7th Annual IMTM Retreat

October 2–4, 2023 / hotel Lanterna Velké Karlovice

INSTITUTE OF MOLECULAR AND
TRANSLATIONAL MEDICINE

IMTM REACTOR

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PROGRAM

Monday, October 2nd

08:00		DEPARTURE FROM OLOMOUC	
10:00	11:00	CHECK-IN	

Chair: Alžběta Srovnalová

11:00	11:20	Juan Bautista de Sanctis	AUTOANTIBODIES, IMMUNE RESPONSE, PREVAL STUDY, LEASONS LEARNED
11:20	11:40	Juan Bautista de Sanctis	ACTIVATION OF LYMPHOCYTES BY CUET. IT IS MORE THAN A SIMPLE EVENT
11:40	12:00	Rastislav Slavkovský	Development of novel diagnostic solutions for genotyping of somatic variants of BCR-ABL1
12:00		LUNCH	

Chair: Ivo Frydrych

13:00	13:20	Narendran Annadurai	Differential seeding by exogenous R2 and R3 fibrils influences autophagic degradation of intracellular tau aggregates in Tau K18 P301S cells
13:20	13:40	Ihor Kozlov	Effect of human microglia on tau fibril proliferation in co-culture with Tau RD P301S biosensor cells
13:40	14:00	Jiří Řehulka	Triazole-based estradiol dimers with five-atom linkers act as inhibitors of microtubule dynamics

Chair: Petr Pavliš

14:00	14:10	Petr Pavliš	Certification ISO 27000, what it means for users
14:10	14:30	Jan Vidlař	Cybersecurity
14:30	14:40	Martin Szotkowski	Introduction to new IMTM ServiceDesk
14:40	14:50	Jan Lošťák	Introduction to new intranet
14:50	15:00	Petr Pavliš	Introduction to new IMTM VPN
15:00	15:20	COFFEE BREAK	

Chair: Jana Stránská

15:20	15:40	Ondřej Bouška	CIRCULATING AND SALIVARY DNA-BASED BIOMARKERS FOR EARLY DIAGNOSIS AND RECURRENCE MONITORING OF OPSCC
15:40	16:00	Pavel Stejskal	Liquid biopsy-based analyses of solid tumors
16:00	16:20	Lucie Kotková	Methylation changes in the sperm cell during the ontological development of an individual

Chair: Miloš Petřík

16:20	16:40	Kateřina Dvořáková Bendová	68Ga-labelled siderophores for imaging of Klebsiella pneumoniae infection
16:40	17:00	Miroslav Popper	Animal Facility Core: Support for In Vivo Studies
17:00	17:20	Barbora Neužilová	Siderophore chirality in molecular imaging applications
17:20	17:40	Zbyněk Nový	Preclinical evaluation of novel PSMA-targeting ligands labelled with gallium-68
19:00		DINNER	

PROGRAM

Tuesday, October 3rd

Chair: Martin Mistrík

09:00	09:20	Zuzana Machačová	POLA complex facilitates PARP inhibition-induced replication fork acceleration
09:20	09:40	Zdeněk Škrott	CuET nanoparticles – from unmarked eppendorf tube to clinical development
09:40	10:00	Matthew Lacey	Exploring the Mechanism of MCOPPB Senolytic Activity
10:00	10:20	Martin Löffelmann	Novel Dithiocarbamate-Copper Complexes Target p97/NPL4 System in Cancer Cells
10:20	10:40	Dávid Lukáč	Role of DNA nucleases in PARPi-Induced ssDNA Gap Processing
10:40	11:00	COFFEE BREAK	

Chair: Petr Džubák

11:00	11:20	Kateřina Ječmeňová	Identification of the mechanism of action of A3 adenosine receptor agonist – PNH173
11:20	11:40	Nikta Ziaei	Preparation of 3D cultures for high-throughput screening
11:40	12:00	Soňa Gurská	HTS screening campaign to identify novel compounds inhibiting coronavirus infections
12:00	12:20	Martin Ondra	Cystic fibrosis drug discovery: Unleashing the power of high-throughput screening with the HiBiT tag
12:20	12:40	Ermin Schadich	Progress Report Meeting October 2023: Anti-SARS-CoV-2 properties of novel terpenes
12:40	13:00	Jiří Hodoň	Triterpenoid pyridines and pyrazines and the study of their mechanism of action
13:00	14:00	LUNCH	
19:00		DINNER	
21:00		PRESENTATION OF IMTM INTERESTING TRAVEL DESTINATIONS	

Wednesday, October 4th

Chair: Tomáš Oždian

09:00	09:20	Jana Václavková	Analysis of exhaled breath condensate from patients infected with Bordetella pertussis
09:20	09:40	Martina Kintlová	Echo MS: a rapid tool for kinases inhibitors search
09:40	10:00	Miroslav Hruška	Claire: interpreting proteomics data using deep probabilistic databases of peptides
10:00	10:20	Dominik Vítek	Stress response in Hypsibius exemplaris
10:20	10:40	COFFEE BREAK	

PROGRAM

Chair: Lukáš Najdekr

10:40	11:00	Pavlo Polishchuk	The first CACHE challenge: searching for hits in ultra-large chemical databases
11:00	11:20	Alina Kutlushina	Repurposing OpnMe.com drug candidates: A call for collaborative evaluation of potential anti-cancer agents
11:20	11:40	Guzel Minibaeva	STRUCTURE-BASED GENERATION OF SYNTHETICALLY FEASIBLE MOLECULES
11:40	12:00	Alexandra Ivanova	In silico modeling and analysis of new estradiol dimers as tubulin inhibitors with anticancer activity
12:00	12:20	Barbora Kalousová	Clonal somatic variants in hematopoietic cells in relation to atherosclerosis and stroke
12:20	12:40	Monika Vidlařová	Influence of morphine analgesia, opioid growth factor receptor and cannabinoid receptor 2 gene expression in tumor tissue on survival of patients with pancreatic cancer
12:40	13:40	LUNCH	
13:40		DEPARTURE TO OLOMOUC	

AUTOANTIBODIES, IMMUNE RESPONSE, PREVAL STUDY, LEASONS LEARNED

Jenny Garmendia, Marian Hajduch, Vladimira Koudeláková, Hana Jaworek, Juan Bautista De Sanctis

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

Abstract

The PREVAL study aimed to ascertain Sars-CoV-2 infection and the immune response of the general population. In the first visit in 2020, when SARS-CoV-2 infections were low, 750 individuals were screened for autoantibodies: 6 % were positive for anti-IFNα2, 7.6 % for anti-IFNγ, 2.8 % for anti-IFNω, 2.6 % for anti-IL-6, 1.3 % for anti-IL-10 and 0.5 % for anti-IL-17. No significant differences were observed with gender; the cohort's median age was 52 years. However, these antibodies were not blocking IFN/ perforin in the biological assays. 572 individuals were followed for 4 visits. Only 48 individuals from the first visit in 2022 were naive from natural infection and/or vaccination; 8 already had autoantibodies that were detected independently of vaccination or infection in the four visits. The rest of the cohort showed a significant increase in autoantibodies after infection (detected by PCR test and/or antibodies to N protein). The increase was anti-IFNα-2> anti-IFNγ> anti-IFNω> anti-IL6 and >anti-IL10 and anti-IL17. Interestingly, 75 % of the infected individuals that secreted IFNγ >250 mIU/ upon viral antigen stimulation did not produce autoantibodies. Reanalyzing the data, IFN secretion upon stimulation was similar in gender and age, and perforin secretion was significantly higher in vaccinated and infected individuals. Autoantibodies refer to non-clinical autoimmunity and polyclonal B cell activation. IFn gamma and perforin levels refer to proper immune response.

