Czech Annual Cancer Research Meeting

November 20–22 2023 | Olomouc, CZ

18th Czech Annual Cancer Research Meeting
former Diagnostic, Predictive and Experimental Oncology Days

18th Molecular Pathology Days

106th Olomouc Histopathology Seminar

2nd Conference of the National Institute for Cancer Research

ABSTRACT BOOK

Thank you!

ONKO PACIENT 2023

The results of your professional work inspire us to pass them on to oncology patients.

See you in spring 2024!

www.vyzkumrakoviny.cz

Czech Annual Cancer Research Meeting

November 20–22
2023 | Olomouc, CZ

Organizers

MedChemBio – cluster
Šlechtitelů 813/21
783 71 Olomouc-Holice

MedChemBio
Cluster of Medicinal Chemistry and Chemical Biology

National Institute
for Cancer Research

NICR
NATIONAL INSTITUTE
FOR CANCER RESEARCH
CZECH REPUBLIC

Program Committee

MUDr. Ludmila Boublíková, Ph.D.
doc. Mgr. Jan Bouchal, Ph.D.
prof. Mgr. Jiří Drábek, Ph.D.
MUDr. Petr Džubák, Ph.D.
doc. MUDr. Marián Hajdúch, Ph.D.
prof. Ing. Michal Hocek, CSc., DSc.
prof. MUDr. Pavel Klener, Ph.D.
prof. MUDr. Zdeněk Kolář, CSc.
Mgr. Vladimíra Koudeláková, PhD.
Prof. RNDr. Ondřej Slabý, Ph.D.
prof. MUDr. Karel Smetana, DrSc.
prof. MUDr. Marek Svoboda, Ph.D.
prof. MUDr. Aleksi Šedo, DrSc.

Scientific Committee

MUDr. Ludmila Boublíková, Ph.D.
doc. Mgr. Jan Bouchal, Ph.D.
prof. Mgr. Jiří Drábek, Ph.D.
MUDr. Petr Džubák, Ph.D.
doc. MUDr. Marián Hajdúch, Ph.D.
prof. Ing. Michal Hocek, CSc., DSc.
prof. MUDr. Pavel Klener, Ph.D.
prof. MUDr. Zdeněk Kolář, CSc.
Mgr. Vladimíra Koudeláková, PhD.
Prof. RNDr. Ondřej Slabý, Ph.D.
prof. MUDr. Karel Smetana, DrSc.
prof. MUDr. Marek Svoboda, Ph.D.
prof. MUDr. Aleksi Šedo, DrSc.

President of the Conference

doc. MUDr. Marián Hajdúch, Ph.D.

Professional Guarantee

The Czech Society for Histochemistry and Cytochemistry

The University Hospital Olomouc

Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry Palacky University Olomouc

Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry Palacky University Olomouc

European Infrastracture for Translational Research (EATRIS-ERIC)

Cancer Research Czech Republic

National Institute for Cancer Research

Czech Association for Cancer Research

Conference Office

Conference Coordinator & Administrator

Peter Vanek, M. Sc.
+420 775 050 355
vanek.peter@icloud.com

Graphic & Visuals Design

Denisa Pavelková

ISBN: 978-80-908348-2-8
ISSN: 2788-1695

The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU

Funded by the European Union
NextGenerationEU

CZECH RECOVERY PLAN

MINISTRY OF EDUCATION YOUTH AND SPORTS

MONDAY 20TH NOVEMBER, 2023

12:30 — 12:45 **OPENING CEREMONY**

Early detection and prevention of cancers: Lung cancer focus 12:45 – 14:45

Chairs: Marián Hajdúch, Jiří Votruba

12:45 – 13:15	Assessing the lung cancer risk reduction potential of novel tobacco and nicotine containing products – A matter of dose response **Peter Harper**
13:15 – 13:45	Epidemy of lung cancer is continuing despite the effort of medical and research specialists **Jiří Votruba**
13:45 – 14:15	Identifying biomarkers for lung cancer and COPD diagnosis by protein analysis of exhaled breath condensate **Jana Václavková, Marián Hajdúch**
14:15 – 14:45	How can we maximize the social value of biomedical innovation? **Jakub Hlávka**

NiCR NATIONAL INSTITUTE FOR CANCER RESEARCH CZECH REPUBLIC

14:45 — 15:00 **COFFEE BREAK**

Molecular pathology of cancers and molecular targets: Hematological malignancies I / 15:00 – 16:15

Chairs: Martin Mistrik, Pavel Klener

15:00 – 15:15	Protein kinase B signaling in lymphomas: the current state and future therapy perspectives **Ondrej Havranek**
15:15 – 15:30	Clonal evolution in mantle cell lymphoma at single cell resolution **Dmitry Kazantsev**
15:30 – 15:45	Foxo1-Rictor axis mediates adaptive increase in Akt activity and cell survival during bcr inhibitor therapy in chronic lymphocytic leukemia **Kryštof Hlaváč**
15:45 – 16:00	Modulation of oncogenic FLT3-ITD kinase in acute myeloid leukaemia cells **Eva Řezníčková**
16:00 – 16:15	ALDH1A as a therapeutic target in acute myeloid leukemia **Mehak Shaikh**

16:15 — 16:45 **COFFEE BREAK**

Molecular pathology of cancers and molecular targets: Hematological malignancies II / 16:45 – 17:45

Chairs: Juan DeSanctis, Ester Mejstříková

16:45 – 17:00	BCL-XL blockage with A1155463 significantly increases efficacy of venetoclax in BCL-2 positive lymphoproliferative malignancies **Alexandra Dolníková**

MONDAY 20TH NOVEMBER, 2023

17:00 – 17:15	Hybrid amyloidosis, R2 (rarest in rare) **Patrik Flodr**
17:15 – 17:30	Molecular dynamics of the lineage switch in acute leukemia: Insight through mass cytometry and CITE-Seq analysis using trajectory inference **Jan Stuchlý**
17:30 – 17:45	Composition of immunocompetent cells in bone marrow and peripheral blood in context of anti-CD19 targeted therapy of B cell precursor leukemia **Ester Mejstříková**

TUESDAY 21ST NOVEMBER, 2023

Molecular pathology of cancers and molecular targets: Genetic predisposition / 9:00 – 10:15

Chairs: Jiří Drábek, Pavel Vodička

9:00 – 9:30	Genetic predisposition to colorectal cancer: Identification of novel cancer susceptibility genes **Asta Försti**
9:30 – 9:45	Genomic Instability in adenomas and in colorectal cancer progression **Pavel Vodička**
9:45 – 10:00	Large-scale categorization of germline missense variants in the checkpoint kinase gene CHEK2 revealed a set of functionally-impaired variants increasing breast cancer risks **Zdeněk Kleibl**
10:00 – 10:15	Precise correction of casual mutations as a treatment strategy for monogenic disorders with increased predisposition to cancer **Lucie Peterková**

NICR NATIONAL INSTITUTE FOR CANCER RESEARCH CZECH REPUBLIC

10:15 — 10:45	**COFFEE BREAK**

Molecular pathology of cancers and molecular targets: Targeted therapies / 10:45 – 12:15

Chairs: Ondřej Slabý, Zdeněk Kleibl

10:45 – 11:15	Real-world precision medicine in oncology: Comparison of results in pediatric and adult patients **Ondřej Slabý**
11:15 – 11:30	Topological stress triggers difficult-to-repair DNA lesions in ribosomal DNA with subsequent formation of PML-nucleolar compartment **Pavla Vasicova**
11:30 – 11:45	Exploring ATR inhibition in MLL-ENL-driven leukemogenesis: threshold and context role of ATR signaling **Pavla Chaloupkova**

TUESDAY 21ST NOVEMBER, 2023

11:45 – 12:00	PPM1D activity promotes the replication stress caused by cyclin E1 overexpression **Libor Macurek**
12:00 – 12:15	The role of autoproteolysis and mitoribosomal proteins in the regulation of LACTB tumor suppressor **Sara Escudeiro-Lopes**
12:15 — 13:15	**LUNCH + POSTER PRESENTATION**

Cancer biomarkers and molecular targets I / 13:15 – 15:00

Chairs: Lucie Kučerová, Josef Srovnal

13:15 – 13:45	Advances in liquid biopsy testing **Mikael Kubista**
13:45 – 14:00	Ultra-sensitive detection of miRNAs related to myelodysplastic syndromes in human blood plasma **Giusy Finocchiaro**
14:00 – 14:15	Novel model of circulating tumor cells for validation of druggable pathways in triple-negative breast cancer **Lucia Kucerova**
14:15 – 14:30	Patient-derived xenografts of pancreatic carcinoma – our experience with adenosquamous form **Tomas Sychra**
14:30 – 14:45	Integrative multi-omics approaches unveil novel insights into vestibular schwannoma pathogenesis **Lucie Pfeiferova**
14:45 – 15:00	PIWIL1-4 in human gliomas and glioblastoma stem-like cells **Elena Garcia Borja**
15:00 — 15:30	**COFFEE BREAK**

Innovative cancer therapies I / 15:30 – 17:00

Chairs: Michal Hocek, Tomáš Etrych

15:30 – 16:00	Automation + miniaturization = acceleration **Alexander Dömling**
16:00 – 16:15	Heterocyclic analogues of lupane triterpenoids trigger apoptosis selectively in cancer cells **Milan Urban**
16:15 – 16:30	Development of degradable PHPMA-based nanogels: preparation procedure and physicochemical properties **Petr Šálek**
16:30 – 16:45	Photocontrolled interleukin/receptor pairs through genetic code expansion **Gustavo Fuertes**
16:45 – 17:00	3,5,7-Substituted pyrazolo[4,3-d]pyrimidine inhibitors of cyclin-dependent kinases and cyclin K degraders **Radek Jorda**

NiCR NATIONAL INSTITUTE FOR CANCER RESEARCH CZECH REPUBLIC

TUESDAY 21ST NOVEMBER, 2023

17:00 — 17:15 **COFFEE BREAK**

Innovative cancer therapies II / 17:15 – 18:30

Chairs: Alexander Dömling, Milan Urban

17:15 – 17:30 Unveiling the potential of Stony Brook taxanes: A promising solution to combat taxane resistance in solid tumors
Radka Vaclavikova

17:30 – 17:45 Engineered antibodies as platforms for cancer therapy
Gargi Das

17:45 – 18:00 Derivatives of trilobolide actívate the cytotoxic response of T cytotoxic and NK cells against tumour cells
Juan Bautista De Sanctis

18:00 – 18:15 Triazole-based estradiol dimers with five-atom linkers act as inhibitors of microtubule dynamics
Jiří Řehulka

18:15 – 18:30 Discovery of novel specific carbonic anhydrase IX inhibitors by HTS compaign
Soňa Gurská

19:00 — 24:00 **SOCIAL EVENT**

WEDNESDAY 22ND NOVEMBER, 2023

Cancer biomarkers and molecular targets II / 8:30 – 10:15

Chairs: Petr Džubák, Helena Kupcová Skalníková

8:30 – 9:00 The development of low input proteomic technologies for the analysis of rare quiescent cancer cells
Silvia Surinova

9:00 – 9:15 Connecting genomic variants and the proteome with peptide level resolution at scale
Willy Peña Büttner

9:15 – 9:30 Proteotype classification of metastatic and localized renal cell carcinomas for prognosis and therapy response
Pavel Bouchal

9:30 – 9:45 Can lipid profiles reflect the true biological age and healthy ageing?
Lukáš Najdekr

9:45 – 10:00 Lactosylceramide synthases B4GALT5 and B4GALT6 and their potential role(s) in colon cancer cells
Jan Vondráček

10:00 – 10:15 The cervical mucus – proteomic characterization and protein-tissue analysis in the healthy patient subset
Tomáš Oždian

WEDNESDAY 22ND NOVEMBER, 2023

10:15 — 10:45 **COFFEE BREAK**

Cancer biomarkers and molecular targets III / 10:45 – 13:00

Chairs: Karel Smetana, Luca Vannucci

10:45 – 11:15	Generation of myeloid-derived suppressor cells (MDSC) and their targeting in cancer **Viktor Umansky**
11:15 – 11:30	Fibroblast heterogeneity and cancer biology **Karel Smetana, Jr.**
11:30 – 11:45	Revealing immune response against melanoma during spontaneous regression in pig model **Helena Kupcová Skalníková**
11:45 – 12:00	Single-cell profiling of native surface glycosphingolipid epitopes opens new dimension for deconvolution of breast cancer intratumoral heterogeneity and phenotypic plasticity **Jiřina Procházková**
12:00 – 12:15	Gut microbiome can influence local and distant immune environment and tissue structures: a germ-free animal lesson perspective also for cancer-microbiome relationships **Luca Vannucci**
12:15 – 12:30	AI in pathology: state of the art and AI-powered image analysis with ImageJ **Kateřina Čížková**
12:30 – 12:45	New prognostic markers and therapeutic strategies in solid tumors **Petr Benes**
12:45 – 13:00	Single cell molecular pathology with Xenium *in situ* **Agnieszka Ciesielska**

NICR
NATIONAL INSTITUTE
FOR CANCER RESEARCH
CZECH REPUBLIC

13:00 — 14:00 **LUNCH + POSTER PRESENTATION**

From molecular pathology to novel therapies of urological cancers 14:00 – 15:15

Chairs: Aleksi Sedo, Jan Bouchal

14:00 – 14:15	Glutamine metabolism as a target in therapy resistant prostate cancer **Holger H.H. Erb**
14:15 – 14:30	Spliceosome component SRSF9 is involved in an rs5918762 allele-specific manner in alternative splicing of androgen receptor variant 7 **Jasper Van Goubergen**
14:30 – 14:45	Molecular basis of cisplatin resistance in testicular germ cell tumors **Laura Matoušková**
14:45 – 15:00	*In vivo* toxicity and therapeutic efficacy of enhanced blood retention and tumor uptake PSMA-targeting ^{225}Ac-labeled radioconjugates **Zbynek Novy**
15:00 – 15:15	A-ring fused heterocyclic derivates of dihydrotestosterone targeting the androgen receptor in prostate cancer **Miroslav Peřina**

WEDNESDAY 22ND NOVEMBER, 2023

15:15 — 15:45 **COFFEE BREAK**

Innovative cancer therapies III: Polymers and nanoprobes
15:45 — 17:15

Chairs: Milada Šírová, Marek Kovář

15:45 – 16:00	Antitumor activity of HPMA copolymer conjugates bearing taxanes and platinum-based drugs for the treatment of head and neck cancer **Katerina Behalova**
16:00 – 16:15	Stimuli-responsive polymer nanoprobes intended for fluorescence-guided surgery **Tomáš Etrych**
16:15 – 16:30	Hydrophilic polymer carriers for tumor targeted delivery of photosensitiser precursors **Kevin Kotalík**
16:30 – 16:45	Advanced glycopolymers as potent inhibitors of galectin-induced tumor progression **Petr Chytil**
16:45 – 17:00	Powerful increase of gemcitabine *in vivo* antitumor activity through drug delivery system based on biocompatible HPMA copolymers: drug release kinetics is crucial **Marek Kovář**
17:00 – 17:15	Targeted drug delivery using polymer carrier with P-gp overcoming capacity for treatment of chemoresistant tumors **Milada Šírová**

17:15 — 17:30 **CLOSING CEREMONY**

Thank you all for your support!

GOLD PARTNERS

BRONZE PARTNERS

Funded by the European Union NextGenerationEU
CZECH RECOVERY PLAN
The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU
INOVAČNÍ CENTRUM OLOMOUCKÉHO KRAJE
Co-funded by the European Union

PARALLEL SESSIONS / Evropa Hall Small

MONDAY 20TH NOVEMBER, 2023

10:00 — 12:30	SALVAGE project meeting	
14:00 — 16:30	NICR administrative meeting	NiCR

TUESDAY 21ST NOVEMBER, 2023

10:00 — 15:00	106th Olomouc Histopathology Seminar	
15:45 — 16:45	NICR research program 1 meeting	NiCR
17:00 — 18:00	NICR research program 3 & 4 meeting	NiCR

WEDNESDAY 22ND NOVEMBER, 2023

15:45 — 16:45	NICR research program 5 meeting	NiCR

PARALLEL SESSIONS / Ventana Lounge

MONDAY 20TH NOVEMBER, 2023

18:00 — 21:00	NICR Board meeting	NiCR

TUESDAY 21ST NOVEMBER, 2023

13:30 — 15:00	NICR research program 2 meeting	NiCR

WEDNESDAY 22ND NOVEMBER, 2023

18:00 — 21:00	NICR International Science and Advisory Board Meeting	NiCR

106. Olomoucký histopatologický seminář

Úterý 21. listopadu 2023 / 10:00 – 15:00 / sál Evropa (malá)

H17824/21
29letý muž s ventrikuloperitoneálním shuntem pro hydrocefalus, s krvácením do mozkových komor, nyní s nálezem nejasného útvaru v levé postranní komoře.
Referuje MUDr. Dominik Hraboš

H5350/23
68letý muž s tumorem pravé nadledviny velikosti 38 mm.
Referuje: MUDr. Daniela Kurfurstová, Ph.D.

H26317/22
19letý muž se suspektním papilomem/fibromem měkkého patra vlevo.
Referuje: MUDr. Jaroslav Michálek, Ph.D.

H18373/23
44letý muž s intradurálním tumorem v oblasti L3-4.
Referuje: MUaDr. Gvantsa Kharaishvili, Ph.D.

H5745/23
59letá žena s hmatnou rezistencí hlavy parietookcipitálně velikosti 28 mm.
Referuje: MUDr. Dominika Fritzová.

14495/23
67letý muž s tumorem pravé ledviny velikosti 35 mm.
Referuje: MUDr. Vladimíra Kaťuchová

H6060/23
Žena ročník 1958 s nodulárním tumorem dolního laloku pravé plíce dle CT cca 14x8 mm, SUV 2,2. Po extraanatomické VATS dodány fragmentované vzorky celkové velikosti do 30 mm.
Referuje: MUDr. Patrik Flodr, Ph.D.

