IMTM REACTOR

6th Annual IMTM Retreat

October 3–5, 2022 / hotel Lanterna Velké Karlovice

INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE

PROGRAM

Monday, October 3rd

08:30		DEPARTURE FROM OLOMOUC	
10:30	12:00	CHECK-IN	

Chair: Hana Jaworek

13:00	13:10	Tomáš Oždian	Welcome Speech
13:10	13:30	Ermin Schadich	Anti-SARS-CoV-2 properties of novel compounds
13:30	13:50	Ondřej Bouška	Pooled testing for Covid-19 surveillance in schools and screening of the asymptomatic population

Chair: Barbora Lišková

14:00	14:20	Soňa Gurská	Compounds with cell type - specific cytotoxicity
14:20	14:40	Jiří Řehulka	Drug repurposing for treatment of neurodegenerative diseases
14:40	15:00	Kateřina Ječmeňová	Path to finding novel adenosine receptors agonists and antagonists
15:00	15:20	Jiří Hodoň	Triterpenoid pyrazines and pyridines
15:20	17:40	Ivo Frydrych	Biological evaluation of betulinic acid derivatives with aryl moiety via Suzuki-Myiaura cross-coupling reaction
15:40	16:00	COFFEE BREAK	

Chair: Pavel Moudrý

16:00	16:20	Dávid Lukáč	DNA synthesis of the leading and lagging strands
16:20	16:40	Tereza Buchtová	Cannabidiol and cannabis extracts antagonize platinum-based chemotherapy via cellular uptake
16:40	17:00	Katarína Chromá	A PARylation siRNA screen to identify novel proteins involved in PAR signaling
17:00	17:20	Zuzana Machačová	The role of PARP1 in DNA replication
17:20	17:40	Matthew Lacey	Senescence: Stable or Irreversible?
19:00		DINNER	

Tuesday, October 4th

Chair: Miloš Petřík

09:00	09:20	Zbyněk Nový	Biological evaluation of 225Ac-PSMA derivatives for alpha therapy in prostate cancer
09:20	09:40	Kateřina Bendová	Positron emission tomography imaging of Burkholderia cepacia complex infection using gallium labelled ornibactin
09:40	10:00	Martin Ondra	The use of nanobit technology in HTS: Novel approach for identification of CFTR correctors and modifiers
10:00	10:20	Agáta Kubíčková	Tracing c-Myc Endogenous Expression by NanoBiT Technology for Small Molecules Identification
10:20	10:40	Alžběta Srovnalová	Cell cycle analysis by high content microscopy and immunofluorescence staining
10:40	11:00	COFFEE BREAK	

PROGRAM

Chair: Viswanath Das

11:00	10:20	Martin Löffelmann	New NPL4 protein inhibitors and their mechanism of action
11:20	10:40	Zdeněk Škrott	Prolonged fever-like mild hyperthermia in cancer treatment
11:40	12:00	Monika Vidlařová	Effect of opioid and cannabinoid receptors expression on survival of
12:20	12:40	Narendran Annadurai	Autophagy induction promotes the clearance of intracellular tau P301S repeat domain aggregates seeded by exogenous tau R3 fibrils
12:40	13:00	Pavel Stejskal	Clinical application of liquid biopsy
13:00	14:00	LUNCH	

Chair: Lukáš Najdekr

14:00	14:20	Jarmila Stanková	Czech multi-omics cohort from a proteomics perspective Nematodes
14:20	14:40	Miroslav Hruška	Claire: an open-source system for deep interpretation of tandem mass spectra
14:40	15:00	Lenka Hrubá	Detection of kinase activity by MALDI-TOF mass spectrometry
18:00		DINNER	
19:00		MUSIC	

Wednesday, October 5th

Chair: Dušana Majera

09:00	09:20	Jan Vidlař	Computer security
09:20	09:40	Pavlis Petr	Data management on IMTM, Object storage and OwnCloud
09:40	10:00	Barbora Koblihová	Clonal somatic variants in hematopoietic cells in relation to atherosclerosis and stroke
10:00	10:20	Miroslav Popper	Advanced Animal Models: Orthotopic Implantation and Transvocal Intratracheal Instillation
10:20	10:40	COFFEE BREAK	

Chair: Tomáš Oždian

10:40	11:00	Pavlo Polishchuk	Application of chemoinformatics in drug design
11:00	11:20	Alexandra Ivanová	A new tool to perform automated molecular dynamics simulation and analysis
11:20	11:40	Guzel Minibaeva	Prediction of MARK4 inhibition by MM-GBSA method
11:40	12:00	Alina Kutlushina	Mapping and exploring MARK4 chemical space
12:00	12:20	Dominik Vítek	Stress response in Hypsibius exemplaris
12:20	12:30	Tomáš Oždian	Concluding remarks
12:30	13:30	LUNCH	
13:30		DEPARTURE TO OLOMOUC	

Anti-SARS-CoV-2 properties of novel compounds

Ermin Schadich[1], Marián Hajdúch[1], Petr Džubák[1], Milan Urban[1]

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

Abstract

Our recent publication showed that natural products could be good source foe development of drug candidates against SARS-CoV-2 virus (SASR-CoV-2). Consistently, our current study was focused on analysis of activity of compounds from proprietary library for antiviral activity against SARS-CoV-2. Forty two compounds were tested for antiviral activity using antiviral assays. Vero 76 cells were infected by virus and treated by compounds at 10.00 μM concertation for 72 hours. Subsequently, selected hit compounds were tested in four-fold serial dilutions within 10.00-0.39 μM concentration range. The results showed that seven compounds had antiviral activity SARS-CoV-2. The activity of three compounds against SARS-CoV-2 but only four compounds were selected as primary hits. Dose response analyses showed that IC50s of these compounds were smaller than 10.00 μM. These four compounds will be used as the scaffolds for synthesis of novel drug candidates.

Acknowledgment

This work was funded by the grants from the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN—LM2018130, EATRIS-CZ—LM2018133) and European Regional Development Fund—Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and IGA_LF_2021_036 (Palacky University in Olomouc).

Citation

Ćavar Zeljković S, Schadich E, Džubák P, Hajdúch M, Tarkowski P. Antiviral Activity of Selected Lamiaceae Essential Oils and Their Monoterpenes Against SARS-Cov-2. Front Pharmacol. 2022 May 2;13:893634. doi: 10.3389/fphar.2022.893634. PMID: 35586050; PMCID: PMC9108200.

Pooled testing for Covid-19 surveillance in schools and screening of the asymptomatic population

Ondřej Bouška[1], Vladimíra Koudeláková[1], Soňa Gurská[1], Kateřina Kubáňová[1], Rastislav Slavkovský[1], Hana Jaworek[1], Jana Vrbková[1], Petr Džubák[1], Marián Hajdúch[1]

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

Abstract

Pooled testing enables increasing testing capacity while saving time and decreasing financial burden during preventive testing. Together with alternative sampling strategies, e.g., gargle lavage self-sampling, it represents an effective strategy for mass Covid-19 surveillance without escalating supply shortages during oscillating Covid-19 waves. The aim of this study was to validate pooling testing and report real-life data on the implementation of pooled testing for Covid-19 surveillance.

Methods

Forty-five SARS-CoV-2 positive gargle lavage samples were enrolled to validate a two-stage pooled testing strategy. Three SARS-CoV-2 detection assays were tested during the validation study. Six- and twelve-sample pools were tested during preventive testing of the asymptomatic population and Covid-19 surveillance in schools.