Acknowledgment

Thank you to The COVID-19 Team, Anna Janošťáková, Jitka Křenová, Zuzana Hlaváčková, Michaela Bendova.

ACTIVATION OF LYMPHOCYTES BY CUET. IT IS MORE THAN A SIMPLE EVENT

Juan Bautista De Sanctis[1], Jana Mastná[2], Vendula Pokorna[2], Viktor Valentini[2], Eva Kominková[2], Jenny Garmendia[1], Marian Hajduch[1]

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Abstract

Two groups have shown the effect of disulfiram and Copper on lymphocyte activation. (EMBO J . 2022 Aug 16;41(16):e110636, Discov Oncol. 2023 Jun 16;14(1):103). On the other hand, we have shown an increase in the cytotoxic response of T and NK cells, human and mouse, against different types of tumours. The response is observed at concentrations between 1 and 10 nM for as early at 15 min to 18 hr. Re-stimulation or costimulation with antigen may render the cells unresponsive. The first mechanism proposed was the activation of the src kinase lck; however, we have observed with CuEt an increase in tyrosine phosphorylation of ERK kinase and an increase in NFKB, leading to an increase in IL-2 secretion. When the secreted IL-2 is captured by the specific antibody or the IL-2 receptor alpha is blocked, the increase in cytotoxic response is not observed, suggesting that CuEt priming involves at least two different cell events. Moreover, the effect of CuEt is stronger in CD8 cells than in CD4 cells, suggesting that lck not linked to antigen receptors may be activated by CuEt. In addition, CuEt enhances NKG2D, a receptor for tumour stress antigens, MICA and ULBP, which NFkB induces. Thus, CuEt induces a multifactorial activation of lymphocytes, enhancing cytotoxic responses against tumour cells, an event; however, that is preferentially observed in not previously activated cells.

Acknowledgment

ENOCH project. PerMed project

Citation

Wang, Q., Zhu, T., Miao, N., Qu, Y., Wang, Z., Chao, Y., Wang, J., Wu, W., Xu, X., Xu, C., Xia, L., & Wang, F. (2022). Disulfiram bolsters T-cell anti-tumor immunity through direct activation of LCK-mediated TCR signaling. The EMBO journal, 41(16), e110636. Zhang, S., Zong, Y., Chen, L., Li, Q., Li, Z., & Meng, R. (2023). The immunomodulatory function and antitumor effect of disulfiram: paving the way for novel cancer therapeutics. Discover. Oncology, 14(1), 103.

Development of novel diagnostic solutions for genotyping of somatic variants of BCR-ABL1

Rastislav Slavkovský[1], Denisa Zermeghová[2], Jakub Vojtíšek[3], Onderková Martina[3], Bezděková Kateřina[3], Petr Brož[3], Pavlína Kostyu[3], Tóthová Iveta[4], Marián Hajdúch[1]

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[2]Faculty of Natural Sciences, Palacky University in Olomouc, Olomouc, Czech Republic
[3]R&D, BioVendor - Laboratorní medicína, Brno Czech Republic
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[5]Bioxsys, Ústí nad Labem

Abstract

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease of hematopoietic tissue that arises from the malignant transformation of bone marrow stem cells and their clonal expansion and accumulation in the peripheral blood. It is caused by a reciprocal translocation also known as Philadelphia chromosome. Disease can be treated with tyrosine kinase inhibitors (TKIs). Secondary molecular changes in kinase domain of ABL1 that cause resistance to TKIs could arise during the progress of disease. These secondary changes, typically non-synonymous SNV, occur in up to 90% of patients, and our goal was to be able to detect these aberrations with the highest possible sensitivity. Successful ABL1 genotypization can be used to CML diagnostics and adjust the patient's treatment.

Methods: Three different artificial plasmids containing partial BCR-ABL1 or ABL1 cDNA sequence were developed to simulate the presence or absence of 7 clinically important variants. Plasmids were diluted from 106 to 10 copies/µl. We implemented qPCR method for BCR-ABL1 pre-amplification and ABL1 genotyping using fastGEN method followed NGS sequencing using MiSeq (Illumina). Sequencing data were processed using dedicated workflow in GENOVESA software.

Results: Optimization of the PCR settings allowed us pre-amplify down to 10 copies of BCR-ABL1. The proposed optimized fastGEN method was able to detect all 7 variants in simulated samples with down to 30 copies of BCR-ABL1 and also in samples with down to 0,03 % BCR-ABL1 IS (international scale) or with 1% of VAF (variant allele fraction).

Conclusion: Further validation using real clinical RNA samples is required to fully implement the method in clinical practice.

Acknowledgment

This work was supported by the project National Centers of Competence, NCK2 Personalised Medicine: From Translational Research into Biomedical Applications PerMed: T2BA (TN02000109) funded by Technology Agency of the Czech Republic.

Differential seeding by exogenous R2 and R3 fibrils influences autophagic degradation of intracellular tau aggregates in Tau K18 P301S cells

Narendran Annadurai[1], Agáta Kubíčková[1,2], Ivo Frydrych[1,2], Marián Hajdúch[1,2], Viswanath Das[1,2]

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Abstract

Aggregation of misfolded tau protein is a common feature of tauopathies. Cells employ diverse mechanisms to eliminate misfolded tau with different conformations, contributing to the varied clinical and pathological manifestations of tauopathies. This study focuses on the clearance of seeded tau aggregates following the induction of biosensor cells with R2 and R3 fibrils that exhibit distinct aggregation kinetics and seeding potencies. We hypothesise that the dissimilarity in time-dependent intracellular seeding induced by R2 and R3 fibrils underlies the variation in autophagy failure. These discrepancies may account for the heterogeneity of pathology and disease progression between 3R and 4R tauopathies, given the absence of R2 in 3R tau isoforms. In R2-induced cells, alterations in p62 and LC3II/I levels, indicative of proteotoxic stress and autophagy failure, occur sooner than in R3-induced cells. Conversely, LAMP1 levels remained unaffected, suggesting a failure in the fusion of aggregate-containing autophagosomes with lysosomes. This autophagic failure may increase seed-dependent intracellular aggregation in induced cells. Consequently, we assessed the impact of autophagy inducers on the clearance of intracellular tau aggregates in induced cells. We provide insights into the distinct mechanisms of autophagy failure and autophagy clearance of intracellular aggregates in cells induced with R2 and R3 fibrils.