17038/21
72letý muž s recidivujícím tumorem na krku vlevo. Exstirpován tumor velikosti vel. 42x32x42 mm.
Referuje: MUDr. Martin Eliáš

H8658/23
76letý muž s laločnatou poměrně dobře ohraničenou expanzí v rektovezikální exkavaci velikosti 63 mm s okrsky kalcifikací.
Referuje: MUDr. Barbora Šopíková

H9180/23
66letá pacientka přijata k biopsii suspektních lézí v močovém měchýři.
Referuje: MUDr. Martina Navrátilová

Notes

Notes

POSTER SECTION

1. Impaired HSC fitness and accelerated leukemogenesis in a mouse model of chronic inflammation
   Srdjan Grusanovic
2. Targeting Plectin in hepatocellular carcinoma
   Zuzana Outlá
3. Cytoskeletal disruption drives dna damage and carcinogenesis
   Petra Novotná
4. Inhibition of P-glycoprotein mediated multidrug resistance and STAT3 signaling pathway by polymeric conjugates bearing HIV protease inhibitor derivatives
   Daniil Starenko
5. Effect of acetylsalicylic acid nanopolymer on tumour antigen expression in human cell lines.
   Jenny Valentina Garmendia
6. Discrimination of resected glioma tissues using surface enhanced Raman spectroscopy and Au@ZrO2 plasmonic nanosensor
   Václav Ranc
7. Characterization of drug release rate: an approach using Surface plasmon resonance, capillary electrophoresis, diffusion-ordered NMR spectroscopy
   Alena Libánská
8. Age prediction from semen samples through the detection of DNA methylation
   Lucie Kotková
9. Differential roles of ARF GTPase-activating proteins GIT1 and GIT2 in regulating microtubule nucleation in glioblastoma cells
   Vadym Sulimenko
10. CD147 – a promising target in head and neck cancer?
    David Kalferť
11. CD38 and tumor associated macrophages in prostate cancer immune microenvironment
    Nino Vashakidze
12. Micelle-forming amphiphilic copolymers for the treatment of resistant solid tumors
    Markéta Frejková
13. Regulation of enterocyte development in the intestinal epithelium by bmp ligands
    Linda Berková
14. Predictive biomarkers testing - from simple tests to complex genomic profiling era
    Barbora Kubová
15. Regulated cell death in 3D glioblastoma spheroids induced by near-infrared photothermal therapy using cationic gold nanorods
    Monika Žárská
16. Oxysterols in pancreatic cancer: A comparative study of their effects on different cell lines
    Tereza Tesarova
17. Circulating and salivary DNA-based biomarkers for early diagnosis and recurrence monitoring of oropharyngeal squamous cell carcinomas
    Ondrej Bouska
18. Positron emission tomography imaging of Klebsiella pneumoniae infection using gallium-labelled siderophores
    Kateřina Dvořáková Bendová
19. Establishment and characterization of preclinical models derived from circulating tumor cells
    Zuzana Kahounová
20. Circulating tumor cell detection and characterization in solid tumors
    Pavel Stejskal
21. Lupane derivatives inhibit Gli1-mediated transcription in human glioblastoma cell line via direct interaction with Gli1
    Ivo Frydrych
22. Protein engineering of humanized antibody 5D3 designed to prostate cancer therapy
    Zora Nováková
23. Diversity of oral and gut microbioma in adenokarcinoma and squamous cell lung carcinoma
    Jozef Škarda
24. Tracing main c-Myc isoforms endogenous expression for targeted anti-cancer therapies
    Agáta Kubíčková
25. Expression-methylation profile diversity of long non-coding RNAs in epithelial ovarian cancer patients with different platinum-free interval
    Karolina Seborova
26. Exploring the role of notch signaling pathway in ovarian carcinoma: potential therapeutic targets and biomarkers
    Alzbeta Spalenkova
27. Antiviral activity of selected lamiaceae essential oils and their monoterpenes against SARS-Cov-2
    Ermin Schadich
28. Cytoplasmic p21 affects caspase-independent cell death pathway – parthanatos – in colon cancer cells following genotoxic stress
    Viswanath Das
29. Morphine analgesia, opioid growth factor receptor and cannabinoid receptor 2 gene expression in tumor tissue improve survival of patients with pancreatic cancer
    Monika Vidlarova
30. Morphological profiling of 2500 bioactive compounds by high throughput screening using cell painting assay
    Alzbeta Srovnalova

POSTER SECTION

31. Amplification of androgen receptor and expression of miR-375 as liquid biopsy markers for monitoring of prostate cancer progression and ARTA therapy failure
Eva Szczyrbová

32. *In vitro* and *in vivo* efficacy of Stony-brook taxanes in paclitaxel resistant ovarian carcinoma
Marie Ehrlichova

33. The role of the checkpoint kinase 1 (Chk1) inhibition in luminal subtype of breast carcinoma
Veronika Bouškova

34. Decreased glioblastoma growth and macrophage infiltration in a disintegrin and metalloproteinase domain-containing protein 8 (ADAM8) knockout mice
Nikola Ternerová

35. Fibroblast activation protein expressing mesenchymal cells influence T cell quantity and function in glioblastoma
Magdalena Houdová Megová

36. Differential seeding potency of exogenous R2 and R3 fibrils influences autophagic degradation of intracellular tau aggregates in Tau K18 P301S cells
Narendran Annadurai

37. Immunomodulatory effect of mesenchymal cells on T cells in a glioblastoma 3D spheroid model
Tereza Šváblová

38. Identification of the mechanism of action of A3 adenosine receptor agonist – PNH173
Kateřina Ječmeňová

39. Towards identification and validation of novel dc-sign ligands
Martin Ondra

40. How can single cell and spatial transcriptomics help in cancer research?
Alice Mášová

41. Trastuzumab induces cell death in tnbc cells: a step towards repurposing the monoclonal antibody
Hussein Sabit

42. Targeted treatment of severe vascular malformations using PIK3CA inhibitor alpelisib in children and young adults – single center prospective case series
Martin Sterba

43. Regulation of AR-V7 by 3'UTR-binding miRNAs
Elisa Morales

44. The role of HUS1 and RAD51 in cisplatin-based chemotherapy resistant bladder cancer
Nils van Creij

45. Clonal somatic variants in hematopoietic cells in relation to atherosclerosis and stroke
Barbora Kalousová

46. Molecular genetics of extraskeletal myxoid chondrosarcoma focusing on a rare alternative TCF12::NR4A3 gene fusion associated with unusual morphologic features
Jiří Lenz

47. Targeting of tyrosine kinases contributing to KMT2A-r mediated leukemogenesis as a therapeutic approach in mixed-lineage leukemia
Nikola Niederlova

48. Potential role of CDK13 in neuroblastoma progressionJ
Jiří Kohoutek

49. Liquid biopsies in multiple myeloma
Sabina Sevcikova

50. Casein kinase 1 alpha inhibition in acute myeloid leukemia
Pavlína Janovská

navify®

Onkologické portfolio

Roche

navify® Tumor Board

Komplexní pohled na onkologického pacienta

navify® Tumor Board je cloudové řešení, které bezpečně integruje, přenáší a zobrazuje pacientská data z různých zdrojů a umožňuje jejich standardizované přehledné zobrazení dle potřeb klinika. Podporuje klinické rozhodnutí a napomáhá onkologickým týmům v jejich spolupráci tak, aby byla efektivně optimalizována péče o každého pacienta.

Roche s.r.o., Diagnostics Division
Na Valentince 3336/4, 150 00 Praha 5 – Smíchov
www.roche-diagnostics.cz

Více informací na: www.NAVIFY.com

MONDAY 20TH NOVEMBER, 2023

Chairs: Marián Hajdúch, Jiří Votruba

Assessing the Lung Cancer Risk Reduction Potential of Novel Tobacco and Nicotine Containing Products – A Matter of Dose Response.

Peter Harper

Guy's and St Thomas' Hospital, London, United Kingdom. Leaders in Oncology Care, London, United Kingdom

Dr Peter Harper MD, Consulting Oncologist, Guy's and St Thomas Hospital. Founder of Leaders in Oncology Care

Assessing the Lung Cancer Risk Reduction Potential of Novel Tobacco and Nicotine Containing Products – A Matter of Dose Response.

Smoking is one of the most important risks for lung cancer and therefore for any smoker quitting is the best approach. Despite all the warnings, information and legislation to make smoking in everyday life increasingly difficult, many smokers do not quit even in the face of serious disease.

Doctors and Public Health authorities have begun to examine the role novel tobacco products (NTP's) can play in reducing the negative impacts of smoking on health, for this group of patients – those who cannot or will not quit. While the availability of epidemiological data and trends may vary by disease the impact that these products may have in reducing the incidence of smoking-related cancer will take decades to fully evaluate. In the absence of long-term disease data, an interim approach is needed to understand the risk reduction potential of these new products relative to cigarettes. For cancer, epidemiology shows that the lower the exposure to carcinogens, the lower the risk of cancer. Given that both industry and independent studies have confirmed that these NTP's contain both fewer toxicants and lower levels of carcinogens found in cigarette smoke we need to better understand how this translates to the risk of developing cancer.

We know that the key mechanisms which drive the development and invasiveness of cancer are: (1) the amount of genetic damage and (2) the level of inflammation, we can quantitatively understand the cancer risk of these products relative to cigarettes, while the epidemiological data are still being generated.

To reduce the risk of smoking-related cancer the best option is to stop smoking. For those who cannot stop smoking, or will not stop smoking we need to look at the data emerging on the risk reduction potential of NTP's. Using the Heated Tobacco Products data recently authorized by the United States Food and Drug Administration (FDA) to illustrate this approach there is a reasonable indication that smokers who cannot quit smoking would be able to reduce their risk of smoking-related cancers such as lung cancer, should they switch to products containing HTP's with a demonstrated lower carcinogen exposure.

Key Words: Novel Tobacco Products, Heated Tobacco Products, Cancer Risk Reduction, Potential Tobacco Harm Reduction

Dr Harper through Harper Consulting Limited provides Consulting Services to Philip Morris International on the topic of Tobacco Harm Reduction

Epidemy of lung cancer is continuing despite the effort of medical and research specialists

Jiří Votruba

I. Clinic of tuberculosis and respiratory diseases, General University Hospital in Prague Czech Republic

Early Lung cancer detection strategies and research, new methods of diagnosis and local treatment and standard and research methods of neoandjuvant imunotherapy will be discussed.

Identifying biomarkers for lung cancer and COPD diagnosis by protein analysis of exhaled breath condensate

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

[1]Laboratory of Experimental Medicine, Institute of Molecular a Translational Medicine, Faculty of Medicine and Dentistry Palacky University, Olomouc, Czech Republic
[2]Department of Pediatrics, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[3]Department of Respiratory Medicine, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

Lung cancer is one of the cancer types with highest prevalence and cancer-caused deaths in the Czech Republic. Unfortunately, the prevalence of lung cancer is still increasing, especially in women. Since 2021 up to date, the preventive screening has included pulmonary function tests and LDCT among the risk groups. However, the research towards developing non-invasive methods, which could complement or replace currently used screening methods, is still requested. In this work, we have studied an exhaled breath condensate (EBC) which is a biological matrix collected non-invasively and considered as a rich source of biomarkers from the respiratory tract.

Exhaled breath condensate collected using Turbo 14 Turbo DECCS System

were prepared and measured by high resolution mass spectrometry (HPLC-MS/MS-LTQ Orbitrap Elite) in 3 technical replicates. Data were evaluated by Statistica, Bioconductor R, Skyline and Proteome Discoverer. The validation phase is done by SureQuant approach for targeted MS analysis using Orbitrap Exploris™ 480 mass spectrometer (Thermo Scientific).

We have focused on non-small cell lung cancer (NSCLC) diagnostics. We have identified 4806 proteins and of them, 4179 proteins were quantified at least in one replicate across 226 individuals' samples measured in triplicates. Combining univariate and multivariate statistical approaches and sensitivity analysis, we have suggested 72 biomarkers that could distinguish NSCLC patients from COPD patients and healthy controls. Our models for NSCLC and COPD biomarker prediction worked well and seem to be promising. The ongoing mass spectrometry based targeted analysis would suggest the resulting biomarker panel which will be used for developing an ELISA-based assay for usage in lung cancer preventive screening.

This work was supported by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2023_025).

How can we maximize the social value of biomedical innovation?

Jakub Hlávka

University of Southern California (Keck)

Bringing biomedical innovation to patients requires interdisciplinary, evidence-based policy making. How can we use health economics and health policy to inform the design of incentives for biotechnology R&D, drug manufacturing and its sustainability, regulatory frameworks at the national and European level, the payment for high-cost and high-value medications, and ultimately, the delivery of health care to patients across multiple disease areas? This lecture will address some of the most significant inefficiencies in the Czech Republic and propose specific ways for addressing them.

MONDAY 20TH NOVEMBER, 2023

Chairs: Martin Mistrik, Pavel Klener

Protein kinase B signaling in lymphomas: the current state and future therapy perspectives

Ondrej Havranek[1,2]

[1.] *BIOCEV, First Faculty of Medicine, Charles University, Prague, Czech Republic.*
[2.] *Department of Hematology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

Non-Hodgkin lymphomas (NHL) are formed by a very heterogeneous group of malignant tumors derived in majority from B lymphocytes. One of the most critical pathways involved in NHL pathogenesis is the PI3K (phosphatidylinositol-3-kinases)/AKT (protein kinase B) signaling pathway. This pathway is critical for regulation of many basic cellular processes, including cell growth and proliferation or metabolism. It is one of the most frequently altered pathways in caner in general. In lymphomas, particularly common are PTEN (phosphatase and tensin homolog, a negative regulator of PI3K/AKT pathway) deletions or PIK3CA (coding for PI3K p110 subunit α) amplifications, less frequent are FOXO1 (forkhead box protein O1) or mTOR (mammalian target of rapamycin) mutations. PI3K/AKT pathway also cross talks with cMyc, one of the hallmark lymphomas associated transcription factor. Functionally, we have previously reported that in substantial proportion of lymphomas, the PI3K/AKT pathway activity originates from the so called “tonic“ B-cell receptor (BCR) signaling. We also found that PI3K/AKT hyperactivation is one of mechanisms mediating resistance to inhibition of the so called “chronic active” BCR signaling. Giving its importance in cancer, multiple PI3K and AKT inhibitors were developed with several already approved for clinical use. For lymphoma treatment, PI3K inhibitors that were clinically approved are idelalisib (PI3Kδ isoform inhibitor), copanlisib (PI3Kα/δ inhibitor), and duvelisib (PI3Kγ/δ inhibitor), with no AKT inhibitor approved for use in lymphomas so far. These inhibitors showed great promise, however, they suffer from low selectivity, serious side effects, lower than expected efficacy, and resistance development. We have promising preliminary data on several opened research questions related to PI3K/AKT pathway inhibition and its lymphoma related biology: What are all sources of PI3K/AKT activation in lymphomas and are these targetable? Are there better and universal approaches to inhibit PI3K/AKT pathway as are better inhibitors or specific combinations? Since the current lymphoma treatment is still based on 50 years old non-specific chemotherapy combinations, new therapy approaches are necessary. We believe that molecular biology-based approaches for PI3K/AKT pathway inhibition may represent one possible approach to decrease toxicity and improve efficacy of lymphoma treatment.

Clonal evolution in mantle cell lymphoma at single cell resolution

Alexandra Dolníková[1], Ladislav Anděra[2,3], Dmitry Kazantsev[1], Magdalena Klánová[1], Eva Pokorná[1], Dana Sovilj[2], Marek Trněný[4], Pavel Klener[1,4]

[1]*Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.*
[2]*Institute of Biotechnology CAS/ BIOCEV, Prague, Czech Republic.*
[3]*Institute of Molecular Genetics CAS, Prague, Czech Republic.*
[4]*First Department of Medicine – Department of Hematology, Charles University General Hospital, Prague, Czech Republic*

Mantle cell lymphoma (MCL) is a clonal B cell proliferative malignancy characterized by translocation t(11;14)(q13;q32), cyclin D1 overexpression and significant chromosomal instability. Despite existing standard treatments, it inevitably relapses and thus considered uncurable. One of the main hypotheses of MCL clonal evolution is therapy-mediated selection of subclones carrying adverse genetical aberrations. Whole exome sequencing analysis of MCL tumors shows that the majority of detected single-nucleotide variants are already present at time of diagnosis.

In the present study, we employed single cell RNA sequencing to characterize subclonal structure of MCL in five patients sampled at diagnosis and at the first relapse after the failure of standard immunochemotherapy. The fresh-frozen cells were sorted to obtain Hoechst− CD45+ population which was processed using 10X NextGEM 3’ protocol. The resulting libraries were paired-end sequenced on Illumina Novaseq 6000 System. We used STARsolo to align reads to GRCh38 reference. Transcript counts were processed and analyzed using tools from the Bioconductor repository.

After the dataset have been subset for undamaged singlet cells, we used immunological reference from Monaco et al. to label and subset the B cells. Malignant B cells were subset based on Cyclin D1 gene expression and unsupervised clustering. The cell cycle phase was inferred by embedding the dataset in the cell cycle position space. The dataset was batch-corrected for sample of origin and cell cycle phase. For unsupervised clustering, we have used an iterative approach to determine the best K nearest neighbors for the optimal number of clusters. These clusters were characterized based on their expression markers scores which were used to determine gene set enrichment statistics per cluster. We performed sample- and cluster-centric differential expression

analysis between timepoints and identified enriched gene sets for each comparison. For copy number inference we have used inferCNV package with normal cells as a reference for each given sample.

Our single cell transcriptomic data shows that while healthy immune cells are phenotypically similar between all our patients, the MCL cells are markedly different between patients. Despite correcting the MCL cell subset for the sample and cell cycle phase effects, we observed distinct clusters even for the cells obtained from the same tissue type of different patients. We have also observed clusters of transcriptionally similar cells originating from multiple patients even after batch-correction. Analysis of differential expression per sample showed markedly different hallmark pathways changes for each sample at relapse compared to diagnosis. Gene set characterization of the cell clusters in combined dataset allowed us to identify three major cluster groups – metabolic, active and dormant MCL cells. Differential expression analysis revealed upregulation of OXPHOS and E2F pathways for active clusters.

Droplet-based single cell sequencing methods are invaluable for robust detection of subclonal population structure. Our data strongly supports therapy selection hypothesis, showing that for the aggressive MCL subtype, no new phenotypes arise, instead, the tumor population is shaped in prospect of more aggressive cells already present at time of diagnosis. Mantle cell lymphoma is a heterogeneous disease, varying in its molecular phenotype between patients, it is also markedly phenotypically heterogeneous on the subclonal level. Our findings highlight the need for a personal approach to MCL diagnosis and treatment.

The study was supported by Grant Agency of the Czech Republic GA23-05474S and National Institute for Cancer Research (EXCELES) LX22NPO5102.

Foxo1-rictor axis mediates adaptive increase in akt activity and cell survival during bcr inhibitor therapy in chronic lymphocytic leukemia

Kryštof Hlaváč[1,2], Laura Ondrišová[1,2], Václav Šeda[1,2], Eva Hoferková[1,2], Giorgia Chiodin[3], Lenka Košťálová[1,2], Gabriela Mladonická Pavlasová[1,2], Daniel Filip[1,2], Pedro Faria Zeni[1], Jan Oppelt[1], Anna Panovská[2], Karla Plevová[2], Šárka Pospíšilová[2], Martin Šimkovič[4], Filip Vrbacký[4], Daniel Lysák[5], Stacey M. Fernandes[6], Matthew S. Davids[6], Alba Maiques-Diaz[7], Stella Charalampopoulou7, Jose I. Martin-Subero[7], Jennifer R. Brown[6], Michael Doubek[2], Francesco Forconi[3,8], Jiří Mayer[2], Marek Mráz[1,2]

[1]Molecular Medicine, CEITEC Masaryk University, Brno, Czech Republic.
[2]Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic.
[3]Cancer Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.
[4]4th Department of Internal Medicine – Haematology, University Hospital Hradec Kralove and Faculty of Medicine Hradec Kralove, Charles University, Prague, Czech Republic.
[5]Department of Haematology and Oncology, University Hospital Pilsen, Pilsen, Czech Republic.
[6]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, USA.
[7]Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain.
[8]Haematology Department, Cancer Care Directorate, University Hospital Southampton NHS Trust, Southampton, United Kingdom

Background

Genetic resistance mechanisms to BCR inhibitors in CLL have been extensively described, but it remains unclear whether non-genetic adaptation might exist in CLL cells allowing for lasting lymphocytosis or resistance. We focused on the possible role of the Akt pathway in adapting to BCR inhibitors since, in mouse models, PI3K-Akt activation is the only known factor that rescues the apoptosis induced by BCR deletion in mature B cells (Srinivasan et al. Cell, 2009).

Methods

We performed transcriptome profiling (Illumina) and analyzed samples obtained from CLL patients before and during ibrutinib or idelalisib therapy (n=70) and performed gene editing in MEC1 cells to reveal the FoxO1/Rictor functional role.