Results

In total, 61,111 samples were tested by the sample pooling strategy. Saving nearly 47,000 reactions (corresponding to 75 % of costs for reagents and consumables) by sample pooling strategy while maintaining high sensitivity (allowing even detection of low positive samples with Ct values > 35) supports its benefit for containing the Covid-19 pandemic. Implementation of pooling testing resulted in up to 3,8 fold increase in testing capacity and reduced time for results delivery. Employment of gargle lavage self-sampling in the testing algorithm allowed more efficient testing capacity utilization compared to the clinician´s taken NPSs.

Acknowledgment

This study was financially supported by the European Regional Development Fund–Project ENOCH (C Z.02.1.01/0.0/0.0/16_019/0000868), by the project HERA (ECDC/HERA/2021/004 ECD.12218), by the Ministry of Health of the Czech Republic, and by the internal grant of Palacky University (IGA_LF_UP_2022_012).

Compounds with cell type - specific cytotoxicity

Soňa Gurská[1], Lenka Lachnitová[1], Michaela Daňková[1], Renata Buriánová[1], Petr Džubák[1], Marián Hajdúch[1]

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

Abstract

In vitro cytotoxicity profiling may be valuable for selecting compounds for further analysis, identifying compounds with particular mechanisms of action and potentially predicting in vivo biological response. High-throughput screening (HTS) technique allows to evaluate the cytotoxic activity of thousands of individual small molecules in a short time.
The MTS assay as a cytotoxicity test is routinely used in IMTM HTS facility. This assay was validated on 10 cell lines (8 cancer cell lines and 2 non-cancer cell lines) in 384 as well as 1536 well plate format. 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, quantitative) 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 type.
Some compounds did not affect the cell viability in any cell type, whereas other were cytotoxic to all cell types at similar concentrations or exhibited cell type – specific cytotoxicity. The presentation will focused on compounds with cell type – specific cytotoxicity. Observed cell line- cytotoxic activity relationships will be discussed.

Acknowledgment

This study was supported by the the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133, and CZ-OPENSCREEN, LM2018130), and the IGA_LF_2022_033 (Palacky University in Olomouc).

Drug repurposing for treatment of neurodegenerative diseases

Jiri Rehulka[1]

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

Abstract

Drug repurposing is an approach that identifies new indications for approved drugs. Because this strategy reduces the cost of the drug development and speeds up the drug discovery process, it is often used for rare and orphan diseases. Among others, this approach has been applied also on neurodegenerative diseases, however several rare indications still miss the effective treatment. In the talk will be presented the cell-based model of a neurodegenerative disease and high-throughput assay for identification of small-molecule inhibitors that could be used for management of rare neurodegenerative disease.

Acknowledgment

This work was supported by the Czech Science Foundation (reg. No. 18-26557Y), by grants from the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133, CZ-OPENSCREEN, LM2018130 and Czech-BioImaging, LM2018129), the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/ 0.0/0.0/16_019/0000868) and IGA_LF_2021_038 (Palacky

Path to finding novel adenosine receptors agonists and antagonists

Kateřina Ječmeňová[1], Soňa Gurská[1], Jana Kotulová[1], Marián Hajdúch[1], Petr Džubák[1]

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

Abstract

Adenosine receptors are G protein-coupled receptors (GPCR). There are four types of adenosine receptors (A1, A2A, A2B and A3). They play roles in several pathological conditions, including cancer. The finding of new agonists and antagonists as potential anticancer drugs is challenging. We use a fluorescence imaging plate reader (FLIPR) to screen newly synthesized compounds on reporter cell lines for adenosine receptors. After activating the receptor by an agonist, calcium release occurs from the endoplasmic reticulum. At the same time, apoaequorin is activated by the addition of coelenterazine. Upon calcium binding, active aequorin in the reporter cell line oxidizes coelenterazine into coelenteramide, causing the production of CO2 and emission of light.
Another approach for receptor studying is cell live-imaging. We use a fluorescent probe CELT-171, an antagonist, to visualise the human A3 receptor. By measuring the fluorescence signal level, we can deduce whether the compound competes for the same binding site on the receptor or not. Although this method is suitable for discovering drugs binding to A3 receptor, we can not distinguish between an agonist and an antagonist. In this case, a combination with FLIPR would provide more accurate results.

Acknowledgment

This project was supported by IGA_LF_2022_033 (Palacky University in Olomouc), the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133 and CZ-OPENSCREEN, LM2018130).

Triterpenoid pyrazines and pyridines

Jiří Hodoň,[a] Ivo Frydrych,[b] Zdeňka Trhlíková,[a] Jan Pokorný,[a] Lucie Borková,[a] Sandra Benická,[a] Martin Vlk,[c] Barbora Lišková,[b] Agáta Kubíčková,[b,d] Martina Medvedíková,[b] Martin Pisár,[a] Jan Šarek,[b] Viswanath Das,[b,d] Anna Ligasová,[b] Karel Koberna,[b] Petr Džubák,[b] Marián Hajdúch,[b] Milan Urban.[*,b]*

[a]Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc, 17. listopadu 1192/12, 771 46 Olomouc, Czech Republic.
[b]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 1333/5, 779 00 Olomouc, Czech Republic.
[c]Czech Technical University in Prague, Faculty of Nuclear Sciences and Physical Engineering, Břehová 7, 115 19 Prague 1, Czech Republic.
[d]Czech Advanced Technologies and Research Institute (CATRIN), Institute of Molecular and Translational Medicine, Palacký University Olomouc, Křížkovského 511/8, 77900 Olomouc, Czech Republic.

Abstract

Triterpenes are natural compounds that have various biological activities including antimalarial [1], antileishmanial [2], anti-HIV [3], anti-inflammatory [4], and many others [5]. Among those activities, antitumor activity is probably the most studied and important. Many research groups have been preparing semisynthetic triterpenes with high and selective cytotoxicity against cancer cells [6]. Triterpenes containing a heterocycle fused to their skeletons are one of the largest and most important classes of such compounds [7–11].

The main aim of this work was to investigate the cytotoxicity of triterpenoid pyridines and pyrazines and to find possible structure-activity relationships between triterpenoid 3-oxoderivatives and their corresponding pyrazines and pyridines. Seven triterpenoid oxocompounds 1a–7a (Figure 1), representatives of five common terpenoid skeletons were chosen because their derivatives were often found to be highly cytotoxic [5].

Citation

[1]e Silva, Luiz Francisco Rocha, et al.; European Journal of Med. Chem. 102 (2015): 398-402.
[3]Sousa, Maria C., et al.; PloS one 9.3 (2014): e89939.
[4]Bednarczyk-Cwynar, Barbara, et al.; Frontiers in Pharmacology 7 (2016)
[5]Dzubak, Petr, et al.; Natural product reports 23.3 (2006): 394-411.
[6]Heller, Lucie, Anja Obernauer, René Csuk.; Bioorg. & Med. Chem. 23.13 (2015): 3002-3012.
[7]Kvasnica, Miroslav, et al.; Natural product reports 32.9 (2015): 1303-1330.
[8]Urban, Milan, et al.; Journal of natural products 70.4 (2007): 526-532.
[9]Haavikko, Raisa, et al.; Medchemcomm 5.4 (2014): 445-451.
[10]Pokorny, Jan, Lucie Borkova, and Milan Urban.; Current Med. Chem. 25.5 (2018): 636-658.
[11]Urban, Milan, et al.; Bioorganic & medicinal chemistry 20.11 (2012): 3666-3674.

