20 **Clonal somatic variants in hematopoietic cells in relation to age and stroke**
Rastislav Slavkovsky

11:20 11:40 **MEG3 as a potential biomarker in meningiomas**
Hanuš Slavík

11:40 12:00 **CTCs detection in GBM patients using CytoTrack instrument**
Alona Řehulková

12:20 12:40 **Assays for classical and novel drug targets**
Jiří Řehulka

12:40 13:00 **DNA replication and cancer**
David Lukáč

13:00 14:00 **LUNCH**

14:00 20:00 **EXPLORATION OF VYSOČINA**

20:00 **MASOŽRAVÁ BERTA**

Wednesday 9th

Chair: Pavlo Polishchuk

9:00 9:20 **Simulation Studies and Free Energy Calculations for Bystin Complexes**
Olena Mokshyna

9:20 9:40 **In-silico search for molecular targets of 5-arylidene-2-(4-hydroxyphenyl)aminothiazol-4(5H)-ones cytotoxic against cancer cell lines**
Mariia Matveieva

9:40 10:00 **De novo design of inhibitors of SARS-CoV-2 main protease**
Aleksandra Nikonenko

10:00 10:20 **Search for new adenosine antagonists molecules by the ligand-based pharmacophore models**
Alina Kutlushina

10:20 10:40 **Claire: an open-source python framework for unsupervised detection of protein variants and in-depth interpretation of shotgun proteomics data**
Miroslav Hruska

10:40 11:00 **COFFEE BREAK**

Chair: Anna Ligasová

11:00 11:20 **A new method for the induction of protein aggregation in cells**
Zdeněk Škrott

11:20 11:40 **CBD protects from CuET treatment via induction of MT2A protein**
Tereza Buchtová

11:40 12:00 **Senolytics efficacy against Senescent cells of varied origins**
Matthew Lacey

12:00 12:10 **Concluding remarks**
Tomáš Oždian

12:10 13:00 **LUNCH**

13:30 **DEPARTURE FROM SKALSKÝ DVŮR**

Inhibition of α-synuclein peptide aggregation *in vitro*

Narendran Annadurai[1] and Viswanath Das[1]

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Abstract

α-synuclein is abundant in human brain and the aggregated α-synuclein is the major constituent of Lewy bodies, a pathogenic hallmark of all synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies and multiple system atrophy. These Lewy bodies are associated with brain cell death. Thus it is important to study the process involved in the aggregattion of α-synuclein and also to find the inhibitors of α-synuclein aggregation.

We have used the 36 a.a. length α-synuclein peptide to study and screen the inhibitors of α-synuclein aggregation. Thioflavin-T based in vitro α-synuclein aggregation assay was designed and optimized. The formation of α-synuclein fibrils was analyzed by using Fluorescence microscopy and atomic force microscopy. The compounds were tested at 1 μM concentration to find the inhibitors of α-synuclein aggregation. Then the seeding ability of α-synuclein fibrils was tested in synuclein biosensor cell lines.

From in vitro assay we found the time dependent increase of α-synuclein aggregation. Then with the fluorescence microscopy and atomic force microscopy we were able to image the fibril formation of α-synuclein aggregates. From our preliminary screening and dose-dependent assays we have identified the compounds that were able to inhibit the formation of α-synuclein peptide fibrils. The presence of intracellular synuclein aggregates confirmed the seeding ability of α-synuclein fibrils.

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

MARK4 inhibitors

Lenka Řeháčková[1]

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

Abstract

Microtubule Affinity Regulating (MARK) protein family is involved in the tau protein phosphorylation. In its physiological state tau protein phosphorylation helps to stabilize microtubules of axons, but in hyperphosphorylated state, neurofibrillary tangles are formed and microtubules disintegrate, which causes neuronal death. Tau protein hyperphosphorylation was found in patients with Alzheimer disease as well as increased expression of MARK4. In our project we have developed method for detection of MARK4 activity and we have identified several molecules which are selective inhibitors of MARK4, they have no toxicity and are able to cross blood brain barrier. These molecules are currently being tested in the cell model.