Results

We observed that Akt phosphorylation (S473) is induced above pre-therapy levels in ~70% of CLL patients treated with ibrutinib within the first 3 months of therapy (n=31; P=0.016), and an additional 10% of patients had stable pAkt[S473] levels. Similarly, pAkt[S473] increased in 55% of patients treated with single-agent idelalisib (n=11). pAkt[S473] was also restored in ibrutinib-treated MEC1 cells, where after an initial drop in Akt phosphorylation, its levels were induced by >10 folds ($P< 0.05$). Importantly, CLL cases with pAkt[S473] induction had a significantly higher lymphocytosis on ibrutinib *in vivo* (month 1 and 3, $P< 0.05$), and cells obtained during ibrutinib therapy were highly sensitive to Akt inhibitor MK2206 (80% apoptosis). To decipher mechanisms leading to Akt activation, we performed RNA profiling of paired CLL samples obtained before and during ibrutinib (n=22) or single-agent idelalisib (n=18) therapy. This identified 16 differentially expressed mRNAs (overlap of both drugs) involved in the PI3K-Akt pathway. RICTOR induction was particularly notable since it is an essential assembly protein for mTORC2, which phosphorylates Akt on S473 (Sarbassov et al. 2005). Rictor was also increased on protein level (n=39, P=0.02 for ibrutinib; n=9,

P=0.027 for idelalisib). RICTOR knock-out in MEC1 or inhibition of mTOR led to a dramatic decrease in basal pAkt[S473] levels as well as ibrutinib-induced pAkt[S473]. RNA sequencing of primary samples obtained during therapy revealed that levels of transcription factor FoxO1 were upregulated during BCR inhibitor treatment (n=31, P=0.005 for ibrutinib; n=9, P=0.002 for idelalisib), which attracted our attention since FoxO1 has been previously shown to activate Rictor in renal cells. Analysis of genome-wide FoxO1 binding (Cut&Run) revealed a clearly increased binding to RICTOR promotor in ibrutinib-treated MEC1 cells, and overall increased binding across the genome (1,190 FoxO1-bound regions in vehicle-treated cells vs. 3,354 regions in ibrutinib-treated cells). Genome-editing experiments and treatment with FoxO1 inhibitor (AS1842856, 0.5 μM) revealed that transcription factor FoxO1 is directly responsible for Rictor/pAkt[S473] activation during ibrutinib and idelalisib treatment. Knock-out of FoxO1 in the MEC1 cell line in 15 independent FoxO1 knock-out MEC1 clones led to a 70% decrease in pAkt[S473] levels (P< 0.0001) and a 40% decrease in Rictor levels (P< 0.001). Moreover, FoxO1 knock-out cells had a growth disadvantage in a competitive growth assay in the presence of ibrutinib or idelalisib. This suggests that the FoxO1-Rictor-pAkt[S473] axis does not require BTK or PI3Kδ activity. FoxO1 inhibitor decreased basal, anti-IgM-induced, and ibrutinib-induced pAkt[S473] levels. Furthermore, FoxO1 inhibitor (0.5 μM) induced apoptosis alone (~40% CLL cell killing) or more potently in combination with ibrutinib or idelalisib (~60% apoptosis; n=7). FoxO1 inhibitor also blocked the proliferation of primary CLL cells induced by T-cell factors in vitro (CD40L+IL21+IL4), and the effect was even more potent in combination with ibrutinib.

Conclusion

Overall, we describe for the first time that the FoxO1-Rictor-pAkt[S473] axis is involved in acute non-genetic adaptation to BCR inhibitors and CLL lymphocytosis during therapy and suggest that FoxO1 is a potential novel therapeutic target. It has been previously shown that Akt activity is a driver of Richter transformation (Kohlhaas et al., 2021). It is plausible that increased pAkt[S473] levels during that BCR inhibitor therapy might accelerate the transformation, and this is the subject of ongoing experimental work.

Supported by: Ministry of Health of the Czech Rep., grant no.NU20-03-00292, NU22-03-00117, and NV18-03-00054; ERC under the European Union's Horizon 2020 Research and Innovation Programme (grant agreement no.802644); National Institute for Cancer Research (Programme EXCELES, ID Project No.X22NPO5102) - Funded by the European Union-Next Generation EU; MH CZ-DRO (FNBr,65269705); MUNI/A/1224/2022.

Modulation of oncogenic FLT3-ITD kinase in acute myeloid leukaemia cells

Eva Řezníčková[1,2], Petra Břehová[3], Soňa Krajčovičová[4], Miroslav Peřina[1], Alexandra Dolníková[5], Pavel Klener[5], Miroslav Soural[4], Radim Nencka[3], Vladimír Kryštof[1,2]

[1]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[2]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic.
[3]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic.
[4]Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[5]Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

Oncogenic mutations in the gene encoding FLT3 kinase are frequently detected in patients with acute myeloid leukaemia (AML), and several potent kinase inhibitors have been developed. However, treatment with FLT3 inhibitors often leads to the resistance development and subsequent relapse, and novel therapeutic strategies to address resistance are thus urgently needed.

Recently, we have explored the novel structural motif of FLT3 inhibitors. Among the prepared compounds, we identified a lead structure that showed nanomolar inhibitory activities against FLT3-ITD and FLT3-D835Y in biochemical assays as well as its selectivity towards FLT3-ITD-positive AML cell lines. Efficacy was confirmed in MV4-11 and MOLM-13 cells as well as in cells harbouring the FLT3-ITD-D835Y mutation. *In vitro* experiments showed suppression of FLT3 autophosphorylation and inhibition of downstream signalling pathways. In addition, treatment of mice with MV4-11 xenografts significantly reduced tumour growth without any adverse effects and confirmed the antileukaemic activity *in vivo.*

Alternatively, targeted degradation of oncogenic protein kinases has emerged as a feasible therapeutic strategy. Therefore, based on previously developed competitive inhibitor of FLT3 and CDK9, we have designed and prepared a novel pomalidomide-based proteolysis-targeting chimera (PROTAC). A series of experiments demonstrated selectivity towards FLT3-ITD bearing AML cells and confirmed a proteasome-dependent mechanism of action. Dual degradation of FLT3-ITD and CDK9 resulted in blockade of FLT3-ITD downstream signalling pathways, activation of apoptosis and cell cycle arrest of FLT3-ITD AML cells. Moreover, the transcriptional repression induced by degradation of CDK9 reduced the expression of crucial genes involved in AML pathogenesis. The results obtained indicate the beneficial impact of simultaneous FLT3-ITD/CDK9 degradation for AML therapy.

This work was supported by the European Union – Next Generation EU (National Institute for Cancer Research, EXCELES, LX22NPO5102).

ALDH1A as a therapeutic target in Acute Myeloid Leukemia

Mehak Shaikh[1,2], Miroslava K Adamcova[1,3], Muhube Yazir[4], Céline Baier[4], Andrada Constantineascu[4], Chloé Fourmond[4], Mileidys Perez-Alea[4], Meritxell Alberich-Jorda[1,3]

[1]Department of Hemato-Oncology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Faculty of Science, Charles University, Prague, Czech Republic.
[3]Childhood Leukemia Investigation Prague, Department of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.
[4]Advanced Biodesign, Lyon, France

Background:

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by a differentiation arrest and accumulation of blasts. Upon initial treatment, most patients exhibit remission, however 50% of all patients who achieved remission relapse over time. We and others have reported that there is an increase in the levels of the enzyme aldehyde dehydrogenase (ALDH1A) in the group of refractory and relapse AML patients, and that high enzymatic activities are associated with drug resistance. ALDH1A oxidizes the toxic aldehydes to carboxylic acids, thus protecting cells from oxidative damage and proteo-toxic stress. DIMATE is an inhibitor of ALDH1A which could be employed to inhibit chemo-resistance in AML. The use of DIMATE in combination with standard of care drugs could promote chemo-sensitivity and provide clinical benefits.

Aims: The aims of this study are (1) to determine the effect of ALDH1A inhibition in AML cells, (2) to investigate the synergistic role of DIMATE with other standard of care drugs and (3) to determine the molecular mechanism causing the synergistic effect.

Methods:

Drug response assay, capillary electrophoresis immunoassay, colony cultures, and murine in-vivo assays. Experiments have been performed using human AML cell lines and murine MLL-AF9 leukemic models.

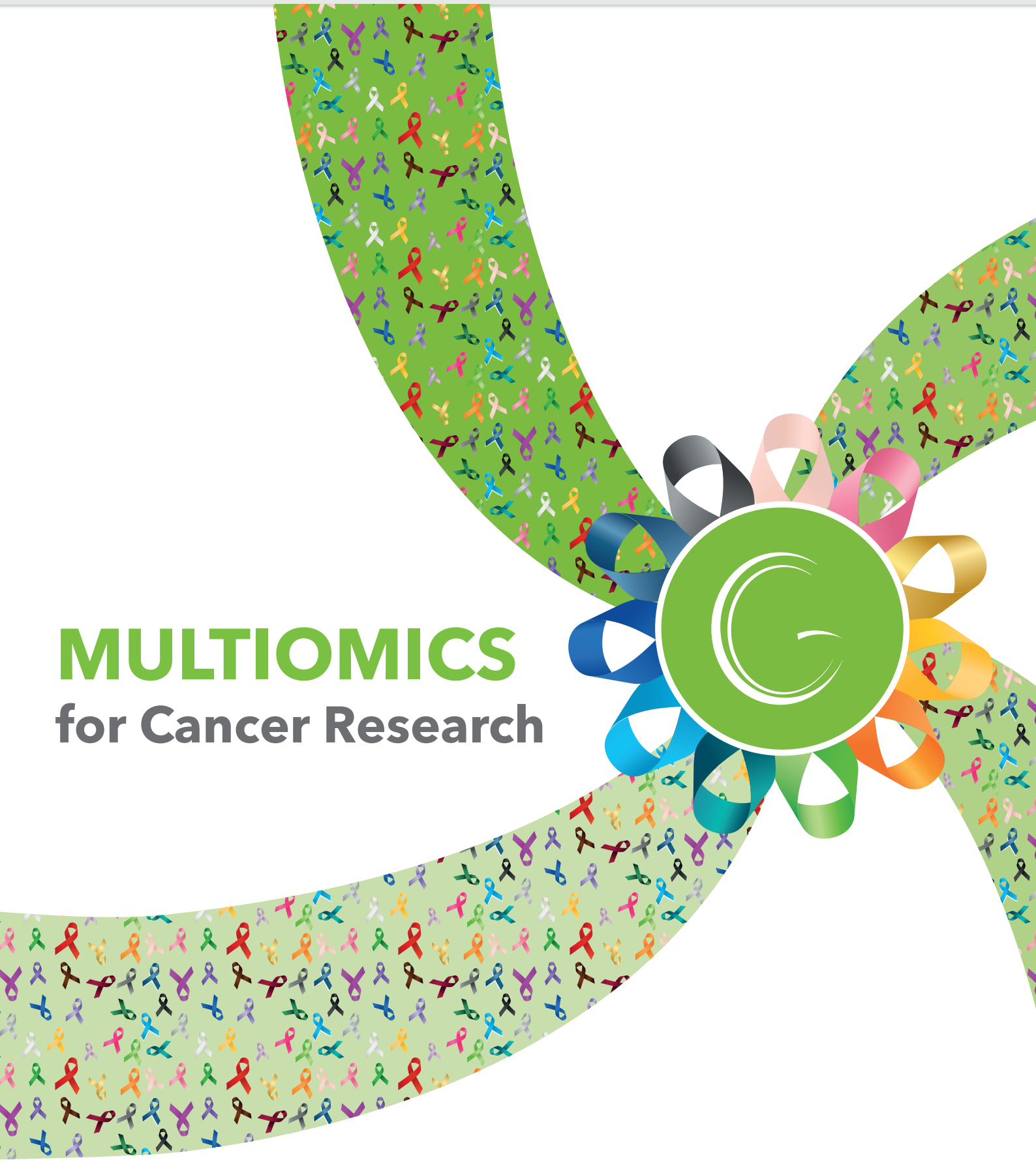
Results:

To understand the effect of DIMATE on AML cell lines, we carried out a drug response assay with different concentrations of DIMATE. We observed that AML cells present high sensitivity to DIMATE leading to reduced viability. Next, colony culture assays of MLL-AF9 leukemic cells demonstrated reduced colony forming potential upon inhibition of ALDH1A. Similarly, treatment of MLL-AF9 leukemic mice with DIMATE reduced the leukemic burden. To understand the cause of apoptosis in these cells, we carried out capillary electrophoresis immunoassays. We found that the Noxa/Mcl-1 axis played a major role and there was a time-dependent regulation of pro-apoptotic and anti-apoptotic proteins in response to DIMATE. Following exposure of AML cells to DIMATE, apoptogenic aldehydes accumulated and apoptosis occurred by simultaneous induction of 1) endoplasmic reticulum (ER) stress, 2) the genotoxic stress sensor GADD45 and 3) reactive oxygen species (ROS). DIMATE induction of ER stress condition was verified by the activation of the eiF2a/ATF4/CHOP sensor axis, known to promote apoptosis, and abrogation of the NFkB signaling pathway. In addition, DIMATE also lead to down-regulation of the proliferative AKT/mTOR pathway. Next, we screened the cytotoxic effect of 10 standard of care drugs in combination with DIMATE in a panel of 10 different AML cell lines. The most synergistic interactions were obtained with DIMATE and hypomethylating agents decitabine and azacytidine. The effect of DIMATE in combination with decitabine, azacytidine and Bcl-2 inhibitor S55746 was further explored using colony forming assays using AML cell lines. Next, the effect of DIMATE was further explored in MLL-AF9 leukemic models and its effect was compared with the chemotherapeutic agent Ara-C.

Summary/Conclusion:

We observed that leukemic cells treated with DIMATE exhibit increased apoptosis, due to upregulation of apoptotic proteins, abrogation of the NFkB signaling pathway, and downregulation of the AKT/mTOR signaling pathway. Further, we determined synergistic effects between DIMATE and hypomethylating agents, which could help in promoting chemo-sensitivity and have a clinical benefit.

Genomics Transcriptomics Metagenomics Epigenomics Oncobiomics Proteomics Microbiomics Pharmacogenomics



MULTIOMICS
for Cancer Research

MONDAY 20TH NOVEMBER, 2023

Chairs: Juan DeSanctis, Ester Mejstříková

BCL-XL blockage with A1155463 significantly increases efficacy of venetoclax in BCL-2 positive lymphoproliferative malignancies

Alexandra Dolníková[1], Ladislav Anděra[2,3], Dmitry Kazantsev[1], Magdalena Klánová[1], Eva Pokorná[1], Dana Sovilj[2], Marek Trněný[4], Pavel Klener[1,4]

[1]Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.
[2]Institute of Biotechnology CAS/ BIOCEV, Prague, Czech Republic.
[3]Institute of Molecular Genetics CAS, Prague, Czech Republic.
[4]First Department of Medicine – Department of Hematology, Charles University General Hospital, Prague, Czech Republic

Mantle cell lymphoma (MCL) is a rare chronically relapsing subtype of aggressive B-cell non-Hodgkin lymphoma characterized by the canonical chromosomal translocation t (11;14) and other recurrent molecular cytogenetic aberrations including overexpression of BCL-2 antiapoptotic protein. Venetoclax (VEN), a BCL-2 inhibitor, has demonstrated activity in MCL both as a monotherapy and in combination with other targeted agents, e.g., ibrutinib. Treatment with single agent venetoclax is, however, hampered by frequent development of drug resistance caused in large part by adaptive upregulation of other anti-apoptotic BCL-2 family members, namely BCL-XL and MCL1. Specific inhibitors of BCL-XL and MCL1 are currently being tested in clinical trials.

Immunodeficient NOD-SCID-gamma mice were xenografted subcutaneously with MCL cell lines and PDX cells established from patients with BCL-2 positive lymphoproliferative disorders. The therapy was given for two weeks (4 days ON / 3 days OFF). VEN was administered by oral gavage (100 mg / kg once daily), A1155463 (a BCL-XL inhibitor) was administered intraperitoneally (10 mg / kg once daily). Western blotting was implemented to evaluate expression levels of BCL-2 proteins, immunoprecipitation was used to analyze the levels of BIM bound to BCL-2 and BCL-XL before and after exposure to VEN. CRISPR-Cas9 was employed to derive clones with BCL-XL knock-out (HBL-2, MAVER-1). In addition, transgenic (over)expression of BCL-XL in the HBL-2 BCL-XL K/O clone was also derived to better analyze the mechanistic role of BCL-XL in mediating susceptibility to VEN. Blood cell counts in mice on A1155463 +/- VEN therapy were analyzed using the Mindray BC-5300 Auto Hematology Analyzer. In this study, we analyzed molecular mechanisms of *in vivo* acquired resistance to VEN using a panel of several murine cell line-based xenografts (CDX, n= 4) of MCL and patient-derived xenografts obtained from patients with MCL (VFN-M1, -M5R1 and -M16), BCL2-positive DLBCL (VFN-D1, -D9 and -D20), B-ALL (VFN-ALL1 and -ALL6) and T-ALL (VFN-ALL7) (PDX, n= 9). First, the mice xenotransplanted with VEN-sensitive cells were subject to monotherapy with VEN until development of VEN-resistant (VEN-R) tumors. The VEN-R tumors, when retransplanted to secondary mice, remained resistant to single-agent VEN. Western blot analysis of VEN-R tumors revealed marked upregulation of BCL-XL in majority of those tumors compared to untreated controls. Other changes included upregulation of MCL1 and downregulation of BIM proteins in several models. Immunoprecipitation experiments confirmed that BCL-XL indeed serves as a buffer for BIM released from BCL-2 after exposure to VEN thereby blocking VEN-triggered apoptosis. Importantly, we demonstrated that the upregulation of BCL-XL caused not only VEN resistance, but also led to BCL-XL-specific pro-apoptotic priming of VEN-R lymphoma cells. *In vitro*, the combination of VEN and A1155463, a specific BCL-XL inhibitor, induced strong cytotoxic synergy on a panel of MCL, DLBCL and ALL cell lines (n= 21) and primary cells (n=14). CRISPR-Cas9-mediated BCL-XL knock-out resulted in marked sensitization to VEN-induced apoptosis in MCL cell lines further confirming critical role of BCL-XL in mediating susceptibility to VEN. *In vivo*, pharmacological blockage of BCL-XL strongly increased sensitivity to VEN. Despite that A1155463 exerted limited anti-lymphoma activity as monotherapy on virtually all tested CDX and PDX models, its combination with VEN was synthetically lethal and exerted significantly enhanced anti-lymphoma activity. Of note, the efficacy of the VEN and A1155463 combination was highly effective even in mice bearing VEN-R. It has been demonstrated by other groups that molecular mechanisms responsible for BCL-XL upregulation include activation of NFkappaB signaling via CD40 and hypoxia. Upregulation of BCL-XL induced by these microenvironmental factors in turn caused VEN resistance *in vivo*. Indeed, we confirmed that upregulation of BCL-XL was observed already after engraftment of VEN-sensitive MCL cells in immunodeficient mice. MCL cells isolated ex vivo from the established CDX tumors were significantly more sensitive to BCL-XL inhibition compared to the corresponding in vitro growing MCL cell lines. Our results thus confirmed increased BCL-XL dependence of MCL cells *in vivo* compared to in vitro. We suggest that these microenvironmental factors were also critical for the selection of VEN-resistant clones during the therapy with VEN eventually leading to overgrowth of BCL-XL overexpressing VEN resistant MCL tumors. Historically, experimental therapy of patients

with chronic lymphocytic leukemia with navitoclax, a combined inhibitor of BCL-2 and BCL-XL, was rater disappointing. The plausible reasons included both low efficacy of navitoclax due to insufficient BCL-2 inhibition, and a dose-limiting thrombocytopenia caused by BCL-XL blockage. We demonstrated that the thrombocytopenia associated with continued therapy with A1155463 could be successfully managed by 4 days ON / 3 days OFF treatment strategy, which cannot be applied in case of fixed dual BCL-2/BCL-XL inhibitors like navitoclax, or AZD4320. In summary, the combined inhibition of BCL-2 and BCL-XL with VEN and A1155463 is a highly effective experimental treatment strategy for BCL-2 positive lymphoproliferative malignancies with a potential translation to the clinical grounds. Financial Support: Grant Agency of the Czech Republic GA23-05474S, National Institute for Cancer Research (EXCELES) LX22NPO5102

Hybrid amyloidosis, R² (rarest in rare)

Patrik Flodr[1], Martina Navrátilová[1], Tomáš Pika[2], Dušan Holub[3], Dominik Hraboš[1], Jiří Minařík[2], Jaroslav Michálek[1], Petr Džubák[3]

[1]Department of Clinical and Molecular Pathology, FH and FMD Palacký University, Olomouc, Czech Republic.
[2]Department of Hemato-oncology, FH Olomouc, Olomouc, Czech Republic.
[3]Department of Molecular and Translational Medicine, FMD Palacký University, Olomouc, Czech Republic

Introduction:

The number of human amyloid proteins increased on 42 in October 2022 (ISA 2022), 20 proteins in systemic and 30 proteins in localised amyloidosis with overlap in 3 amyloids, 4 candidate proteins are under investigation. In general amyloidosis is a heterogeneous acquired (30 subtypes), hereditary (17) or both (4), systemic or localised disease that results from an abnormal deposition of beta-sheet fibrillar protein aggregates in various tissues with variable distribution in extracellular space. Hybrid amyloidosis is rarest in rare form with variable fibril protein combinations.