Biological evaluation of betulinic acid derivatives with aryl moiety via Suzuki-Myiaura cross-coupling reaction

Ivo Frydrych[1], Lucie Borková[1], Barbora Vránová[2], Barbora Lišková[1], Soňa Gurská[1], Petr Džubák[1], Marián Hajdúch[1], Milan Urban[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2]Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic

Abstract

In this work, a large set of betulinic acid derivatives modified with various aromatic substituents at the position C-3 were prepared via Suzuki-Myiaura cross-coupling. All compounds were tested for their in vitro cytotoxic activity in 8 cancer and 2 healthy cell lines. Derivatives 6h, 6i, and 6o had the lowest IC50 in the CCRF-CEM cell line (0.69 – 4.0 μM), therefore, they were selected for the evaluation of the mechanism of action. First, the effect of 6h, 6i, and 6o on cell death induction was studied. To our surprise, we have not detected almost any apoptotic cells, even following a long-time exposure of CCRF-CEM cells to the compounds. On the other hand, a dramatic cell number decrease was observed, proportional to the time of the compound's exposure. Based on this data it was concluded that the effect of compounds is cytostatic rather than cytotoxic, which was further confirmed by subsequent studies of the impact of 6h, 6i, and 6o on the cell cycle. Detailed cell cycle analysis revealed a block in the G1 phase accompanied by reduced expression of phosphorylated forms of the RB protein as well as cyclin A protein. Evaluation of the pharmacological properties of the most promising compounds revealed their high stability in the presence of phosphate buffer, human plasma, and microsomes and limited permeability determined using permeability through artificial membrane (PAMPA) and cell permeability assay: Caco-2 and MDCK-MDR1 cell lines.

Acknowledgment

This project was paid by the Ministry of Health of the Czech Republic (NV19-03-00107).

DNA synthesis of the leading and lagging strands

[1] *Dávid Lukáč,* [1] *Zuzana Machačová,* [1] *Pavel Moudrý*

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

Abstract

DNA synthesis of the leading and lagging strands works independently and cells tolerate single-stranded DNA generated during strand uncoupling if it is protected by RPA molecules. Natural alkaloid emetine is used as a specific inhibitor of lagging strand synthesis, uncoupling leading and lagging strand replication. Here, by analysis of lagging strand synthesis inhibitors, we show that despite emetine completely inhibiting DNA replication: it does not induce the generation of single-stranded DNA and chromatin-bound RPA32 (CB-RPA32). In line with this, emetine does not activate the replication checkpoint nor DNA damage response. Emetine is also an inhibitor of proteosynthesis and ongoing proteosynthesis is essential for the accurate replication of DNA. Mechanistically, we demonstrate that the acute block of proteosynthesis by emetine temporally precedes its effects on DNA replication. Thus, our results are consistent with the hypothesis that emetine affects DNA replication by proteosynthesis inhibition. Emetine and mild POLA1 inhibition prevent S-phase poly(ADP-ribosyl)ation. Collectively, our study reveals that emetine is not a specific lagging strand synthesis inhibitor with implications for its use in molecular biology.

Acknowledgment

Czech Science Foundation grant no. 20-03457Y.

Cannabidiol and cannabis extracts antagonize platinum-based chemotherapy via cellular uptake

Tereza Buchtova[1], Lucie Bereseova[1], Katarina Chroma[1], Martin Mistrik[1]

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

Abstract

Cannabinoids and cannabidiol belong among constituents of cannabis sp. (marijuana plant). The substances alone or as a part of cannabis products are frequently used by cancer patients to attenuate the chemotherapy-induced side effects. Every year, the presence of cannabis products on the public market grows while the illegality status weakens in an increasing number of countries. The FDA has approved several cannabis-derived drugs for various diseases and chemotherapy-induced nausea and vomiting. Numerous cannabinoid-mediated anticancer effects have been reported, further motivating cancer patients to support their therapy with such co-treatment. However, here we present alarming data that CBD and cannabis extract effectively counteract platinum-based chemotherapy. We show that even low concentrations of cannabinoids reduced the toxicity of the drugs. Platinum drug resistance was accompanied by a decrease in various stress markers. Additional experiments based on analysis of intracellular platinum content suggest a substantial effect on cellular transport or retention of platinum drugs.

This study provides essential information about the potential interactions of platinum-based drugs with cannabis products, which patients and physicians should consider. Furthermore, this project may evoke further cancer research on the double-edged properties of cannabis products in cancer treatment.

Acknowledgment

The work was supported by project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), Grant agency of the Czech Republic: GACR 20-28685S, Internal grant of University of Palacky IGA_LF_2022_038, and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

A PARylation siRNA screen to identify novel proteins involved in PAR signaling

Katarína Chromá[1], Pavel Moudry[1]

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

Abstract

Emetine is a natural product alkaloid, many decades known as a specific chemical inhibitor of Okazaki fragments synthesis. Inhibition of lagging strand can cause replication stress, which is considered as a source of genome instability. One of the markers of the replication stress is formation of single-stranded DNA (ssDNA). Accumulation of ssDNA can occur on either leading or lagging strand by uncoupling the polymerases or by unregulated unwinding by helicase exposing the strands. In this work we propose the idea that DNA replication inhibition by emetine is not caused by strand uncoupling but rather with protein synthesis inhibition. Our in vitro studies focused on strand uncoupling markers after emetine exposure in comparison to adarotene, inhibitor of PolA. Our results revealed that emetine does not cause ssDNA accumulation followed by chromatin bound RPA32 loading and activating DNA damage response pathway. In line with this, emetine did not activate replication checkpoint. Moreover, inhibition of protein synthesis precedes inhibition of DNA replication after treatment with emetine. Collectively, we showed that emetine completely blocks DNA replication and should not be used as lagging strand inhibitor.

Acknowledgment

Czech Science Foundation grant no. 20-03457Y.

The role of PARP1 in DNA replication

Zuzana Machačová[1], Pavel Moudrý[1]

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

Abstract

Chromosomal DNA replication is an essential and strictly regulated cellular process. In human cells, many replication origins are activated during the S-phase of the cell cycle, from which the two strands unfold to form the replication fork. In unperturbed S-phase cells, poly(ADP-ribosyl)ation (PARylation) signal is detected and localized within sites of DNA replication. Enzymes with the ability to catalyze the transfer of ADP-ribose to target proteins are called poly(ADP-ribose)polymerases (PARPs) and plays an important role in various cellular processes, including DNA synthesis and repair. Inhibition of PARPs causes aberrant acceleration of replication fork progression and leads to DNA damage, however, the mechanism by which PARP affects fork speed is poorly understood. Due to the clinical use of PARP inhibitors in the treatment of BRCA mutated ovarian and breast cancer, it is necessary to identify the mechanism of their action on replication fork dynamics in order to make cancer treatment more effective.

Acknowledgment

This project was supported by the Czech Science Foundation (grant no. 20-03457Y).

Senescence: Stable or Irreversible?

Matthew Lacey[1], Martin Mistrík[1]

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

Abstract

Senescence is a stable form of cell cycle arrest which is commonly defined as an irreversible state of cell cycle arrest. Senescence in the context of human health is a complex topic, with senescent cells being implicated in a number of age related diseases and the general decline of health as a person ages. The possibility that senescence could be reversed is a contentious field, which does not appear to enjoy a large degree of publicity.

This talk describes the research into the reversion of cells from stress induced premature senescence and their return into the cell cycle, also discussing the challenges and implications of this line of study.

Acknowledgment

This work was financially supported by the IGA_LF_2022_038 grant.

Biological evaluation of 225Ac-PSMA derivatives for alpha therapy in prostate cancer

Zbynek Novy[1]

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

Abstract

One of the targets in fight with the prostate cancer is so called prostate specific membrane antigen (PSMA). Specific proteins (such as PSMA-11) binding PSMA could be radiolabeled and used as diagnostic or therapeutic radiopharmaceuticals. Current approach in this field is to employ alpha emitters; such approach is called target alpha therapy (TAT). This study presents results of the biological evaluation of four new 225Ac-labeled PSMA ligands for TAT.