AZV – 15-31984A

Targeting the Sonic Hedgehog Signaling Pathway with Betulinic Acid Derivatives

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[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

The sonic hedgehog (Shh) represents evolutionary important signaling pathway. Aberrant activation of the Shh pathway has been shown in a variety of human tumours, including basal-cell carcinoma, medulloblastoma, glioblastoma, rhabdomyosarcoma, leukemias, and cancers of the breast, lung, pancreas, and prostate. The canonical Shh pathway contains several key components. The most important effectors comprise transmembrane member smoothened (SMO) and glioma-associated oncogene homolog (GLI) family of transcription factors. Both are considered to be relevant targets for cancer therapeutics. Based on literature data, there is evidently a high effort dedicated towards pharmacologically targeting SMO. To date, only two inhibitors (GANTs and arsenic trioxide) specifically targeting GLI transcription factors are in active clinical trials. Screening for small molecular GLI inhibitors thus represents attractive goal. It has been previously shown, that betulinic acid (BA) induces apoptosis and inhibit Shh signalling in rhabdomyosarcoma. Inspired by this finding, we screened small library of structurally unrelated BA derivatives. We examined the activity in NIH3T3 Cell Line with a Gli-dependent firefly luciferase reporter. The active compounds were studied in detail.

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).

Bystin regulates c-myc on protein level

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

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

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

IGA LF_2020_007

CRISPR/Cas9 technology - not only a genome editing tool

Agáta Kubíčková[1] and Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

These days CRISPR/Cas9 technology is widely used in the field of genomic engineering for generation of cellular and animal models by editing 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.

This project is supported by the European Regional Development Fund - Project ENOCH (No.CZ.02.1.01/0.0/0.0/16_019/0000868) and by grant IGA_LF_2020_007.

Literature

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Profiling of novel anti-cancer agents from the perspective of adenosine receptors

Jana Kotulová[1], Petr Džubák[1] and Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

Being G-protein coupled receptors, large number of biological processes could be modulated by targeting adenosine receptors (AdoRs), including cancer. Given the complexity of adenosine signalling per se and the interplay between adenosine and AdoRs, potential of adenosinergic system is yet to be unrevealed and fully understood.

Four AdoR subtypes (A1, A2A, A2B, and A3) are involved in different signalling pathways, having both pro- and anti-cancer properties. Besides other parameters, these properties also depend on the nature of interacting molecule.

Our aim was to develop a pipeline of assays for identification of novel AdoR agonists and antagonists and to study further the mechanism of action of the promising compounds. We identified several potent modulators of AdoRs and the most encouraging findings will be presented.

The study was supported by the Internal Grant of Palacky University (IGA_LF_2020_019); GACR (19-081248) and CZ-OPENSCREEN (LM 2018130).

High-throughput screening of chemical compound libraries in Nematodes

Dominik Vítek[1], Tomáš Fürst[2], Marián Hajdúch[1] and Jiří Voller[1]

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[2]Department of mathematical analysis and applications of mathematics, Faculty of science, Palacký university

Abstract

Whereas screening chemical compounds on cell lines provides invaluable information regarding their toxicity and primary effect of the compound, it doesn't reveal how they may react in the organism. Caenorhabditis elegans provides a convenient stepping stone between testing of compounds in cell lines and mice. Because of its fortunate phylogenic placement, it has most organ systems analogous to mammals, short developmental cycle and relatively simple cultivation. Due to these factors C. elegans has been extensively researched and used as a model organism. We intend to take advantage of this vast knowledge and use C. elegans as a model in anti-aging activity screening of chemical compound library.

As a preliminary step in the screening for anti-aging activity we decided to test the toxicity of the candidate compounds. Compounds able to inhibit development and survival of C. elegans can be repurposed as potential nematicides. This approach also allows step-wise optimization of microscopy and image analysis methods as the development/death related phenotypes are readily recognized.

We report our progress in the optimization of culture and screening conditions for not only C. elegans but also for two important sheep parasites Haemonchus contortus and Teladorsagia circumcinata.

INTER-COST project of Czech Ministry of Education (LTC19030)

Development of a novel high throughput screening tool for CFTR modifiers discovery

Martin Ondra[1], Daciana Catalina Dumut[2], Juhi Shah[2], Amanda Centorame[2], Danuta Radzioch[2] and Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2]Faculty of Medicine, McGill University, Montreal, QC, Canada

Abstract

CFTR is an epithelial membrane protein with the function of ions and water transport regulation. Mutations affecting function, localization or stability of CFTR cause Cystic fibrosis. Antibodies against CFTR, electrophysiology and overexpression of fluorescent recombinant proteins are techniques used to monitor processing, trafficking, recycling and function of CFTR. Although sophisticated, these methods have their limitations. Recently, a new approach to monitor physiological levels of protein has been explored. CRISPR/Cas9 mediated knock-in of HiBiT tag into the genomic locus of the extracellular loop of CFTR could be the new approach in monitoring localization of CFTR in the membrane.