Material and methods:

Our file contains 789 FFPE and native analysed samples with 379 positive specimens diagnosed between the years 2007-2023 in variable tissues and organs stained with Congo red and/or Saturn red as a diagnostic step with consequent immunohistochemical analysis (IHC) a proteomic analysis (laser captured microdissection-liquid chromatography/tandem mass spectrometry - LMD-LC/MS/MS) as a typing steps with completely analysed 263 specimens. Multiplex fluorescent IHC assay was applied in three-hybrid amyloidosis.

Results:

In 263 fully analysed specimens 11 hybrid amyloidosis were found, two-hybrid subtypes - 7x AL/AH (6x lambda/IgG, 1x kappa/IgG), 1x AL kappa/ATTR, 1x AL lambda/AApoAIV, 1x AH IgG1/ATTR, and 1x three-hybrid AH/dual AL (IgG1, lambda, kappa). The most common underlying disease was AL amyloidosis with a localized B-cell neoplasia of undetermined significance (LBCN US) in two-hybrid amyloidosis 1x AL kappa/AApoAIV, 1x AL lambda/AH IgG3, and in three-hybrid amyloidosis 1x AH IgG1/dual AL (kappa, lambda) and this last one case was also analysed with multiplex fluorescent IHC assay which discovered variably combined overlaping positivity for IgG, kappa and lambda. One case with extranodal marginal zone lymphoma (ENMZL MALT-type) was accompanied by AL kappa/AH IgG1. All coresponding results will be shown in tables and figures.

Discussion:

Detected hybrid types of amyloid are highly important in differential diagnosis of coincidental diseases both producing amyloid deposits (commonly AL amyloidosis + another one) and are also challenging the correct interpretation of amyloid protein deposition process e.g. a/ deposits from different specimens or organs in a same patient, b/ deposits from the same specimen and organ in diverse microspaces, c/ deposits from the same specimen and organ in the same microspace. Applied multiplex fluorescent IHC assay may be helpful in a clarification of overlaping topography of hybrid amyloid deposits with retained spatial context in an one slide also in a neighbouring cells (LBCN US) with variable co-expressions. Detected microanatomical variabilities may reveal distinct subtypes of amyloid fibrilogenesis (simple coincidence of variable amyloidosis, true two/three hybrid amyloidosis, combined seeding and fibrilogenesis). Analysis of amyloid deposits irrespective of origin and localization is appealing for diagnostic and experimental precising including also amyloid signature proteins (SAP, HSPG, SAP, apo-AI, apo-AIV, apo-E and others). Presented algorithm shows highly valid method with crucial impact on novel therapeutic decisions (siRNA, antisense mRNA, anti-human-SAP antibodies, monoclonal antibody biding amyloid protofilaments, accelerators of fibrillization, etc.) which are different in particular amyloidosis and concurrent diseases (e.g. ATTRwt amyloidosis with MGUS/ MM without AL amyloidosis, and more combinations exist). Polymorphisms and mutation burden is another horizon in amyloid deposits survey.

References:

1. Buxbaum JN, Dispenzieri A, Eisenberg DS, Fändrich M, Merlini G, Saraiva MJM, Sekijima Y, Westermark P. Amyloid nomenclature 2022: update, novel proteins, and recommendations by the International Society of Amyloidosis (ISA) Nomenclature Committee. Amyloid. 2022 Dec;29(4):213-219. doi:10.1080/13506129.2022.2147636. Epub 2022 Nov 24. PMID: 36420821.

2. Sanchorawala V, Blanchard E, Seldin DC, O'Hara C, Skinner M, Wright DG. AL amyloidosis associated with B-cell lymphoproliferative disorders: frequency and treatment outcomes. Am J Hematol. 2006 Sep;81(9):692-5. doi:10.1002/ajh.20635. PMID: 16795060.

3. Telio D, Bailey D, Chen C, Crump M, Reece D, Kukreti V. Two distinct syndromes of lymphoma-associated AL amyloidosis: a case series and review of the literature. Am J Hematol. 2010 Oct;85(10):805-8. doi: 10.1002/ajh.21814. PMID: 20872959.

4. Stuhlmann-Laeisz C, Schönland SO, Hegenbart U, Oschlies I, Baumgart JV, Krüger S, Röcken C. AL amyloidosis with a localized B cell neoplasia. Virchows Arch. 2019 Mar;474(3):353-363. doi: 10.1007/s00428-019-02527-7. Epub 2019 Jan 24. PMID: 30680453.

5. Yabe M, Ozkaya N, de Jong D, Aypar U, Ritorto MS, Barbé E, Miedema IHC, Sen F, Chapman JR, Landau HJ, Dogan A. Localized Peritumoral AL Amyloidosis Associated With Mantle Cell Lymphoma With Plasmacytic Differentiation. Am J Surg Pathol. 2021 Jul 1;45(7):939-944. doi: 10.1097/PAS.0000000000001684. PMID: 33739787; PMCID: PMC8192423.

6. Matsumoto Y, Masuda T, Nishimura A, Horie H, Harada K, Yoshida M, Shimura K, Kaneko H, Taniwaki M. A case of AL amyloidosis associated with follicular lymphoma with plasmacytic differentiation. Int J Hematol. 2020 Feb;111(2):317-323. doi: 10.1007/s12185-019-02734-7. Epub 2019 Sep 21. PMID: 31542852.

7. Holub D, Flodrova P, Pika T, Flodr P, Hajduch M, Dzubak P. Mass Spectrometry Amyloid Typing Is Reproducible across Multiple Organ Sites. Biomed Res Int. 2019 Jan 31;2019:3689091. doi: 10.1155/2019/3689091. PMID: 30834260; PMCID: PMC6374819.

8. Wechalekar, AD, Gillmore, JD, Hawkins, PN. Systemic amyloidosis. Lancet 2016; 387(15): 2641–2654.

9. Gertz, MA. Immunoglobulin light chain amyloidosis: 2022 update on diagnosis, prognosis, and treatment. Am J Hematol. 2022; 97(6): 818-829. doi:10.1002/ajh.26569.

10. Habib El-Khoury, David J Lee, Jean-Baptiste Alberge, Robert Redd, Christian J Cea-Curry, Jacqueline Perry, Hadley Barr, Ciara Murphy, Dhananjay Sakrikar, David Barnidge, Mark Bustoros, Houry Leblebjian, Anna Cowan, Maya I Davis, Julia Amstutz, Cody J Boehner, Elizabeth D Lightbody, Romanos Sklavenitis-Pistofidis, Mark C Perkins, Stephen Harding, Clifton C Mo, Prashant Kapoor, Joseph Mikhael, Ivan M Borrello, Rafael Fonseca, Scott T Weiss, Elizabeth Karlson, Lorenzo Trippa, Timothy R Rebbeck, Gad Getz, Catherine R Marinac, Irene M Ghobrial. Prevalence of monoclonal gammopathies and clinical outcomes in a high-risk US population screened by mass spectrometry: a multicentre cohort study. The Lancet Haematology. Volume 9, Issue 5, 2022, pages e340-e349, ISSN 2352-3026, https://doi.org/10.1016/S2352-3026(22)00069-2.

Supported by AZV-16-31156A, NU22-08-00306 and LF_2022_004 from Palacky University Olomouc

Molecular dynamics of the lineage switch in acute leukemia: insight through mass cytometry and CITE-Seq analysis using trajectory inference

Jan Stuchlý, Barbora Vakrmanová, Aneta Skotnicová, Michaela Reiterová, Michael Svatoň, Martina Vášková, Markéta Žaliová, Eva Froňková, Ester Mejstříková, Tomáš Kalina

CLIP, Dept. Paediatric Haematology/Oncology, 2nd Faculty of Medicine Charles University Prague, Prague, Czech Republic

Introduction:

Our study takes an in-depth look at lineage switching in B-cell precursor acute lymphoblastic leukemia (BCP ALL), a process that significantly influences minimal residual disease (MRD) monitoring (Novakova et al. Haematologica 2020). Switching ALL (swALL) is characterized by significant change of phenotype towards monocytic lineage and gradual loss of B-cell markers during early phase of the treatment. Transdifferentiated blasts are difficult to distinguish from mature monocytes. SwALLs are genetically characterized by rearrangements of the ZNF384 and DUX4 gene and a mutation in the transcription factor PAX5-P80R.

Employing a combination of mass cytometry, RNA-Seq analysis, and state-of-the-art computational methods, we aimed to enhance our understanding of the transdifferentiation process and its association with varying genetic aberrations.

Methods:

We tested samples from the early phase of the therapy with simultaneous presence of both lymphoid and myeloid suspect blasts. We built a mass cytometry panel combining myeloid and lymphoid markers. In addition, the intracellular version contained transcriptional factors PAX5 and PU.1. CITE-Seq was used to enable simultaneous analysis of clonal rearrangements with immunophenotypic and transcriptomic profile on single cell level.

Patients:

By mass cytometry panels we measured paired samples (diagnostic and on treatment sample) from patients with a monocytic switch (n=5) with the following genotypes: DUX4r (n=3), PAX5-P80R (n=1), ZNF384r (n=1). One patient with genotype ZNF384r ((diagnostic sample) was analyzed by CITE-Seq.

Results:

We have developed an integrated computational trajectory inference (TI) framework tviblindi (https://github.com/stuchly/tviblindi/) which allows for the tracking of the evolution of markers both on transcriptomic and protein levels. TI methods are designed to discover the dynamical processes in the

static snapshot obtained by the single-cell measurements. The cells can be ordered along the discovered development trajectories which introduces the pseudotemporal information. We can thus interrogate the orchestration of specific markers which govern the dynamics of the switch.

We observed the expected lag of protein expression when compared to the changes on transcriptomic level. In practice this means that we detect a significant subset of cells which have already switched into the monocytoid program on RNA level, while still having the immunophenotype of B-cell blasts. Our findings highlight specific immunophenotypic shifts during lineage switching, with CD33 being the earliest marker to increase both on RNA and protein level across all genetic subtypes. However, significant variations in marker expressions were observed across different genetic subsets, suggesting subtype-specific dynamics in lineage switching.

Additionally, in one patient with the genotype ZNF384r, we observed a notable increase in IKZF1 on RNA level just prior to the lineage switch, hinting at a potential role in transdifferentiation. However, further investigation is necessary to solidify this relationship. In this patient, we were also able to track the VDJ recombination on single cell level up to the point of lineage switch. After the switch the cells quickly ceased to express the VDJ which correlated with the cessation of expression of IGHM. This fact affects the application of VDJ tracking on RNA level for MRD monitoring.

Conclusion:

So far with available technologies we had only limited insight in the transcriptomic profile of lymphoid and myeloid populations. Our preliminary data suggest that the transcriptome precedes the intrinsic leukemia cell phenotype in a number of markers. Even with extended myeloid panels, the distinction between healthy monocytes and monocytoids was elusive, underscoring the complexity of lineage switching. Our study highlights the potential of advanced analytical tools and methods in the detailed understanding of lineage plasticity in leukemia and could lead to the development of improved diagnostic strategies and targeted treatments.

[1] Novakova M, Zaliova M, Fiser K, Vakrmanova B, Slamova L, Musilova A, Brüggemann M, Ritgen M, Fronkova E, Kalina T, Stary J, Winkowska L, Svec P, Kolenova A, Stuchly J, Zuna J, Trka J, Hrusak O, Mejstrikova E. DUX4r, ZNF384r and PAX5-P80R mutated B-cell precursor acute lymphoblastic leukemia frequently undergo monocytic switch. Haematologica. 2021 Aug 1;106(8):2066-2075.

Supported by NPO NÚVR and partly by NU23-05-00353.

Composition of immunocompetent cells in bone marrow and peripheral blood in context of anti-CD19 targeted therapy of B cell precursor leukemia

Ester Mejstříková[1], Martina Vášková[2], Tomáš Kalina[1], Ondřej Hrušák[1], Michaela Reiterová[1]

[1]Department of Pediatric Hematology and Oncology LF2 UK and Motol, Prague, Czech Republic. [2]Department of Pediatric Hemaotlogy and Oncology University Hospital Motol, Prague, Czech Republic

Introduction. Acute lymphoblastic leukemia is the most common malignancy in childhood. Each year approximately 80 children are diagnosed with ALL in Czechia. The predominant subtype is B cell precursor leukemia. Recently targeted therapies were introduced for both frontline and relapsed patients. Blinatumomab which is anti-CD19/anti-CD3 construct is used in frontline patients in medium and high risk arm during the consolidation phase of the therapy. Chemotherapy leads to elimination of blasts and gradual reconstitution of hematopoiesis. During the therapy patients suffer from variable degree of immunosuppression. A prerequisite for success of blinatumomab therapy is sufficient amount of functional T cells. So far only limited knowledge about dynamics of various cellular subsets during the therapy is available. Methods and samples. We developed a polychromatic flow cytometry panel to analyze bone marrow (BM) and peripheral blood (PB) using the Cytek Aurora instrument. This instrument allows for the simultaneous measurement of >20 fluorochromes, reducing sample volume requirements and enabling comprehensive evaluation of large cell populations. The optimized final monoclonal antibody combination includes a total of 26 features for immunophenotyping and two features for material type identification (bone marrow or peripheral blood). It allows detailed analysis of subpopulations of T cells, B cells including its progenitors, NK cells, monocytes, dendritic cells and basophils. We track selected time points of patients in the blinatumomab treatment arm and the control arm, both before and after blinatumomab therapy alone. In total we analyzed 240 samples during ALL therapy. Preliminary results. Detailed analysis is ongoing including development of novel analytical tools. Blinatumomab leads to variably lasting B cell aplasia together with activation of T cells. Dynamics of cell subsets depends on intensity of the therapy. It is currently debated whether blinatumomab means a worse chance of eventual effectiveness of CAR T cell therapy in refractory patients. In our cohort one patients with infant ALL treated with blinatumomab was later treated with CAR T cells. So far the follow up is short and we observe response under CAR T cell therapy. Of note patient with exceptional CD19 positive refractory T ALL relapse to conventional chemotherapy did not respond and even after blinatumobab blasts with preserved CD19 expression increased in peripheral blood. Conclusion. The

intensity of ALL treatment affects the composition of cell subpopulations. Targeted anti-CD19 treatment leads to varying lengths of B-lineage aplasia.

Supported by NPO and partly by NU23-05-00353.

TUESDAY 21ST NOVEMBER, 2023

Chairs: Jiří Drábek, Pavel Vodička

Genetic predisposition to colorectal cancer: Identification of novel cancer susceptibility genes

Asta Försti[1,2], Kari Hemminki[3,2]

[1]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany.
[2]German Cancer Research Center (DKFZ), Heidelberg, Germany.
[3]Charles University, Pilsen, Czech Republic

[1]Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.
[2]Institute of Biotechnology CAS/ BIOCEV, Prague, Czech Republic.
[3]Institute of Molecular Genetics CAS, Prague, Czech Republic.
[4]First Department of Medicine – Department of Hematology, Charles University General Hospital, Prague, Czech Republic

About 15% of colorectal cancer (CRC) patients have first-degree relatives affected by the same malignancy. However, for most families the cause of familial aggregation of CRC is unknown. To identify novel high-to-moderate-penetrance germline variants underlying CRC susceptibility, we performed whole exome (WES) and whole genome sequencing (WGS) in Polish CRC families showing a Mendelian inheritance pattern. After WES or WGS, we used our in-house developed Familial Cancer Variant Prioritization Pipeline (FCVPP) to identify novel cancer predisposition variants. We identified both nonsense, missense and 5'UTR variants involved in the regulation of innate immune response (SLC15A4), apoptosis and AKT pathway (PTK7), reactive oxygen species and mucus biology (CYBA, TRPM4), Wnt signaling (APCDD1) and histone modification (HDAC5) and in a protooncogene (SRC). Some of the identified variants may show they effect according to a synergistic or polygenic model. Our findings contribute to the identification of unrecognized genetic causes of familial CRC.

Genomic instability in adenomas and in colorectal cancer progression

Pavel Vodička[1,2,3], Michal Kroupa[1,3], Kristýna Tomášová[1,3], Anna Šišková[1,2], Kateřina Balounová[1], Soňa Vodenková[1,3], Veronika Vymetálková[1,2,3], Rajiv Kumar[1], Safaa Andarawai[1,3], Saba Selvi[1], Stefanie Brezina[4], Andrea Gsur[4], Kari Hemminki[3], Ludmila Vodičková[1,2,3]

[1]IEM ASCR, v.v.i, Prague, Czech Republic.
[2]First Medical faculty, Charles University, Prague, Czech Republic.
[3]Medical faculty in Pilsen, Charles University, Pilsen, Czech Republic.
[4]Medical University of Vienna, Vienna, Austria

In solid cancers both impaired DNA repair and disrupted telomere length (TL) homeostasis are key culprits in cancer initiation, progression and prognosis. Telomere attrition resulting in replicative senescence, simultaneously by-passing cell cycle checkpoints, is a hallmark of cellular malignant transformation. Telomerase, ubiquitous in advanced solid cancers, is fundamental to cell immortalisation. Human solid neoplasms often exhibit chromosomal instability (CIN), which generates either abnormal aneuploid karyotypes, or continually expands phenotypic heterogeneity as tumor cell populations undergoing cell divisions. We searched for the CIN markers in the adenoma-adenocarcinoma transition and in CRC progression. Understanding the mechanisms and dynamics of tumor genomic diversification, where DNA damage response and telomere homeostasis are important players, is critical in understanding carcinogenesis and overcoming drug resistance. A part of the above search is the comparison of telomere homeostasis genetics (based on GWAS study) with TL in 7,000 patients with sporadic CRC.

The mitochondrial dysfunction, another cancer hallmark, is linked with DNA repair capacity and compensate for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN reported ambiguous and inconsistent results for various cancer types. Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing CIN, while TL maintenance characterized by telomerase expression is required for cancer cell proliferation and tumour growth. The reports on TL as a biomarker for cancer risk, patient therapy response and/or survival are contradictory as well. Our investigations were also focused on mtDNA_CN in CRC tissues and adjacent mucosa.