Acknowledgment

This work was supported in parts by the IGA_LF_2022_033, CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053, (EXCELES, ID Project No. LX22NPO5102 and EXCELES, ID Project No. LX22NPO5107) - Funded by the European Union - Next Generation EU from the Ministry of Education, Youth and Sports of the Czech Republic (MEYS), and the GACR (23-06301J).

Effect of human microglia on tau fibril proliferation in co-culture with Tau RD P301S biosensor cells

Ihor Kozlov, Narendran Annadurai, Viswanath Das

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Abstract

With the increasing number of tau pathologies and the average age of the world's population, the need for an affordable and reliable model for basic and translational research is increasing. Such a model should include not only a positive component, such as cells that reflect the spread of pathological forms of tau protein but also elements potentially counteracting this process. These elements are microglia, but today there is evidence both in favor of their preventing the spread of misfolded tau, and the opposite. Therefore, the system must be flexible and potentially include different forms of microglia (aging, dystrophic, with gene knockout, etc.) and new tau mutations, allowing for qualitative and quantitative analysis of tau pathology. As a basis for such, the work here describes studies with immortalized human microglial cell line HMC-3 modified with fluorescent marks in co-culture with Tau RD P301S biosensor cells. A confocal scanning system was used to display the process of tau fibrils and whole cells uptake by microglia with further calculation of the number of tau protein aggregates and showed that HMC3 cells were unable to slow down seeding even after LPS activation.

Acknowledgment

This work was supported in parts by infrastructural projects (CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053), the projects National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102) and National Institute for Neurological Research (Program EXCELES, ID Project No. LX22NPO5107) - Funded by the European Union - Next Gener

Citation

1. Panda, C. et al. Aggregated Tau-PHF6 (VQIVYK) Potentiates NLRP3 Inflammasome Expression and Autophagy in Human Microglial Cells. Cells 10, 1652 (2021). 2. Brelstaff, J. H. et al. Microglia become hypofunctional and release metalloproteases and tau seeds when phagocytosing live neurons with P301S tau aggregates. Sci. Adv. 7, eabg4980 (2021). 3. Odfalk, K. F., Bieniek, K. F. & Hopp, S. C. Microglia: Friend and foe in tauopathy. Prog. Neurobiol. 216, 102306 (2022).

Triazole-based estradiol dimers with five-atom linkers act as inhibitors of microtubule dynamics

Jiří Řehulka[1], Michal Jurášek[2], Lenka Hrubá[1], Aleksandra Ivanová[1], Soňa Gurská[1], Olena Mokshyna[1], Pavel Trousil[2], Lukáš Huml[2], Pavel Polishchuk[1], Marián Hajdúch[1], Pavel B. Drašar[2], Petr Džubák[1]

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Abstract

Steroid dimers containing two steroid skeletons can be rarely found in nature, however they can be prepared using click chemistry. We explored the effect of selected five-atom linkers on the biological activity of the estradiol dimer. Set of thirteen new dimers with carbon, nitrogen or oxygen in the linker centre was subjected to cytotoxicity assay and cell cycle profiling. The cytotoxicity of the active dimers was highly comparable with natural estradiol metabolite 2-methoxyestradiol. Cell cycle analysis and immunofluorescence proved the interference of dimers with microtubule assembly and mitosis. The measured results as well as proposed *in silico* model indicated that the activity of the estradiol dimers can be modulated by structural changes in the linker.

Acknowledgment

This work was supported by CEREBIT (Project No. CZ.02.1.01/0.0/0.0/16_025/0007397), an internal grant of the UCT Prague No. A1_FPBT_2022_007 and by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90140), infrastructural projects (CZ-OPENSCREEN – LM2018130; EATRIS-CZ – LM2018133) and National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation

CIRCULATING AND SALIVARY DNA-BASED BIOMARKERS FOR EARLY DIAGNOSIS AND RECURRENCE MONITORING OF OPSCC

Ondrej Bouska[1], Vladimira Koudelakova[1], Zuzana Horakova[2], Marian Hajduch[1]

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

Oropharyngeal squamous cell carcinoma (OPSCC) incidence has more than tripled during the last 30 years in the Czech Republic. Almost 800 OPC cases were newly diagnosed in 2021, making OPSCC more prevalent than etiologically related cervical cancer. Although, OPSCCs are divided into two groups: human papillomavirus (HPV) related OPSCCs and non-HPV OPSCCs. As well as in cervical cancer, risk stratification, early diagnosis, and locoregional recurrence monitoring methods are needed. This study aims to validate the applicability of liquid biopsies and DNA biomarkers for early OPC diagnosis and its recurrence. In this study, newly diagnosed OPSCC patients and patients in remission were enrolled. HPV tumor status was determined by HPV detection and p16 IHC. Pre & post-treatment HPV testing in gargle lavage (GL), oropharyngeal swabs (OPS), and plasma were performed, followed by regular testing according to the standard follow-up protocol. HPV-related OPSCC was diagnosed in 87 % of cases, and HPV16 was the only tumor-derived HPV genotype. GL and OPS's sensitivity (SE) and specificity (SP) for newly diagnosed HPV-related OPSCCs were 80 %, 90.5 %, and 100 %, 100 %, respectively. Detection of circulating tumor HPV DNA showed 92% SE and 100% SP. Post-treatment/follow-up HPV infection persisted in 9.5 % of OPSCC patients. In conclusion, HPV-related OPSCCs are predominant compared to non-HPV OPSCCs. At diagnosis, combined HPV detection in saliva and plasma showed excellent sensitivity.

Acknowledgment

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Liquid biopsy-based analyses of solid tumors

Pavel Stejskal, Josef Srovnal, Alona Řehulková, Veronika Černohorská, Marián Hajdúch

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Abstract

Thanks to the development of molecular, genetic, and computational methods, many molecular-genetic associations applicable to liquid biopsy (LB) have been described during the last decade. In contrast to the anatomically limited and invasive classic biopsy, LB enables repeated and non-invasive detection and characterization of tumors via biomarkers circulating in the body fluids, e.g., peripheral blood. Circulating tumor cells (CTCs), circulating tumor nucleic acids, and circulating extracellular vesicles are among the most frequently discussed LB biomarkers. 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 CTC detection using CytoTrack CT11TM device based on semiautomatic, immunofluorescent, and pre-enrichment-free direct CTC capture from peripheral blood samples. We also focused on the analysis of circulating tumor nucleic acids.