Human bronchial epithelial (HBE) cells expressing WT CFTR were used for CRISPR/Cas9 knock-in of HiBiT. Electroporation efficiency and cutting efficiency of four different crRNAs and four different templates for knock-in of HiBiT into specific genomic locus of CFTR were tested. Specificity and accuracy of HiBiT knock-in were tested by luminescence assays, western blotting and sequencing. Our results show the design and validation of this new tool for high throughput screening. HiBiT tag was successfully introduced by CRIPSR/Cas9 into 2 different positions of the 4th extracellular loop of CFTR. Detection of the luminescence signal of CFTR in total cell lysate and also on the surface of live cells with endogenous protein expression was achieved.

This project was supported by Mobility Support at UP - CZ.02.2.69/0.0/0.0/16_027/0008482.

Literature

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The MicroScale Thermophoresis: Principles and advantages of technology

Jarmila Stanková[1]

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

Abstract

MicroScale Thermophoresis is a biophysical quantitative analysis of interactions between biomolecules. The technique is based on the detection of a temperature-induced change in fluorescence of a target as a function of the concentration of a non-fluorescent ligand. The changes in fluorescence are caused by two different effects, temperature related intensity change (TRIC) and thermophoresis. TRIC is a function of fluorescent molecules to change their fluorescence intensity in different temperatures. On the other hand, thermophoresis is a function of all biomolecules to move along temperature gradients. MST profile of a protein typically differs significantly from MST profile of protein-ligand complex due to binding-induced changes (size, charge and solvation entropy). As a ligand can be used various types of molecules including proteins, DNA, RNA, peptides, small molecules, fragments and ions. Moreover, MST, in comparison with common binding affinity methods, is beneficial in low sample consumption and it can be performed in almost any buffer, even in plasma and cell lysate. The real cases of protein-ligand interaction and binding affinity analysis will be described in the presentation.

This project is supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and by grant IGA LF_2020_019.

Fatty acids enhance expression of Killing receptors in human NK cells of young and old volunteers

Juan Bautista De Sanctis[1], Danuta Radzioch[1, 2] and Marián Hajdúch[1]

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Abstract

(1) Background NK cells are involved in innate and adaptive immune responses. In healthy ageing, NK cell number is not modified; however, spontaneous cytotoxicity may be decreased. Metabolic changes may be responsible for this effect. (2) Methods: The fatty acid profile of normolipemic: 30 young (23±4 years, BMI 22.1±1.3) and 30 old (63 ±5 years, BMI 22.9±2.5) healthy donors were evaluated along with the expression, assessed by flow cytometry, of NK killing receptors (KR). at basal level and after cultivation with fatty acids for 24 hr. (3) Results: A significant increase in oleic ($P<0.01$), arachidonic ($P<0.001$), lignorenic ($P<0.001$), and nervonic acid ($P<0.0001$) and a significant decrease in docosahexaenoic acid ($P<0.01$) were recorded in elderlies as compared to younger donors. At basal levels, significant differences in KR were encountered between the groups ($P<0.0001$). After fatty acid incubation, KR expressions were significantly enhanced ($P<0.001$) by saturated fatty acids and arachidonic acid. Docosahexaenoic acid and eicosapentaenoic acid produced the contrary effect. Conclusions NK cell KR and KIR receptors expression differ with age. Fatty acids modulate the expression of KR receptors in vitro and hence NK cell function.

The work was supported by a grant from the Ministry of Education, Youth and Sport, Czech Republic: Molecular and Cellular Clinical Approach to Healthy Ageing, ENOCH (European Regional Development Fund Project No. CZ.02.1.01/0.0/0.0/16_019/0000868, IMTM #869/V19).

Antibiotic, antiparasitic and antiviral activity of novel compounds

Ermin Schadich[1]

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

Abstract

Infectious diseases such as leishmaniases, tuberculosis and coronavirus disease 2019 (COVID 19) 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 three diseases. Screening of 1,280 compounds from BigLopac, a commercial chemical library of bioactive compounds, for 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 while the dose-response analyses showed 12 active compounds. It is also worth to mention that the in vitro analyses of compounds against severe acute respiratory syndrome coronavirus 2 are calibrated and one compound was found to be active.