Abstract

The compounds (FR54, FR55, FR94 and FR96) were tested in mice model using human prostate cell line LNCaP. Ex vivo biodistribution was evaluated in various time points (1, 4, 24, 48, 72 and 120 h p.i.) by dissecting the animals and measuring radioactivity in 12 different organs. The liver, kidneys and tumors were then examined by means of histology (H&E staining, PSMA, gamma H2AX and Ki67).
Results: The principal organs accumulating tested PSMA ligands were tumor and kidneys with vastly higher uptake compared to other evaluated organs (up to 120 %ID/g). Tumor-to-blood ratio was 1 490 in case of FR94. Histology showed necrotic lesions in the tumors, high PSMA expression in tumor tissue, DNA damage in FR94/96 treated tumors and higher cellular proliferation in untreated tumors.

Conclusion

Biodistribution study revealed favorable biodistribution of all tested PSMA ligands with extremely high tumor-to-blood ratios. The histology confirmed promising properties of these new potential TAT compounds.

Acknowledgment

The work was supported by IGA LF 2020_007, by the Ministry of Education, Youth and Sports of the Czech Republic (EATRIS-CZ LM2015064) and by the European Regional Development Fund–Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Positron emission tomography imaging of Burkholderia cepacia complex infection using gallium labelled ornibactin

Bendova K.[1], Novy Z.[1,2], Hajduch M.[1,2], Raclavsky V.[3], Novotny R.[3], Petrik M.[1,2]

[1] *Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic*
[2] *Czech Advanced Technology and Research Institute, Palacky University Olomouc*
[3] *Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University Olomouc*

Abstract

Bacteria from *Burkholderia cepacia* complex (BCC) may cause severe hospital acquired pneumonia in immunocompromised patients. Due to these infections being quite unpredictable and often resistant to various antibiotics and disinfectants, it is necessary to diagnose the causative agent quickly, so that adequate treatment could be initiated. In this study, we present the use of radiolabelled siderophore ornibactin, chelator produced by BCC for iron scavenging, for imaging of BCC infections by positron emission tomography.

Ornibactin was radiolabelled with gallium-68 with high radiochemical purity, that was determined on RP-HPLC. ^{68}Ga-Ornibactin showed high stability in human serum, low protein binding values and hydrophilic properties. The complex showed high *in vitro* specificity for bacteria from BCC compared to other respiratory pathogens. *Ex vivo* and *in vivo* results from experiments showed rapid pharmacokinetics and renal excretion of the complex. In BCC infection models, the accumulation of the complex in the site of infection was displayed by PET imaging.

Given the optimal *in vitro* characteristics and *in vivo* biodistribution, ^{68}Ga-Ornibactin complex seems to be a promising compound for BCC infection imaging.

Acknowledgment

The authors received financial support from the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), the European infrastructure for translational medicine EATRIS-ERIC-CZ (No. LM2018133), the Internal Grant Agency of Palacky University (Project number: IGA_LF_2022_012) and the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

THE USE OF NANOBIT TECHNOLOGY IN HTS: NOVEL APPROACH FOR IDENTIFICATION OF CFTR CORRECTORS AND MODIFIERS

Martin Ondra1,2, Amanda Centorame3,4, Lukáš Lenart1, Daciana Catalina Dumut2,3, Juan B. De Sanctis1,2, Danuta Radzioch1,3,4, Marian Hajduch1,2,

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

[2] *Czech Advanced Technology and Research Institute, Palacky University Olomouc*

[3] *Faculty of Medicine, McGill University, Montreal, QC, Canada*

[4] *RI-MUHC, Montreal, QC, Canada*

Abstract

Cystic fibrosis is a lethal autosomal recessive disease caused by mutations in CFTR protein. CFTR is an epithelial membrane protein with the function of ions and water transport regulation. Mutations in CFTR can affect its function, localization, or stability. Up to now, more than 2 000 mutations in CFTR have been identified. New approaches to monitoring physiological levels of CFTR have been explored to create new feasible HTS models for such a large number of mutations. CRISPR/Cas9 mediated knock-in of HiBiT tag1 into the genomic locus of the extracellular loop of WT-CFTR could be the new approach to monitoring the localization of CFTR in the membrane. Cells expressing WT-CFTR-HiBiT can further be used for a second CRISPR/Cas9 modification creating various screening tools for given mutations. Human bronchial epithelial cells (16HBE14o-) expressing WT CFTR were used for the CRISPR/Cas9 knock-in of the HiBiT tag. HiBiT tag was successfully introduced by CRISPR/Cas9 into two different positions of the 4th extracellular loop of CFTR and functionally validated. Furthermore, clones expressing the HiBiT tag were tested and validated for HTS. The approach for inserting CFTR pathogenic mutations into WT-CFTR-HiBiT with endogenous expression has been established.

Acknowledgment

This project was supported by IGA_LF_2022_012, the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133), Project ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868.

Citation

Schwinn, Marie K., et al. "CRISPR-mediated tagging of endogenous proteins with a luminescent peptide." ACS chemical biology 13.2 (2017): 467-474.

Tracing c-Myc Endogenous Expression by NanoBiT Technology for Small Molecules Identification

Agáta Kubíčková[1], Marian Hajdúch[1]

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

Abstract

Although c-Myc is well known as a proto-oncogene, its structure and function as a transcription factor make it a problematic therapeutic target. To determine c-Myc inhibitors, we have developed a high-throughput screening (HTS) system established on a combination of CRISPR/Cas9 and NanoBiT technologies. This system uses a cell-based assay to detect c-Myc inactivation in an HTS format. It arised from two pure clones of a stable osteosarcoma cell line. In each clone, one of the main c-Myc isoforms is tagged (p64 or p67). A short tag known as HiBiT, consisting of only a few amino acids, was integrated at the C-terminus of the c-Myc protein using CRISPR/Cas9, resulting in the expression of the two tagged protein isoforms from the endogenous c-Myc locus. Using chemiluminescence readout as a surrogate for c-Myc expression, we validated the gained cellular models using siRNA and known small molecule modulators of c-Myc expression. We will use this system to perform quantitative HTS against approximately 2,600 existing bioactive compounds from two different chemical libraries. Our work has also revealed the unique advantage of combining novel technologies in accelerating drug discovery for c-Myc-targeted anti-cancer therapies.

Acknowledgment

Supported by EATRIS, OpenScreen and IGA_LF_2022_033.

Citation

Schwinn, Marie K., et al. "CRISPR-mediated tagging of endogenous proteins with a luminescent peptide." ACS chemical biology 13.2 (2017): 467-474.

Cell cycle analysis by high content microscopy and immunofluorescence staining

Alzbeta Srovnalova[1], Jiri Rehulka[1], Sona Gurska[1], Petr Dzubak[1], Marian Hajduch[1]

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

Abstract

The cell cycle and its regulation is a fundamental process in every single cell. The tumor cells are capable to overcome the precision control of the cell cycle mechanism leading uncontrolled replication. For this reason, many anticancer drugs are designed to block the tumor cells' division. The reporter system of fluorescent ubiquitination cell cycle indicator (FUCCI) based on two oscillating fluorescently tagged proteins Cdt1 DNA licensing factor and its inhibitor Geminin enables the analysis of each cell cycle phasis. Based on this method, a lot of potential cell cycle modulators can be measured in live cells. A comparison of two methods was performed in two cancer cell lines. The cell cycle analysis of the CEM cell line was performed using flow cytometry, while analysis of cell cycle changes in the U2OS cell line was performed using high-content microscopy. Groups of chemicals exhibiting an IC50 of less than 10 micromolar in the CEM cell line were selected. The group of benzodiazepine derivatives was chosen for further analysis. The alpha-tubulin immunodetection was performed and significant changes in the mitotic spindle were observed. The palbociclib bounded to carborane moiety expressed cytotoxic effect in CEM cell lines, while it was non-toxic in U2OS, but effective as a G1 phase blocker. The high-throughput screening of active molecules based on FUCCI system can reveal new drugs with potency to block the cell cycle in one of the phases (G1, G1/S, S-G2/M).