Large-scale categorization of germline missense variants in the checkpoint kinase gene *CHEK2* revealed a set of functionally-impaired variants increasing breast cancer risks

Zdeněk Kleibl[1], Lenka Stolařová[2], Petra Kleiblova[1], Petra Zemánková[1], consortium CZECANCA[1], consortium ENIGMA[1], Libor Macůrek[2]

[1]Laboratory of Oncogenetics, Institute of Medical Biochemistry and Laboratory Diagnostic, First Faculty of Medicine, Charles University & General University Hospital in Prague, Prague, Czech Republic.
[2]Laboratory of Cancer Cell Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Introduction and Purpose: Germline pathogenic variants (protein truncations, including large intragenic rearrangements and spliceogenic variants) in the *CHEK2*

gene, encoding the checkpoint kinase *CHK2*, confer a moderately increased breast cancer (BC) risk (odds ratio; OR ~ 2.5) that qualifies carriers for enhanced BC screening. In addition to pathogenic variants, dozens of missense *CHEK2* variants of uncertain significance (VUS) have been identified, hampering the clinical utility of germline genetic testing, as variants in the *CHEK2* gene are the second most common germline alteration in patients in the EU and US. To reduce the diagnostic uncertainty of clinical germline genetic testing, we performed a robust functional analysis of germline *CHEK2* missense variants in a representative set of VUS.

Methods: Functional characterization was performed using *CHEK2* complementation assays quantifying KAP1 phosphorylation and *CHK2* autophosphorylation in human RPE1-*CHEK2* knockout cells expressing variant *CHK2* isoforms. The assays were performed using high-content microscopy, which also allows precise tracking of the intracellular localization of the variants analyzed. We initially collected 460 *CHEK2* missense VUS identified by the ENIGMA consortium in 15 countries, including 51 variants reported by the national CZECANCA consortium. Concordant results in both functional assays were then used to categorize *CHEK2* VUS from 12 ENIGMA case-control datasets of 73 048 BC patients and 88 658 ethnicity-matched controls. This dataset included 4 436 carriers of 377 unique *CHEK2* missense variants identified in 3.4% of BC patients and 2.2% of controls.

Results: A total of 430/460 VUS were successfully analyzed, of which 340 (79.1%) were concordant in both functional assays and categorized as functionally impaired (N=102), functionally intermediate (N=12), or functionally wild type-like (N=226). Impaired nuclear localization was found in two variants. We then examined the association with BC risk in a case-control analysis including 3,660 (82.5%) carriers of *CHEK2* missense variants that were concordantly categorized. The OR and 95%CI (confidence intervals; p-value) for carriers of functional, intermediate, and wild-type-like variants were 2.83 (2.35-3.41; 2.4E-30), 1.57 (1.41-1.75; 9.1E-17), and 1.19 (1.08-1.31; 3.6E-4), respectively. The meta-analysis of population-based datasets showed similar results. The risk (OR) for the most common known pathogenic variant c.1100delC in the case-control dataset was 2.73; 95%CI 2.29-3.26.

Discussion: We have developed a robust functional assay for the analysis of germline variants affecting the *CHEK2* gene, which we have used to perform the largest categorization of *CHEK2* missense variants to date. Our analysis will allow definitive clinical classification of *CHEK2* germline variants (in ClinVar). We determined the functional consequences for the majority of *CHEK2* missense VUS found in BC patients (3 660/4 436; 82.5%). Carriers of functionally impaired missense variants accounted for 0.5% of BC patients and were associated with a moderate risk similar to that of truncating *CHEK2* variants. In contrast, 2.2% of all BC patients carried functionally wild-type/intermediate missense variants with no clinically relevant BC risk in heterozygous carriers. Our study will significantly contribute to improving the outcome of germline genetic testing on a global scale.

Acknowledgment:

Supported by the grant projects of the Ministry of Education, Youths and Sports of the Czech Republic grant EXCELES no. LX22NPO05102 funded by EU, Czech Ministry of Health NV19-03-00279, DRO-VFN-64165; Charles University projects COOPERATIO and SVV260516; Lenka Stolarova was partially supported by Czech Academy of Science (PPPLZ project L200522201).

Precise correction of causal mutations as a treatment strategy for monogenic disorders with increased predisposition to cancer

Lucie Peterková[1], Markéta Racková[1], Michael Svatoň[1], Petr Říha[2], Drahuše Novotná[3], Petr Sedláček[2], Lucie Šrámková[2], Karolina Škvárová Kramarzová[1]

[1]Childhood Leukaemia Investigation Prague, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic.
[2]Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic.
[3]Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Fanconi anemia (FA) is a rare genetic disorder resulting from mutations in genes involved in the repair of DNA inter-strand crosslinks. Most FA patients develop bone marrow failure (BMF) already during childhood and require allogeneic transplantation of hematopoietic stem and progenitor cells (HSPC). Furthermore, FA patients have an increased cancer predisposition, particularly to cancers of head and neck, ovarian, breast, prostate cancer, and leukemia. To date, 23 FA complementation groups and their corresponding genes causal of FA were identified and the most common both in Czech Republic and worldwide is FANC-A in which the FANCA gene is mutated. As a final curative treatment of BMF, patients undergo allogenic hematopoietic stem and progenitor cell (HSPC) transplantation which is associated with significant morbidity and mortality. The recent development in the field of gene therapy and gene editing could provide a beneficial

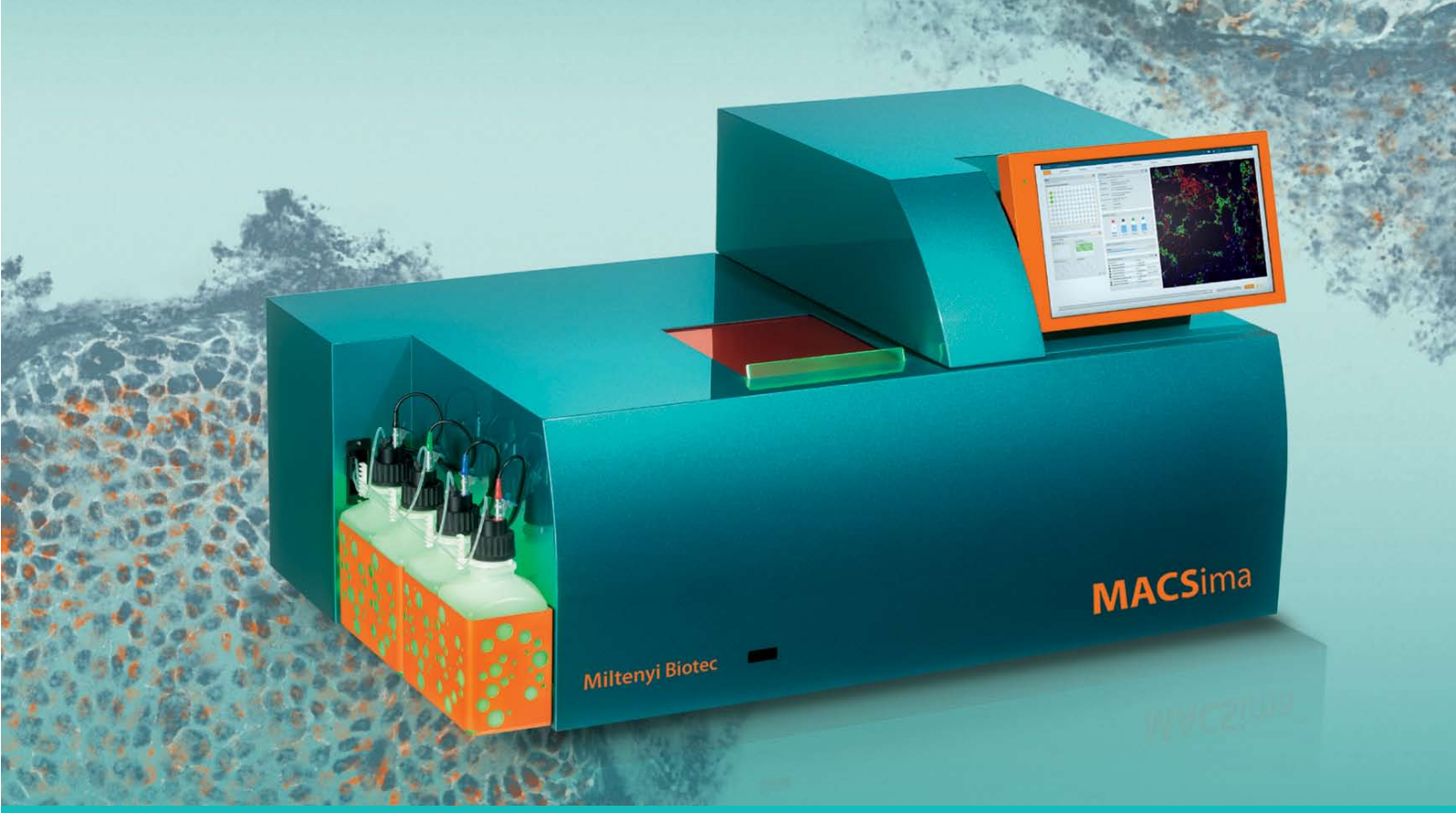
alternative for FA patient. Patient´s HSPCs could be corrected ex vivo with the use of genome-editing technologies followed by reinfusion of the corrected cells into patient. This treatment alternative would not require a HSPC donor and reduce pre-transplantation conditioning as well as the risk of development of graft-versus-host disease that increases the risk of cancer later in life in FA patients. Prime editing is an advanced genome-editing technology capable of precise correction that is considered to hold great promise for development of gene therapies.

Our research focuses on two unrelated FANCA patients (patient 1: FANCA c.1A>G and c.4010+1-c.4010+18del; patient 2: FANCA c.1A>G and c. 3788_3790delTCT), specifically on their shared c.1A>G mutation. To correct this mutation, we designed and prepared pegRNAs carrying both the targeting sequence and correcting template and introduced them into fibroblasts from these patients along prime editor and nicking sgRNAs by electroporation. Efficiency of prime editing was analysed by next generation sequencing, recovery of production of full-length FANCA protein was detected by immunoblotting.

We obtained correct editing of up to 25% in cells from both unrelated patients and we were able to detect full-length FANCA protein. We also observed an increase in the amount of corrected DNA over time up to 55% in culture in agreement with the hypothesized functional restoration of FA pathway and associated proliferation advantage of corrected cells.

Our results indicate that prime editing can be successfully and effectively used in cells with FA genetic background and support further testing of this gene editing method for the development of therapeutical approach for patients with other inherited bone marrow failure disorders. Such approach could significantly decrease the genotoxic burden of the current treatment strategies and decrease the incidence of cancer in these patients.

Supported by KOG-202108-00904 and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.



MACSima™ Imaging System

The easy way to ultrahigh-content imaging

- **Stain hundreds of markers on a single sample using the principle of iterative stain, image, erase cycles**
- **Freedom to analyze any type of fixed sample, including tissue, adherent and suspension cells**
- **Complete walk-away system through fully automated processing and imaging**

For more information please contact:

BIOHEM
produkty pre medicínu

BIOHEM a.s.
Zlatovská 2211 | 911 01 Trenčín | Slovakia
Petra Kupková | +420 737 977 668 | kupkova@biohem.sk

▸ **miltenyibiotec.com**

18th Czech Annual Cancer Research Meeting

November 20-22, 2023 | Olomouc

Miltenyi Biotec

TUESDAY 21ST NOVEMBER, 2023

Chairs: Ondřej Slabý, Zdeněk Kleibl

Real-world precision medicine in oncology: Comparison of results in pediatric and adult patients

Ondrej Slaby[1,2], Petra Pokorna[3], Michal Eid[4], Jaroslav Sterba[4]

[1]Masaryk University, Faculty of Medicine, Department of Biology, Brno, Czech Republic. [2]University Hospital Brno, Department of Pathology, Brno, Czech Republic. [3]CEITEC, Masaryk University, Brno, Czech Republic. 4University Hospital Brno, Brno, Czech Republic

Recent advances in cancer research and modern therapies have significantly extended the therapeutic options for these diseases. Success has been achieved even in those malignancies that until recently were considered uncontrollable by systemic treatment. Thus, the prognosis of cancer patients is improving, including those with metastatic disease, and the logical goal of clinical research is to transform disseminated disease from a fatal to a chronic disease. Behind this progress and this ambition, besides cancer immunotherapy, is mainly the application of knowledge from molecular pathology and its use for individualized therapeutic planning. Applying this knowledge takes us from the histopathological evaluation of tumors to the next level, which considers the biological behavior of individual malignancies. This enables a higher level of individualization of cancer treatment, where we use technologies that will allow comprehensive genomic profiling (next-generation sequencing, NGS), which we call precision oncology. For precision oncology, a multidisciplinary approach in the form of a molecular tumor board (MTB) is also essential; in Czech, such an interdisciplinary panel can be referred to as a molecular oncology indications committee. Typically, specialties such as clinical oncologist, pathologist, molecular biologist (molecular pathologist), clinical geneticist, and clinical pharmacologist are represented. The panel's role is then to find an appropriate and highly individualized treatment plan beyond standard treatment based on the evaluation of comprehensive genomic analyses. In our communication, we will introduce you to the functioning and results of two molecular oncology indication committees at the University Hospital Brno, the committee for childhood and adult tumors. We will compare typical characteristics and results achieved by precision oncology in children and adults and point out their possible secondary use for cancer research. Supported with the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) – Funded by the European Union – Next Generation EU.

Topological stress triggers difficult-to-repair DNA lesions in ribosomal DNA with subsequent formation of PML-nucleolar compartment

Alexandra Urbancokova[1], Terezie Hornofova[2], Josef Novak[1], Sarka Salajkova[1], Monika Zarska[1], Zdenek Hodny[1], Pavla Vasicova[1]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic. [2]Institute of Molecular Genetics of the Czech Academy of Sciences, prague, Czech Republic

Promyelocytic leukemia protein (PML) is a scaffold for accumulating proteins in specific nuclear sites. This membraneless compartment is essential for regulating various nuclear processes such as DNA repair, telomere maintenance, or chromatin modification. Under specific genotoxic stress, PML interacts with the nucleolus, forming the PML-nucleolar associations (PNAs), which undergo dynamic structural changes reflecting the activity of RNA polymerase I (RNAPI). We reveal that this interaction is promoted by chemotherapeutics that share the ability to inhibit topoisomerases and RNAPI simultaneously. The inhibition of DNA double-strand break (DSB) repair augmented the occurrence of PNAs linking the stimulus for PNAs formation to unresolved DNA damage. The most potent treatment, doxorubicin, introduced DSBs into the rDNA locus. We showed that PNAs colocalized with damaged rDNA and, after reactivation of RNAPI, promoted its sequestration from active nucleoli. Using rDNA locus cleavage by I-PpoI, we proved that rDNA damage is a potent PNAs-inducing stimulus.

Our findings have implications for genome stability and diverse diseases and indicate that PNAs form when difficult-to-repair rDNA DSBs occur in nucleoli, highlighting the interplay between the PML/PNAs and rDNA alteration caused by deficiencies in topoisomerases, inhibition of RNAPI, and rDNA DSBs. Altogether, these findings highlight the role of the PML-nucleolar compartment in rDNA maintenance and extend the knowledge about this multitasking protein.

Exploring ATR inhibition in MLL-ENL-driven leukemogenesis: threshold and context role of ATR signaling

Pavla Chaloupkova[1], Jan Gursky[1], Zuzana Jurtik Korbasova[1], Nikola Niederlova[1], Pavla Koralkova[1], Jiri Bartek[2,3], Vladimir Divoky[1]

[1]Department of Biology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic.
[2]Danish Cancer Society Research Center, Copenhagen, Denmark.
[3]Department of Medical Biochemistry and Biophysics, Science for Life Laboratory, Division of Genome Biology, Karolinska Institute, Stockholm, Sweden

Targeted therapy interfering with multi-step process of leukemia development is one of the most important challenges in leukemia research. In our group, we are testing experimental targeting of acute myeloid leukemia (AML) with translocations of the mixed lineage leukemia 1 (MLL1, also known as KMT2A) gene. Despite considerable progress in leukemia treatment, mixed lineage leukemia (MLL) remains a disease with dismal prognosis; five-year survival rates are around 50%, much lower compared with other types of acute leukemia. Targeted treatment of MLL is challenging; we are particularly focused on the efficacy of synthetic lethal interactions in the DNA damage response (DDR) pathway in MLL cells.

Preclinical models published earlier suggested that inhibition of some of the key components of canonical and non-canonical DDR pathways may be lethal for MLL-rearranged myeloid progenitors. Severe MLL-oncogene induced replication stress (RS), resulting in the activation of ATR/Chk1- and ATM/Chk2-mediated signaling/checkpoint, seemed to provide rationale for the potential use of ATR inhibitors (or ATM inhibitors) in MLL therapy. However, many initial phase I clinical trials utilizing these approaches failed. It has become clear that novel synthetically lethal therapeutic strategies in leukemogenesis would need to be tested using faithful preclinical models and that fusion partners in MLL fusions could determine therapeutic responses.

We previously described a mouse model of MLL-rearranged leukemia, with AML evolving from chronic myeloproliferation (preleukemia state) with a long latency period (Takacova et al, PMID: 22516260). In this model, the Mll-ENL oncogene has been introduced by homologous recombination and is controlled by the endogenous Mll promoter, thus, expressed at physiological levels. Due to the fusion with the estrogen receptor ligand binding domain (ERtm), the activity of Mll-ENL-ERtm protein is dependent on the continuous provision of tamoxifen (TAM). In this model, inflammatory microenvironment triggers oxidative stress and accumulation of damaged DNA in myeloid progenitors, which together with MLL oncogene-induced RS results in preleukemia, with ATR/Chk1- and ATM/Chk2-mediated DDR/checkpoint activation forming intrinsic biological barrier against transformation and preventing full leukemia development. Only experimental suppression of this barrier by the ATR/ATM inhibitor caffeine promoted development of leukemia from a preleukemia condition.

We hypothesized that varying extent of ATR inhibition by pharmacologically characterized molecules ceralasertib (AZD6738) and elimusertib (BAY-1895344) using *in vivo*/*in vitro* preclinical models might provide novel insights into the complexity and consequences of DDR targeting in MLL.

As mentioned, our murine Mll-ENL strain represents an AML model with long latency of leukemia development (mean survival 592 ± 112 days of oncogene expression). During the course of multistep leukemogenesis, critical events take place at 7 months of Mll-ENL expression: cooperation between the oncogene-induced DDR and inflammatory cytokine network activates natural antitumor barrier based on the induction of a senescence phenotype. Those cells that breach the barrier and continue the oncogene-driven leukemogenesis presumably rely on the functional RS response and DNA damage signaling, dominantly orchestrated by ATR. To assess the potentially antagonistic roles of ATR in antitumor barrier triggering vs. tumor-supportive pathway, we analyzed the consequences of ATR inhibition at early stage of leukemogenesis (7 months on TAM). First, the provision of orally-available ATRi ceralasertib for 6 months resulted in increase in immature c-kit+/Mac-1+ cells population in both BM and spleen, accompanied by an increase in the myeloid Mac-1+/Gr-1+ in the spleen. Consistently with increased Mac1+ proliferating rates in Mll-ENL BM and spleen, our data suggest the weakening of antiproliferative barrier as a result of DDR signaling attenuation by ATR inhibition. Notably, 6 months treatment with ceralasertib leads to increased load of γH2AX foci, however elevated level of DNA damage seems not to be cytotoxic. Second, the administration of pharmacologically superior ATRi elimusertib for 1 months resulted in severe hematopoietic toxicity with 50 % reduction of hemoglobin and hematocrit.