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

Cytotoxic activity of triterpenoid pyrazines and their bioisosteric pyridines

Jiří Hodoň[1,2]

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

Abstract

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. The conjugates these triterpenoids with glucose, tetraacetyl glucose and other molecules were prepared using Huisgen cycloaddition in order to increase their solubility and bioavailability. Cytotoxic activities of all prepared compounds were measured. The most active derivates were further examined for their mechanism of action: First, influence of the active compounds on cell cycle was studied, then influence on apoptosis and nucleic acid synthesis using flow cytometry methods. Active compounds with the most promising therapeutic index was selected and equipped by biotin in order to be used in pull down assays evaluated by quantitative proteomics and SILAC. A set of proteins was found that bind selectively to the active molecule which may be responsible for the activity.

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 internal grants of Palacky University (IGA_LF_2019_019 and IGA_PrF_2019_027)

Study of the basic pharmacokinetic properties of a new carboranes

Barbora Lišková[1], Martina Medvedíková[1], Pavlína Řezáčová[2], Bohumír Grüner[3] and Marián Hajdúch[1]

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[2]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic
[3]Institute of Inorganic Chemistry of the Czech Academy of Sciences, Řež, Czech Republic

Abstract

Infectious diseases such as leishmaniases, tuberculosis and coronavirus disease 2019 (COVID 19) 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 three diseases. Screening of 1,280 compounds from BigLopac, a commercial chemical library of bioactive compounds, for 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 while the dose-response analyses showed 12 active compounds. It is also worth to mention that the in vitro analyses of compounds against severe acute respiratory syndrome coronavirus 2 are calibrated and one compound was found to be active.

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

Identification of compounds with CYP2W1-specific cytotoxic activity

Soňa Gurská[1], Petr Džubák[1], Katharina Klöditz[2], Magnus Ingelman-Sundberg[2] and Marián Hajdúch[1]

[1] Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
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Abstract

The cytochrome CYP2W1 belongs to the youngest members of the P450 family of enzymes. Multiple expression analyses showed significant expression of CYP2W1 in fetal colon and exclusively in 30% of colon cancers in adults. Therefore CYP2W1 is a promising therapeutical target for colon cancer treatment. The aim of the study was to identify prodrugs that are activated by CYP2W1 and subsequently cause specific tumor toxicity.

Cells SW480-CYP2W1 and SW480-mock were treated with compounds from commertial libraries (LOPAC, Prestwick and ENZO) as well as from IMTM proprietary library at 3 different concentrations (1, 10 and 50 μM). The effect of the compounds was measured after 72-hour treatment by the MTS assay.
For each compound and concentration the PI (percentage of inhibition) value was calculated by Dotmatics software. To compare the response of both cell lines the ratio (R) and subtraction (S) of PI values for each compound was calculated. On the basis of the set threshold for R and S the active compounds (hits) were selected. Obtained results will be presented and discussed.

Study was supported by grants: MSMT-CZ - EU-OPENSCREEN (LM2015063)

Proteomic signature in exhaled breath condensates for a non-invasive diagnostics of lung cancer and COPD

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

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[3] *Department of Pediatrics, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University Olomouc*

Abstract

The exhaled breath condensate (EBC) is a promising source of biomarkers which is collected non-invasively. Most of the EBC consists of condensed water vapor. However, it is rich with biomarkers such as small molecule mediators, protons, various ions, lipids, oxidative stress markers, proteins, oxidants, nucleotides, DNA, miRNA and exosomal RNA. Thus, it could serve as a biological matrix where the biomarkers of various respiratory and systemic diseases can be found. In the future, the analysis of exhaled breath could complement invasive diagnostic approaches. Our work is focused on protein signature of early diagnosis of lung cancer. We described a gel-free mass spectrometry based approach which showed in-depth characterization of the EBC proteome.

Proteins in the EBC sample are solubilized, denatured, reduced and trypsin digested. 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™ 2.4 software. Proteins detected in at least 75 % of at least one subgroup (subgroups are controls, lung cancer and COPD) were further statistically evaluated with software R and Bioconductor.

We have collected and analyzed samples from 90 controls, 163 lung cancer patients and 77 COPD patients. Our approach led to identification of 7925 proteins across all samples and 1384 of them were used for statistical analyses. The protein signatures of individual subgroups were established.