Acknowledgment

This work was supported by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2022_038).

Citation

Sakaue-Sawano et al. Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. Cell. 2008 Feb 8;132(3):487-98. doi: 10.1016/j.cell.2007.12.033. PMID: 18267078
Koh et al. A quantitative FastFUCCI assay defines cell cycle dynamics at a single-cell level. J Cell Sci. 2017 Jan 15;130(2):512-520. doi: 10.1242/jcs.195164. Epub 2016 Nov 25. PMID: 27888217.

New NPL4 protein inhibitors and their mechanism of action

Martin Loffelmann[1], Zdeněk Škrott[1], Pavel Štarha[2], Martin Mistrík[1]

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

[2]Department of Inorganic Chemistry, Faculty of Science, Palacky University in Olomouc, Olomouc, Czech Republic

Abstract

Protein degradation systems are crucial for the proper function and viability of cells. Cancer cells are even more dependent on these systems due to an excess of proteins with an incorrect conformation or mutations. One of these systems is p97 pathway, which cooperates with the ubiquitin-proteasome system. p97 has several cofactors, among which Nuclear protein localization homolog 4 (NPL4) plays an essential role. Dithiocarbamates (DTCs) are small organic compounds that are used in various therapies and agriculture. One of their features is the ability to form complexes with metals. A DTC-copper complex (bis(diethyldithio-carbamate)-copper (CuET) is an inhibitor of NPL4, which causes aggregation of NPL4, heat shock response, and accumulation of polyubiquitinated proteins. Sixteen other compounds from the group of DTCs were selected as potential NPL4 inhibitors. The DTC-copper complexes were screened for their effect on NPL4 and cytotoxicity. The screening resulted in a positive correlation. Both effects, the cytotoxicity and the effect on NPL4, were detected in ten of sixteen DTC-copper complexes. The interaction between DTC-copper complexes and NPL4 was confirmed by Drug affinity responsive target stability (DARTS) method, which was performed on the isolated and purified protein.

Acknowledgment

This work was financially supported by IGA_LF_2022_038 (Palacky University in Olomouc) and 20-28685S (GAČR).

Prolonged fever-like mild hyperthermia in cancer treatment

Jiří Řehulka[1], Zdenek Skrott[1], Katarina Chroma[1], Marketa Dvorakova[1], Lucie Beresova[1], Tereza Buchtova[1], Pavel Moudry[1], Martin Mistrik[1]

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

Abstract

Due to malignant transformation, cancer cells are characterised by elevated levels of genotoxic, replication or proteotoxic stresses, which makes them vulnerable to various external conditions, including increased temperature. Hyperthermia, i.e. exposure of the tumour to higher temperatures, represents a very effective therapeutic modality usually combined with standard chemotherapy and radiation. Established thermal therapy is usually based on the heating of tissues to 40-45°C for a short period, either to sensitise the tumour to conventional treatments or to induce its regression. However, the potentials and possible usefulness of continuous mild fever-like thermotherapy (below 40°C) are largely unexplored. Here, we present an intriguing efficacy of prolonged mild hyperthermia in various cancer models, together with the identification of affected cellular pathways and potential combinations with clinically used drugs.

Acknowledgment

The study was supported by an Internal grant of Palacky University (IGA_LF_2022_038).

Effect of opioid and cannabinoid receptors expression on survival of patients with pancreatic cancer

[1]MONIKA VIDLAŘOVÁ, [1]JOSEF SROVNAL, [1,2]EMIL BERTA, [3]LUBOMÍR VEČEŘA, [1]SOŇA GURSKÁ, [1]ALONA ŘEHULKOVÁ, [1]PAVLA KOUŘILOVÁ, [1]MARIÁN HAJDÚCH

[1] Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2] Ringerike Hospital, Hønefoss, Norway
[3] Tomas Bata Regional Hospital, Zlin, Czech Republic

Abstract

Pancreatic cancer is one of the most common causes of cancer-related death in the world and is characterized by frequent metastases. In patients with pancreatic cancer, opioids and cannabinoids are often used to treat pain, nausea and side effects of chemotherapy, but they can affect patients survival. These analgetics work though opioid and cannabinoid receptors. Receptors and their pathways affect tumor progression and methastases. In our study, we investigated expression of opioid receptor mu (OPRM), kappa (OPRK), delta (OPRD) and nociceptin (OPRL) and cannabinoid receptor 2 (CNR2) in the tumor tissue of patients with pancreatic cancer and we analyzed relationship between their gene expression and patients survival. We also analyzed the effect of the used analgesia (piritramide/morphine) on patients survival.

Methods

Gene expression of OPRM, OPRK, OPRD, OPRL and CNR2 was analyzed in RNA purified from tumor tissues in 71 pacients with pancreatic cancer. RNA purification was done using precipitation method from TRIzol lysates. Expression of markers was detected using real-time RT-PCR on LightCycler 1536 from Roche. B-actin gene expression was used for gene expression normalization. Specific cut-off values were calculated for each marker using maxstat R software, ver. 3.3.1. Relationship between expression of OPRM, OPRK, OPRD, OPRL and CNR2 in tumor tissue and patients survival was analyzed using COX regression, Kruskal-Wallis/ANOVA test and Kaplan-Meier method.

Results

We investigated 71 patients (31 females and 40 males, average age 63 years), in clinical stadium I-III and radicality R0 and R1. On univariate survival analysis, we found that patients receiving morphine in the postoperative period had statistically significantly longer overall survival than patients receiving piritramide (log-rank test, $p = 0.003$). The univariate survival analysis revealed that OPRD negative patients (i.e. with expression of OPRD receptor on pancreatic cancer cells lower than 9.425) had significantly longer overall survival (log-rank test, $p = 0.007$). For all other studied receptors the lower/higher expression had no statistically significant effect on overall survival.

Conclusion

It appears that new approaches targeting opioid receptors may have a future, however, they require further investigation and validation.

Acknowledgment

This study was supported by Ministry of Health of the Czech Republic [NV18-03-00470]; Palacky University Olomouc [LF 2022_011]; European Regional Development Fund [ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868) and Programme EXCELES LX22NPO5102.

Autophagy induction promotes the clearance of intracellular tau P301S repeat domain aggregates seeded by exogenous tau R3 fibrils

Narendran Annadurai[1], Viswanath Das[1]

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

Abstract

Limited proteolysis of pathogenic proteins such as tau and α-synuclein plays a critical role in the pathogenesis of neurodegenerative diseases. In addition, the autophagy-lysosomal system is impaired in patients with primary tauopathies. Our study focused on how the exogenous tau R3 fibrils dysregulate the lysosome-autophagy pathway and how activating these cellular degradation mechanisms eliminates the intracellular tau aggregates in tau biosensor cells.

Methods

Biosensor cells were seeded with tau R3 fibrils, and cells were collected time-dependently and processed for Western blot (WB) to detect the level of autophagy activity. We performed an MTT to find the IC50 of the autophagy inducers and inhibitors. Then, the seeding experiment was carried out in the presence or absence of compounds. Finally, the level of triton-insoluble fractions of intracellular tau aggregates in seeded biosensor cells in the presence or absence of compounds was analysed.

Results

WB results showed that the LC3A/B-II (autophagy marker) level was not increased in R3 fibrils seeded biosensor cells. An increase in p62 level and no change in LAMP1 level was observed. IC50 of the compounds were determined. Treating R3 fibrils seeded biosensor cells with autophagy inducers but not autophagy inhibitors reduced intracellular tau aggregation. R3 fibrils seeding induced accumulation of triton-insoluble tau aggregates, and autophagy induction reduced the level of triton-insoluble tau aggregates.

