IMTM REACTOR

INSTITUTE OF MOLECULAR AND TRANSLATIONAL MEDICINE

IMTM REACTOR

8th Annual IMTM Retreat

October 21–23, 2023 / hotel Medlov

PROGRAM

Monday, October 21

08:00		Departure from Lékařská Fakulta Bus station	
10:00		Arrival to Hotel Medlov	

Chair: Pavel Moudrý

10:30	10:45	**Lucie Berésová**	A new versatile method for studying shear stress and circulating tumor cells *in-vitro*
10:45	11:00	**Matthew Lacey**	MCOPPB Mechanism - Autophagy Inhibition Drives Senolysis
11:00	11:15	**Martin Loffelmann**	Copper ionophores and their mechanism of action in the cuproptotic type of cell death
11:15	11:30	**Dávid Lukáč**	Exploring ssDNA Gaps in a BJ RAS Model of Early Stage Cancer Development
11:30	11:45	**Zuzana Machačová**	Uncoupling PARP trapping from inhibition of PARP activity to study replication gaps and replication fork acceleration
11:45	12:00	**Dominik Vítek**	Stress response to ionizing radiation and heavy metals in Hypsibius exemplaris
12:00	13:00	LUNCH	
13:00	15:00	**Petr Pavliš**	IT training
15:00	15:30	COFFEE BREAK	

Chair: Miloš Petřík

15:30	15:45	**Katarína Hajduová**	Preclinical assessment of enhanced blood retention and tumor uptake PSMA-targeting alpha emitters-labeled radioconjugates
15:45	16:00	**Jiří Voller**	Kinetin derivatives for therapy of neural system disorders
16:00	16:15	**Kristýna Krasulová**	68Ga siderophore siderotyping for the imaging of Pseudomonas aeruginosa infections
16:15	16:30	**Barbora Neužilová**	The interaction of B. pertusis (BP) with its human host results in inflammation
16:30	16:45	**Viktor Valentini**	Will add later but it will be focused on LCK and CuEt
16:45	17:00	**Ermin Schadich**	Antimycobacterial property of compounds from proprietary library
17:00	17:15	BREAK	

Chair: Viswanath Das

17:15	17:30	**Pavel Stejskal**	Liquid biopsy in solid tumors
17:30	17:45	**Monika Vidlařová**	Validation of a method for circulating tumor cell detection in ovarian cancer
17:45	18:00	**Ihor Kozlov**	Effect of inhibitors on the aggregation of wild-type and mutated forms of tau monomers
18:00	18:15	**ALLDI CHARANRAJ GOUD**	Advanced Surface Plasmon Resonance (SPR) Technology for Drug Discovery in Neurodegenerative Disorders: Targeting the R2R3 Peptide Regions of Tau Protein

18:15	18:30	**Mária Nováková**	Advanced human stem cell models of Alzheimer's disease
18:30	18:45	**Jan Sebastian Novotný**	microRNAs (miRNAs) in Alzheimer's disease
18:45	19:00	**Jana Zelinková**	Amyloid Precursor Protein (APP) as a candidate molecular inducer and regulator of reactive astrogliosis
19:00		DINNER	

Tuesday, October 22

Chair: Dušan Holub

9:00	9:15	**Martina Kintlová**	Advancing Kinase Inhibitor Discovery: SCIEX Echo® MS Integration for Rapid HTS and Therapeutic Insights
9:15	9:30	**Denisa Kroupová**	Characterization of Intact Carbonic Anhydrase 9 (CAIX) Using SPR and LC-MS
9:30	9:45	**Miroslav Hruška**	Claire: a cloud-enabled software for detecting rare peptides, with a focus on peptide variants
9:45	10:00	**Tomáš Oždian**	Is the Tear Proteome Suitable Source for Cancer Biomarker Studies
10:00	10:15	**Jarmila Stanková**	Advanced Proteomics Analysis of Plasma Samples in an Enigma Cohort Study
10:15	10:30	**Jana Václavková**	Mass spectrometry-based analysis of exhaled breath condensate
10:30	11:00	COFFEE BREAK	

Chair: Soňa Gurská

11:00	11:15	**Lucie Kotková a Barbora Kalousová**	Clonal somatic variants in hematopoietic cells of the healthy population with link to epigenetic age prediction model MethAge performance
11:15	11:30	**Ondřej Bouška**	Circulating and Salivary DNA-based Biomarkers for Early Diagnosis and Recurrence Monitoring of HPV-Related Oropharyngeal Cancer
11:30	11:45	**Lenka Hrubá**	Nucleoside-based drugs resistance in oncology
11:45	12:00	**Kateřina Ječmeňová**	Live imaging of A3 adenosine receptor interactions with a fluorescent antagonist probe
12:00	12:15	**Lukáš Lenart**	Precision-Crafted Cystic Fibrosis Models: Generation of Novel Tools for Advanced High-Throughput Screening
12:15	12:30	**Martin Ondra**	Now you see me: boosting regeneration capacity of corneal endothelial cells by drug repurposing
12:30	12:45	**Alžběta Srovnalová**	Morphological profiling and advancel cell cycle analysis using high throughput microscopy and robotic platform
12:45	13:00	**Matěj Šamaj**	Validation of Potential Molecular Targets of PNH173 and PNH192 Identified by Genome-Wide CRISPRi Screening in Human Lymphoblastoid Cells
13:00	14:00	LUNCH	
14:30	16:00	HOTEL EXCURSION IN CZECH	
16:15	17:45	HOTEL EXCURSION IN ENGLISH	
19:00		DINNER	

Wednesday, October 23

Chair: Ivo Frydrych

9:00	9:15	**Hendrik Vercammen**	Forecasting a clear vision: How QSAR modeling catalyzes small molecule development in the field of corneal endothelial regeneration
9:15	9:30	**Nikta Ziaei**	Optimizing high-throughput screening: Optimized 3D spheroid models for cutting-edge drug discovery
9:30	9:45	**Aleksandra Ivanová**	GenCReM: de novo generation of synthetically feasible compounds based on genetic algorithm
9:45	10:00	**Guzel Minibaeva**	Crem-dock: comparison to Reinvent4 and fragment expansion study
10:00	10:15	**Jan Macháň**	PySPRESSO: Simple Python Pipeline for Multi-Batch LCMS Data processing
10:15	10:30	**Jiří Hodoň**	Triterpenoids are natural compounds with various biological activities
10:30	11:00	COFFEE BREAK	

Chair: Milan Urban

11:00	11:15	**Antonio Conte**	Discovery and Development of Novel DYRK1A Inhibitors Using a Multi-Component Approach
11:15	11:30	**Riccardo Fusco**	Virtual screening is a promising approach that accelerates the discovery of pharmacological agents
11:30	11:45	**Zeinab Saedi**	Targeting factor XII with small molecule inhibitors: A novel approach to safer antithrombotic and anti-inflammatory therapy
11:45	12:00	**Samatha Masineni**	Innovative Design and Synthesis of SOS1/RAS Degraders
12:00	12:15	**Marziyeh Mousazadeh**	Precision medicine: Developing chemical inhibitors for KRAS G13C-mutant cancers
12:15	12:30	**Mayur Mukim**	Targeting the K-Ras G13C Mutation with Covalent Small Molecules
12:30	12:45	**Kaoud Salama**	MraY Enzyme: Crucial Target for Innovative Antibiotics
12:45	13:00	**Vijayendar Venepally**	Multicomponent Reaction Strategy for the Synthesis of Small-Molecule Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists
13:00	14:00	LUNCH	
14:00		DEPARTURE FROM HOTEL MEDLOV	

A new versatile method for studying shear stress and circulating tumor cells *in-vitro*

Lucie Béresová [1], Jan Víteček [3,8,9], Iva Protivánková [1]), Michal Dudka [6], Katarína Chromá [1,2], Zdeněk Škrott [1,2], Tereza Buchtová [1], Kamila Poláková [3], Jan Novotný [7], Ludmila Novaková [7], Jiri Bártek [1,4,5] and Martin Mistrík [1*]

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[8] International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic

[9] Department of Biochemistry, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Abstract

In the metastatic process, cancer cells navigate through various conditions, including mechanical shear stress in the bloodstream. This study introduces a novel device - The Shear Stress Generator (SSG) - which effectively mimics these conditions by applying shear stress to cells. The SSG generates shear stress levels comparable to those found in human veins and capillaries, providing a simpler and more accessible alternative to traditional microfluidic systems. Designed to be compatible with standard cell culture flasks and incubators, this method exposes much larger numbers of cells to mechanical shear stress, thereby expanding analytical possibilities and reducing the loss of suspension cells typically seen in microfluidic systems. Using this device to investigate the responses of various human cell lines to shear stress, we identified a previously unknown, reversible pre-cytokinetic block in cells that lose anchorage during mitosis and are kept under constant shear stress. Interestingly, some cancer cell lines can bypass this unconventional arrest, suggesting it may serve as a safety checkpoint to limit the proliferation of cancer cells in the bloodstream and reduce their potential for spreading.

Acknowledgment

MCOPPB Mechanism - Autophagy Inhibition Drives Senolysis

Matthew Lacey [1], Lucie Béresová [2], Martin Mistrík [2]

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

Abstract

Cellular senescence, a stable form of cell cycle arrest, has been linked to a number of age-related pathologies. In an effort to combat diseases of aging, the targeted treatment and removal of senescent cells using senolytic compounds is an ongoing area of research. MCOPPB was previously identified as a senolytic compound via the screening of a chemical library, our data indicates that MCOPPB's senolyitc activity appears to be independent of its role as a Nociceptin receptor agonist. Instead, it appears as if the senolytic activity of MCOPPB is tied to autophagy inhibition. Further research has shown that other autophagy inhibitors can also function as senolytic compounds.