For functional experiments with transformed leukemia cells, six cell cultures of Mll-ENL-ERtm positive (MEER) cells were derived from c-Kit-positive bone marrow (BM) precursors by induction of the oncogene with 4-hydroxytamoxifen (4-OH-TAM). All derived MEER cell cultures were first cultivated in the presence of SCF and then fully adapted to FLT3-ligand. Transcriptome gene set enrichment analyses suggested that the MEER cells represent transformed leukemia rather than preleukemia disease state.

We determined the cytotoxic effect of both ATRis on MEER cells; our assays revealed a substantial

cytotoxic effect of both tested ATR inhibitors on MEER cells *in vitro*. Additionally, there was a pronounced influence on the extent of inhibition of the ATR pathway. As anticipated, elimusertib demonstrated a notably lower IC50 value, substantiating its heightened proficiency in eliciting a stronger suppression of the ATR signaling pathway. Thus, ATRis appear to have the potential to kill transformed MLL cells at concentrations inhibiting the anti-tumor barrier (or at toxic but sublethal concentrations) as observed *in vivo* in the preleukemic state.

To further describe the mechanism of action of ATRi on MEER cells, we analyzed cell cycle progression, replication rate and γH2AX induction upon ATRi treatment. We hypothesized that MEER cells with perturbed S-phase may rely more on ATR-dependent S/G2 checkpoint, thus becoming highly vulnerable to ATRi. As anticipated, upon ATRi treatment accumulation of cells in S-phase together with decrease in the number of proliferating cells/ increase in sub-G1 phase cells was observed.

In summary, we show ceralasertib-induced acceleration of leukemogenesis in preleukemia MLL mouse model, as mild/moderate ATR inhibition in progenitors that suffer DNA damage and activate the DDR barrier resulted in a gradual accumulation of immature myeloid cells. Elimusertib, more potent ATRi, revealed *in vivo* severe toxicity to hematopoiesis, including red cells. The MEER cell model, representing a more transformed cell state, reveals an apparent addiction to the ATR checkpoint. This is consistent with the high proliferation rate and overall rewired program in these cells, and hence the dependence on ATR signaling is likely to be high for complete replication and prevention of chromosome breakage and cell death. Thus, while checkpoint addiction of transformed leukemia cells with DDR defects offers synthetic lethal vulnerability with therapeutic potential, attenuation of DNA damage checkpoint with an inhibitor may promote development of leukemia from coexisting preleukemia subclones. Grant support: EXCELES-LX22NPO5102; GACR-23-05462S; AZV-NU21-03-00338

PPM1D activity promotes the replication stress caused by cyclin E1 overexpression

Libor Macurek[1], Andra Martinikova[1], Miroslav Stoyanov[1], Anna Oravetzova[2], Jana Dobrovolna[1], Pavel Janscak[1], Marcel Vugt[3]

[1]IMG, Prague, Czech Republic.
[2]IMG, Prague, Denmark.
[3]UMCG, Groeningen, Netherlands

Oncogene-induced replication stress has been recognized as a major cause of genome instability in cancer cells. Increased expression of cyclin E1 caused by amplification of the CCNE1 gene is a common cause of replication stress in various cancers. The PPM1D phosphatase is a negative regulator of p53 and has been implicated in termination of the cell cycle checkpoint. Amplification of the PPM1D gene or frameshift mutations in the last exon of the PPM1D promote tumorigenesis. Here, we show that PPM1D activity further increases the replication stress caused by overexpression of cyclin E1. In particular, we demonstrate that cells expressing a truncated mutant of PPM1D progress faster from G1 to S phase and fail to complete licensing of the replication origins. In addition, we show that transcription-replication collisions and replication fork slowing caused by cyclin E1 overexpression are exaggerated in cells expressing the truncated PPM1D. Finally, replication speed as well as accumulation of the focal DNA copy number alterations caused by induction of cyclin E1 expression was rescued by pharmacological inhibition of PPM1D. We propose that increased activity of PPM1D suppresses the checkpoint function of p53 and thus promotes genome instability in cells expressing the CCNE1 oncogene.

The role of autoproteolysis and mitoribosomal proteins in the regulation of LACTB tumor suppressor

Sara Escudeiro-Lopes, Juan Gonzales-Morena, Zuzana Keckesova

IOCB, Prague, Czech Republic

Tumor suppressors represent one of the first lines of defense against malignant transformation and their inactivation in cells leads to onset of tumorigenesis. Cancer cells employ a variety of ways to inactivate cellular tumor suppressors, such as their epigenetic silencing or mutagenesis. Less understood are mechanisms by which cancer cells inactivate tumor suppressors post-translationally. In our study we uncovered a novel post-translational strategy cancer cells use to inactivate potent mitochondrial tumor suppressor LACTB in breast cancers. We uncovered that substrate of LACTB is LACTB itself; that LACTB possesses autoproteolytic ability, which is important for its tumor suppressor activity. We show that cancer cells misuse this feature of LACTB to force LACTB into self-degradation. This is mechanistically realized through upregulation of mitochondrial MRPS34 protein, which, through interaction with LACTB, is a positive regulator of LACTB's autoproteolytic activity and a negative regulator of LACTB.

TUESDAY 21ST NOVEMBER, 2023

Chairs: Lucie Kučerová, Josef Srovnal

Advances in liquid biopsy testing

Mikael Kubista[1], Anders Ståhlberg[2], Erik Larsson[2]

[1]Insitute of Biotechnology, Prague, Czech Republic.
[2]Gothenburg University, Gothenburg, Sweden

The liquid biopsy is changing the way we monitor treatment response and progression of cancer patients, predict the outcome therapies and survey for actionable mutations, relapse and most recently also for primary screen. Many different biomarkers can be measure in blood, which opens for multimarker diagnostics. In plasma samples, most popular biomarker is circulating tumor DNA and we also see rapid development on exosomes and microRNAs. In may talk I will present several new methods for liquid biopsy testing, where focus is on actionable biomarkers and cost savings, to make the tests routinely affordable. These includes Two-Tailed PCR and SiMSen Sequencing. I will also present a new biomarker in liquid biopsy testing and a new platform for highly cost efficient multiplexing of currently up to 30 biomarkers.

Ultra-sensitive detection of miRNAs related to myelodysplastic syndromes in human blood plasma

Giusy Finocchiaro[1], Tomáš Špringer[1], Markéta Bocková[1], Zdeněk Krejčík[2], Michaela Dostálová-Merkerová[2], Jiří Homola[1]

[1]Institute of Photonics and Electronics, Prague, Czech Republic.
[2]Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Myelodysplastic syndromes (MDS) are a heterogeneous group of hematological malignancies that can progress to acute myeloid leukemia. The diagnosis of MDS involves two subsequent steps: i) the blood counts of the peripheral blood of the patient; and ii) the examination of bioptic samples of the bone marrow of the patient. Due to the invasiveness of bone marrow biopsy, less invasive approaches such as liquid biopsy are developed for MDS diagnosis. For instance, multiple miRNAs have been related to MDS as potential biomarkers, and thus their detection in patients' blood plasma can be perspectively used for MDS diagnosis. However, the detection of miRNAs remains challenging due to their low level in human blood plasma.

Here, we present an extremely sensitive optical biosensor-based approach for the detection of miRNAs and demonstrate the detection of miRNAs related to MDS (e.g., hsa-miR-150-5p and hsa-miR-451a) in human blood plasma. The approach is based on the oligonucleotide-triggered release of nanoparticles from a sensor surface [1]. To improve the detection performance of the approach, we study three strategies: i) size of nanoparticles, ii) shape of nanoparticles (e.g., spheres and rods), and iii) multi-level sandwich structures on a sensor surface. We demonstrate that the proposed approach enables us to detect miRNAs in human blood plasma with a limit of detection (LOD) at a low attomolar level, which is the best LOD for miRNAs achieved so far using a label-free optical biosensor. Furthermore, this detection performance is close to the sensitivity of the standard polymerase chain reaction (PCR) method, with the advantage that the proposed approach involves a simpler procedure of blood plasma pretreatment without requiring miRNA extraction from the sample.

This work is supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

[1] T. Špringer, Z. Krejčík, J. Homola, Biosensors and Bioelectronics 194, 113613 (2021)

Novel model of circulating tumor cells for validation of druggable pathways in triple-negative breast cancer

Lucia Kucerova[1,2], Jana Plava[2], Svetlana Miklikova[2], Michal Mego[3]

[1]Translational Research Unit, Faculty of Medicine, Comenius University; and National Cancer Institute, Bratislava, Slovakia.
[2]Cancer Research Institute, Biomedical Research Center, Bratislava, Slovakia.
[3]2nd Department of Oncology, Faculty of Medicine, Comenius University and National Cancer Institute, Bratislava, Slovakia

Metastatic dissemination remains the main reason for high cancer mortality in women diagnosed with breast cancer. Even though the tumour cell dissemination via circulating tumour cells (CTC) which are released from the primary site is a very ineffective process, distant metastases appear in 46% of triple-negative breast cancer (TNBC) patients corresponding to the disease aggressiveness. Laboratory models for functional testing which mimic the spread of metastatic cells are needed for efficient investigation of the underlying mechanisms and therapeutic intervention.

Human fluorescently labelled model TNBC cells MDA-MB-231 werre used to inject immunocompromised mice. Tumour cells from lung metastases were isolated, propagated and repeatedly injected intravenously. Subsequently, metastatic cells from lungs and CTC were detected and analysed.

Here, we describe novel isogenic variants LMC3 and CTC3 of TNBC model cell line. No

significant changes were detected in proliferation, cytoskeletal organization or chemoresistance in newly derived cells. However, these variants have increased migration potential, altered expression profiles, and elevated tumorigenic potential. We observed upregulation of MMP9 and downregulation of CDH11, CST7, CXCR4 and TNFSF10 expression from in the Tumour Metastasis Gene Array. We identified 5 upregulated genes - COL6A1, COL6A2, MMP9, SPARC, SSP - and 11 downregulated genes - ADAMTS1, COL12A1, COL15A1, COL8A1, ECM1, FN1, ICAM1, ITGB5, MMP11, THBS1 and THBS2 in Human Extracellular Matrix and Adhesion Molecules Array. Moreover, cell line CTC3 readily produces metastases in the lungs and bone marrow, as confirmed by histological examination, and detectable viable circulating tumour cells in the blood within 3-4 weeks post inoculation as confirmed by live-cell imaging.

This model enables rapid *in vivo* screening of candidate strategies limiting CTC levels in the blood thus potentially decreasing metastatic dissemination. It may be exploited for mechanistical studies to unravel molecules and signalling pathways contributing to CTC presence in the blood and hence tumour dissemination.

Grant support: VEGA 2/0124/21, APVV-20-0158, VEGA 1/0349/21

Patient-derived xenografts of pancreatic carcinoma – our experience with adenosquamous form

Tomas Sychra[1,2,3], Radka Vaclavikova[3,4], Arpad Szabo[2,1], Alzbeta Spalenkova[3,4], Karolina Seborova[3,4], Tereza Tesarova[3,4], Kamila Koci[1], Bhavana Hemantha Rao[4], Viktor Hlavac[4,3], Pavel Soucek[3,4], Martin Oliverius[2,1]

[1]Third Faculty of Medicine Charles University, Prague, Czech Republic.
[2]University Hospital Kralovske Vinohrady, Prague, Czech Republic.
[3]National Institute of Public Health, Prague, Czech Republic.
[4]Medicine Faculty in Pilsen Charles University, Pilsen, Czech Republic

Introduction and objectives: Adenosquamous pancreatic (ASPC) carcinoma is the aggressive rare type of pancreatic cancer (PC) with worse prognosis than more frequent but also dismal PDAC (pancreatic ductal adenocarcinoma). Incidence of ASPC is around 0.5-4% of all PC with high morbidity and mortality rates, 5-year OS is 4-18 months and most patients die as a result of the chemoresistance of the tumor cells and its microenvironment. Therapeutic options are limited and highly empiric because of a lack of strong data. The aim of our research team is to contribute to the personalization of PC therapy using an *in vivo* model.

Methodology: Our team created Patient Derived Xenografts (PDX) with ASPC. Whole tumor tissue with ASPC cells and its microenvironment was obtained from patient operated at the Surgical Clinic of 3FM CU and UHKV and implanted subcutaneously into immunodeficient NOD/SCID and NU/NU mice. After successful tumor engraftment and re-transplantation, we received a sufficient number of individuals to administer and measure effects of various types of conventional and experimental cytostatic therapy. We harvested tumor tissue and other organs for histopathological and molecular genetic examination between retransplantations and after therapeutic treatment. All experimental work was done in compliance with and governed by the existing regulations and guidelines, approved experimental project MZDR 37099 /2021-5/OVZ was followed.

Results: We have successfully established PDX models of ASPC with a success rate of tumor growth 89% - 73 from 82 individuals in ten generations, two of which were therapeutic. The average tumor growth time to a size of approximately 150mm3 was 48 days. We have collected 60 tumor samples for histopathologic examination and RNA sequencing.

Conclusion

We have evaluated the effects of different treatment regimens compared to the untreated control group of mice and each other. We will evaluate the stability and behavior of the PDX model throughout all generations but also the effect of the treatment regimens using imaging and molecular genetic methods to optimize the whole process for use in the precise personalized treatment of pancreatic cancer patients.

Sponsored by GAUK project No. 308223; Donatio Facultatis Medicae Tertitae; MŠMT programme INTER-EXCELLENCE; subprogramme INTER-ACTION-LUAUS23164.; GAČR project No. 21-14082S.

Integrative multi-omics approaches unveil novel insights into vestibular schwannoma pathogenesis

Lucie Pfeiferova[1], Michaela Tesarova[2,3], Lenka Peterkova[2,3], Monika Stastna[1], Jana Sachova[1], Miluse Hradilova[1], Radovan Hynek[4], Karel Smetana, Jr.[3,5], Lukas Lacina[3,5,6], Michal Kolar[1], Zdenek Fik[2]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Department of Otorhinolaryngology and Head and Neck Surgery, 1st Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic.
[3]Institute of Anatomy, 1st Faculty of Medicine, Charles University, Prague, Czech Republic.
[4]Institute of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Czech Republic. [5]BIOCEV, Biotechnology and Biomedicine Centre, Vestec, Czech Republic.
[6]Department of Dermatovenereology, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

Background:

Vestibular schwannoma (VS), also known as acoustic neuroma, is a slow-growing benign tumor arising from Schwann cells in

the vestibular nerve. While it is generally non-life-threatening, the clinical manifestations of VS can significantly impact patients' quality of life. To enhance our understanding of VS pathogenesis and identify potential therapeutic targets or diagnostic and prognostic markers, a comprehensive analysis of the molecular landscape is essential. In recent years, emerging technologies such as spatial transcriptomics, single-cell analyses, and proteomics have offered unprecedented insights into complex biological processes.

Methods:

In this study, we employed a multi-omics approach to unravel the intricate molecular mechanisms underlying VS development and progression. We utilized spatial transcriptomics to obtain spatially resolved gene expression profiles within VS tissue samples. Additionally, single-cell analyses were performed to dissect the cellular heterogeneity and identify distinct cell populations within the tumor microenvironment. Furthermore, proteomic analyses were employed to characterize the proteome alterations associated with VS.

Results:

Our spatial transcriptomic analysis revealed the spatial organization of gene expression patterns within the VS, providing insight into the cellular composition and functional states of different regions within the tumor. Through single-cell analyses, we identified novel cell populations, including Schwann cell subtypes, immune cells, and stromal cells, contributing to VS pathogenesis. Furthermore, proteomic profiling allowed for the identification of differentially expressed proteins and potential signaling pathways dysregulated in VS.

Conclusion:

The integrative analysis of spatial transcriptomics, single-cell analyses, and proteomics has enabled us to gain a comprehensive view of the molecular landscape of VS. Our findings shed light on the cellular heterogeneity, the interplay between cell types, and dysregulated molecular pathways involved in VS pathogenesis. These results offer novel insights into the development of potential diagnostic and prognostic markers of VS.

PIWIL1-4 in human gliomas and glioblastoma stem-like cells

Elena Garcia-Borja[1], Rosana Mateu[1], František Siegl[2], Jiří Šána[2], Aleksi Šedo[1], Petr Bušek[1]

[1]Laboratory of Cancer Cell Biology, Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, Czech Republic
[2]Central European Institute of Technology, Masaryk University, Brno, Czech Republic

PIWI proteins constitute a germline-specific subfamily of Argonaute proteins that bind to short non-coding RNAs known as piRNAs. The PIWI/piRNA complexes play crucial roles in silencing transposable elements and regulating gene expression to maintain genome stability in stem cells. Recently, PIWI proteins were shown to participate in the pathogenesis of several types of cancer. Here we analyzed the expression pattern of *PIWIL1-4* in human gliomas and glioblastoma stem-like cells (GSCs), which are considered key to glioma progression, treatment resistance and recurrence.

We derived 24 paired GSC and non-GSC cultures from freshly resected human glioblastomas (GBMs) and assessed the expression of the four human PIWI proteins. *PIWIL1* and *PIWIL3* were undetectable or were detected at very low levels in all samples. *PIWIL2* expression was significantly higher in GSCs compared to non-GSCs and decreased in GSCs upon serum-induced differentiation. In contrast, *PIWIL4* was detected at similar levels in GSCs and non-GSCs. Furthermore, *PIWIL4* and *PIWIL2*, but not *PIWIL1* or *PIWIL3*, were expressed in orthotopic GSC-derived tumors in immunodeficient mice.

Consistent with the results in cell cultures, *PIWIL3* expression was not detected in a pilot analysis of 30 GBMs and was therefore omitted from further studies. *PIWIL1, 2* and 4 expression was then analysed in 160 GBMs (grade IV), 20 low-grade gliomas (grade II astrocytomas and oligodendrogliomas) and 12 non-tumorous brain tissues (pharmacoresistant epilepsy). *PIWIL1* was only detected in half of the GBM tissues and at very low levels. *PIWIL2* and *PIWIL4* were detected in most of the samples and their expression was lower in GBMs compared to low-grade gliomas and non-tumorous brain.

Collectively, our results challenge the previously reported upregulation of *PIWIL1* in GBMs and its presumed significance in GSCs. Rather, our data suggest that *PIWIL2* or *PIWIL4* may be involved in the maintenance of the stem cell phenotype of glioma cells.

Supported by the Ministry of Health, Czech Republic grant no. NV19-03-00501 and National Institute for Cancer Research (LX22NPO5102).

TUESDAY 21ST NOVEMBER, 2023

Chairs: Michal Hocek, Tomáš Etrych

Automation + miniaturization = acceleration

Alexander Dömling

Palacky University, Olomouc, Czech Republic

The mantra 'Automation + Miniaturization = Acceleration' is successfully applied in many research areas and technologies, but not in synthetic chemistry, which is largely believed to be not automatable. However, with the potential to accelerate discoveries while flattening costs, increase safety, streamline data generation, enhance reproducibility, and lower the environmental footprint, automation and miniaturization are two promising approaches to synthesis and are worthwhile to invest in.

Since 2018 we have introduced acoustic droplet ejection (ADE) and iDOT for the automated synthesis of small molecules on nanoscale. [1] We showed that organic chemistry can be generally performed on nanoscale e.g. by producing large numbers of novel boronic acids, [2] isoquinolines, [1] quinazolines, [3] iminopyrrolidines [4], and covalent inhibitors. [5] Moreover, we showed that libraries based on multiple chemistries in parallel can be performed on the ADE platform, approaching degrees of diversity comparable to large screening libraries. [6] Notably, the synthesis of 1536 unprecedented drug-like small molecules based on 16 different scaffolds consumed only 20 mg of building blocks inclusive solvent. Thus, ADE is suitable to considerably reduce the ecological footprint of synthetic chemistry. Clearly, running reactions on a small scale is not only more sustainable and greener, safer, producing less waste, but also more rapid and economical.