Acknowledgment

The work was supported by the European Regional Development Fund (ENOCH, CZ.02.1.01/0.0/0.0/16_019/0000868).

Clinical application of liquid biopsy

Pavel Stejskal[1,2], Josef Srovnal[1,3], Alona Řehulková[1,3], Veronika Černohorská[1], Marián Hajdúch[1]

[1] Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2] Department of Biochemistry and Biophysics, University of California San Francisco
[3] University Hospital Olomouc, Olomouc, Czech Republic

Abstract

Over the past decade, the liquid biopsy (LB) concept has been in the spotlight and became the subject of translational research. It is based on the analysis of tumors using biomarkers circulating in the body fluids, e.g., peripheral blood. Circulating tumor cells (CTCs), circulating tumor DNA/RNA (ctDNA/ctRNA), and circulating extracellular vesicles (ctEVs) are among the most frequently discussed LB biomarkers. This minimally invasive promising tool can facilitate early detection as well as monitoring of minimal residual disease and treatment efficacy. However, the lack of standardization and validity makes its clinical implementation challenging.
The purpose of this study is to exploit recent methodological progress and to contribute to LB standardization and relevancy. In this context, we aim for the integration of different biomarkers and increase the understanding of their pathophysiology. Here, we demonstrate the CTCs detection using CytoTrack CT11TM device based on the EpCAM immunofluorescence and pre-enrichment independent CTCs capture from the whole blood samples. We also focused on ctDNA quantification as well as its release mechanisms.

Acknowledgment

This work was financially supported by grants awarded by Palacky University Olomouc (LF 2022_012), ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868, Ministry of Health of the Czech Republic (NV18-03-00470), Ministry of Education, Youth and Sport of the Czech Republic (LM2018132) and European Union - Next Generation EU (LX22NPO5102).

Czech multi-omics cohort from a proteomics perspective

Jarmila Stanková[1], Jana Vrbková[1], Dušan Holub[1], Petr Džubák[1], Marián Hajdúch[1]

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

Abstract

Czech multi-omics cohort consisting of healthy blood donors (n=127) was sampled for multi-omics analysis. In this study, we focused on plasma samples suitable for proteomics analysis. The samples were prepared with Filter-Aided Sample Preparation protocol, peptides were purified on OMIX tips (Agilent Technologies) and measured by LC-MS with HCD fragmentation and precursor/fragment mass detection by Orbitrap. During LC-MS analysis, spiked-in standards were used to follow a quality across the multiple runs. Data were searched by ProteomeDiscoverer 2.5 (Thermo), including the INFERYS rescoring node. We were able to identify 3014 proteins in total, and 2918 in total were also quantified. The following data analysis includes a comparison of technical replicates, the influence of missing values, and the dataset's normalization. We compare a group of samples collected from male and female donors to study the differential protein expression related to sex. The quality of obtained data was evaluated on several levels.

Acknowledgment

This study was supported by the European Regional Development Fund (Project ENOCH No. CZ.02.1.01/0.0/0.0/16_019/0000868), the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133), and IGA_LF_2022_033 (Palacky University in Olomouc).

Claire: an open-source system for deep interpretation of tandem mass spectra

Miroslav Hruska[1, 2], Dusan Holub[1], Petr Dzubak[1], Marian Hajduch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2]Department of Computer Science, Faculty of Science, Palacky University Olomouc

Abstract

Detection of peptides from mass spectra of proteome resides at the core of computational proteomics analyses. Although various detection methods exist, severe problems of false negatives and false positives arise when focusing on detecting rare peptides. To improve this situation, we have developed Claire: an open-source Bayesian system incorporating peptide prior probabilities into detection. In practice, Claire allows detection against large pre-computed peptide databases while utilizing three-level data indexation to enable fast and memory-efficient interpretation of mass spectra. Claire substantially outperformed state-of-the-art software when employed for detecting amino acid substitutions originating from single nucleotide variants. Further, our mathematical framework derived posterior probabilities that were much more precise than those of other commonly used approaches. Although we primarily investigated human protein data, Claire's ability to interpret large databases allows unrestricted analyses against all known organisms—an interesting venue for further research.

Acknowledgment

This work was supported in parts by the Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/0000868, CZ.01.1.02/0.0/0.0/16_084/0010360, LM2018130, LM2018131), Technology Agency of the Czech Republic (TE02000058, TN01000013), Ministry of Health of Czech Republic (NV16-32318A, NV16-32302A).

Detection of kinase activity by MALDI-TOF mass spectrometry

Lenka Hrubá[1], Petr Džubák[1], Marián Hajdúch[1]

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

Abstract

Protein kinases transport phosphate group from ATP to substrate molecules, specially at serine, threonine or tyrosine residues, thereby affecting their function. Phosphorylation can be activating as well as inhibitory. Kinases have a number or irreplaceable functions in the human organism and are interesting targets in the development of target therapy. Kinase activity can be measured by various methods, such as western blotting, enzyme-linked immunosorbent assay (ELISA), intracellular flow cytometry, ATP assay etc. In our project we developed method for kinase activity detection by MALDI-TOF mass spectrometry. Method is based on direct detection of reaction substrate and product, whereas each phosphate group creates shift in m/z 80 Da. Thanks to this, we are able to distinguish the number of phosphorylated sites. Advantages of our method is its simplicity of implementation, accuracy in the identification of substrate and product of enzyme reaction, high sensitivity of detection compare to colorimetric methods and suitability for high throughput screening. Method was optimized for all four isoform of MARK kinases (MARK1-4) and for glycogen synthase kinase-3 alfa and beta.

Acknowledgment

The work was supported by the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN – LM2018130 and EATRIS-CZ – LM2018133), Grant Agency of Czech Republic (19-08124S), an internal grant of Palacky University (IGA_LF_2022_033), and the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Computer security

Jan Vidlař[1]

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

Abstract

Biref report about network and computer secuity at the IMTM. Informations for users about basic rules for work in the network. Introduction into the remote connection rules and possibilities.

Data management on IMTM, Object storage and OwnCloud

Peter Pavliš[1]

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

Abstract

Data produced on IMTM must be protected against accidental failures as well as deliberate attacks. The scientific projects generate data of various types: personal, configuration data, raw data, calculated data, metadata. To ensure the security and continuity of scientific operations, each type of data requires a slightly different way of handling. Users can use the existing IMTM information infrastructure to secure their data. There are several specific backup devices available. In some cases, the user can choose the degree of redundancy of the stored data. Recently, a highly scalable object storage CEPH with a capacity of 2.5PB was put into operation. It provides API for machine access using the S3 protocol, or the more user-friendly OwnCloud technology.

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

Barbora Koblihová[1], Rastislav Slavkovský[1], Jiří Drábek[1], Ivo Frydrych[1], Marián Hajdúch[1], Robert Mikulík[2], Michal Haršány[2]

[1] Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2] 1st Department of Neurology, St. Anne's University Hospital Brno, Brno, Czech Republic

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. 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.
Methods: In this study, we are detecting somatic mutations of blood cells in 4 cohorts of patients aged >70 years (presence/absence of carotid atherosclerosis or stroke). In the case of 24 patients, we also analyzed mutations of cells from carotid plaques. CHIP mutations were identified by the method of massive parallel sequencing using a targeted DNA custom panel (Qiagen) containing 38 CHIP-related genes.

Results and conclusions

It was shown that ~70 % of sequenced patients (n=226) are positive, with mutations observed most often in genes DNMT3A and TET2 (~50 % of all mutations), regardless of the study cohort. The average cumulative frequency of variants is the highest in the group of patients with both atherosclerosis and stroke (av. cum. VAF = 8.3 %), and the lowest in the control group of patients without stroke and atherosclerosis (av. cum. VAF = 3 %). The presence of CHIP mutations was also confirmed in patient samples of plaques. As a next step, statistical analysis will be done to find any clinical association and CHIP.