Acknowledgment

Copper ionophores and their mechanism of action in the cuproptotic type of cell death

Martin Loffelmann [1], Zdeněk Škrott [1,2], Lucie Béresová [1], Martin Mistrík [1]

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[2]*Laboratory of Genome Integrity, Institute of Molecular and Translational Medicine, Czech Advanced Technology and Research Institute, Palacky University Olomouc, Olomouc, Czechia*

Abstract

Cuproptosis is a newly discovered type of cell death dependent on the presence of copper ions. These ions are important as cofactors of enzymes involved in metabolic pathways and detoxification processes. However, the abundance of copper ions in cells can have detrimental effects. Characteristic markers of the cuproptotic type of cell death were described as oligomerization of lipoylated dihydrolipoyl transacetylase (DLAT) proteins and the iron-sulphur cluster protein loss leading to proteotoxic stress. Furthermore, cells dependent on mitochondrial metabolism are more sensitive to this type of cell death. Cuproptosis is mediated by ferredoxin 1 (FDX1) reductase, which is responsible for protein lipoylation and the reduction of copper ions that are subsequently responsible for DLAT oligomerization. We tested two copper ionophores in our experiments – bis(diethyldithiocarbamate)-copper (CuET) and elesclomol (Ele) – to see their effectiveness in causing cuproptosis in numerous cancer cell lines. We observed that Ele had higher toxicity and that the phenotypes were the same as those caused by CuET, such as unfolded protein response, the heat shock response, abrogation of the p97/NPL4 pathway, and the accumulation of polyubiquitinated proteins. On the contrary, we struggled to detect typical cuproptotic phenotypes in cells treated with Ele or CuET. This indicates that cuproptosis may be cell line specific or that its mechanism may be different and is associated with a distinct proteotoxic stress pathway.

Acknowledgment

Exploring ssDNA Gaps in a BJ RAS Model of Early Stage Cancer Development

Dávid Lukáč [1], *Pavel Moudrý* [1] *and Martin Mistrík* [1]

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Abstract

Precancerous lesions are abnormal tissue changes that can progress to cancer, often driven by genetic mutations like the activation of the RAS oncogene. In this preliminary work, we used immortalized human foreskin fibroblast BJ cells with an introduced RAS oncogene as a model for precancerous lesions, as the RAS oncogene is a well-known driver of oncogenesis. Our focus is to determine whether activation of the RAS oncogene via a doxycycline-inducible system causes replication stress and toxic ssDNA gaps. We employ immunofluorescence analysis with ScanR scanning microscopy using an undenatured CldU staining assay and a modified DNA fiber protocol utilizing S1 nuclease, which specifically cuts single-stranded DNA regions. Our goal is to uncover how RAS-induced replication stress impacts early cancer development, which may offer new insights into mechanisms that drive tumor progression.

Acknowledgment

Uncoupling PARP trapping from inhibition of PARP activity to study replication gaps and replication fork acceleration

Zuzana Machačová [(1)], *Iva Protivánková* [(1)], *Pavel Moudrý* [(1)]

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Abstract

PARP inhibitors are a promising class of anticancer drugs commonly used to treat breast cancer and ovarian cancer, among others. They target poly(ADP-ribose) polymerase (PARP), an important protein involved in multiple cellular processes including DNA repair and DNA replication. It was previously thought that PARP inhibitors induce replication fork stalling and collapse leading to DNA double-strand breaks and eventually cell death in cells with defective homologous recombination (HR) repair and/or replication fork protection (FP) pathways. In 2018, we confronted this model with the observation that PARP inhibitors do not cause fork stalling, and rather induce replication fork acceleration even in HR/FP defective cells. It is also known that PARP inhibitors can induce PARP trapping onto DNA, however, it remains unclear if trapping or blocking PARP activity, is responsible for high fork velocity and induction of single-stranded DNA gaps.

Using high-content imaging screen of commercially available PARP inhibitors, we aim to investigate which ones show the strongest ability to trap PARP1 and compare their capability to induce fork acceleration, inhibition of PARylation and formation of ssDNA gaps. Our goal is to define which effect of PARPi, whether trapping or activity block, is crucial for replication fork acceleration, induction of replication gaps and cellular sensitivity.

Acknowledgment

Stress response to ionizing radiation and heavy metals in Hypsibius exemplaris

Dominik Vítek [1], Matthew Lacey [1], Jiří Voller [1,2], Marián Hajdúch & Rastislav Slavkovský [1]

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[2] *Laboratory of growth regulators, Czech Republic*

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 several time-course experiments, showing the progress of mRNA transcription during the first several days after exposure to ionizing radiation.

In recent developments of the study, we have not only closely monitored the rise of individual DNA damage repair genes within the organism, but also expanded the study beyond the original 48 hours scheme. In addition, we began to screen the resistance to presence of heavy metal compounds, such as lead, zinc, and iron in Hypsibius exemplaris and Caenorhabditis elegans for comparison.

Acknowledgment

Kinetin derivatives for therapy of neural system disorders

Jiří Voller [1,2], Barbara Maková [2] , Václav Mik [2], Barbora Lišková [1], Martin Sládek [3], Markéta Dokonalová [2], Lenka Drašarová [4], Jana Kotulová [1], Martina Medvedíková [1], Alena Sumová [3], Petr Džubák [1], Marián Hajdúch [1], Miroslav Strnad [5]

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Abstract

The plant hormone kinetin has demonstrated protective effects in various animal models and shows potential for slowing aging in human cells and model invertebrates. It is an active ingredient in therapeutic cosmetics with clinically proven efficacy and is currently undergoing clinical trials as an oral treatment for familial dysautonomia. In animal cells, kinetin can be converted into kinetin riboside triphosphate, which serves as a substrate to enhance the activity of the neuroprotective kinases PINK1 and casein kinase 2. This lecture summarizes the testing of a large library of kinetin derivatives in in vitro models of familial dysautonomia, Friedreich's ataxia, and Parkinson's disease, as well as the effects of these compounds on circadian rhythm. Mechanistic studies (transcriptomics, kinase inhibition and interaction with adenosine receptor), biotransformation analysis as well as ADME in vitro results are also reported.

68Ga siderophore siderotyping for the imaging of Pseudomonas aeruginosa infections

Kristýna Krasulová [(1)], Barbora Neužilová [(1)], Kateřina Dvořáková Bendová [(1)], Marián Hajdúch [(1,2)], Miloš Petřík [(1,2)]

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Abstract

Pseudomonas aeruginosa (P.a.) is a prominent opportunistic pathogen responsible for a wide range of infections, particularly in immunocompromised patients. The development of novel therapeutic and diagnostic strategies is being intensively pursued. One of the promising diagnostic strategies for detecting and localising P.a. could be the use of its siderophores. Siderophores are small iron-chelating molecules produced by almost all microorganisms. P.a. produces a group of siderophores called pyoverdines. The iron in the siderophore structure can be replaced by gallium-68, a positron-emitting radionuclide suitable for positron emission tomography (PET) imaging. These radiolabeled siderophores mimic the natural iron acquisition process of the bacteria, allowing for specific and efficient targeting of the pathogen. Meyer et al. analysed pyoverdines and classified them into three different siderophore types. Type I was identified with the type strain P.a. ATCC15692 (PVD I, PAO1), type II was identical with P.a. ATCC27853 (PVD II, PA27) and third group reacted as P.a. DSM3602 (PVD III, PA6). Here we report on the 68Ga-pyoverdines siderotyping for the imaging of P.a. infections. Radiolabeling of selected siderophores (PVD I,II,III) with Ga-68 was performed using acetate buffer. *In vitro* uptake of 68Ga-siderophores was tested in various characterised P.a. model strains and also patient isolates to confirm P.a. three-types siderotyping. Mouse infection models with different P.a. strains were developed to evaluate the use of 68Ga-pyoverdines for the detection of bacterial infections caused by different P.a. strains.

All studied pyoverdines were labeled with 68Ga with high (>95%) radiochemical purity. 68Ga-pyoverdines displayed different levels of uptake in tested P.a. strains corresponding to the three-group distribution among characterised strains and patient isolates. PET/MRI imaging of selected 68Ga-pyoverdines in P.a. infected animals showed focal accumulation of 68Ga-pyoverdines in P.a. infected tissue. The radioactive signal from each of the used pyoverdins accumulated specifically at the site of infection corresponding to the respective group strain. No radioactive signal was detected in S. aureus infections, sterile inflammation, heat-inactivated samples, or saline injections. Siderotyping of the Pseudomonas aeruginosa strains into three groups was confirmed by *in vitro* testing of the uptake of three pyoverdines by a number of different P.a. strains. Three siderophores characterized three groups and were successfully used to imagine infection using PET/MRI system in mice. By combining all three siderophores, a cocktail was created that allowed the detection of infection caused by any Pseudomonas aeruginosa strain.

Acknowledgment

PET/CT imaging of Bordetella pertussis infection

Barbora Neužilová [(1)], Kristýna Krasulová [(1)], Kateřina Dvořáková Bendová [(1)], Peter Sebo [(2)], Jana Holubová [(2)], Marián Hajdúch [(1,3)], Miloš Petřík [(1,3)]

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Abstract

The interaction of B. pertusis (BP) with its human host results in inflammation, activation of the immune response and damage to host tissues. [1, 2] The use of siderophores and the haem utilisation systems of BP are genetically characterised and share key regulatory features. We investigated the imaging of BP by positron emission tomography (PET) using radiolabelled siderophore [68Ga]Ga-desferrioxamine-B (DFO- B). We monitored BP infection in a nasal, lung and muscle model.