Highly automated and miniaturized synthesis at a nanoscale can also be incorporated into drug discovery: we realized for the first time the incorporation of HT synthesis and HT protein crystallography for the discovery of potent covalent SARS-CoV-2 3CLpro inhibitors; [7] we showed the discovery of menin-MLL antagonists; [8] and most recently we discovered Crbn degrading glues with extraordinary cellular potency in a HT-phenotypic screen. [9] We strongly believe that early medicinal chemistry will depart from currently mostly performed manual mmol scale synthesis towards automated and highly miniaturized synthesis and will help to accelerate future drug discovery.

Literature:

[1] Y. Wang et al., ACS Central Science 2019, 5, 451. [2] C. G. Neochoritis et al., Sci. Adv. 2019, 5, DOI: 10.1126/sciadv.aaw4607. [3] M. Hadian et al., Green Chemistry 2020, 22, 2459-2467. [4] A. Osipyan et al., Angew. Chem. Intl Ed. Engl. 2020, 59, 12423. [5] F. Sutanti et al., Sci. Adv. 2021, 7, DOI: 10.1126/sciadv.abd9307 [6] L. Gao eta., Green Chemistry, 2023, DOI: 10.1039/D2GC04312B. [7] F. Sutanto et al., Angew. Chem. Intl Ed. Engl. 2021, 60, 18231. [8] K. Gao et al. RSC Med. Chem. 2021,12, 809. [9] Z. Wang et al. unpublished.

Heterocyclic analogues of lupane triterpenoids trigger apoptosis selectively in cancer cells

Milan Urban[1], Jiří Hodoň[2,1], Ivo Frydrych[1], Karel Koberna[1], Anna Ligasová[1], Vlastimil Mašek[1], Barbora Lišková[1], Václav Ranc[1]

[1]IMTM, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Olomouc, Czech Republic.
[2]Dept. of Organic Chemistry, Faculty of Science, Palacký University, Olomouc, Czech Republic

Lupane triterpenoids are natural compounds with unique biological activities.[1,2] Triterpenoid heterocycles that we prepared earlier in our lab had proven high and selective cytotoxicity in various cancer cell lines.[3] Some of them were more cytotoxic in leukemic cells resistant to daunorubicin or paclitaxel, which is interesting for further development of anti-cancer drugs usable in the treatment of resistant leukemia.[3]

The main aim of this work was to determine their mechanism of action. Therefore we analyzed cell cycle and expression of the pro- and anti-apoptotic proteins in cells treated with our cytotoxic compounds. From the results it is clear, that most of them cause apoptosis via mitochondrial pathway.[3] In addition, we used fluorescent microscopy combined with co-localization experiments and could see the accumulation of fluorescently labelled molecules in mitochondria. The same result was obtained using Raman confocal microscopy and deuterated analogues. We used this method since deuterium labelling does not interfere with the biological activity of the parent compounds as expected in much larger fluorescent labels. Last, not least, the impact of one of the compounds on mitochondria was visualized using electron microscopy.[3] The optimization of the pharmacological parameters of this set of derivatives yielded medoxomil prodrugs that had IC_{50} of 26-43 nM in K-562 cells, which makes them the most active compounds from our research to date.3 Structure-activity relationships and comparison of all visualization techniques will be discussed.

ACKNOWLEDGEMENT

This project was supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

REFERENCES

1. P. Džubák, M. Urban, J. Šarek, Nat. Prod. Rep. 2006, 23, 394.

2. L. Borková, J. Hodoň, M. Urban, et al., Asian J. Org. Chem. 2018, 7, 1542.

3. J. Hodoň, I. Frydrych, M. Urban, et al., Eur. J. Med. Chem. 2022, 243, 114777.

Development of degradable PHPMA-based nanogels: preparation procedure and physicochemical properties

Jana Kousalová, Petr Šálek, Ewa Pavlova, Rafał Konefał, Olga Kočková, Tomáš Etrych

Ústav makromolekulární chemie AV ČR, v. v. i., Prague, Czech Republic

Nanogels represent three-dimensional nanoscale structures with physically, covalently crosslinked polymer networks. These nanosystems are characterized by their hydrophilicity, an ability to swell in aqueous media, softness, porous structure, biocompatibility, stimuli-responsive behavior, such as change of pH, temperature, or ionic strength, as well as biodegradability. There are a variety of constituents, such as monomers, synthetic and natural polymers, serving as building blocks for final polymer nanogels. For instance, poly[*N*-(2-hydroxypropyl) methacrylamide] (PHPMA) is a hydrophilic and biocompatible polymer derived from *N*-(2-hydroxypropyl) methacrylamide (HPMA) monomer that belongs to the group of methacrylate monomers. Due to its unique and tunable properties, PHPMA is typically used in biomedical and pharmaceutical applications, such as drug delivery, targeted therapy, imaging, and tissue engineering.

In our work, we employed dispersion polymerization for the preparation of degradable and covalently crosslinked PHPMA-based nanogels with a diameter below 100 nm. As first, we optimized the conditions of dispersion polymerization in terms of polymerization medium polarity and crosslinking co-monomer concentration. Therefore, we studied the effects of concentration of *N,N´*-bis(acryloyl) cystamine (BAC) as crosslinking co-monomer varying from 5 to 25 wt%, and different ratios of H_2O/MetCel and H_2O/EtCel as polymerization media on the quality, size, particle size distribution, morphology, and composition of the final nanogels. The physicochemical properties of PHPMA-based nanogels were studied transmission (TEM) and cryogenic transmission electron microscopy (cryo-TEM), dynamic light scattering (DLS), asymmetric flow field-flow fractionation (A4F), and nuclear magnetic resonance spectroscopy (NMR). The degradability of the nanogel was introduced by BAC crosslinking comonomer possessing disulfide bonds that can undergo cleavage under physiologic reducing conditions. Besides, HPMA and BAC were also copolymerized with a reactive 6-methacrylamido hexanoyl hydrazide (BMH) co-monomer containing hydrazide groups. After the optimization, we innovately obtained PHPMA-BMH-BAC nanogels crosslinked with 20 wt% BAC by dispersion polymerization in H_2O/MetCel mixture (80/20 w/w) with number-average diameter Dn = 30 nm and Z-average hydrodynamic diameter D_z = 49.3 nm. The degradability of the developed PHPMA-BMH-BAC nanogels was investigated by TEM after the incubation in the presence of glutathione solution. Our results showed that the reductant triggered the degradation of the nanogel.

ACKNOWLEDGEMENTS:

The authors wish to thank the project „National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU“ for the financial support.

Photocontrolled interleukin/receptor pairs through genetic code expansion

Phuong Ngoc Pham[1], Jiří Zahradník[2,3], Lucie Kolářová[1], Bohdan Schneider[1], Gustavo Fuertes[1]

[1]Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic.
[2]Charles University, Prague, Czech Republic.
[3]Weizmann Institute of Science, Rehovot, Israel

Cytokines are key modulators of the immune and inflammatory responses. To date, several cytokines have been approved for cancer immunotherapy in the clinic, e.g. interleukin-2 (IL-2). However, the therapeutic application of cytokines is often hindered by toxicities and/or modest efficacies. Therefore, the spatiotemporal control of interleukin-receptor interactions may help to expand the utility of these biomolecules as protein-based therapeutics. Here, we report on a light-induced ON-switch IL-24/IL-20R2 heterodimer assembly based on genetically encoded photocaged tyrosines. Using a combination of biophysical, molecular biology, and cell-based assays, we show that a single ortho-nitrobenzyl-tyrosine residue introduced at position 70 of recombinant IL-20R2 significantly reduces the binding affinity towards a sequence-optimized version of IL-24. Mild irradiation with UV light removes the caging group thus enabling complex formation and activation of the JAK/STAT signaling cascade. These results provide a proof-of-concept for the rational design of photoactivatable interleukin/receptor pairs directed against several conditions such as oncology, autoimmune disorders, and viral infections.

3,5,7-Substituted pyrazolo[4,3-d]pyrimidine inhibitors of cyclin-dependent kinases and cyclin K degraders

Radek Jorda[1], Libor Havlíček[2], Miroslav Peřina[1], Veronika Vojáčková[1], Tomáš Pospíšil[3], Stefan Djukic[4], Jana Škerlová[4,5], Jiří Grúz[1], Nicol Renešová[6], Pavel Klener[6,7], Pavlína Řezáčová[4,5], Miroslav Strnad[8], Vladimír Kryštof[1]

[1]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[2]Isotope Laboratory, Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic. [3]Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[4]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic.
[5]Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic.
[6]First Faculty of Medicine, Institute of Pathological Physiology, Charles University, Prague, Czech Republic.
[7]First Department of Internal Medicine-Hematology, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic.
[8]Palacký University and Institute of Experimental Botany, Czech Academy of Sciences, Olomouc, Czech Republic

3,5,7-Trisubstituted pyrazolo[4,3-d] pyrimidines have been identified as potent inhibitors of cyclin-dependent kinases (CDKs), which are established drug targets. Herein we describe their further structural modifications leading to novel nanomolar inhibitors with strong antiproliferative activity. We determined the crystal structure of fully active CDK2/A2 with 5-(2-amino-1-ethyl)thio-3-cyclobutyl-7-[4-(pyrazol-1-yl)benzyl]amino-1(2) H-pyrazolo[4,3-d]pyrimidine (24) at 1.7 Å resolution, confirming the competitive mode of inhibition. Biochemical and cellular assays in lymphoma cell lines confirmed the expected mechanism of action through dephosphorylation of retinoblastoma protein and RNA polymerase II, leading to induction of apoptosis. Importantly, we also revealed an interesting ability of compound 24 to induce proteasome-dependent degradation of cyclin K both *in vitro* and in a patient-derived xenograft *in vivo*. We propose that 24 has a dual mechanism of action, acting as a kinase inhibitor and as a molecular glue inducing an interaction between CDK12 and DDB1 that leads to polyubiquitination of cyclin K and its subsequent degradation.

TUESDAY 21ST NOVEMBER, 2023

Chairs: Alexander Dömling, Milan Urban

Unveiling the potential of Stony Brook taxanes: A promising solution to combat taxane resistance in solid tumors

Radka Vaclavikova[1,2], Karolina Seborova[1,2], Alzbeta Spalenkova[1,2], Marie Ehrlichova[1], Tereza Tesarova[1,2], Tomas Sychra[3,4], Martin Oliverius[3,4], Lei Chen[5], Hersh Bendale[5], Iwao Ojima[5], Cristian Sandoval-Acuña[6], Jaroslav Truksa[6], Pavel Soucek[1,2]

[1]National Institute of Public Health, Prague, Czech Republic.
[2]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic.
[3]Third Faculty of Medicine, Charles University, Prague, Czech Republic.
[4]Department of Surgery, University Hospital Kralovske Vinohrady and Third Faculty of Medicine, Charles University, Prague, Czech Republic.
[5]Institute of Chemical Biology & Drug Discovery, Stony Brook University - State University of New York, Stony Brook, NY, USA.
[6]Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV Research Center, Vestec, Czech Republic

Introduction: Taxanes are widely used anticancer drugs. However, the resistance of cancer cells to conventional taxanes (paclitaxel, docetaxel) is a serious problem in the successful treatment of solid tumors. New experimental taxanes (Stony Brook taxanes; SB-Ts) seem to be potent agents against solid tumors with the most aggressive and drug-resistant phenotype. The aim of our research was to use in vitro methods for the evaluation of the activity of SB-Ts in cancer cell lines and select and analyze just the most effective agents in subsequent *in vivo* (CDX and PDX) preclinical models and recommend the most effective derivatives for the next clinical testing.

Methods and Results: At first, in vitro efficacy, proliferation, apoptosis of tested cell lines and uptake of SB-Ts in sensitive and resistant models of ovarian cancer cells were investigated. SB-T taxanes demonstrated 50 to 1000-times higher cytotoxicity than paclitaxel and significantly higher accumulation in resistant tumor cells. Furthermore, SB-T taxanes were effective at 20-times lower concentrations compared to paclitaxel in blocking of the cell cycle at G2/M. For the most efficient taxane analogues SB-T-121605 and SB-T-121606, their effect and toxicity *in vivo* was also analyzed using mouse cell-derived xenograft (CDX) models and also in patient-derived xenograft (PDX) models of solid tumors. The incorporation of SB-T-121605 and SB-T-121606 into the regimens containing paclitaxel was effective in suppressing tumor growth in ovarian carcinoma resistant CDX mouse models at small doses (≤3mg/kg), where their adverse effects were decreased. PDX models of highly aggressive subtypes of pancreatic carcinoma were established and the efficacy of SB-Ts was verified at small doses (≤3mg/kg) in this *in vivo* model as well. Molecular mechanisms behind SB-Ts action were studied by mRNA profiling. Key pathways and genes associated with the molecular mechanism of action of SB-Ts were identified (e.g., NOTCH and AhR signaling pathways, CPS1 and TRIP6 genes).

Conclusion: Using in vitro technologies, the most effective SB-T analogues SB-T-121605 and SB-T-121606 were selected for evaluation of their effectivity in mouse *in vivo* tumor models. Both SB-Ts were effective in suppression of tumor growth. In addition, the deregulation of gene expression profile linked to taxane treatment enabled identification of candidate pathways and genes, which now are under active investigation, as potential therapeutic targets in human cancers.

Acknowledgments: This study was supported by projects of the Czech Science Foundation no. 21-14082S, the Czech Ministry of Education, Youth and Sports: INTER-ACTION, project no. LUAUS23164, the Grant Agency of Charles University in Prague, project No.308223 and program Cooperatio "Surgical Disciplines" no. 207043 and by the National Institutes of Health (NIH), U.S.A. grant R01 CA103314.

Engineered antibodies as platforms for cancer therapy

Gargi Das[1,2], Zora Nováková[2], Jakub Ptacek[2], Cyril Bařinka[2]

[1]Charles University, Department of Cell Biology, Prague, Czech Republic.
[2]Institute of Biotechnology, BIOCEV, Vestec, Czech Republic

Prostate Cancer (PCa) is the leading cause of death amongst men according to cancer statistics 2023. The present therapeutics are not sufficiently effective in eliminating all PCa cells and hence, new approaches are being actively researched.

Prostate-specific membrane antigen (PSMA) is an established biomarker upregulated in the neoplastic and metastatic form of PCa. Hence, we have developed an anti-PSMA antibody 5D3 with nanomolar affinity for PSMA, for imaging and therapy for PCa. The single chain variable fragment (scFv) of 5D3 is linked to the cyclic peptide 33 (CP33) to engage macrophages to PSMA (+) cells leading to activation of the immune cells and in turn specific elimination of cancer cells. This is achieved by the release of reactive oxygen species and simultaneous phagocytosis of the tumor target by activated macrophages.

In our work, we have successfully cloned and purified the 5D3-scFv-CP33 construct and shown that it is efficient in mediating the killing

of tumor targets in a concentration dependent manner. Therefore, this bispecific fusion can provide a platform suitable for cancer immunotherapy.

Derivatives of Trilobolide actívate the cytotoxic response of T cytotoxic and NK cells against tumour cells

Alex Puskasu[1], Michal Jurášek[2], Jenny Garmendia[1], Petr Dzubak[1], Marián Hajduch[1], Juan Bautista De Sanctis[1]

[1]IMTM. Palacky University, Olomouc, Czech Republic.
[2]Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Prague, Czech Republic.

Trilobolide is a natural product found in Laserpitium trilobun, Thapsia transtagana, and Thapsia garganica. Most of the studies on Trilobolide and its derivatives are related to tumour cell death. We decided to analyze the effect of different Trilobolide derivatives on human lymphocytes. Lymphocytes were incubated for 18 hr with 10 up to 100 nM of the compounds, washed and challenged to different tumour cell lines K562, HCT116, Jurkat, susceptible to killing and HT29, K562TAX, and A549, resistant to spontaneous cytotoxicity. The treated cells with TB91 and 92 [JM1] (thapsigargin derivatives) were more effective ($P<0.01$) in killing the susceptible and resistant cell lines than the controls and the cells treated with TB87 and TB89. The enhanced cytotoxic effect parallels the increase in perforin secretion for compounds TB91 and TB92, which is absent for compounds TB87 and TB89. The four compounds induced cell activation, but the biological effects differed. It is concluded that the compounds derived from Trilobolide can be interesting leading chemical structures for future studies.

[JM1]thapsigargin derivatives

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], Pavel Trousil[2], Lukáš Huml[2], Pavel Polishchuk[1], Marián Hajdúch[1], Pavel B. Drašar[2], Petr Džubák[1]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University and University Hospital in Olomouc, Olomouc, Czech Republic.
[2]Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Prague, Czech Republic

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.

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 EU.

Discovery of novel specific carbonic anhydrase IX inhibitors by HTS compaign

Soňa Gurská[1], Margaréta Volníková[1], Jiří Brynda[2], Pavlína Řezáčová[2], Miloš Petřík[1], Petr Džubák[1], Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic.
[2]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

The HTS (high-throughtput screening) technique accelerates the screening of a large numbers of potential biological modulators against selected and specific targets. This approach is widely used in the pharmaceutical industry and in academic institutions as a primary tool for early-stage drug discovery. The aim of this screening compaign was to identify novel inhibitors of human carbonic anhydrase IX (CA IX). Since this enzyme plays a key role in cancer cell proliferation and metastasis, and its overexpression in hypoxic tumors is associated with malignant progression and poor treatment outcome, inhibition of CA IX activity could be a promising approach for novel anticancer therapies.

To identify small molecule inhibitors of CA IX in HTS conditions a new fluorescence-based assay was designed. In this assay, pyranine was used as a fluorescent indicator of pH change. In the primary screen, the inhibition activity of over 10.000 unique compounds from IMTM Proprietary Library was analyzed at one concentration (10 μM). The PI (percentage of inhibition) values were calculated for all tested compounds and 175 compounds with PI value > 70% were selected as hits. In secondary screening, the inhibitory activity (IC50) of the hits against CA IX was determined. To evaluate the specificity of selected hits and the most potent inhibitors, the inhibitory activity of these compounds against

CA II was also tested. To quantify the suitability of the assay in HTS, the Z-factor was determined for each plate. Data were analyzed by Dotmatics software.

Results obtained from the primary and secondary screening compaigns as well as most promising selected hits will be presented and discussed.

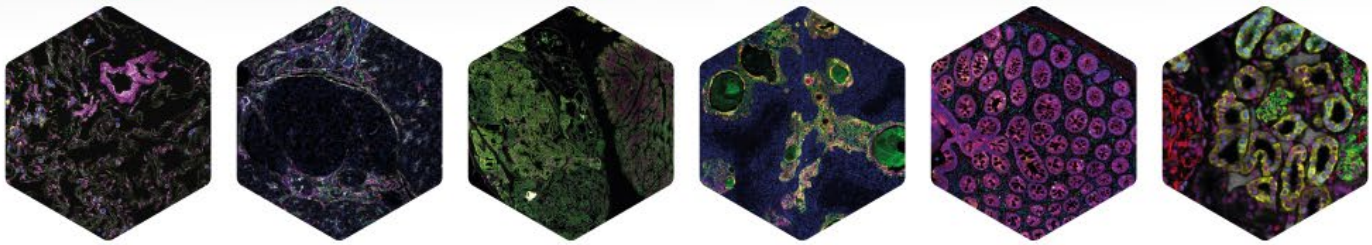
This work was supported by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2023052, EATRIS-CZ - LM2023053), and by the Internal Grant of Palacky University Olomouc (IGA_LF_2023_025).