Acknowledgment

This work was financially supported by grants awarded by the European Union-Next Generation EU (LX22NPO5102) and Palacky University Olomouc (LF2023_006).

Methylation changes in the sperm cell during the ontological development of an individual

Lucie Kotková, Jiří Drábek

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Abstract

Changes in methylation levels in specific CpG areas are associated with the chronological age of an individual. This phenomenon can be used for forensic phenotyping. Predicting the age of an unknown trace donor can narrow down the pool of potential suspects in cases where no match is found among the suspects or in the police database.

Due to the tissue specificity of DNA methylation, different types of samples must be processed specifically. Semen samples are common on the crime scene, but age-associated methylation changes in germ cells are much less researched than in somatic cells. Furthermore, sperm cells and their progenitors undergo an extra wave of excessive methylation remodeling, compared to somatic cells.

The ontological development of human sperm cells from conception to the mature sperm cell capable of another conception is quite fragmented in the literature. Here, I will present the steps of whole sperm development in a comprehensive manner, with a special focus on DNA methylation changes and epigenetic heritability.

Acknowledgment

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68Ga-labelled siderophores for imaging of Klebsiella pneumoniae infection

Kateřina Dvořáková Bendová[1], Michaela Zappeová[1], Patrik Mlynárčik[2], Kristýna Krasulová[1], Barbora Neužilová[1], Miloš Petřík[1]

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[2] Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic

Abstract

Klebsiella pneumoniae (KP) is an opportunistic bacterial pathogen that can be a cause of a broad spectrum of diseases (e. g. pneumonia, urinary tract infections, surgical wound infections). With the growing threat of increasing antimicrobial resistance, this pathogen becomes progressively more dangerous, especially in the medical setting. It is necessary to diagnose infected patients quickly and accurately, so that adequate treatment could be initiated and further spread through the hospital environment prevented. In this study, we present the use of radiolabelled siderophores for imaging of KP infections by positron emission tomography. All siderophores were labelled with high radiochemical purity (> 95%). Overall siderophore uptake was higher in cultures cultured in minimal medium and the highest uptake values were achieved with [68Ga]Ga-Ferrirubin. In normal mice, the main route of siderophore excretion was though the kidneys for most siderophores. In murine model of acute myositis, the highest signal accumulation in the site of infection was assessed for [68Ga]Ga-Ferrirubin. In rat model of acute pneumonia, we observed the accumulation of the radiolabelled siderophore in the infected lung. We have demonstrated that radiolabelled siderophores can be used for KP infection imaging. Furthermore, from the selected siderophores, [68Ga]Ga-Ferrirubin appears to be the most perspective one.

Acknowledgment

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Animal Facility Core: Support for *In Vivo* Studies

Miroslav Popper, Zbyněk Nový, Kateřina Dvořáková-Bendová, Kristýna Krasulová, Barbora Neužilová, Kateřina Frantíková, Tereza Schneiderová, Katarína Hajduová, Miloš Petřík

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

Abstract

The animal facility of the Institute of Molecular and Translational Medicine of Palacky University provides preclinical in vivo efficacy models for numerous diseases. Our team provides technical expertise in the creation and imaging of sophisticated models of diseases. The animals are operated on according to the principles of good surgical practice under the guidance of our animal welfare officer, which include an aseptic working method, minimal surgical trauma, anesthesia (injection or inhalation), and analgesia, if necessary, peri- and post-operative care and detailed reporting. In vivo studies are very complex, expensive, and laborious. You must precisely determine your hypothesis and schedule, but at the same time, you must identify critical points where you can expect different outcomes. At that point, you are forced to modify the schedule. However, this must be done and written in the project proposal before ordering laboratory animals. Having some in vitro results before is good because this is a legitimate reason to conduct in vivo studies. In the project proposal, you must select an appropriate in vivo animal model, the number of animals, methods, and techniques that will be used, and you must describe all operations and activities carried out on animals. The experienced technical team provides all animal care and observation of health status. Do not worry; The Animal Facility core team is ready to help and support you at every step of your in vivo project.

Acknowledgment

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

Siderophore chirality in molecular imaging applications

Barbora Neužilová, Kristýna Krasulová, Kateřina Dvořáková-Bendová, Zbyněk Nový, Miroslav Popper, Miloš Petřík

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

Abstract

Siderophores are low molecular mass iron chelators produced by many microorganisms for iron acquisition and storage. The iron in siderophores is possible to be replaced by gallium-68 without loss of activity and to allow molecular imaging by positron emission tomography (PET)(Petrik et al). Ferrirhodin (FRH) and ferrirubin (FR) are microbial ferrichrome-type isomeric siderophores. Ferrirhodin has trans configuration of the anhydromevalonic acid as acyl groups and ferrirubin has cis (Fidelis et al). It was shown that the chirality of siderophores plays an important role in the cell recognition and uptake (Brillet et al, Raymond et al). Here we study the influence of siderophore chirality on their use for molecular imaging of infection. FR and FRH were labelled with gallium-68 using acetate buffer. In vitro characteristics of 68Ga-FR and of 68Ga-FRH were determined. Ex vivo biodistribution studies were performed at 30 and 90 min after injection as well as in vivo PET/CT imaging in healthy Balb/c mice. Tested siderophores were labelled with 68Ga with high (>95%) radiochemical purity. The resulting complexes differed in their in vitro characteristics. 68Ga-FRH is more hydrophilic and has higher protein binding, which also correlates with ex vivo biodistribution in mice, where it showed a moderate retention in the blood as seen on PET/CT images.

Acknowledgment

We gratefully acknowledge the financial support of the project National institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU and the European Regional Development Fund (Project ENOCH No. CZ.02.1.01/0.0/0.0/16_019/0000868).