DFO-B was labelled with gallium-68. The radiochemical purity of complex was measured on RP-HPLC. We study BP – Wild type (WT). In vitro uptake of [68 Ga]Ga-DFO-B was evaluated in bacteria, which were cultivated in Stainer-Scholte medium with - where is significantly reduced absorption of the 68 Ga-siderophore - and without ferrous sulfate heptahydrate. The heat-inactivated bacterial cultures displayed also diminished uptake of 68 Ga siderophore. Muscle, lung and nasal infection models were used to assess [68Ga]Ga-DFO as a radiotracer of B. pertussis infection using PET/CT imaging. In vivo PET/CT imaging was performer 45 min after [68 Ga]Ga-DFO-B injection in BP infected Balb/c mice and 5 h post-infection. [68Ga]Ga-DFO became the preferred candidate for PET infection imaging showing high accumulation at the site of infection in tested infection models.

Acknowledgment

[1.] Bickman, Timothy J., Tomoko Hanawa, Mark T. Anderson, Ryan J. Suhadolc a Sandra K. Armstrong. Differential expression of Bordetella pertussis iron transport system genes during infection. Molecular Microbiology [online]. 2008, 70(1), 3-14. ISSN 0950-382X. doi:10.1111/j.1365-2958.2008.06333.x

[2.] Adam M. Smith, Carlos A. Guzmán, Mark J. Walker, The virulence factors of Bordetella pertussis: a matter of control, FEMS Microbiology Reviews, Volume 25, Issue

The effects of CuEt on Lck phosphorylation and cytotoxic activity

Viktor Valentini [1], Juan Bautista De Sanctis [1], Jenny Garmendia [1], Claudia De Sanctis [1], Hana Duchová [1], Marián Hajdúch [1].

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Abstract

Introduction: The involvement of LCK (leukocyte-specific kinase) in T-cell receptor signaling and NK cell activation, particularly through the phosphorylation of tyrosine residues that modulate its activity, is well established. CuEt, a compound with promising anti-cancer properties, has been shown to activate T-cells at nanomolar concentrations. However, the specific activation pathway responsible for this effect has not been fully elucidated. This study aims to investigate the impact of a 1nM concentration of CuEt on CD8+ T cells (memory and naive) and NK cells, with a focus on the phosphorylation of LCK at tyrosine 394 (activating) and 505 (inhibitory) sites. Additionally, the study will assess the involvement of downstream signaling molecules such as Zap70, LAT, and ERK1/2.
Material and Methods: Lymphocytes were isolated from buffy coat using Ficoll hypaque. Then, CD8 and NK cells were separated using nano-bead-labeled antibodies and were subsequently stimulated for 30 minutes. The impact of CuEt stimulation was further examined in the presence of the tyrosine kinase inhibitor dasatinib. Additionally, cytotoxic assays were conducted by exposing the stimulated cells to different cell lines, including HCT116, HCT116KO, and A549.

Results: Western blot analysis revealed that CuEt induces phosphorylation of LCK at tyrosine 394 and 505 in CD8+ T cells and NK cells. Phosphorylation on Tyr192 and serine residues wasn't detected. Stimulated cells are more cytotoxic to 2D cultures of cancer cell lines than spheroids. Dasatinib inhibits the cytotoxic effect of CuEt. Cells re-stimulated by CuEt could not respond; hence, they may be exhausted.

Conclusions: CuEt induces activated LCK activity independently of antigen presentation. The double-phosphorylated form of Lck recorded may be related to CD45-independent activation. These processes may be important for eliminating solid tumors.

Acknowledgment

Antimycobacterial property of compounds from proprietary library

Ermin Schadich [1], Soňa Gurská [1,3], Barbora Lišková [1], Pavel Znojek [1], Pavlo Polishchuk [1], Miroslav Soural [1,2], Petr Džubák [1,3], Marián Hajdúch [1,3]

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Abstract

In the last decade, we assessed the antimicrobial activity profiles of compounds from proprietary library against Leishmania parasites and different bacterial species including Mycobacterium bovis, as well as their antiviral activity profiles. While our studies were focused mainly on acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during three-year pandemic (2020-2023), the other pathogens including bacterium Mycobacterium tuberculosis become more significant pathogens in post-pandemic period. Tuberculosis, a disease caused by M. tuberculosis remains a serious health problem due to high incidence and mortality globally. The emergency of drug-resistant strains of M. tuberculosis and the increase in disease incidence, stresses the need for development of novel drugs. The objective of this study was to identify the novel compounds with activity against three different bacteria including two Mycobacterium bovis BCG substrains, M. bovis BCG substrain Danish and M. bovis BCG substrain Russia and multidrug resistant M. tuberculosis. The activity of compounds from proprietary library were tested for activity against M. bovis BCG substrain Danish and M. bovis BCG substrain Russia in a high throughput growth inhibition assay. The primary high throughput screen of 4800 proprietary library compounds for antimycobacterial activity against M. bovis BCG Russia showed that fifty compounds were identified as the primary hits, which at concentration of 50 µM inhibited the bacterial growth at the rate of ≥ 50%. When this library was screened for activity against M. bovis BCG substrain Danish, the results showed that sixty compounds were identified as the primary hits.

The dose-response analysis showed that forty two compounds had the activity against both strain with IC50 smaller than 10 µM. However, as the seventeen hits were ignored due to their previously published activity and eighteen hits were cytotoxic to human BJ fibroblast and/or mouse J774 cells, only seven secondary hits were tested for activity against intracellular M. bovis substrain Russia in the infected mouse J774 cells. Five secondary hits with the IC50 against intracellular bacteria smaller than 10 µM were selected as the final hits. Four of these final hits were also active against multidrug resistant M. tuberculosis in dose response assays. Further studies will be focused on these four compounds.

Acknowledgment

Liquid biopsy in solid tumors

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

[1] *Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Czech Advanced Technology and Research Institute, Palacky University and University Hospital in Olomouc, Czech Republic*

[2] *University Hospital in Olomouc, Olomouc, Czech Republic*

Abstract

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, typically in peripheral blood. Circulating tumor cells (CTCs), circulating tumor DNA and RNA (ctDNA and ctRNA) 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 solid tumors. 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. The ctDNA/ctRNA profiling was also optimized for cancer patient plasma samples. The established protocols will be applied to larger cohorts of patients whose enrolment is ongoing.

Acknowledgment

Validation of a method for circulating tumor cell detection in ovarian cancer

Vidlařová Monika [1], Stejskal Pavel [1], Kubíčková Agáta [1], Frydrych Ivo [1], Srovnal Josef [1], Hajdúch Marián [1]

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

Abstract

Introduction: Ovarian cancer is the fifth leading cause of cancer-related death in women in Europe and the leading cause of death among gynecological malignancies. About 80 % of ovarian cancer patients have disseminated disease at the time of diagnosis. Circulating tumor cells (CTCs) may be precursors of distant metastatic spread and can act as an independent prognostic and predictive biomarker.

Methods: For validation IGROV-1, OVCAR-3 and OVCAR-5 cell lines were used. Cells were stained with pan-cytokeratin, EpCAM and CD45 antibody and DAPI. Cell lines OVCAR-3 and OVCAR-5 were selected to validate of ovarian cancer CTCs detection in blood samples. From two healthy volunteers three peripheral blood samples were collected into Cell-Free DNA BCT® (Streck, Inc.). Into each whole blood sample 100 cells (3xOVCAR-3, 3xOVCAR-5) were spiked in. Samples were processed, stained and smeared on Cytodiscs. The CTCs were identified using the CytoTrack CT11 (2/C, Denmark), a semi-automated immunofluorescence microscopy detecting the pan-cytokeratin and EpCAM signals.

Results: Detection of circulating tumor cells in ovarian cancer was validated with using OVCAR-3 and OVCAR-5 cell lines. In samples with the OVCAR-3 cell line an average of 48 cells out of 100 were detected. In samples with the OVCAR-5 cell line an average of 60 cells out of 100 were detected.

Conclusion: The selected antibody mixture can be used to detect CTCs in ovarian cancer patients.

Acknowledgment

Effect of inhibitors on the aggregation of wild-type and mutated forms of tau monomers

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Abstract

Tauopathies are characterized by a diversity of causes, several mutations associated with changes in the rate of progression of neurodegeneration, and mechanisms of fibril seed propagation between neurons and glia. Therefore, it is important to test new potential drugs in models reflecting all or most of the pathologic factors leading to aggregate formation. Small molecules can potentially slow the aggregation of mono- and oligomers by binding to preformed beta structures stabilizing them or leading to the degradation of pathological oligomers. Other molecules may prevent the further spreading of seeds. The work describes the results of the seeding experiments with Tau RD P301S biosensor cells. A confocal microscope system was used to calculate the number of tau protein aggregates and the area infected by aggregates. Cells were transfected with preformed tau fibrils previously prepared from monomers of Tau protein consisting of a 62 amino acid site with mutations in the repeats responsible for polymerization.