Acknowledgment

Funded by ENOCH project CZ.02.1.01/0.0/0.0/16_019/0000868 and IGA LF UP 2022_012.

Advanced Animal Models: Orthotopic Implantation and Transvocal Intratracheal Instillation

Miroslav Popper[1]

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

Abstract

The variety of the specific research models of laboratory mice is wide. Sometimes you need to administer an agent or a test item systematically, but in other cases, you must apply an agent or a test item locally. A few advanced techniques for the administration of agents are shown here.
In the first group are the orthotopic implantation laboratory models.
Intrasplenic administration is suitable for administering agents in the liquid form up to 50 µL of volume. The spleen can be removed 1-2 minutes after administration. It is a good model for metastatic studies with the use of tumor cells (1.).
Intracecal administration is suitable for the administration of agents in the liquid form up to 20 µL of volume or xenograft up to the size of 4 x 4 mm (2.).
Intrarectal administration is suitable for the administration of agents in the liquid form up to 10 µL of volume or xenograft up to the size of 4 x 4 mm (2.).
In the second group are the orthotopic infection laboratory models.
Transvocal intratracheal instillation is suitable for the administration of agents in liquid or bead form for up to 50 µL of volume and up to 200 µM of bead size (3.).
The orthotopic application techniques in the animals are a bit complicated to process, so you must prepare a suitable protocol.
The orthotopic laboratory animal models provide an organ-specific and/or a tissue-specific tumor growth environment or infection environment to study the onset and course of infection.

Acknowledgment

Support of mobility at Palacky University Olomouc II., 3 – Junior abroad (LF_10): CZ.02.2.69/0.0/0.0/18_053/0016919.

Citation

1. Dafflon C et al. An Intrasplenic Injection Model for the Study of Cancer Stem Cell Seeding Capacity. doi: 10.1007/978-1-0716-0747-3_20. PMID: 32705651.

2. Kasashima H et al. Mouse model of colorectal cancer: orthotopic co-implantation of tumor and stroma cells in cecum and rectum. doi: 10.1016/j.xpro.2021.100297. PMID: 33554135; PMCID: PMC7843928.

3. Lawrenz MB et al. Intubation-mediated intratracheal (IMIT) instillation: a noninvasive, lung-specific delivery system. doi: 10.3791/52261. PMID: 25490457; PMCID: PMC4354010.

Application of chemoinformatics in drug design

Pavel Polishchuk[1]

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

Abstract

In silico tools are widely used to facilitate discovery of new promising biologically active molecules. They reduce costs by replacing of some wet-lab works, speed up the overall progress by selecting the most promising objects for further investigation, explain observed structure-activity relationship and help to design new molecules. We will overview the most typical cases of applications of chemoinformatics approaches and discuss their applicability, limitations, possible outputs and contribution to drug discovery projects. These will be approaches for discovery of primary hits, explaining of observed structure-property relationships, optimization of compound properties, target fishing, protein-protein interactions, etc.

Acknowledgment

This research was funded by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), ELIXIR CZ research infrastructure project (MEYS Grant No: LM2018131), Technology Agency of the Czech Republic (project TN01000013), and LTARF18013 INTER-EXCELLENCE grant of MEYS.

A new tool to perform automated molecular dynamics simulation and analysis

Aleksandra Ivanová[1], Olena Mokshyna[1], Pavel Polishchuk[1]

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

Abstract

Molecular dynamics is a commonly used method to explore conformational space, to investigate ligand pose stability and to estimate binding affinity of protein-ligand complexes. Although to run a simple simulation requires some effort and knowledge since there is no common convenient tool to combine all preprocessing and analysis steps together.
In our study we implemented a sequential pipeline of the preparation, running, analysis and energy calculation steps to easily run simulations of the most popular molecular systems, such as proteins, protein-ligand complexes and protein-ligand-cofactor systems. Such an automated pipeline allows not only to reduce complexity of the method but also minimizes human involvement and increases robustness of the procedure.

Acknowledgment

The work was supported by the Czech Ministry of Education, Youth and Sports projects CZ-OPENSCREEN (LM2018130), EATRIS-CZ (LM2018133) and by the European Regional Development Fund project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868). The study was partially supported by the MEYS of the Czech Republic through the e-INFRA CZ (ID:90140).

Prediction of MARK4 inhibition by MM-GBSA method

Guzel Minibaeva[1], Aleksandra Ivanová[1], Pavel Polishchuk[1]

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

Abstract

Betulinic acid is one of the most investigated pentacyclic triterpenes because some of its derivatives are selectively cytotoxic against cancer cells, another serve as anti-HIV or antiprotozoal agents. Conjugates of cytotoxic derivatives of betulinic acid have been used to reach all three goals – to obtain compounds of high activity, high bioavailability, and to study their mechanism of action. All compounds prepared in this study were tested in vitro for their cytotoxic activity by standard MTS assay. Compounds with m-aminophenyl, m-hydroxyphenyl or p-hydroxyphenyl substituent in the position 3 were the most active compounds with cytotoxicity in the range 0.69 – 3.9 μM on CCRF-CEM cell line. Also, these compounds had a cytotoxicity of less than 10 M against the CEM-DNR and K562-TAX resistant cell line.

Knowledge of the ADME properties of semisynthetic triterpenes can be decisive for the selection of leading candidate(s) in our drug discovery program. Potential candidates showed sufficient stability in hepatic microsomes with low category of intrinsic clearance and low ability to diffuse through an artificial cellular membrane in PAMPA. The compounds with m-hydroxyphenyl substituent demonstrated good stability in human plasma and showed a small improvement in cellular permeability in our PAMPA model and cell permeability assay: Caco-2 and MDCK-MDR1 cell lines.
We have more answers for finding which type of the substituent in which position (o, m, or p) gives better result.

Acknowledgment

Supported by IGA_LF_2021_038 (Palacky University in Olomouc).

Mapping and exploring the MARK4 chemical space

Alina Kutlushina[1], Pavel Polishchuk[1]

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

Abstract

MARK4 is one of the popular targets against early- and mid-stage Alzheimer's disease. Strong and significant elevation of both of MARK4 expression levels and of MARK4–tau interactions in Alzheimer's disease brains has been observed. They lead to neurofibrillary tangles inside nerve cell bodies and are therefore associated with early-stage Alzheimer's disease.
In order to explore the MARK4 chemical space we took 814, 1278 and 24 tested molecules from IOCB, LOPAC and Enamine datasets respectively, from which 62, 9 and 1 molecules showed IC50 ≤ 2 μM against MARK4. Additionally, we collected 782 molecules with known activity to MARK4 from ChEMBL v. 30, 69 of them have IC50 less than 2 uM. As reference molecules we used molecules with known activity to kinases from ChEMBL v.30 and molecules from Enamine. The compounds were mapped by TMAP and visualised by Faerun. We discovered that the most studied chemical scaffold is 7H-pyrrolo[2,3-d] pyrimidine derivative. 63 compounds with this scaffold have an activity up to 20 μM, but 698 compounds have IC50 ≥ 50 uM. 2 compounds with pyrido[2,3-d]pyrimidine derivative are highly active MARK4 inhibitors with IC50 ≤ 0.005 uM, and 11 molecules with the same core have IC50 more than 2 uM. 10 molecules with pyrido[2,3-b]pyrazine derivative are highly active (IC50 ≤ 2 μM). Furthermore, we identified at least 5 unique scaffolds of discovered MARK4 inhibitors, around which the chemical space is poorly studied.