Citation

Petrik M et al. (2021) 68Ga-labelled desferrioxamine-B for bacterial infection imaging. Eur J Nucl Med Mol Imaging 48: 372-382. Fidelis K et al. (1990) Structure and molecular mechanics of ferrirhodin. Acta Crystallogr C 46 (Pt 9):1612-7. Brillet K et al. (2011) Pyochelin Enantiomers and Their Outer-Membrane Siderophore Transporters in Fluorescent Pseudomonads: Structural Bases for Unique Enantiospecific Recognition. J. Am. Chem. Soc. 133, 41, 16503–16509. Raymond K et al. (2015) Coordination Chemistry of Microbial Iron Transport. Acc. Chem. Re

Preclinical evaluation of novel PSMA-targeting ligands labelled with gallium-68

Zbyněk Nový, Katarína Hajduová, Miloš Petřík

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

Abstract

The prostate-specific membrane antigen (PSMA) is well-known target in prostate cancer and is investigated for last 20 years. In this study, we have focused in preclinical testing of three novel PSMA inhibitors. Compounds P15 and P16 possesses different binding motif compared to PSMA-617, meanwhile compound P19 is based on PSMA-617.Methods included basic stability test in PBS (0-2h), stability in human plasma (0-2h), determination of log D and evaluation of plasma protein binding for three tested compounds and for PSMA-617 as golden standard. The labelled ligands were tested in vitro to reveal their binding to LNCaP cells as well as their internalization into these cells. The ex vivo biodistribution studies were performed in LNCaP-tumor bearing mice (1, 2 h p.i.). Finally, tumor mice were injected with studied compounds and PET/CT imaging was done 1 h p.i. . The plasma protein binding of PSMA-617 and P19 was in level of 50-60%, nevertheless P15 and P16 displayed much lower values (10-40%). Ex vivo biodistribution study showed the highest accumulation of the tracer in kidneys in case of PSMA-617, meanwhile the three tested ligands had much lower kidney uptake, but the tumor uptake was lower as well. Tumor uptake of P19 was 3,52% ID/g, compared to 5,25 %ID/g for PSMA-617. All tested compounds were able to image tumor using PET/CT. P19 was the only compound vere tumor/blood ratios were comparable to PSMA-617. PET/CT showed favorable tumor contrast only for P19 and PSMA-617.

Acknowledgment

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POLA complex facilitates PARP inhibition-induced replication fork acceleration

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Abstract

Poly(ADP-ribose) polymerase inhibitors (PARPi) represent a promising class of anti-cancer drugs targeting crucial cellular processes, including DNA repair and replication. Previous understanding suggested that PARPi triggered replication fork stalling, leading to DNA double-strand breaks and cell death. However, we have recently contradicted this model, revealing that PARPi induces replication fork acceleration rather than stalling. Even though the therapeutic potential of PARPi is well recognized, the precise molecular mechanism behind PARPi-induced replication fork acceleration remains not fully understood. This study clarifies that the effect of PARPi on DNA replication is not a reduction in origin activity but rather an increase in replication fork speed. To deepen our understanding, we systematically depleted the catalytic subunits of all 16 human DNA polymerases and assessed their impacts on PARPi-induced fork acceleration. Our findings identify POLA and PRIMPOL as crucial mediators of PARPi-induced fork acceleration. Disrupting POLA1 activity through chemical inhibition or protein depletion in various human cell lines reduces PARPi-induced fork acceleration. Moreover, POLA1 depletion sensitizes cells to PARPi via induction of ssDNA gaps and activation of DNA damage response. These results establish POLA1 as a newly discovered key player in PARPi-induced fork acceleration and provide suggestions for possible targeting of POLA1 in BRCA1-mutated tumours.

Acknowledgment

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CuET nanoparticles – from unmarked eppendorf tube to clinical development

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Abstract

Disulfiram, a drug used to treat alcoholism for decades, is a promising candidate for drug repurposing to treat cancer. However, despite intensive pre-clinical research, epidemiological evidence and numerous case reports, the results of a few conducted clinical trials are rather disappointing. We have demonstrated that not disulfiram itself, but its copper complex CuET is responsible for its anticancer activity. Given that the conversion of disulfiram to CuET in the human body is rather minimal and influenced by a plethora of confounding factors, the circulating levels of CuET are extremely variable. This may explain the varied results observed in clinical trials a represent a crucial obstacle preventing successful repurposing of disulfiram for cancer. As a possible solution, we have developed CuET nanoparticles, that overcome major limitations of disulfiram. These nanoparticles are easy to prepare and show great stability, a good pharmacokinetic profile and anticancer activity in vivo, making them a possible drug candidate suitable for further development.

Acknowledgment

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Exploring the Mechanism of MCOPPB Senolytic Activity

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Abstract

Cellular senescence is defined as a stable form of cell cycle arrest. While senescence is involved in a number of beneficial processes within the body, the accumulation of senescent cells with age has been associated with age related pathologies. As such, senescent cells are a promising target for treatment as the destruction and removal of senescent cells (senolysis) is seen as potential avenue to combat diseases of aging. MCOPPB is an opioid receptor agonist specifically acting as a ligand to the Nociceptin receptor, the senolytic properties of which were originally discovered via the screening of a chemical library, whose effectiveness has been proven in both in vitro and in vivo mouse models. The purpose of this study has been to explore the mechanism by which MCOPPB induces senolysis. Our data indicates that MCOPPB causes senescent cells to undergo an autophagy linked and caspase independent form of cell death, apparently triggered independently to the Nociceptin receptor agonist activity of MCOPPB.

Acknowledgment

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Novel Dithiocarbamate-Copper Complexes Target p97/NPL4 System in Cancer Cells

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Abstract

Proteostasis is responsible for maintaining a balance between the proteosynthesis and degradation of proteins. Cancer cells are highly dependent on proteostatic systems, where the p97 pathway plays an important role. Protein NPL4 (Nuclear protein localization homolog 4) is one of p97's crucial cofactors, being responsible for binding to substrates meant for degradation. Recent studies point to NPL4 as a potential target in cancer treatment. Possible NPL4 inhibitors could be found in the group of dithiocarbamates (DTCs). DTCs are small organic sulphur-containing compounds with the ability to bind metal ions, especially copper. One of these DTC-copper complexes – bis(diethyldithiocarbamate)-copper (CuET) – was recently identified as an NPL4 protein inhibitor causing its aggregation and showing anticancer activity. However, it was not known whether this activity was associated only with CuET or with other DTCs as well. We prepared and tested 20 DTCs-copper complexes for their ability to immobilize NPL4 and 13 scored positively. The NPL4 aggregation was also associated with heat shock response, unfolded protein response, p97 immobilization, and accumulation of poly-ubiquitinated proteins. The same phenotypes that were previously observed for CuET. We also performed a cytotoxic screening and found a positive correlation between cytotoxicity and NPL4 aggregation. These findings propose DTCs-copper complexes for further studies with possible anticancer therapy applications.