Acknowledgment

Advanced Surface Plasmon Resonance (SPR) Technology for Drug Discovery in Neurodegenerative Disorders: Targeting the R2R3 Peptide Regions of Tau Protein

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Abstract

This study presents an innovative approach to drug discovery for neurodegenerative disorders, utilizing advanced Surface Plasmon Resonance (SPR) technology to identify novel therapeutic compounds targeting the Tau protein. Specifically, the focus is on the R2R3 peptide regions of Tau, which are critical in the pathology of tauopathies, including Alzheimer's disease and other neurodegenerative disorders. The study explores how these peptide regions, both in their wild-type form and a well-characterized mutant, respond to potential therapeutic compounds. A panel of compounds—Purpurin, Oleocanthal, Entacapone, and Rosmarinic acid—was screened for their binding affinities using SPR. The results demonstrated favourable interactions with wild-type and mutant R2R3 peptides, suggesting these compounds may have therapeutic potential in mitigating Tau-related neurotoxicity. For the wild-type R2R3 peptides, Purpurin exhibited a dissociation constant (KD) of 5.45×10^{-6} M, with association (Kon) and dissociation (Koff) rates of 113.63 $M^{-1}s^{-1}$ and 6.197×10^{-4} s^{-1}, respectively. Oleocanthal and Entacapone also showed strong binding, with KD values of 2.786×10^{-6} M and 1.69×10^{-6} M, respectively, reflecting significant potential for therapeutic development. The P301L mutant peptide interactions revealed even stronger binding for Rosmarinic acid, with a KD of 6.31×10^{-7} M, alongside Purpurin, which exhibited a KD of 1.03×10^{-5} M. Advanced SPR techniques such as Multi-Injection Cycle Kinetics (MICK) and Titration Cycle Kinetics (TCK) were employed to validate these interactions, offering more profound insights into the binding dynamics. These results demonstrate the robustness of SPR in identifying promising drug candidates and pave the way for functional studies using proteomics to explore further the molecular mechanisms by which these compounds exert their effects on Tau pathology. This study represents a significant step forward in discovering novel therapeutics for neurodegenerative diseases, particularly those characterized by abnormal Tau aggregation.

Acknowledgment

Advanced human stem cell models of Alzheimer's disease

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Abstract

Stem cell technology has the potential to revolutionize Alzheimer´s disease (AD) research. With the ability to self-renew and differentiate into various cell types, stem cells are valuable tools for disease modeling. However, recent evidence indicates that differentiation of CNS cell lineages requires intimate exchange of growth and transcription factors between differentiating cells. Cell to cell communication is essential for coordination of cellular events, better development and homeostasis. We have developed a novel indiscriminate protocol for optimal maturation and physiological function of CNS cell lineages. Neurons generated using the indiscriminate approach exhibited thinner dendritic branches, less frequent, but significantly larger pre- and post-synaptic boutons, delayed electrophysiological maturity, robust calcium responses and decreased vulnerability to glutamate-induced cell death. In terms of their transcriptional profile, these neurons were 98% identical to the neurons harvested from week 20 human fetal brain cortex. Astrocytes showed enriched branching, robust calcium responses and significant transcriptional similarities with astrocytes harvested from fetal brain cortex, while the oligodendrocytes demonstrate least changes. All preliminary experiments show, that the new 2-D in vitro model is likely to become preferred tool for AD research, drug screening, and cell therapy.

Acknowledgment

Identification of blood miRNAs altered in Alzheimer's disease: joint evidence from meta-analysis and co-expression analysis

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Abstract

A number of studies have demonstrated the role of microRNAs (miRNAs) in Alzheimer's disease (AD). However, the roles of individual miRNAs are often controversial in different studies, and the miRNAs identified as playing a role in AD also vary between studies. Several meta-analytic studies have previously reported a unifying view on the most relevant AD-related miRNAs. However, many of the original studies reported only significantly differentially expressed miRNAs and only these values were then often included in subsequent meta-analyses. This may lead to biased results due to overestimation of the significance of the miRNAs in question, given the lack of data on instances (studies) where these miRNAs did not show a significant difference in AD. The aim of this meta-analytic study was to investigate the pooled effect of miRNAs in AD based only on studies that provided a complete set of results from differential expression (DE) analysis results (including also non-significantly DE miRNAs) or raw expression data of human samples. We performed a meta-analysis of 15 datasets containing data for 2808 miRNAs and a weighted differential co-expression network analysis. We identified 162 miRNAs with significant and most stable roles in AD (such as hsa-miR-194-1, hsa-miR-5703-3p, hsa-miR-6501, hsa-miR-10392-5p, hsa-miR-6867 and others), whose targets were related to, e.g., intrinsic pathway for apoptosis or cytokine signaling in immune system. Differential co-expression analysis subsequently detected 12 clusters of miRNAs. 4 clusters with the largest average fold-change in AD turned to be related to e.g. hippocampus development, regulation of neuroinflammatory response, myelin maintenance, protein phosphorylation or negative regulation of apoptotic process. These results further expand and refine our understanding of the role of miRNAs in AD and provide a basis for further experimental research and choice of potential targets for therapeutic action.

Acknowledgment

Morphological changes of astrocytes in Alzheimer´s disease

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Abstract

During progression of neurodegenerative diseases such as Alzheimer's disease (AD), astrocytes display morphological changes, a hallmark of reactive astrogliosis, which reflect functional alterations resulting in pathological consequences. Our recently published paper presents the Amyloid Precursor Protein (APP) as a candidate molecular inducer and regulator of reactive astrogliosis. However, behaviour of APP in human astrocytes in AD remains poorly understood.

Taking into account these observations, we focused on the morphology of astrocytes in AD and examined APP changes in reactive astrocytes in AD within the areas without presence of amyloid in human samples. Astrocytes from postmortem human brain sections which included 3 healthy subjects and 3 AD patients were subjected to detailed morphological analysis focusing on parahippocampal gyrus. GFAP stained sections show reactive astrocytes in AD which manifest significant differences in the majority of the observed parameters in comparison to healthy subjects. The next ongoing step is the investigation of the relationship between APP and IFNg in reactive AD astrocytes. This analysis will guide future studies of the role of APP in astrocytes and beyond.

Advancing Kinase Inhibitor Discovery: SCIEX Echo® MS Integration for Rapid HTS and Therapeutic Insights

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Abstract

Kinases are integral to numerous biological processes, making them prime targets in high-throughput screening for drug discovery. Identifying novel kinase inhibitors is crucial for developing treatments for various diseases. Previously, we relied on MALDI-TOF analysis for early-stage drug discovery. Still, to enhance the speed and capacity of our workflow and overcome MALDI-TOF's limitations, we have now integrated the SCIEX Echo® MS System into our laboratory. This cutting-edge platform enables rapid, chromatography-free MS/MS analysis using acoustic sample ejection, allowing direct sample introduction to electrospray from the plate and supporting sample dilution. The 6500+ Triple Quad can detect compounds in the 5–2000 Da mass range, even in complex matrices, making it highly effective for high-throughput, quantitative studies. The Echo® MS assay has been optimized for Aurora and cyclin-dependent kinases (CDKs), key targets in cancer therapy, and MARK kinases, potential therapeutic targets for Alzheimer's disease. Notably, a set of derivatives has recently been identified as potent inhibitors of MARK enzymes. These derivatives, which also influence the cell cycle, have shown activity when tested against Aurora and CDK kinases. Therefore, the further improvement of the selectivity of this class of the compounds will be needed.

Acknowledgment

Characterization of Intact Carbonic Anhydrase 9 (CAIX) Using SPR and LC-MS

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Abstract

The research focuses on characterizing the intact form of carbonic anhydrase 9 (CAIX) using SPR and LC-MS (Vanquish NEO-Orbitrap Exploris 480). LC-MS enables the analysis of post-translational modifications and the dynamics of enzyme conformation. This data provides valuable insights into the functional properties and stability of the enzyme under various conditions. SPR offers real-time monitoring of CA9 interactions with its ligands, contributing to a deeper understanding of the mechanisms of enzymatic catalysis and inhibition. This methodology allows us to examine the binding kinetics and specificity of CA9 interactions with potential inhibitors, which is crucial for developing therapeutic strategies. This protocol will subsequently serve as a foundational methodological approach for characterizing other intact proteins.

Acknowledgment

Claire: a cloud-enabled software for detecting rare peptides, with a focus on peptide variants

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Abstract

Detection of peptides and their forms represents a starting point for most bottom-up proteomics analyses. Although a reliable detection of reference peptides is a simple and well-established procedure, its complexity disproportionately grows as the interest shifts toward rare peptide forms. Herein, we discuss the developments made over the last year in Claire—our software system utilizing deep probabilistic peptide databases for peptide detection. The internal developments centered around introducing several statistical models to allow more robust reasoning over the detected peptides. These included the solidification of a target-decoy strategy, improvements in evaluating peptides' sequencing support, and a model for assessing peptides' origin. Further, we substantially improved the ability to quantify peptides, also serving to distinguish somatic variant peptides from protein synthesis errors. The external developments included finalizing the cloud computing environment and generalizing the web user interface. Altogether, these developments bring Claire several steps closer to allow a reliable detection of rare peptide forms, based on solid theoretical foundations.

Acknowledgment

Is the Tear Proteome Suitable Source for Cancer Biomarker Studies

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Abstract

The search for biomarkers in body fluids, especially through non-invasive methods, has garnered increasing interest. While this approach is applicable to many tissues and diseases, the brain presents a unique challenge due to its encapsulation within the skull and protection by the blood-brain barrier. Cerebrospinal fluid (CSF) is the only body fluid that directly reflects brain activity, but its collection is invasive. Another potential, though underutilized, source of biomarkers is tear fluid. Tears, which wash the eye, are connected to the brain via the optic nerve. The tear proteome has been investigated in relation to various diseases, including Parkinson's disease, breast cancer, and colon cancer, demonstrating its diagnostic potential. In our preliminary study, we compared two groups: patients with retrobulbar neuritis and healthy volunteers. In the retrobulbar neuritis group, approximately 600 proteins per sample were identified in the initial analysis. Subsequently, the protocol for the healthy volunteers was significantly optimized and applied to state-of-the-art Orbitrap instruments with ion mobility separation, leading to the identification of around 3,000 proteins.

Tissue expression analysis of these results revealed 20 proteins specific to brain tissue. This finding, along with documented crosstalk between tears and cerebrospinal fluid, suggests the presence of neuronal proteins in the tear proteome. These results support the suitability of tear fluid as a promising source of biomarkers for brain malignancies.