Acknowledgment

The work was supported by the Czech Ministry of Education, Youth and Sports projects ELIXIR-CZ (LM2018131), CZ-OPENSCREEN (LM2018130), EATRIS-CZ (LM2018133), MSMT-5727/2018-2 and by the European Regional Development Fund project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Stress response in Hypsibius exemplaris

Dominik Vítek[1], Tadeáš Frycák[2], Tomáš Fürst[3], Matthew Lacey[1], Martin Vavruša[2], Jiří Voller[1,2], Marián Hajdúch[1], Rastislav Sladkovský[1]

[1] Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2] Laboratory of growth regulators, Czech Republic
[3] Department of mathematical analysis and applications of mathematics, Czech Republic

Abstract

Tardigrades show remarkable resilience to diverse forms of stress, including desiccation, gamma irradiation, and heat shock. Molecular basis of this phenomenon remains unexplained as it is also unknown if the resistance to individual stress modalities has a common underpinning. In order to study protective mechanisms, we carried out transcriptomic profiling of H. exemplaris recovering from exposure to sublethal dose of ionizing radiation. We observed a uniquely high overexpression of genes (up to x*) related to several DNA damage response pathways. On the other hand, no remarkable change was observed in the genes encoding antioxidant defences.
Furthermore, we developed a method for the construction of dose-response curves from microscopy images of animal populations in 384-well plates. Classification of live/dead phenotypes is performed by a convolutional neural network. Using this method, we analysed if previous exposure to stress modifies the resistance to subsequent exposure to the same and other stressors. These experiments also suggest that induction of antioxidant mechanisms is not the principal defence mechanism of H. exemplaris against ionizing radiation.

Acknowledgment

Student’s grant competition - IGRÁČEK: DSGC-2021-0085: A study of radiotolerance of a tardigrade and evaluation of radioprotective activity of its extracts.

Importance of autoantibodies in SARSCoV-2 infection

Juan Bautista De Sanctis[1], Jenny Garmendia[1], Marian Hajduch[1]

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

Abstract

The majority of individuals, around 80 %, that have been infected by the SARS-CoV-2 virus can clear the pathogen; however, around 20 % develop moderate to severe disease with a 1-2 % mortality depending on age, sex and comorbidities. After the virus has been cleared, 10-15 % still have medical symptoms related to Covid infection (prolonged Covid). A substantial amount of individuals, 10 % of the population essentially male elders, have autoantibodies against cytokines, primarily IFN alpha and omega. Female elder women also have autoantibodies against other cytokines IL6 and other antigens, antinuclear antibodies. These autoantibodies impair the proper anti-viral responses facilitating viral escape and inducing cytokine storm. We found autoantibodies in a small cohort of patients; anti-IFNalpha2 and anti-IL6 autoantibodies, and we want to do a major screening of the samples to ascertain the importance of these autoantibodies in the population and its possible relationship to prolonged Covid.

Acknowledgment

ENOCH; project: CZ.02.1.01/0.0/0.0/16_019/0000868
EXCELS, project No. LX22NPO5103

Adaptative memory cell responses against SARS-CoV-2 virus

Juan Bautista De Sanctis[1], Ermin Schadich[1], Ivo Frydrych[1], Alexis García[2], Hana Jaworek[1] and Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University in Olomouc, Olomouc, Czech Republic
[2]Institute of Immunology. Faculty of Medicine. Universidad Central de Venezuela

Abstract

We evaluated samples from 60 adult individuals (30 from CR and 30 from Venezuela) who were: 1) not exposed to the virus (according to their knowledge), 2) suffered the infection, 3) were vaccinated and no Covid, 4) vaccinated and Covid. We monitored, CD154/IFNg (CD4 memory), CD8/CD107a (CD8 memory), CD79a (memory B cells), CD314 (NKG2D). Whole blood, 0.5 ml, viral peptides or 10 pfu of inactivated virus, and incubated for 18 hr. Positiveness was considered when the values were> 2 % as compared to the negative control. In young non-vaccinated individuals, good memory responses 30 % CD8, 20 % CD4, and 20 % B. Positive correlation CD314 memory CD8 response. Post-Covid, n=10, all memory responses were low in 3 patients even after 60 days after infection. The vaccinated group no Covid: good memory responses CD4 85 %, CD8 70 % of the individuals, B cell 50 %. A subgroup (10) compensated memory response with NK or NKT cells. CD314 decreased with time after vaccination, but CD8 memory did not fall as fast as CD4. Two individuals did not have good CD8 responses. Post-Covid and vaccinated n=4, low normal CD4 memory and low CD8 and B cell memory responses, $p < 0.01$ as compared to the vaccinated ones. Conclusions: Memory CD4 and CD8 responses can be efficiently achieved by vaccination in most cases, and protective memory responses can be observed in non-vaccinated populations. Memory responses post-Covid were poor, and vaccination may partially actívate immune cell memory.

Acknowledgment

Covid program at the Institute of Immunology. Caracas. Project FW03010472 Studium účinnostních funkcí experimentálních vakcín proti COVID2 na zvířecích modelech s pracovním názvem „covid vakcíny".

Citation

Mayora S, Zabaleta-Lanz M, Martínez W, Toro F, De Sanctis JB, García A. Lymphocyte subpopulations in Venezuelan patients infected with SARS CoV-2. Gac Méd Caracas 2020;128 (Supl 1):S74-S78

In vivo evaluation of enhanced blood retention and tumor uptake PSMA-targeting 225Ac-labeled radioconjugates

Zbynek Novy[1], Milos Petrik[1], Katerina Bendova[1], Falco Reissig[2], Krisof Zachler[2], Constantin Mamat[2], Daniela Kurfurstova[3], Marian Hajduch[1]

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

[2]Helmholtz-Zentrum Dresden-Rossendorf, Institute for Radiopharmaceutical Cancer Research, Germany

[3]Institute of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University Olomouc

Abstract

We investigated the *in vivo* behavior of two novel macropa-based PSMA ligands (namely [225Ac]FR94, [225Ac]FR96) modified with albumin binding moieties. The performed in vivo studies involved ex vivo biodistribution studies and subsequent immunohistochemical examinations of selected organs using LNCaP-tumor bearing mice. Tissues were dissected, weighed, and the accumulated activity was quantified in a gamma-counter at 1, 4, 24, 48, 72, and 120 h (plus 168 h for [225Ac]FR96) post-injection to determine the radiotracer uptake as a percentage injected activity (dose) per gram of the corresponding organ (%ID/g). Kidneys, liver and tumor were examined using immunohistochemical staining methods to detect PSMA expression, DNA damage (γH2AX), proliferation status (Ki67) and necrosis (H&E). The highest accumulation of radioactivity was measured in the LNCaP tumors at 168 h p.i. for [225Ac]FR96 (153.48 ± 37.76 %ID/g) and at 128 h p.i. for [225Ac]FR94 (46.04 ± 7.77 %ID/g). The second most prominent organ of radioligand uptake were kidneys with the highest accumulation of 67.92 ± 20.67 %ID/g (4 h p.i.) for [225Ac]FR94 and 59.90 ± 6.46 %ID/g (48 h p.i.) for [225Ac]FR96. Blood clearance of radioactivity was slower compared to the corresponding counterparts without albumin binders. In vivo experiments in tumor-bearing animals confirmed slower pharmacokinetic behavior of studied PSMA ligands like longer retention in blood and kidneys, caused by the presence of albumin binding moieties.

Acknowledgment

This research was funded by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102 funded by the European Union – Next Generation EU) and by the European Regional Development Fund via project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Citation

Ferlay, J. et al. Cancer statistics for the year 2020: An overview. International Journal of Cancer (2021) doi:10.1002/ijc.33588.

INSTITUTE OF MOLECULAR AND
TRANSLATIONAL MEDICINE