Acknowledgment

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Role of DNA nucleases in PARPi-Induced ssDNA Gap Processing

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Abstract

Single-stranded DNA (ssDNA) gaps induced by Poly ADP-Ribose Polymerase (PARP) inhibitors, rather than double-stranded breaks (DSBs), are responsible for cancer cell vulnerability. PARP inhibitors disrupt the DNA repair machinery which potentially leads to toxic ssDNA gaps accumulation in cells deficient in ssDNA gap repair or suppression pathways. If left unrepaired, ssDNA gaps pose a significant threat to genomic integrity as they can transform into other DNA lesions, including DSBs. This project aims to uncover the mechanism responsible for processing PARP inhibitor (PARPi)-induced ssDNA gaps, with the goal of identifying a specific DNA nuclease responsible for ssDNA break processing into ssDNA gaps. Our approach involves detecting PARPi-dependent ssDNA gaps through CldU incorporation into the template or nascent DNA strand, followed by signal detection using immunofluorescence microscopy. We plan to knockdown a set of DNA nucleases and observe if it results in a decrease in CldU signal after PARP inhibitor treatment. This research aims to provide insights into the enzymes involved in ssDNA gap processing, thereby enhancing our understanding of the molecular mechanisms of PARPi-induced cancer cell vulnerability.

Acknowledgment

This project was supported by the MEYS CR (Large RI Project LM2018129 - Czech-BioImaging) and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Identification of the mechanism of action of A3 adenosine receptor agonist – PNH173

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Abstract

A nucleoside-based A3 adenosine receptor agonist, PNH173, exhibits significant cytotoxic effects against several cancer cell lines derived from tumors of various histogenetic origins, with almost no cytotoxic activity against normal human fibroblasts. PNH173 demonstrates good pharmacological properties in non-clinical ADME tests, reduces tumor growth, and enhances overall survival in in vivo mice experiments. By live imaging microscopy, the competitive assays between CELT-171228, fluorescent-labeled A3 adenosine receptor antagonist, and the agonist PNH173 confirmed the binding to the orthosteric binding site on A3AR. The transcriptomic and proteomic profiling and western blotting were performed after treatment with PNH173. The changes were observed in the Wnt, MAPK, and PI3K/Akt signaling pathways, which are the most affected pathways by A3AR, and also eukaryotic translation initiation factor 4E (eIF4e) showed changes. Furthermore, the activity of Wnt/β-catenin and two main isoforms of c-myc transcription factor activity upon PNH173 treatment were analyzed using five reporter systems at various time points.

Acknowledgment

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Preparation of 3D cultures for high-throughput screening

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Abstract

The 3D cell culture system bridges the gap between 2D cell culture models and complex tissue. Among 3D cultures, spheroids, spherical aggregates of tumor cells, can mimic the microenvironment of cells with higher fidelity. Compared to 2D-cultured cells, spheroids provide more vivid and cost-efficient models with a higher degree of relevance to clinical and biological applications. The spheroid is used in a broader range of in vitro studies, including proteomics, metabolomics, and drug screening. In this study to investigate the influence of anticancer drugs on tumor cells, spheroids were cultured in agarose-coated 384-well plates using GFP-labelled HCT-116 colorectal cancer cells and treated with four different concentrations (1μM, 20μM, 50μM, 100μM) of the anticancer drug Irinotecan. The image analysis technique and viability assay used to conduct the experiment. Signal Image Artist software (SIMA) is used to analyze changes in spheroids size and intensity. A luminescent viability kit (CellTiter-Glo, Promega) quantitatively evaluated spheroids viability under distinct conditions. Our results indicate that with increasing the concentration of anticancer drugs, the viability and size of spheroids statistically decrease.

Acknowledgment

Institute of Molecular and Translational Medicine (IMTM)

HTS screening campaign to identify novel compounds inhibiting coronavirus infections

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Abstract

Coronaviruses (CoVs) are a large family of viruses with a variety of susceptible hosts. When infecting humans, CoVs usually cause mild to moderate upper – respiratory tract illnesses. However, some of them cause severe and fatal diseases. Therefore, the development of effective antiviral drugs is a global public health priority. Our screening campaign is focused on discovering novel antiviral compounds that inhibit CoV infection. The goal is to identify compounds that block the binding of the SARS-CoV-2 S-protein Receptor Binding Domain (RBD) to human angiotensin-converting enzyme 2 (hACE2), which is essential for infection. It is one of the key targets in the first phase of the SARS-Cov-2 replication cycle, where binding of CoV to hACE2 or TMPRSS2 transmembrane protease serine 2 (TMPRSS2) allows entry into the host cell. This method is based on the enzymatic activity of hACE2 and has been successfully used in a 96 – well plate format. To screen an EU – Openscreen library of 95.000 compounds, the assay needs to be miniaturized and implemented into HTS platform. The process of the method transfer to the IMTM HTS platform will be presented and discussed.

Acknowledgment

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Cystic fibrosis drug discovery: Unleashing the power of high-throughput screening with the HiBiT tag

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Abstract

Cystic fibrosis is a severe autosomal recessive disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR), resulting in disrupted CFTR protein biosynthesis, trafficking, or function. The insertion of the HiBiT tag (Schwinn et al., 2017) in the 4th extracellular loop of WT-CFTR enabled the monitoring of CFTR plasma membrane localization and its trafficking in live cells. The 11-amino acid HiBiT tag binds with high affinity to a large inactive subunit (LgBiT), generating a reporter luciferase with bright luminescence. Following the validation of the reporter cell line (B38) with the endogenous expression of HiBiT tagged WT-CFTR, we proceeded with CRISPR/Cas9-mediated insertion of the most common CFTR mutation – the ΔF508. Several different approaches were applied to assess the CRISPR efficiency. To conclude, this work describes a novel reporter cell line with the potential to be an ultimate building block for developing unique cellular CF models.

Acknowledgment

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Citation

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

Progress Report Meeting October 2023: Anti-SARS-CoV-2 properties of novel terpenes

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Abstract

Our recent publication showed that terpene libraries could be good source for development of novel drug candidates against SARS-CoV-2 virus (SASR-CoV-2). Consistently, our current study was focused on antiviral property against SARS-CoV-2 of compounds from proprietary terpene library. So, far, the forty four compounds were tested for antiviral activity using antiviral assays. Vero 6 cells were infected with the virus and treated by compounds at 10.00 μM concertation for 72 hours. Subsequently, the selected hit compounds were tested in four-fold serial dilutions within 10.00-0.39 μM concentration range. The results showed only four compounds were selected as primary hits. Dose response analyses showed that three of these four compounds were active against SARS-CoV-2 (Their IC50s < 10.0 μM). This was promising and provided the scaffold for synthesis of further terpene derivatives. So, recently, the terpene library was upgraded with the forty four newly synthesized compounds that are not cytotoxic to Vero 6 cells. Further ongoing analyses are focused on their antiviral properties.

Acknowledgment

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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.