This work was supported by grants from the Ministry of Health of the Czech Republic (16-32302A and 16-32318A), from the Czech Ministry of Education, Youth and Sports (LM2018133), European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and by the internal grant of Palacky University Olomouc (IGA_LF_2020_019).

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López-Sánchez L. M., Jurado-Gámez B., Feu-Collado N., Valverde A., Cañas A., Fernández-Rueda J. L., Aranda E., Rodríguez-Ariza A.: Am. J. Physiol. Lung Cell. Mol. Physiol. 313, L664 (2017).

Identification of biomarkers in bronchoalveolar lavage of Bordetella pertussis infected mice

Dušan Holub[1], Petr Džubák[1], Marián Hajdúch[1], Jiří Mašín[2], Radim Osička[2] and Peter Šebo[2]

[1] Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2] Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Abstract

Bordetella pertussis is bacteria that cause pertussis, also known as whooping cough(1). 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 new-borns 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, which was focused on identification of potential host and/or pathogen protein biomarkers in bronchoalveolar lavage of Bordetella pertussis infected mice. The bronchoalveolar lavage samples were collected from infected and control mice. Liquid chromatography and mass spectrometry analysis allowed to quantify 721 proteins from the bronchoalveolar lavage 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. Additionally, we identified specific chaperonin protein from Bordetella pertussis, which could be potential pathogen biomarker.

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Behavioral research laboratory: IMTM and FNUSA-ICRC cooperation

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Abstract

The behavioral research laboratory was created through cooperation in two institutes - IMTM and FNUSA-ICRC.

The Animal Center (AC) behavioral research laboratory offers services and expertise in the design of in vivo experimental plan in neuroscience research and, supervising the procedures. As well as AC supports the project license and authorization process from the Ethical Committee.

The team of AC provides carrying out procedures on rodents with the use of sophisticated instruments as IntelliCage, PhenoMaster, and MotoRater. These systems give the possibility to perform tests of animals' activity, their emotionality, learning and memory, social behaviors, locomotor activity, metabolic parameters as feeding and drinking analysis, body weight monitoring, behavioral changes in pharmacological treatment, both short-term and long-term observations, up to several weeks in one experimental process and, phenotyping of new transgenic animal models. The equipment lets provide procedures with animals and observe their behavior with a minimum of experimenter intervention and human bias.

All these matters allow for more reproducible data, minimizing stress and improving animal welfare according to 3R rules.

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Preclinical tumor imaging with 89Zr-labeled monoclonal antibody ramucirumab

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Abstract

The aim of this study was to prepare ramucirumab (RAM) radiolabeled with positron emissiontomography (PET) imaging radionuclide zirconium-89 and determine its *in vitro* and *in vivo* biological properties. The conjugation of RAM with the chelatorp-SCN-Bn-deferoxamine (DFO) was carried out in sodium bicarbonate. DFO-RAM was radiolabeled with (89Zr)Zr-oxalate in sodiumacetate solution. The binding affinity of prepared 89Zr-DFO-RAMto VEGFR2 was tested *in vitro* on prostate adenocarcinoma (PC-3) and ovary adenocarcinoma (SK-OV-3) cell lines.The PET/CT imaging and ex vivobiodistribution studies were performed in mice engrafted with PC-3 and SK-OV-3 tumors. The *in vitro* experiments showed preserved bindingability of 89Zr-DFO-RAM to VEGFR2 with the KD values38.93±2.04 and 36.59±8.36 nM for PC-3 and SK-OV-3 cells respectively. Theobtained *ex vivo* biodistribution data revealed the activity uptake inPC-3 and SK-OV-3 tumors at about 8.7±0.2 and 12.1±1.6 %ID/g respectively. The tumor-to-bloodratio for 1, 3 and 6 days p.i. was 0.4, 0.6 and 0.8 for PC-3 and 0.5, 1.0 and1.3 for SK-OV-3 tumors respectively. The PET/CT showed highradioactivity accumulation in the tumors starting already at first day p.i.The labeling of DFO-RAM with Zr-89 providedanti-VEGFR2 radiopharmaceutical with promising binding to the target receptorsboth *in vitro* and *in vivo*. The results of *in vivo* experiments proved the potencyof 89Zr-DFO-RAM to target and image VEGFR2-positive tumors.