Acknowledgment

Advanced Proteomics Analysis of Plasma Samples in an Enigma Cohort Study

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Abstract

The Enigma cohort study is a comprehensive multi-omics investigation, with proteomics being one of its key perspectives. Its goal is to enhance our understanding of the plasma proteome using high-resolution mass spectrometry. By employing the cutting-edge Orbitrap Ascend platform and an optimized Data-Independent Acquisition (DIA) workflow for plasma, the study provides in-depth insights into protein expression patterns. The cohort consists of 1,104 plasma samples, of which around 800 have been analyzed so far. Initial results demonstrate high reproducibility and stringent quality control, including metrics such as the number of proteins identified per sample, batch effect evaluation, and technical variations like sample order. Preliminary principal component analysis (PCA) demonstrates clear proteomic distinctions between female and male samples, as well as variability associated with age. These results provide early insights into biological and technical factors influencing plasma proteomics. A multi-omics sub-study on 127 samples from the Enigma cohort further reveals that among the most variable plasma proteins are ApoL1, Alpha-1-antitrypsin, and Immunoglobulin heavy chains, with significant implications for personalized medicine and disease biomarker discovery. Notably, Alpha-1-antitrypsin SNP variants were identified using two independent raw data analysis workflows—IMTM's proprietary software and Proteome Discoverer with the full Uniprot-TREMBL database, underscoring the power of integrated proteomic and genomic approaches.

Acknowledgment

Mass spectrometry-based analysis of exhaled breath condensate

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Abstract

We investigated exhaled breath condensate (EBC) as a non-invasive biological matrix for biomarker discovery, particularly as a promising source of respiratory tract biomarkers. Building upon our already published mass spectrometry-based method, we enhanced it for high resolution and reproducibility in protein identification. EBC was collected using the Turbo 14 Turbo DECCS System (Medivac, Italy) from both healthy and diseased adults who breathed into the collection device for 10 minutes. Proteins in the sample were solubilized, denatured, reduced, digested with trypsin, and purified using StageTip technology. Samples were analysed via high-resolution mass spectrometry (HPLC-MS/MS-LTQ Orbitrap Elite) in three technical replicates. Protein searches were conducted using Proteome Discoverer software (Thermo Scientific) and the data were further analyzed using Statistica and Bioconductor R – package.

In the first part of the work, we focused on non-small cell lung cancer (NSCLC) diagnostics. We have identified 4,806 proteins across 226 individuals' samples measured in triplicates. Combining univariate and multivariate statistical approaches and sensitivity analysis, we identified biomarkers capable of distinguishing NSCLC patients from those with COPD and from healthy individuals. This protein signatures are currently undergoing validation via targeted mass spectrometry, using the SureQuant approach.

In the second part of the work, we compared two EBC collection methods: a resuscitation mask attached to the collection device versus the standard mouthpiece. The mask based method, designed primarily for collecting EBC from individuals infected with Bordetella pertussis, also allowed for sampling from both nasal and oral fractions. We analysed EBC of healthy individuals, identifying 1,844 proteins across 100 individuals' samples measured in triplicates. Differences between the collection methods were observed and could guide future EBC collection strategies for various analyses.

Acknowledgment

Clonal somatic variants in hematopoietic cells of the healthy population with link to epigenetic age prediction model MethAge performance

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*Both authors contributed equally.

Abstract

Introduction: Clonal Hematopoiesis of Indeterminate Potential (CHIP) is characterized by the accumulation of somatic mutations in cells of the hematopoietic system. Although CHIP is often asymptomatic, it has been identified as a potential precursor to malignant neoplasms such as leukemia. Additionally, CHIP has been linked to an increased risk of cardiovascular diseases, including atherosclerosis and stroke. As CHIP is an age-related condition, we hypothesise that the epigenetic age prediction model MethAge may detect accelerated aging in patients with a higher proportion of CHIP variants. This study explores the prevalence of CHIP in the Czech population and its potential link to epigenetic aging, as assessed by the MethAge model.

Methods: The study included over 150 healthy individuals from the ENIGMA cohort, aged 19 to 63 years. Mutations in 38 CHIP-related genes were identified from blood samples using the highly sensitive method of massively parallel sequencing (MPS). Epigenetic age was evaluated using the MethAge method, which involved bisulfite conversion, qPCR, tagmentation, and MPS.

Results and conclusions: We observed CHIP in the healthy population, even in individuals under 40 years of age. However, no mutations were identified in participants younger than 30. Preliminary results of correlation analysis between CHIP positivity and methylation-based age prediction error indicate that CHIP does not affect methylation at the selected loci. This suggests that the MethAge model is not affected by CHIP and retains its ability to estimate chronological but not biological age.

Acknowledgment

Circulating and Salivary DNA-based Biomarkers for Early Diagnosis and Recurrence Monitoring of HPV-Related Oropharyngeal Cancer

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Abstract

Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) has surged in recent decades worldwide. In the Czech Republic, annual OPSCC cases have more than tripled over 30 years, with nearly 800 new cases diagnosed in 2021, surpassing cervical cancer. OPSCC is now predominantly HPV-related, with about 25% of patients experiencing recurrence within five years. Liquid biopsies (plasma and saliva) are being investigated for their potential in diagnosis, treatment monitoring, and recurrence detection through DNA-based biomarkers.

Methods: HPV tumor status was assessed in newly diagnosed OPSCC patients and those in remission using HPV DNA detection in primary tumor tissue and p16 immunohistochemistry. Only cases positive for both HPV and p16 were classified as HPV-related OPSCC. HPV testing was conducted on liquid biopsies (gargle lavage, oropharyngeal swabs, and plasma) before and after treatment, with regular follow-up sampling.
Results: A total of 151 OPSCC patients were enrolled in the study, with 81.8% classified as HPV-related, predominantly involving the HPV16 genotype (98.9% of cases). Pre-treatment analysis in liquid biospies showed high sensitivities: 91.7% in gargle lavage, 96% in oropharyngeal swabs, and 95.8% for circulating tumor HPV DNA (ctHPV DNA) in plasma. Among recurrent HPV-related OPSCC cases, ctHPV DNA was detected in 75% (3/4) of cases, with 50% (2/4) also testing positive for oral HPV DNA.

Conclusion: This study seeks to validate liquid biopsy collection and DNA-based biomarker detection for early diagnosis and recurrence monitoring in HPV-related OPSCC patients. Preliminary results are promising, showing potential for early detection, even in early-stage OPSCC, and its clinical applicability.

Acknowledgment

Nucleoside-based drugs resistance in onkology

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Abstract

Drug resistance poses a significant challenge in oncological therapy for both solid tumors and hematological malignancies. It can complicate initial treatment due to primary resistance of tumor cells or cause relapse when secondary resistance develops[1]. In vitro cellular models are essential for understanding the mechanisms by which cancer cells acquire this resistance, discovering new therapeutic approaches to overcome resistant phenotypes, and enhancing treatment efficacy.

Over the past three decades, our institute has developed several drug-resistant cellular models. Recently, we have focused on elucidating resistance mechanisms of nucleoside-based drugs, including cytarabine, fludarabine, 6-thioguanine, and new experimental compounds, primarily PNH173. Clarifying the mechanism of action and resistance of novel substances is a complex task requiring multidisciplinary expertise, encompassing analyses of DNA/RNA, proteins, and metabolites. We are focusing mainly on protein pathways involved in drug resistance and identifying new predictive markers.

We have recently evaluated the mechanisms of action for cytarabine, fludarabine, and 6-thioguanine, including the influence of multidrug resistance proteins (Pgp, MPRs, BCRP) and markers significantly impacting treatment efficacy. For PNH173, we are investigating proteins potentially linked to drug resistance based on CRISPR-Cas9 analysis results, as well as mTOR and metabolic pathways.

Acknowledgment

Live imaging of A3 adenosine receptor interactions with a fluorescent antagonist probe

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Abstract

Introduction: Adenosine receptors (ARs), particularly the A3 adenosine receptor (A3AR), are G-protein-coupled receptors that are highly expressed in cancer cells, making them valuable therapeutic targets. However, current live-cell imaging techniques for ARs, including visualizing receptor expression, tracking their membrane localization, or studying interactions with AR modulators (such as competition and competitive displacement), remain limited. A deeper understanding of these receptors is essential to enhance the success of adenosine receptor drug discovery. In this study, we present a microscopic technique for visualizing and functionally studying A3AR using CELT-228, a novel A3AR-specific fluorescent probe. This approach can be applied in living cells and competitive binding assays to identify and validate new A3AR agonists and antagonists.

Material and methods: The reporter cell line overexpressing A3AR was treated with the A3AR-specific fluorescent probe (fluorescently labeled A3AR antagonist, CELT-228; Celtarys, Spain), and fluorescent images were captured using spinning disc confocal microscopy (Yokogawa CV7000) at various time points and concentrations. The localization and intensity of the fluorescent signal were then analyzed using Signals Image Artist (SImA) software. A competitive assay utilizing the CELT-228 probe was developed to study interactions between potential A3AR agonists or antagonists, and it was employed for High Content Screening (HCS) of newly synthesized nucleoside-based compounds. This assay was also used to assess interactions of newly identified A3AR agonists or antagonists in cancer cell lines derived from tumors of diverse histogenetic origins expressing A3AR. Additionally, the probe was used to stain spheroids derived from the Hep-3B, hepatocellular carcinoma cell line, which also express the A3 adenosine receptor.

Results: The fluorescent signal of the bound probe was localized on the cell membrane of the reporter cell line and most of the cancer cell lines. Our data from competition assays indicated that the newly synthesized potential anticancer compound binds to the same (orthosteric) binding site on the A3 receptor and competes for it with the fluorescent-labeled probe.