Triterpenoid pyridines and pyrazines and the study of their mechanism of action

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Abstract

Triterpenoids are natural compounds with various biological activities. Our research group is mostly focused on their cytotoxicity.[1] Triterpenoid pyridines and pyrazines, such as 1a and 1b had high cytotoxicity of IC50 0.5 – 1.5 μM in leukemic cell lines (CCRF-CEM, K-562).[2] In addition, they had higher activity against daunorubicin and taxol resistant cells (CEM-DNR, K562-TAX) which makes them promising alternatives for the treatment of resistant leukemias.[2]
First of all, we investigated the mechanism of action of the most interesting compounds. Cell cycle studies, Western blot analysis of apoptosis and cell cycle - related proteins combined with the visualization of the cellular damage using fluorescent and electron microscopy proved that 1a and 1b trigger apoptosis via intrinsic pathway.[2] We found, that the compounds accumulate preferentially in the resistant cells, although the reason for that has to be uncovered yet. Unfavorable pharmacological parameters of the parent compounds motivated us to prepare two sets of prodrugs (e.g. 2, 3). This improved the pharmacological parameters and in addition, medoxomil-type prodrugs 3 surprised us by an extreme selective cytotoxicity against K-562 cells with IC50 26 – 43 nM.[2]
New methods for investigating the mechanism of action of active triterpenes will be introduced along with synthesis and future goals.

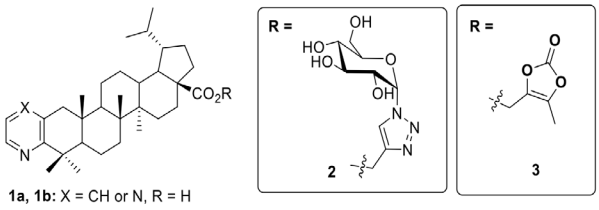

Fig 1: General formula of pyridines, pyrazines and their prodrugs

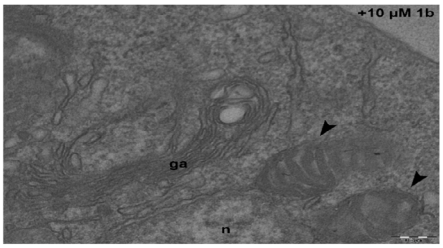

Fig 2: EM analysis of mitochondria after treatment with 1b

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Citation

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Analysis of exhaled breath condensate from patients infected with Bordetella pertussis

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Abstract

Collection of exhaled breath condensate (EBC) is a cheap and non-invasive method to obtain samples from the human respiratory tract. The development of new non-invasive methods for the early detection of lung diseases, injuries and infections would be highly beneficial. EBC represents a rich source of biomarkers which can provide valuable information about respiratory and systemic diseases. Proteomic analysis of EBC is a prospective method to detect early changes in the status of the respiratory system and possibly other organs. Here we focused on biomarkers of whooping cough, which is caused by Bordetella pertussis infection. We evaluated EBC samples from heathy volunteers who were nasally inoculated with B. pertussis and subsequently received antibiotic therapy to eradicate colonisation either 14 days post-inoculation or upon symptom onset, whichever came first. We performed our analysis using mass spectrometry-based proteomics and employed an optimized methodology that includes gel-free sample preparation, Orbitrap based HPLC-MS analysis and powerful search tool to achieve a high number of protein identifications. We analysed patients' samples both pre-and post- B. pertussis inoculation at multiple time points. We have identified over 4000 proteins across a cohort of 89 individuals, whose samples were measured in 3 technical replicates. Among the identified proteins, the biomarkers of whooping cough were selected and suggested.

Acknowledgment

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Echo MS: a rapid tool for kinases inhibitors search

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Abstract

Kinases with their diverse biological functions are important targets in high-throughput screening. The identification of novel kinase inhibitors is a crucial step in the development of therapeutic strategies for many diseases. So far we have used MALDI-TOF analysis as primary tool for early-stage drug discovery. To increase the speed, capacity and overcome the MALDI-TOF limitations we are introducing the SCIEX Echo® MS System to our laboratory. Echo® MS System is a new platform for rapid, chromatography-free MS/MS analysis with acoustic sample ejection. The system allows sample dilution and introduction to electrospray direct from the plate. 6500+ Triple Quad can detect chemical compounds with mass range of 5-2000 Da in the challenging matrices that predicts this system for high-throughput, quantitative work. The Echo® MS assay is being optimized for cyclin-dependent kinases and is readily adaptable for the study of other kinases as well.

Acknowledgment

This work was supported by EU – Programme EXCELES, ID Project No. LX22NPO5102; the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN: LM2023052, EATRIS-CZ: LM2023053); the internal grant of Palacky University Olomouc (IGA_LF_2023_025).

Claire: interpreting proteomics data using deep probabilistic databases of peptides

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Abstract

Reliable detection of peptides from tandem mass spectra is pivotal in advancing proteomics research. Over the last years, we have been developing Claire—a software suite for detecting peptides with amino acid substitutions. Recent computational and statistical developments, however, allow for broader detection scenarios, such as unrestricted detection of post-translational modifications or non-tryptic peptides. Herein, we will discuss the improvements in constructing deep peptide databases, the exact calculation of p-values, and the statistical evaluation of candidate peptides. Finally, we will describe the software developments providing Claire as a cloud service, allowing fast remote interpretation of tandem mass spectra against vast databases—typically in the terabyte scale.

Stress response in Hypsibius exemplaris

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Abstract

Tardigrades show remarkable resilience to diverse forms of stress, including acidity, 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. In previous transcriptome genotyping study, we observed uniquely high overexpression of genes related to several DNA damage response pathways. These results were further confirmed during a time-course experiment, showing the progress of mRNA transcription during the first 48 hours after exposure to ionizing radiation. Since then, we have further expanded upon the study by comparing how varying doses of ionizing radiation reflect on the transcription of mRNA. Furthermore, we have tested if starvation of H. exemplaris compromises its ability to respond to ionizing radiation via gene overexpression. Lastly, we have ran some preliminary experiments regarding tardigrade response to adjustments of pH in its living environment and presence of heavy metal compounds, such as lead, zinc, and iron.

Acknowledgment

Student’s grant competition - IGRÁČEK: DSGC-2021-0085

The first CACHE challenge: searching for hits in ultra-large chemical databases

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Abstract

The CACHE initiative (Critical Assessment of Computational Hit-finding Experiments) was created to improve and accelerate development of approaches for primary hit finding. The first competition involved 25 leading groups in computational chemistry and chemoinformatics from all over the world to find promising hit molecules for the WD40 repeat (WDR) domain of leucine-rich repeat kinase 2 (LRRK2) which is the most commonly mutated gene in familial Parkinson's Disease. The goal was to find hits among compound supplied by the Enamine company which maintains the database of about 2.5 million of synthesized compounds and a Enamine REAL Space which includes more than 10 billion of virtually enumerated synthetically accessible molecules. The 3D structure of the protein was resolved recently, however, no highly active hits were known for this protein that created an additional challenge. We developed and used a multi-step pipeline to enable fast searching of potential hits in a database of billions of molecules. It included de novo generation of query molecules, similarity searching in a large database, a consensus scoring approach incorporated molecular docking and calculation of binding free energy by MM-GBSA, etc. After the first stage of the CACHE challenge we found 8 experimentally confirmed hits, which brought us to top 3 teams. These hits were further optimized during the second stage. In the talk we will describe advantages of our strategies in mining of ultra-large linraries.