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Clonal somatic variants in hematopoietic cells in relation to age and stroke

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Abstract

Clonal hematopoiesis of indeterminate potential (CHIP) has recently been described as a common age-related condition manifested by the accumulation of somatic mutations in cells of the hematopoietic system. Although CHIP is characterized by the expansion of certain cell clones, no other criteria for hematologic neoplasia are met. This state is a potential precursor of malignant transformation, but more interestingly, it can also increase the risk for diseases such as atherosclerosis and ischemic stroke.
To explore this phenomena, we performed a pilot study using advanced deep sequencing of 38 CHIP-related genes in individuals with or without stroke (Qiagen QIAseq Targeted DNA Custom panel; Illumina NovaSeq). Blood samples of 51 stroke patients aged > 65 years were analyzed and compared with a control group of elderly people > 80 years (n = 24) and healthy donors < 30 years (n = 24).

When using molecular barcodes, we achieved average consensus coverage of target areas of 2700x. Thanks to barcodes we increased average base quality to Q > 46 in comparison to situation without barcodes Q ~ 36 and reached the detection limit of somatic variants at a frequency of 0.5 %. So far, we are able to find parameters for the detection of somatic variants in elderly people (mutations observed as expected especially in DNMT3A and TET2), and at the same time we correctly do not observe variants in the control cohort - in young donors.

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MEG3 as a potential biomarker in meningiomas

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Abstract

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. MEG3 is one of the potential candidate biomarker. It is 1,6 kb lncRNA with downregulation in tumors and strong expression in CNS. Also, the MEG3 gene is localized on 14q32 chromosomal region, which is often disturbed in meningiomas. According to our RNA-seq data, overall expression of MEG3 is not changed among investigated groups (recurrence vs non-recurrence; WHO grade I vs II vs III; mesoderm vs neural crest; male vs female). However, there is strong deregulation in MEG3 isoforms within the investigated groups. Also, we performed RISH-based analysis of localization and signal distribution of total MEG3 within the meningioma tissues. Most of the signals are present in spread form of single molecules (80 - 95%), but there are also clusters. This clusters differ between recurrent and non-recurrent patients. Small clusters (1 – 15 px) are more often presented in recurrent patients ($p = 0.026$). Bigger clusters (15 – 30 px) are more often presented in non-recurrent patients ($p = 0.012$). Clusters bigger than 60 px are slightly present (less than 1%). This study shows the importance of complex lncRNAs investigations.

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CTCs detection in GBM patients using CytoTrack instrument

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Abstract

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. Glioblastoma multiforme (GBM) is the most aggressive subtype of malignant tumors of the central nervous system. Cases of primary tumor cell dissemination into peripheral blood have been reported in a few articles. In this study, we validated the detection of CTCs on the CytoTrack CT11TM platform and analyzed the peripheral blood samples from a few patients with GBM.

In-vitro detection of CTCs was performed in the validation phase. U87-MG cell line was added to the whole blood samples and staining was performed with a home-made stain mix of GFAP, Vimentin, CD-45, and DAPI. Furthermore, 7 peripheral blood samples from GBM patients were analyzed.

The U87-MG cell line was selected for the validation experiment. For the staining validation mix of anti-GFAP, anti-Vim, anti-CD45, and DAPI were used. Subsequently, the cells were detected on the instrument. In analyzing the patient's samples, a two-cell cluster, and a single CTC were detected in one of the seven samples.

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Assays for classical and novel drug targets

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Abstract

The talk will cover current cell-based assays available at our screening facility with emphasis on fluorescent proteins. The screening is focused on identification of new compounds that modulate cellular pathways altered in cancer and rare neurodegenerative diseases. In addition, a simple method for determination of microtubule binding site will be presented.

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DNA replication and cancer

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Abstract

Replication of chromosomal DNA is a fundamental process that requires stringent regulation to ensure accurate, efficient DNA synthesis that duplicates the entire genome. In human cells, thousands of replication origins are activated during S phase of cell cycle and two DNA strands are locally unfolded, forming a structures called replication forks. Regulation of replication fork speed and activation of origins are main means that allow replication of entire chromosomal DNA. Speed of replication fork progression is controlled by multiple mechanisms, including proteins PARP and p53. PARylation in unperturbed cells also signals unprocessed Okazaki fragments. To gain insight into the role of lagging strand processing in regulation of replication fork speed we plan to interfere with lagging strand synthesis and test the effects on replication fork speed. Emetine is used for many decades as a specific chemical inhibitor of Okazaki fragments synthesis, uncoupling leading and lagging strand replication. However, our preliminary data using DNA combing shows different effect of emetine on DNA replication. The aim of the project is to clarify effect of emetine on DNA replication.