Conclusion: This technique offers a powerful tool for observing A3AR expression, monitoring small molecule competition, and confirming the binding of novel compounds under native conditions. Our method has the potential to enhance understanding of adenosine receptors and improve the success rate of AR-targeted drug discovery.

Acknowledgment

Precision-Crafted Cystic Fibrosis Models: Generation of Novel Tools for Advanced High-Throughput Screening

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Abstract

Cystic fibrosis (CF) is a severe autosomal recessive disorder, caused by mutations in a cystic fibrosis transmembrane conductance regulator (CFTR) gene. Contemporary CF treatment relies on CFTR modulators, a group of small compounds that aim to restore the CFTR localization and function of an ion channel. Currently, up to 85% of CF patients, mostly carrying the most frequent CFTR mutations, are eligible for treatment with the highly efficient combination of CFTR modulators Trikafta®. However, patients with rare CFTR mutations remain without efficient modulator-based treatment, highlighting the need for the development of novel CFTR modulators. The establishment of innovative high throughput screening (HTS) assays, and relevant cell models endogenously expressing CFTR with rare mutations will dramatically accelerate the screening of potential CFTR modulators. Previously, a reporter cell line enabling the detection of endogenous CFTR levels based on the HiBiT tag has been developed from human bronchial epithelial cells 16HBE14o- (Ondra et al. 2024). This cell line can be further modified by the CRISPR/Cas9 technology to carry desired CFTR mutations, giving rise to novel HTS models with endogenous expression of mutant-CFTR on the isogenic background. As a proof of concept, a monoclonal cell line carrying the most common CF-causing mutation ΔF508 in both CFTR alleles has been prepared and validated.

Acknowledgment

ONDRA, M., L. LENART, A. CENTORAME, D. DUMUT, A. HE, S. ZAIDI, J. HANRAHAN, J. DE SANCTIS, D. RADZIOCH, M. HAJDÚCH. CRISPR/Cas9 bioluminescence-based assay for monitoring CFTR trafficking to the plasma membrane. Life Science Alliance. 2023, 7(1), e202302045, ISSN: 2575-1077, PMID: 37918963.

Now you see me: Boosting regeneration capacity of corneal endothelial cells by drug repurposing

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Abstract

The preservation of corneal transparency relies heavily on the functionality of corneal endothelial cells. Mechanical eye trauma or diseases affecting the corneal endothelium can impair vision and eventually lead to corneal blindness. Currently, the only treatment option for patients with endothelial dysfunction is corneal transplantation. Our research focuses on a new approach to promote the endogenous regeneration capacity of corneal endothelial cells, specifically through drug repurposing. This investigation broadens our understanding of how pharmacological agents can complement or potentially replace donor-dependent therapies. The significance and impact of this project are underscored by its potential to address the critical global shortage of corneal graft tissue through the proposal of pharmacological alternatives to traditional endothelial keratoplasty. Specifically, we have employed our published research methodology [1] to establish a framework for high-throughput screening of large chemical libraries to identify unique compounds that enhance corneal cell regeneration. To date, we have screened over 2,000 compounds. This innovative approach is poised to expedite the discovery of effective treatments, bringing hope for restoring vision and minimizing the necessity for surgical interventions. Undoubtedly, our research paves a promising path forward in the field of regenerative ophthalmology.

Acknowledgment

[1.] Vercammen, H.; Ondra, M.; Kotulova, J.; De La Hoz, E.; Witters, C.; Jecmenova, K.; Le Compte, M.; Deben, C.; Dhubhghaill, S.; Koppen, C.; Hajduch, M.; Van Den Bogerd; B. "Keep on ROCKin": Repurposed ROCK inhibitors to boost corneal endothelial regeneration. Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie (2024), 174, 116435.

Morphological profiling and advancel cell cycle analysis using high throughput microscopy and robotic platform

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Abstract

Introduction: Morphological profiling with the cell painting assay uses high-content, multiplexed imaging to analyze cellular morphology. Fluorescent stains target specific organelles, enabling automated image analysis to provide quantitative and qualitative insights at the single-cell level. This technique helps identify the effects of chemical compounds and supports predictive models to evaluate drug candidates and mechanisms of action, even without prior knowledge of the drug target. It also plays a key role in identifying disease-specific phenotypes and predicting compound toxicity.

For advanced cell cycle analysis, the FUCCI reporter system was used, with fluorescently tagged proteins Cdt1 and Geminin visualizing each cell cycle phase. Tumor cells bypass the cell cycle's precise control, leading to uncontrolled replication, which many anticancer drugs aim to block. We added a far-red probe for histone H2B to ensure comprehensive cell detection. The FUCCI system's versatility, with plasmids for various fluorescent markers, combined with advanced morphological profiling offers deeper insights into cell cycle regulation.
Methods: Six fluorescent probes were used to label eight organelles, and imaging was performed using the CellVoyager 8000 system across four channels. HepG2 cells were treated with 25,000 compounds from the EU-OPENSCREEN library in four replicates, followed by fixation, staining, and robotic analysis. Image analysis software extracted features, generating detailed cellular profiles and identifying subtle phenotypes in response to chemical perturbations.

For FUCCI reporter system, U2OS cells were transfected with green-red and blue-red plasmids, with an additional plasmid for H2B detection. Palbociclib was used as a positive control for G1 phase blockade, Bleomycin for S/G2/M phase blockade detection, and Nocodazole for the detection of mitotic cells. Multinucleated cells were observed following treatment with Paclitaxel. Images were acquired using Yokogawa CV8000, with SIMA software for analysis.

Results and conclusions: Image analysis generated morphological profiles for 25,000 compounds, with high correlation to positive controls and new insights into chemical effects. The FUCCI system detected expected cell cycle effects, and combined with morphological profiling, identified distinct cell populations and states like mitosis, apoptosis, and multinucleation. This approach enhances high-throughput screening of cell cycle modulators and deepens understanding of cellular responses to treatments, offering potential for discovering novel drugs targeting specific cell cycle phases.

Acknowledgment

[1.] Vercammen, H.; Ondra, M.; Kotulova, J.; De La Hoz, E.; Witters, C.; Jecmenova, K.; Le Compte, M.; Deben, C.; Dhubhghaill, S.; Koppen, C.; Hajduch, M.; Van Den Bogerd; B. "Keep on ROCKIn": Repurposed ROCK inhibitors to boost corneal endothelial regeneration. Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie (2024), 174, 116435.

Validation of Potential Molecular Targets of PNH173 and PNH192 Identified by Genome-Wide CRISPRi Screening in Human Lymphoblastoid Cells

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Abstract

PNH173 and its structural analog, PNH192, are 7-deazaadenine derivatives exhibiting strong anticancer activity. A genome-wide CRISPRi screen was performed in the K562 dCas9-KRAB BSD cell line to reveal the mechanisms of action of these nucleoside derivatives PNH173 and PNH192. Based on the screening results phosphorylase kinase regulatory subunit beta (PHKB) and kinesin family member 24 (KIF24) were identified as candidate molecular targets of the tested compounds. Cell lines with upregulated or downregulated expression of PHKB and KIF24 were created and subjected to their further validation. These modified cell lines are expected to display either resistance or increased sensitivity when treated with the studied compounds. Cytotoxicity testing of PNH173 and PNH192 on the established cell lines is being employed to verify the involvement of the candidate genes obtained by the whole-genome CRISPRi screen. Further investigation will explore the downstream pathways influenced by PHKB and KIF24 to better understand their roles in mediating the compounds' anticancer effects.

Acknowledgment

Forecasting a clear vision: How QSAR modeling catalyzes small molecule development in the field of corneal endothelial regeneration

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Abstract

Corneal endothelial dysfunction causes corneal blindness, currently treatable only by transplantation, a solution limited by donor tissue scarcity. Therefore, we seek alternative treatment solutions, envisioning a pharmacological treatment that boosts the endogenous regeneration capacity of corneal endothelial cells. Nevertheless, traditional drug development is both expensive and time-consuming. To overcome these obstacles, we developed a hybrid screening strategy by employing a quantitative structure-activity relationship (QSAR) consensus model that integrates four distinct regression models. After optimizing model weights, we applied this model to the virtual Enamine® Hit Locator Library. Next, a consecutive compound selection was made for experimental validation using an in vitro image-based live cell imaging proliferation assay on B4G12 cells. The in vitro results aligned with the QSAR-predicted biological activities and exhibited trends similar to Y-27632, a ROCK inhibitor used as a positive control for stimulating corneal endothelial cell proliferation and migration [1]. These findings validate the predictive power of our QSAR model and highlight its relevance for future screening of additional compounds using a hybrid screening approach. This strategy offers a cost-effective and efficient alternative to traditional drug development methods, potentially accelerating the hit identification stage of pre-clinical small molecule development focused on corneal endothelial regeneration.

Acknowledgment

[1.] Vercammen, H.; Ondra, M.; Kotulova, J.; De La Hoz, E.; Witters, C.; Jecmenova, K.; Le Compte, M.; Deben, C.; Dhubhghaill, S.; Koppen, C.; Hajdúch, M.; Van Den Bogerd; B. "Keep on ROCKIn": Repurposed ROCK inhibitors to boost corneal endothelial regeneration. Biomedicine & pharmacotherapy (2024).

Optimizing high-throughput screening: Optimized 3D spheroid models for cutting-edge drug discovery

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Abstract

The 3D cell culture system serves as an advanced intermediary between traditional 2D models and complex in vivo tissues, offering a more representative platform for studying cellular behaviors. Among these, spheroids—spherical clusters of tumor cells—stand out for their ability to replicate the natural microenvironment with greater accuracy. Their enhanced cell-cell and cell-matrix interactions render them invaluable tools in drug discovery and toxicity testing.