Acknowledgment

The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic through INTER_EXCELLENCE II grant LUAUS23262, the e-INFRA CZ (ID:90254) and projects ELIXIR-CZ (LM2023055) and CZ-OPENSCREEN (LM2023052). We also acknowledge the contributions from the project ENOCH (CZ.02.1.01/0.0/0.0/16_019/0000868).

Repurposing OpnMe.com drug candidates: A call for collaborative evaluation of potential anti-cancer agents

Alina Kutlushina[1], Vincent-Alexander Scholz[2], Matthias Welsch[2], Pavel Polishchuk[1], Johannes Kirchmair[2]

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Abstract

The path from molecule discovery to drug development is fraught with obstacles, often sidelining potentially valuable candidates before their therapeutic benefits can be fully explored. OpnMe.com, a groundbreaking initiative from Boehringer Ingelheim, offers a collection of these well-characterized molecules, initially designed for specific proteins but halted before commercial development [1]. Our team embarked on a mission to unearth alternative therapeutic pathways for the molecules presented by OpnMe.com. With their known characteristics in mind, repurposing these compounds could provide a swift avenue to new drug developments. To decipher potential repurposing routes, we utilized cutting-edge computational methods, including the Rapid Overlay of Chemical Structures (ROCS) and detailed protein sequence analysis. Notably, we have ordered a selection of these molecules directly from Boehringer Ingelheim, and they are now available at our institute for further research. Given the potential of some of these molecules as effective anti-cancer agents, we encourage the biological community to collaborate with us. Together, we can explore the biological activity of these promising compounds, bridging the gap from molecule discovery to tangible medical solutions.

Acknowledgment

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STRUCTURE-BASED GENERATION OF SYNTHETICALLY FEASIBLE MOLECULES

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Abstract

De novo molecular design is a growing method for generating new compounds with desired pharmacological properties, creating valuable intellectual property. However, a significant limitation of this approach is the need to ensure that the designed molecules can be feasibly synthesized. In the current work, we have developed a tool for creating drug-like compounds within protein binding sites. This tool includes the use of the CReM method to generate ligand structures and molecular docking by 3 available programmes (AutoDock Vina, vinardo and gnina) to assess their binding to a target protein. For de novo compound generation we docked a preliminary created set of starting fragments from ChEMBL compounds and grew them iteratively. We implemented several strategies to select molecules on each iteration: greedy, Pareto or clustering-based selection that should affect diversity of final molecules. The developed tool was used to de novo generation of ligands form CDK2 and other targets frequently used in benchmarking studies to investigate dependency of diversity, synthetic accessibility, docking score and other properties of generated molecules from a chosen setup. This tool was also successfully applied in the first CACHE challenge were the goal was to find promising LRRK2 inhibitors in a database of 16B compounds.

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In silico modeling and analysis of new estradiol dimers as tubulin inhibitors with anticancer activity

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Abstract

Estradiol dimers (ED) are a class of compounds that have been shown to be promising anticancer agents by binding in colchicine's site and inhibiting tubulin polymerization and mitosis formation. In this study we analyzed binding modes of new derived estradiol dimers that showed strong cytotoxic effects on cancer cell lines and were comparable with initial estradiol dimer, a known tubulin inhibitor. To confirm the stability of established docking poses and identified key interactions we performed 150 ns molecular dynamics simulations and calculated binding free energies. The calculated free energies well corresponded to measured tubulin polymerization speed and showed correlation value as 0.93. This supports that identified binding poses can be valid and confirm our conclusions about protein-ligand interactions.

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

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Abstract

Introduction: Clonal hematopoiesis of indeterminate potential (CHIP) is a common age-related condition manifested by the accumulation of somatic mutations in cells of the hematopoietic system. CHIP was recently described as a vascular risk factor associated with higher cardiovascular mortality. The aim of our study was to determine whether CHIP contributes to the etiopathogenesis of ischemic stroke.

Methods: The study included 588 patients aged 70+ years who were assigned into 4 cohorts based on the presence of ischemic stroke and atherosclerosis: (1) stroke with carotid stenosis (N=134), (2) carotid stenosis only (N=69), (3) ischemic stroke only (N=309), and (4) no stroke or stenosis (N=76). Mutations in blood cells were identified in 38 CHIP-related genes using the sensitive method of massively parallel sequencing. Results and conclusions: CHIP positivity in patients was ~75% regardless of the study cohort. Mutations were most commonly observed in genes DNMT3A (230/588; 39%) and TET2 (160/588; 27%). Patients with ischemic stroke, irrespective of the etiology, had a higher cumulative variant allele fraction (VAF) than the patients without a stroke history (4.2%; IQR 1.9-13.5 vs. 3.2%; IQR 1.4-9.7; p=0.045). Also, stroke patients were significantly more likely to have mutated ASXL1 compared to controls (11.7% vs. 4.8%; p=0.025). Based on these findings, somatic mutations in the ASXL1 gene in blood cells could potentially be used as a predictor of stroke in the future.

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Influence of morphine analgesia, opioid growth factor receptor and cannabinoid receptor 2 gene expression in tumor tissue on survival of patients with pancreatic cancer

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Abstract

Introduction: Pancreatic cancer (PDAC) is one of the most common causes of cancer-related death in the world. PDAC patients are often treated with opioid analgesia after surgery. These drugs act through opioid and cannabinoid receptors, which pathways are involved in tumor progression and metastases and can negatively affect the survival of patients.

Methods: Gene expression of opioid/cannabinoid receptors was analyzed in RNA purified from tumor tissues of 130 PDAC patients using real-time RT-PCR on LightCycler 384 Multiwell plates. B-actin gene expression was used for gene expression normalization. Statistical analysis was performed using R software. Relationship between opioid/cannabinoid receptors expression in tumor tissue and patients survival was analysed using COX regression, Kruskal-Wallis/ANOVA test and Kaplan-Meier method. Results: Of the 71 analysed patients, 48 received morphine analgesia and 23 received piritramide analgesia in the postoperative period. Patients receiving morphine analgesia had significantly longer cancer specific survival (CSS) than those receiving piritramide analgesia ($p = 0.04$). Of the studied receptors, high OGFR and CB2 expression have positive influence on length of overall survival (OS; $p = 0.009$ and $p = 0.027$, respectively). Conversely, high delta opioid receptor expression shortened OS ($p = 0.041$). Gene expression of CB1, and CB2 was decreasing significantly with higher stage of disease ($p = 0,003$ and $p = 0,0002$, respectively).

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