Simulation Studies and Free Energy Calculations for Bystin Complexes

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Abstract

Previously our colleagues have shown experimentally that bystin plays an important role in Diamond Blackfan anemia (DBA). In silico studies allowed to identify two main binding sites. The range of ligands binds was docked into the primary binding site with the sufficiently high scores. Among those of special interest were the corticosteroids – nowadays they are commonly accepted treatment for DBA, though their mechanism of action until now remained elusive.

In our previous computational studies (using molecular dynamics (MD) simulations) we were able to detect two shallow binding sites on the protein surface. Ligands of interest were binding mostly to the binding site close to ARG372.

To further explore the binding mechanisms and computationally confirm the binding site we employed several enhanced sampling approaches. Firstly, we used extensive replica exchange simulations (with 74 replicas) to further explore conformational states of the unbound protein. Secondly, we used the metadynamics approach – another popular enhanced sampling technique that allows to explore large and complex conformational spaces. We focused on two main ligands of interest – dexamethasone and one of the IMTM ligands. Due to the openness of the binding site, we used a few simple coordinate variables (CV) - distance from the center of mass of the binding site towards ligands' center of mass and dihedral angle of ARG372 residue.

INTER-EXCELLENCE LTAARF18013, SPF 441100011

In-silico search for molecular targets of 5-arylidene-2-(4-hydroxyphenyl)aminothiazol-4(5H)-ones cytotoxic against cancer cell lines

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Abstract

Among 4-thiazolidinones 5-ene-2-arylimino/amino-4-thiazolidinones are of special interest as one of most perspective groups in anticancer drug discovery process. At the same time, very few attempts were made to outline the main molecular modes of their action [1,11]. In this study we perform docking simulations of a series of 5-ene-2-amino(imino)-4-thiazolidinones showing in vitro inhibition of cancer cell lines proliferation, as well as cell cycle changes in CCRF-CEM cells. The aim is to investigate putative targets which could cause mitosis and DNA/RNA synthesis inhibition: tubulin and mitotic kinases (Aurora A-B, CDK1-2, Polo-like kinase 1, MAP kinase et al.). The results of docking together with prior MTS-assay and cell-cycle analysis allows us to propose most probable molecular targets for the compounds studied.

The work was supported by the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN – LM2018130 and EATRIS-CZ – LM2018133).

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De novo design of inhibitors of SARS-CoV-2 main protease

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Abstract

Spread of SARS-CoV-2 virus caused a lot of deaths in many countries. Finding of promising compounds able to inhibit the virus and its replication became one of the major tasks for research community. In this study we developed our own tool for de novo compound generation. The tool is based on iterative generation and selection of the best candidates by molecular docking. The main protease (3C-like protease) was chosen as the target. 30 available X-ray experemental complexes of the main protease with drug-like fragments were chosen. The ligands from the selected complexes were grown and tested whether they adopt the same position of the parent fragment inside the binding pocket according to molecular docking. Most fitted compounds were selected for the next iteration. Total 1240535 structures were generated within 5 iterations. Based on docking scores, drug-likeness and complexity of compounds we selected 41 candidates promising for further evaluation.

This research was funded by the Ministry of Education, Youth and Sports of the Czech Republic within the INTER-EXCELLENCE LTARF18013 project.

Search for new adenosine antagonists molecules by the ligand-based pharmacophore models

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Abstract

Pharmacophore models are widely used in the early stages of drug development to identify potential matches in large datasets. These models encode the spatial arrangement of features that are important for protein-ligand interactions.

We have developed ligand-based pharmacophore models for adenosine A1, A2a, A3 receptors antagonists using psearch software. Based on the results of the test sample, we have selected representative pharmacophore models. Selected pharmacophore models were screened on the IMTM datasets, namely proprietary library, chemical library, ENZO, LOPAC, Prestwisk and Enamine datasets. Hit lists retrieved for individual models were analyzed. We investigated how diverse the compounds found by the representative pharmacophore models were. Molecular weight and ratio of hydrophobic to charged centers molecules for each compound were studied. Based on this analysis, we selected compounds from the datasets for activity analysis against the adenosine targets by biologists.