In this study, we utilized spheroids as 3D models, specifically formed from the HCT116 cell line in 384-well plates, to optimize high-throughput screening (HTS) protocols across various spheroid types. First, we focused on standardizing the preparation of uniform spheroids, followed by refining the procedure for precise and reproducible delivery of drug compounds at varying concentrations using the ECHO liquid handler. Subsequently, spheroid plates were analyzed using the Cell Voyager CV8000 High-Content Screening System to capture detailed changes in growth, structure, and morphology in response to the treatments. Finally, cell viability was assessed using the MTS assay, providing robust quantitative data on the cytotoxic impact of the tested compounds.

By systematically optimizing each step, this study presents a streamlined approach for high-throughput drug screening in 3D spheroid models, enhancing both the precision and biological relevance of preclinical testing.

Acknowledgment

GenCReM: de novo generation of synthetically feasible compounds based on genetic algorithm

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Abstract

De novo generation methods offer a promising alternative to traditional virtual screening by providing a more efficient means to explore the vast chemical space, which is too large for exhaustive enumeration and screening. However, ensuring the synthetic feasibility of de novo generated compounds remains a key challenge for many existing approaches. In our work, we utilized the recently developed CReM[1] approach, which implicitly controls the synthetic feasibility of generated compounds, along with a genetic algorithm and molecular docking to navigate chemical space effectively. The main objective of our tool is to generate compounds meeting specific criteria defined by a custom fitness function. The parameters which can be controlled are docking scores, physicochemical and drug-likeness properties, ligand-protein interaction fingerprints to preserve important protein-ligand interactions, etc. The implemented pipeline can be used mainly for scaffold decoration and exploration of local chemical space around a lead compound. To some extent it can be applied for unrestricted de novo generation and exploration of wider chemical space.

We demonstrated the applicability of the approach to optimize structures of moderately active compounds and to generate analogs with improved properties. We also showed that synthetic accessibility of designed compounds depends primarily on CReM settings rather than on the complexity of a protein pocket or protein family. The designed molecules will require more comprehensive prediction of their affinity to select the most promising compounds for further synthesis and experimental validation.

Acknowledgment

Crem-dock: Comparison to Reinvent4 and fragment expansion study

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Abstract

Nowadays de novo approaches are increasingly used to search for new biologically active molecules. In this case, new compounds with desired pharmacological properties are assembled in the target cavity guided by the general principles of intermolecular interaction. One of the problems of de novo design tools is difficulty to control synthetic feasibility of generated compounds.

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 [1] to generate ligand structures and molecular docking by EasyDock [2] (3 available programs: AutoDock Vina, smina and gnina) to assess their binding to a target protein. The developed tool has two modes: i) iterative growing of a fragment co-crystallized with a protein preserving the position of the parent part of the molecule and ii) de novo compound generation. In the latter case we use a preliminary created set of starting fragments from ChEMBL compounds. This starting set of fragments is docked and iteratively grown. We implemented several strategies to select molecules on each iteration: greedy, Pareto or clustering-based selection that affect diversity of final molecules.

To validate the ability to generate compounds de novo we compared CReM-dock with the state-of-the-art generative tool Reinvent4 [3], which is a recurrent neural network trained on SMILES combined with reinforcement learning to explore chemical space using docking. For benchmarking we chose the proteins belonging to different families (CDK2, BACE1, DRD2, ESR1, HDAC2, PARP1), which are frequently used in other studies. Compounds designed by CReM-dock had comparable or better docking scores and possessed higher novelty in comparison with Reinvent structures while both tools resulted in comparably well synthetic accessibility of designed molecules.

To validate the ability of CReM-dock to expand small co-crystallized ligands within a binding site to designed compounds with higher affinity we performed a retrospective study. We chose corresponding pairs of smaller and larger ligands having X-rays structures of complexes and apply our tool to the smaller ligands. In some cases we were able to exactly reproduce the larger ligands, in some cases highly similar structures were generated. These results confirm applicability of CReM-dock to design synthetically sensible molecules and explore relevant chemical space.

Acknowledgment

[1] Polishchuk P., Journal of Cheminformatics 2020, 12:28 [doi: 10.1186/s13321-020-00431-w]

[2] Minibaeva G., Ivanova A., Polishchuk P., Journal of Cheminformatics 2023, 15:102 [doi: 10.1186/s13321-023-00772-2]

[3] Loeffler, H.H., He, J., Tibo, A. et al. Reinvent 4: Modern AI−driven generative molecule design. Journal of Cheminformatics 16, 20 (2024) [doi.org/10.1186/s13321-024-00812-5]

PySPRESSO: Simple Python Pipeline for Multi-Batch LCMS Data processing

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Abstract

We want to present an open-source off-line Python module: PySPRESSO, that is designed to simplify the processing of large "multi-batch" LCMS data. This tool includes various filters, correction methods, and statistical tools that allow users to customize settings to the specific needs of their data. We conceived the pipeline as an all-in-one solution for data processing following the use of "peak-picking" software such as MZmine. Streamlining the process of data analysis will not only help us in the multi-omics characterization of endometriosis but will also speed up any other future projects. We hope to gather a community around the PySPRESSO module, that would enrich its functionality and help us find more users. Functions from PySPRESSO module effectively address the problems of batch effect and other analytical errors. In addition to data processing, the main advantages are the ability to generate images suitable for presentations or publications and the subsequent creation of a reporting PDF file that allows users to follow each process applied onto the data step by step. The ability to work off-line with "multi-batch" LCMS data, including those with many missing values, is particularly useful for cases of confidential or excessively large files that are not suitable for uploading to popular online tools. The user can run the workflow locally and interpret the data very quickly by themselves. The code of the pipeline is written entirely in Python, ensuring accessibility and easy implementation of new filters and data transformations. With Python as the main machine learning language, it is also ready to integrate various AI models for feature prediction, data classification, or other correction methods such as SERRF, for example. This pipeline can contribute not only to accelerate data analysis and evaluation, but also to improve reproducibility and unify approaches across scientific teams thanks to its fully accessible code.

Acknowledgment

Triterpenoids are natural compounds with various biological activities.

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Abstract

Our research group primarily focuses on their cytotoxicity. [1] Triterpenoid pyridines and pyrazines had high cytotoxicity of IC50 0.5 – 1.5 µM in leukemic cell lines (CCRF-CEM, K-562). [2] Medoxomil-type prodrugs surprised us with an extreme selective cytotoxicity against K-562 cells with IC50 26–43 nM. [2] The mechanism of action of active derivatives is still unknown. To get more insight into the mechanism of action, it is essential to identify the target proteins of the studied compounds. For these purposes, conjugates of active triterpenes with a bifunctional linker containing diazirine and an alkyne moiety for photocrosslinking with interacting proteins were prepared. New methods for investigating the mechanism of action of active triterpenes will be introduced along with synthesis and future goals.

[1.] Borkova, L., Hodon, J., Urban, M. (2018). Asian J. Org. Chem., 7.8, 1542.
[2.] Hodoň, J., Frydrych, I., Urban, M. (2022). Eur. J. Med. Chem., 243, 114777.

Acknowledgment

Discovery and Development of Novel DYRK1A Inhibitors Using a Multi-Component Approach

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Abstract

Dual-specificity tyrosine-regulated kinase 1A (DYRK1A), a member of the CMGC kinase family, plays a critical role in various cellular processes, including neuronal development and cell cycle regulation. Aberrant activity of DYRK1A has been implicated in diseases such as Down syndrome and neurodegenerative disorders, highlighting its potential as a therapeutic target. This research focuses on the discovery and development of novel inhibitors for DYRK1A using a multi-component approach. We employ a combination of structure-based drug design, molecular docking, and high-throughput screening techniques to identify and characterize potential small-molecule inhibitors.

Our approach pursues two strategies:
First, we are designing ATP-competitive kinase inhibitors. By employing macrocyclization, we aim to enhance selectivity and potency. Macrocyclization constrains the conformation of the inhibitor, promoting interactions with unique regions of the kinase and reducing off-target effects associated with the conserved nature of ATP-binding pockets across the kinome.

Second, we are designing and synthesizing substrate mimics. Recognizing the challenge of designing selective ATP-competitive inhibitors due to the high conservation of the ATP-binding pocket, we explore an alternative strategy targeting the substrate-binding site. By designing molecules that mimic the substrate, we aim to compete with substrate binding, offering potential for higher selectivity. Analysis of the substrate peptide bound to DYRK1A (PDB ID: 2WO6) reveals that Arg6 plays a crucial role in binding, clamped between Glu291 and Glu353. In a fragment-based drug discovery (FBDD) approach, we propose screening pyrimidin-2-amine derivatives for binding to the Arg6 site. Upon establishing binding—confirmed, for example, by crystallography—the fragment can be elaborated using multi-component reaction (MCR) chemistry to extend across the substrate pocket and interact with phosphorylated Tyr321.

Early-stage findings suggest promising interactions, though optimization and further investigation are ongoing to enhance specificity and efficacy. Our work aims to establish a robust framework for the rational design of DYRK1A inhibitors, offering new avenues for therapeutic intervention in diseases linked to DYRK1A dysregulation.