The work was supported by Ministry of education, youth and sport of Czech Republic (MSMT-5727/2018-2) and Russian Federal Target Programme (Project 14.587.21.0049) collaborative project "Improve the output of primary screening of biologically active compounds using computational models".

Claire: an open-source python framework for unsupervised detection of protein variants and in-depth interpretation of shotgun proteomics data

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Abstract

The unsupervised detection of protein variants from tandem mass spectra of proteome has a wide range of biomedical applications. For instance, we have recently shown the ability to identify hypermutated colorectal tumors purely from protein-level data, which has clinical implications for the recommendation of suitable therapy. To detect the protein variants, we have developed an in-house cluster-powered system—claire. In recent months, claire went through major structural changes. Foremost, claire is now an open-source project, capable of running on most modern personal computers. Besides the unsupervised detection of protein variants, claire allows for the deep interpretation of mass spectra and scales well for protein databases on the order of hundreds of gigabytes. In summary, claire thus became much more broadly usable, enables third-party development of modules, and its functionality extends beyond the detection of protein variants.

This work was supported by the Ministry of School, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/0000868, LM2015064, LM2015047), Technological Agency of the Czech Republic (TE02000058), Ministry of Health of Czech Republic (NV16-32318A, NV16-32302A) and by an internal grant of Palacky University (IGA LF_2020_007).

A new method for the induction of protein aggregation in cells

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Abstract

Proteostasis is an integral part of cellular physiology and proteotoxic stress is implicated in several diseases such as cancer or neurodegenerative disorders. Despite its importance, no methodology exists to study protein unfolding, aggregation and chaperone function in cells in live time in definite sub-cellular regions. Heat shock is an established and commonly used approach to induce unfolding and aggregation of proteins. Studying responses to thermal damage of proteins on the level of a single living cell or even subcellular level represents a significant challenge due to the lack of available methods allowing precise and fast delivery of the heat to the target structure at the micrometer scale. Here, we present such single-cell method compatible with laser-scanning microscopes allowing unprecedented spatiotemporal analysis of thermal damage relevant for degenerative diseases, with broad applicability in biomedicine.

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CBD protects from CuET treatment via induction of MT2A protein

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Abstract

CuET is a copper-containing disulfiram metabolite with anticancer activity. Screening for chemical modulators of CuET activity identified cannabidiol (CBD), belonging to the cannabinoid family, as a compound significantly decreasing the cytotoxic effects of CuET. Cannabinoids are often used as supportive therapy by oncological patients. Thus, such information by itself is of high importance as concomitant CBD/CuET treatment can negatively affect the outcome.

Mechanistic insight into this rescuing effect revealed that CBD does not alter the CuET uptake. Instead, it increases the transcription of genes involved in the metallothionein pathway. Metallothioneins are proteins involved in many cellular processes, including homeostasis of biogenic metals. A very high affinity of metallothioneins for metals or metal-containing substances also protects the cell from the negative impact of heavy metals. Here we show that the overexpression of metallothioneins such as MT2A, which the CBD treatment increases levels, reduces the toxic effect of CuET significantly. Vice versa, inhibition of metallothionein pathway renders the cells hypersensitive to CuET.

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Senolytics efficacy against Senescent cells of varied origins

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Abstract

Cellular senescence is typically defined as a stable form of cell cycle arrest. A cell can enter the senescence state via multiple different mechanisms, including: replicative senescence (RS) caused by telomere shortening; Stress-Induced Premature Senescence (SIPS), as a result of irreparable DNA damage; Oncogenic senescence (OS) caused by the activation of specific oncogenes. Cellular senescence has a complex role within an organism: documented as both beneficial and detrimental to health. The most apparent benefit of senescence is inhibition of oncogenesis; however, senescent cells are also the driving factor in a number of health-threatening conditions, including age-related degenerative diseases. As a result, there has been a marked interest in senescent cells as potential targets for treatment.

Senolytics, compounds that are preferentially lethal to senescent cells, are an avenue for such treatment. Senolytics already have proven positive health effects in animal models; the elimination of senescent cells resulted in delayed onset of age-related diseases, prolonged life span and general fitness. However, senolytic therapies have been shown to have variable effectiveness. Even well-established senolytic compounds were found to be ineffective under different experimental conditions.

This study explores the effectiveness of various senolytics against different types of senescent cells (SIPS and OS) to identify whether the origin of senescence alters senolytic efficacy.
This work was financially supported by the grant IGA_LF_2020_023