Acknowledgment

Virtual screening is a promising approach that accelerates the discovery of pharmacological agents

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Abstract

Virtual screening is a promising approach that accelerates the discovery of pharmacological agents, significantly reducing costs and time in drug development. This method is particularly advantageous in emerging fields such as ligand-RNA targeting. However, the selection of the docking engine is crucial for successful outcomes. Various re-docking benchmarks have been proposed over recent years, focusing on both proteins and RNAs. . However, these benchmark focuses on a specific subset of PDB IDs chosen by the authors yielding averaged results more than PDB dependent results. Here we present Multi(NA)DockPy. A fully automated Python-based pipeline capable of performing re-docking experiments of RNA-Ligand complex using a user-defined list of PDB IDs on multiple engines (SMINA, GNINA, rDOCK, LeDock, Plants, nlDOCK, DOCK6). From the results of the re-docking experiments the pipeline is capable of automatically analyze various metrices (e.g. docking power, docking accuracy) in order to assets the best docking engine in a context dependent way based on the provided PDB IDs. In such a way the user can wisely choose a specific engine for it's own virtual screening routine. To validate the pipeline, a set approximately 400 RNA-Ligand complexes from the highly curated Hariboss Database was used.

Acknowledgment

Targeting factor XII with small molecule inhibitors: A novel approach to safer antithrombotic and anti-inflammatory therapy

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Abstract

Thrombosis is a significant global health issue that contributes to ischemic heart disease, ischemic stroke, and venous thromboembolism (VTE). The Global Burden of Diseases, Injuries, and Risk Factors (GBD) Study 2010 documented that ischemic heart disease and stroke collectively accounted for one in four deaths worldwide. This toll is rising, with aging of the populations, increasing rates of obesity and sedentary lifestyles, highlighting the urgent need for improved prevention and treatment strategies for thrombosis.

The search for effective antithrombotic therapies has led to the exploration of Factor XII (FXII) as a promising target. Factor XII, also known as Hageman factor, plays a complex role in blood coagulation and thrombosis. While it is primarily known for initiating the intrinsic pathway of the coagulation cascade, leading to clot formation, its role in thrombosis prevention is less straightforward. Interestingly, individuals with Factor XII deficiency do not typically exhibit excessive bleeding tendencies but have been observed to have a lower risk of thrombotic events. This paradox suggests that Factor XII may contribute to pathological thrombosis more than to normal hemostasis. Current research is exploring the inhibition of Factor XII as a potential therapeutic strategy to prevent thrombosis without significantly increasing the risk of bleeding. It provides a safer alternative to conventional anticoagulants, which often pose a high bleeding risk. Additionally, FXII inhibitors have shown potential in attenuating inflammatory responses and fibrinolysis, further broadening their therapeutic applications.

Our project focuses on the development of small molecule covalent inhibitors of FXII, emphasizing post-screening structure optimization to develop more selective compounds. The inhibitory activity of synthesized compounds toward FXII, also their selectivity has been assessed through a chromogenic substrate hydrolysis assay, and in vitro plasma coagulation assays (aPTT and PT). The analysis of the potential covalent inhibitor/FXII complexes will be performed utilizing Mass spectrometry and X-ray crystallography.

Acknowledgment

Innovative Design and Synthesis of SOS1/RAS Degraders

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Abstract

My research centers on developing a novel class of Proteolysis Targeting Chimeras (PROTACs) designed to degrade SOS1 and RAS, two key regulators of the RAS-MAPK signaling pathway implicated in cancer progression. This study is groundbreaking in two main aspects: first, the dual targeting of SOS1 and RAS for degradation is unprecedented and is expected to have a synergistic effect on inhibiting cancer growth; second, the integration of multi-component reaction (MCR) chemistry with high-throughput screening methodologies is a novel approach in this field. MCR chemistry enables the efficient and rapid synthesis of chemically diverse libraries, streamlining the discovery process of potential therapeutic compounds. When combined with high-throughput screening, this strategy accelerates the parallel synthesis and optimization of PROTAC candidates, enhancing the exploration of chemical space and increasing the likelihood of identifying potent and selective SOS1 degraders. Targeting the SOS1/RAS complex using PROTACs represents an innovative therapeutic strategy, particularly for cancers driven by abnormal RAS-MAPK signaling. By selectively degrading these proteins, this approach has the potential to disrupt oncogenic pathways, overcome resistance mechanisms, and offer a new avenue for precision cancer therapy.
To date, a dozen testable PROTAC compounds have been synthesized through a short, convergent route. These compounds will undergo testing in isogenic cell lines to assess their ability to degrade SOS1 and RAS. Based on the degradation results, further optimization of these compounds for potency and pharmacokinetic/pharmacodynamic (PKPD) parameters will be pursued. Patent research indicates "freedom to operate," and a patent filing is planned for the near future.

In conclusion, the integration of MCR chemistry with high-throughput synthesis in PROTAC development marks a significant advance in drug discovery, enabling more efficient targeting of previously undruggable proteins. This work underscores the importance of innovative synthetic methodologies in accelerating therapeutic breakthroughs in oncology.

Acknowledgment

Precision medicine: Developing chemical inhibitors for KRAS G13C-mutant cancers

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Abstract

The therapeutic potential of novel small molecules targeting the KRAS G13C mutation, a key driver in several aggressive cancers, including non-small cell lung cancer and colorectal cancer is investigated in this research. Based on the WHO statistics, lung and colorectal cancer with 2.5 and 1.9 million new cases worldwide are placed in the first and third rank among all the cancers. The G13C mutation results in the substitution of glycine with cysteine at codon 13 of KRAS, leading to constitutive activation of oncogenic signaling. Developing chemical inhibitors that specifically target this mutation presents a promising approach for precision therapy in patients with KRAS-driven cancers.

The study focuses on synthesizing and evaluating a series of novel compounds designed to selectively and covalently bind to the mutant cysteine residue of KRAS G13C, thereby inhibiting its oncogenic activity. To identify lead compounds, a comprehensive screening funnel is employed, combining virtual screening, biochemical assays, and high-content cellular analysis. The initial screening step involves virtual docking and molecular dynamics simulations to prioritize compounds for experimental validation. Selected candidates are then evaluated using biochemical assays to confirm covalent binding to the G13C mutation and inhibition of GTPase activity. Compounds demonstrating robust biochemical activity undergo cell-based assays using cancer cell lines expressing the KRAS G13C mutation, assessing their impact on proliferation, apoptosis, and key downstream signaling pathways such as MAPK and PI3K. The final stage involves structural biology techniques such as X-ray crystallography and co-crystallography of the KRAS and the final compound to elucidate the binding mode and conformational changes in KRAS.

Given the historical challenges associated with targeting KRAS, the selective inhibition of KRAS G13C represents a significant advancement in targeting this previously "undruggable" oncogene. This research aims to contribute to the growing body of work focused on developing targeted therapies for KRAS-driven cancers, which remain an area of significant unmet medical need.

Acknowledgment

Targeting the K-Ras G13C Mutation with Covalent Small Molecules

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Abstract

KRAS is one of the most commonly mutated oncogenes in human cancers, driving abnormal cell growth. Despite being known for nearly 50 years, drugs targeting the KRAS G12C mutation, like sotorasib and adagrasib, were only recently developed. While G12C is predominant, the G13C mutation also occurs in about 3% of lung cancer cases. Targeting G13C is difficult due to the high affinity of GTP for its binding pocket, which is considered 'undruggable.' Our work focuses on designing small molecules that mimic GTP and covalently bind to C13. These molecules aim to overcome the challenges posed by GTP's strong binding affinity and high cellular concentration. We're using structure-based drug design to develop and test these molecules, hoping to pioneer G13C inhibitors and lay groundwork for targeting other GTP-binding proteins.

Acknowledgment

MraY Enzyme: Crucial Target for Innovative Antibiotics

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Abstract

The enzyme phospho-MurNAc-pentapeptide translocase (MraY) is crucial for bacterial cell wall synthesis, making it an attractive target for antibacterial drug development. Given the rise of antibiotic-resistant bacteria, there is an urgent need for new inhibitors targeting this enzyme. Many natural MraY inhibitors feature uracil and uridine as key components, which inspired their use as anchors in the design of new synthetic inhibitors. In this project, we are designing and synthesizing new inhibitors of the MraY enzyme based on uracil and uridine anchors. The selection of these two anchors is based on their presence in the majority of natural compounds known to inhibit MraY, as well as their structural compatibility with the enzyme's active site. Our strategy involves functionalizing these cores to optimize their inhibitory potency and pharmacokinetic properties. With methods of high throughput experimentation (HTE) synthesis is performed in 96-well plates to obtain high-quality products. Structural analysis has confirmed that our synthesized compounds possess the intended molecular structure, validating the synthetic approach. Biological screening of these compounds is currently pending, but early indications are promising based on their chemical profiles and preliminary binding affinity studies.

Acknowledgment

Multicomponent Reaction Strategy for the Synthesis of Small-Molecule Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists

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Abstract

Multicomponent reactions (MCRs) have emerged as a versatile synthetic strategy in medicinal chemistry, offering significant advantages for drug discovery and development by enabling the combination of three or more reactants in a single, one-pot process. As a key tool in modern drug design, MCRs accelerate the discovery of innovative small molecules with potential therapeutic applications, such as glucagon-like peptide-1 receptor (GLP-1R) agonists, which are gaining recognition as promising treatments for type 2 diabetes mellitus (T2DM) and obesity. Danuglipron, a small-molecule GLP-1R agonist currently undergoing clinical trials, has shown strong efficacy but faces challenges due to its high incidence of gastrointestinal side effects. These adverse effects may be related to the target or influenced by the presence of a carboxylic acid group, which likely impacts its pharmacokinetics, dosing regimen, and causes direct gastrointestinal irritation. In this study, we describe the development of MCR-derived small-molecule GLP-1R agonists that closely parallel the clinical candidate danuglipron. This approach offers a practical MCR-based pathway for generating novel GLP-1R agonists inspired by danuglipron, aiming to overcome its limitations while retaining its therapeutic potential.

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