RAREcyte

Spatial biology

SOLUTION FOR PANCANCER RESEARCH

Lung | Pancreas | Ovary | Thymus | Colon | Kidney



ACCELA

Accelerate your biomedical research

WEDNESDAY 22ND NOVEMBER, 2023

Chairs: Petr Džubák, Helena Kupcová Skalníková

The development of low input proteomic technologies for the analysis of rare quiescent cancer cells

Silvia Surinova

University College London, London, United Kingdom

Many cancers are characterised by the presence of a rare subpopulation of quiescent stem-like cells which are a source of heterogeneity, phenotypic therapy resistance, relapse and further disease evolution. The mechanisms controlling quiescence remain largely unknown, and transcriptomic analysis of quiescent cells has yielded only limited insights. Proteomic analysis of rare cancer stem cells requires the development of a low-input methodology to capture global protein changes and differentially expressed post-translational modifications.

We developed a robust proteomic workflow for the analysis of hundreds to thousands of FACS-sorted cells by combining simple sample processing, multiplexed isobaric labelling and phosphopeptide enrichment to simultaneously identify protein and signalling networks. With the means of this workflow, we were able to characterise a pre-existing, treatment-naïve quiescent state in patient-derived cell lines and a somatic mouse model of glioblastoma, and show that these cells exhibit self-renew capacity and increased therapy resistance.

Connecting genomic variants and the proteome with peptide level resolution at scale

Willy Peña Büttner

Seer

The Proteograph TM Product suite, a technological advancement enabling deep plasma proteomics are enabling high-resolution measurement of plasma proteoforms, which may reveal a rich source of novel biomarkers previously concealed by other proteomics methods. Here, we present data on plasma proteomes from non-small cell lung cancer subjects (NSCLC) and controls identifying NSCLC-associated protein isoforms by examining differentially abundant peptides as a proxy for isoform-specific exon usage. We find four proteins comprised of peptides with opposite patterns of abundance between cancer and control subjects. One of these proteins, BMP1, has known isoforms that can explain this differential pattern, for which the abundance of the NSCLC-associated isoform increases with stage of NSCLC progression. The presence of cancer and control associated isoforms suggests differential regulation of BMP1 isoforms. The identified BMP1 isoforms have known functional differences, which may reveal insights into mechanisms impacting NSCLC disease progression. In Summary, the Proteograph™ workflow interrogates the plasma proteome by addressing challenges of previously impractical workflows, providing a combination of scale, depth, and coverage, which enables the discovery of novel biomarkers and development of improved classification models. This new workflow includes a Cloud-enabled infrastructure for large cohort proteomics data analysis enabling novel biological insights empowering disease classification and biomarker discovery.

Proteotype classification of metastatic and localized renal cell carcinomas for prognosis and therapy response

Jan Šimoník[1], Pavla Bouchalová[1], Richard Štefaník[1], Petr Lapčík[1], David Potěšil[2], Vlad Popovici[3], Ján Podhorec[4,5], Milan Hora[6], Alexandr Poprach[4,5], Ondřej Fiala[7], Pavel Bouchal[1]

[1]Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic.
[2]Central European Institute for Technology, Masaryk University, Brno, Czech Republic.
[3]RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic.
[4]Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, Czech Republic.
[5]Department of Comprehensive Cancer Care, Faculty of Medicine, Masaryk University, Brno, Czech Republic.
[6]Department of Urology, Faculty of Medicine and University Hospital Pilsen, Charles University, Pilsen, Czech Republic.
[7]Department of Oncology and Radiotherapy, Faculty of Medicine and University Hospital Pilsen, Charles University, Pilsen, Czech Republic

Renal cell carcinoma (RCC) represents a serious oncological disease with the second highest incidence in the Czech Republic across the world. Reliable molecular predictive and prognostic biomarkers for RCC are mostly unavailable, namely at protein level. To quantify proteins associated with pro-tumorigenic and pro-metastatic mechanisms in RCC, we first generated a comprehensive RCC-specific spectral library of targeted proteomic assays for 7960 protein groups (FDR=1%) [1]. Second, we have applied data independent

acquisition mass spectrometry (DIA-MS) on QExactive HF-X LC-MS system and analyzed a well-characterized set of metastatic RCC tumors (training cohort n=53, validation cohort n=22) and adjacent non-cancerous tissues (n=17) a part of which responded and a part did not respond to tyrosine kinase inhibitor (TKI) treatment. We have identified and validated single protein biomarker with a poor response to TKI but not with tumor grade. Functional assays using CRISPR/Cas9 knockdown confirmed its role in metastatic potential of 786-0 cells. Third, we analyzed a well-characterized set of initially localized RCC tumors (n=86) of which a half exhibited a relapse in <5 years after diagnosis. We have identified a single potential biomarker as well as protein classifiers able to predict the relapse, for which we have developed targeted proteomics assays for further validation and routine quantification. CRISPR/Cas9 knockdown confirmed the role of the key protein in cell migration in 786-0 cells, supporting its role in metastatic potential of RCC. In a summary, next generation proteomics based on DIA-MS is a powerful tool to classify RCC tissues, to identify prognostic biomarkers and alternative therapeutic targets. Supported by Ministry of Health of the Czech Republic, project No. NV19-08-00250, all rights reserved. CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2018127, is gratefully acknowledged for the financial support of the measurements at the CEITEC Proteomics Core Facility. Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

[1] Lapcik, P., et al., Proteomics 2022; 22(7):e2100228.

Can lipid profiles reflect the true biological age and healthy ageing?

Lukáš Najdekr, Marián Hajdúch

Institute of Molecular and Translational Medicine, Palacký University Olomouc, Olomouc, Czech Republic

Lipidomics, the comprehensive analysis of lipid molecules within biological systems, has emerged as a powerful tool for understanding the complex interplay between lipids and human health. This work explored lipidomic profiles as potential indicators of true biological age and healthy ageing.

We collected human plasma samples (n = 1104) of various ages (19 – 73 y.o.) from a Transfusion department of University Hospital Olomouc (CZ). All samples can be considered healthy without significant pathologies, creating the perfect sample collection for studying ageing effects. The human plasma was analysed using ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). The data matrix was processed using Compound Discoverer 3.3 SP1 (Thermo Fisher Scientific, USA).

By approach of untargeted lipidomics, we have putatively annotated 182 lipids in positive ionisation mode and 130 lipids in negative ionisation mode (MSI ID Level 2). Furthermore, on MSI ID Level 3, we annotated 602 and 175 lipids in positive and negative ionisation modes, respectively. The first glimpse at the data set did not reveal any „one-molecule“ markers. Still, we have observed trends associated with age in groups of phosphocholines (PC), ceramides (Cer) and hexosylceramides (HexCer). This finding is in concordance with the latest trends in small molecule research, where it is more effective to focus on a combined panel of molecules rather than a single entity. As expected, we have also observed differences in lipid profiles based on gender.

By unravelling the intricate relationship between lipids and ageing, lipidomics offers a unique opportunity to identify novel therapeutic targets and develop personalised interventions to mitigate age-related pathologies. Further investigation is warranted to validate and refine these findings, enabling the translation of lipidomic-based approaches into clinical practice for assessing and monitoring the ageing process.

Lactosylceramide synthases B4GALT5 and B4GALT6 and their potential role(s) in colon cancer cells

Jan Vondráček[1], Tereza Šošolíková[1,2], Alena Hyršlová Vaculová[1], Gerardo Vázquez-Gómez[1], Ondrej Kováč[3], Miroslav Machala[3]

[1]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic.
[2]Masaryk University, Brno, Czech Republic.
[3]Veterinary Research Institute, Brno, Czech Republic

The disruption of lipid metabolism has been shown to be associated with several steps of colon cancer development. Glycosphingolipids (GSLs) represent an important group of bioactive lipid molecules found to be deregulated in colon cancer cells, which may affect the progression of the disease. Nevertheless, our knowledge about the roles of individual enzymes and GSL species in colon cancer development and progression remains scarce. In the present work, we studied early key step of GSL synthesis, synthesis of lactosylceramides, which is mediated by β4-galactosyltransferases (lactosylceramide synthases) We tested selective targeting of B4GALT5 and B4GALT6, two enzymes found to be upregulated in colorectal carcinoma cells. We evaluated applicability of pharmacological inhibition with the D-PDMP inhibitor, siRNA-mediated knockdown, and CRISPR/Cas9

gene knockout, using DLD-1 human colon adenocarcinoma cells. The efficacy of inhibition of selected enzymes or their downregulation was verified by a combination of several approaches: detection of decrease in levels of lactosylceramides and related GSL products (LC-MS/MS), changes of expression at the level mRNA (RT-qPCR) and protein (Western blotting). Our present data indicate that down-regulation of lactosylceramide synthase B4GALT6 in DLD-1 cells did not alter cell proliferation or cell cycle progression (only a minor effect at early stages after cell seeding was observed). Interestingly, we observed that downregulation of expression of this enzyme in DLD-1 cells increased their sensitivity to the cytotoxic effects of oxaliplatin, a drug frequently used in the colorectal cancer treatment. The reduction of B4GALT6 was associated also with other GSL species deregulation. We continue to evaluate functional roles of B4GALT5 enzyme in modulation of colon cancer cell phenotype or in regulation of colon cancer cell sensitivity to cytostatics. [Supported by the Czech Ministry of Health, grant no. NU21-03-00421].

The cervical mucus – proteomic characterization and protein-tissue analysis in the healthy patient subset

Tomáš Oždian[1], Jan Vodička[2], Jiří Dostál[2], Dušan Holub[1], Michal Ješeta[3], Pavla Kouřilová[1], Pavel Ventruba[3], Igor Crha[3], Radovan Pilka[2], Petr Džubák[1], Marián Hajdúch[1]

[1]Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Olomouc, Czech Republic.
[2]Department of Gynecology and Obstetrics, University Hospital in Olomouc, Olomouc, Czech Republic.
[3]Department of Gynecology and Obstetrics, University Hospital Brno, Brno, Czech Republic

The cervical mucus is the mucous substance produced by the cervix uteri, which changes its properties during the menstrual cycle and pregnancy dramatically. At the same time, it is the only body fluid produced by the upper female reproductive tract, which could be non-invasively obtained. At the same time, there is an evidence, that uterus has peristaltic properties. The cervical mucus could thus be enriched by the secretion from different parts of the female reproductive tract and the peritoneum. Thus, cervical mucus could be potentially rich source of biomarkers for wide variation of diseases. To verify this hypothesis, we have performed two experiments. In the first one, we have compared subset of healthy women undergoing artificial reproduction with artificial reproduction with natural or stimulated menstrual cycles and we have identified 4370 proteins and 621 of them were differentially expressed in the samples with natural or stimulated cycles. In the second experiment, we have focused on the tissue specific proteins and we have found proteins with expression specific to the testis, liver, placenta, and neural tissues. Those findings further support the hypothesis of cervical mucus as the potential source of biomarkers for future studies.

This work was supported by Czech Health Research Council (NV-18-02-00291), by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN – LM2023052, EATRIS-CZ - LM2023053), and by the internal grant of Palacky University Olomouc (IGA_LF_2023_025) and European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

WEDNESDAY 22ND NOVEMBER, 2023

Chairs: Karel Smetana, Luca Vannucci

Generation of myeloid-derived suppressor cells (MDSC) and their targeting in cancer

Viktor Umansky

German Cancer Research Center (DKFZ), Heidelberg, Germany

Myeloid-derived suppressor cells (MDSC) represent a heterogeneous myeloid cell population that is accumulated and activated in tumor microenvironment under chronic inflammatory conditions and substantially contributes to immunosuppression, representing thereby a valuable therapeutic target. It has been recently demonstrated that such MDSC enrichment could be mediated not only by a long-term production of soluble inflammatory factors but also by extracellular vesicles (EV) secreted by tumor cells. Importantly, EV contain a broad range of proteins, mRNA, microRNA and lipids and are considered as mediators of intercellular communication.

We analyzed polymorphonuclear (PMN) and monocytic (M) MDSC subsets regarding their immunosuppressive capacity and recruitment mechanisms in murine melanoma. The CXCR2/CXCL1 axis was identified as a mediator of PMN-MDSC migration. Inhibition of CXCR2 resulted in a decreased infiltration of tumors with PMN-MDSC and increased survival of melanoma bearing mice. Furthermore, adjuvant treatment of mice with resected tumors reduced the infiltration of pre-metastatic sites with PMN-MDSC and the occurrence of distant metastasis. The decrease in PMN-MDSC infiltration was accompanied by an increase in NK cells. Another possibility of MDSC targeting is based on the inhibition of the transcription factor STAT3, orchestrating MDSC accumulation and acquisition of immunosuppressive properties. The STAT3 inhibitor Napabucasin abrogated the capacity of murine MDSC to suppress T cell proliferation. It induced apoptosis in murine MDSC and significantly increased expression of molecules associated with antigen processing and presentation on these cells. Melanoma bearing mice treated with Napabucasin showed prolonged survival accompanied by a strong accumulation of tumor-infiltrating antigen-presenting cells and activation of CD8 and CD4 T cells. In melanoma patients, circulating M-MDSC strongly expressed activated STAT3 that was associated with a worse progression free survival (PFS), indicating the role of STAT3 as a promising therapeutic target in these patients and as a predictive marker for their clinical outcome.

Fibroblast heterogeneity and cancer biology

Karel Smetana, Jr.[1], Lukáš Lacina[1,2], Pavol Szabo[1], Lucie Pfeiferová[3], Michal Španko[1,4], Michal Kolář[3]

[1]Charles University, 1st Faculty of Medicine, Institute of Anatomy, Praha 2, Czech Republic.
[2]Charles University, 1st Faculty of Medicine and General University Hospital, Department of Dermatovenerology, Praha 2, Czech Republic.
[3]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Praha 4, Czech Republic.
[4]Charles University, 1st Faculty of Medicine and General University Hospital, Dental Clinic, Praha 2, Czech Republic

Fibroblasts represent a ubiquitous cell type in the human body. They are the principal cells of connective tissues and are present in virtually all organs. Fibroblasts produce molecules of extracellular matrix providing anchoring to numerous cell types, e.g., in the form of basement membranes. Developmentally, fibroblasts originate from either the mesoderm or ectomesenchyme – a neuroectodermal neural crest derivative. Phenotypically, cells expressing vimentin in conjunction with the absence of the usual differentiation markers of other cell types, such as keratins, CD34, CD45, tubulin-3, GAFP, and HMB45/tyrosinase, are considered to be fibroblasts. However, they can be further classifieds into many subtypes. Under physiological conditions, fibroblasts can be more closely classified into many subcategories as revealed by currently available robust genomic technologies such as single-cell sequencing. This observation likely results from the influence of other cell types engaging in intercellular interaction in organs and tissues *in situ*. Some of these fibroblasts closely resemble mesenchymal stem cells, including, to some extent, features like differentiation plasticity. In the context of various pathological conditions, such as wound healing, inflammation, and tumours, the fibroblast classifications can become even more complicated. Robust evidence confirmed that activated fibroblasts significantly influence all these processes. In such a pathological context, it was proposed that fibroblasts can also originate by transdifferentiation from other cell types, such as mesenchymal stem cells, pericytes, endothelial cells, macrophages, and cancerous epithelial cells. This heterogeneity of origin is likely to be also reflected in their phenotype. This is particularly well evidenced for so-called cancer-associated fibroblasts (CAFs), where some fibroblasts produce extracellular matrix, others secrete inflammation-supporting factors, and other cells can participate in antigen presentation. Robust data indicate that paracrine products of these fibroblasts participate in the pre-metastatic niche formation. Their products can also be linked to cancer wasting in terminal cancer patients suffering from metastatic disease. From this point of view, CAFs represent a promising target cell population for potential therapeutic

manipulation in cancer patients.

Supported by project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)-funded by the European Union-Next Generation EU, and by Charles University project Cooperatio ONCO.

Revealing immune response against melanoma during spontaneous regression in pig model

Veronika Čížková[1,2], Salim Abuhajiar[1,2], Jakub Červenka[1,2,3], Vratislav Horák[1], Kateřina Vodičková Kepková[1], Petr Vodička[1], Helena Kupcová Skalníková[3,1]

[1]Laboratory of Applied Proteome Analyses and Research Centre Pigmod, Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Libechov, Czech Republic.
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic.
[3]Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, Prague, Czech Republic

Spontaneous regression is a rare phenomenon when cancer partially or completely disappears without treatment. In melanoma, spontaneous regression is accompanied by tumor flattering, reduction in size and depigmentation on macroscopic level, as well as with tumor infiltration by immune cells and deposition of connective tissue on microscopic level. Melanoma-bearing Libechov Minipig (MeLiM) is a large animal model of hereditary melanoma with spontaneous regression occurring in approximately 75% of affected animals. The rest of the affected pigs develop melanoma progression, with metastasizing into lymph nodes and inner organs and cachexia. The progressive melanoma development can be reversed by devitalization, i.e. tumor ischaemization by sutures leaving the tumor *in situ*. Both spontaneous and devitalization-induced regression are accompanied by tumor infiltration by T-cells and by partial or almost complete skin and bristle depigmentation. The aim of our work was to characterize the process of spontaneous regression in the MeLiM model. We mapped the tumor infiltration by immune cells (cytotoxic and helper T-cells, macrophages, B-cells, granulocytes, etc.) during spontaneous regression. We also studied secreted factors (e.g. growth factors, chemokines, cytokines) in the intercellular communication and regulation of proliferation and migration of cells in the melanoma microenvironment. We identified approx. 10 times increased interleukin 6 (IL-6) expression in melanoma tissue compared to surrounding healthy skin, as well as highly elevated IL-6 levels in blood plasma of melanoma-bearing animals compared to melanoma-free controls. The effects of IL-6 on immune cell infiltration, malignant cell migration, as well as its possible roles in the regression process deserve further studies. Our results on pig melanoma model may participate in elucidation of changes in tumor tissue during the spontaneous regression, reveal the role of immune system in control of tumor growth and may lead to identification of targets of interest to study in human disease.

Acknowledgement: This study was supported by the European Union – Next Generation EU as part of the Czech Recovery Plan (project No. LX22NPO5102) and by the Operational Program Research, Development and Education (project No. CZ.02.1.01/0.0/0.0/16_019/0000785).

Single-cell profiling of native surface glycosphingolipid epitopes opens new dimension for deconvolution of breast cancer intratumoral heterogeneity and phenotypic plasticity

Jiřina Procházková[1], Radek Fedr[1,2], Barbora Hradilová[1,3], Barbora Kvokačková[1,2,3], Josef Slavík[4], Miroslav Machala[4], Pavel Fabian[5], Jiří Navrátil[5], Simona Kráčalíková[6], Monika Levková[6], Petra Ovesná[7], Jan Bouchal[6,8], Karel Souček[1,2,3]

[1]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic.
[2]International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic.
[3]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic.
[4]Veterinary Research Institute, Brno, Czech Republic. 5Masaryk Memorial Cancer Institute, Brno, Czech Republic.
[6]Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Olomouc, Czech Republic.
[7]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic.
[8]Department of Clinical and Molecular Pathology, University Hospital, Olomouc, Czech Republic

In this study, we asked a question if cellular profiles of glycosphingolipids (GSLs) alter with epithelial-to-mesenchymal transition (EMT) status and if so, can flow cytometric single-cell profiling of GSL-related epitopes present in non-tumor/tumor breast tissue serve as a tool for reproducible description of BCa-related heterogeneity and phenotypic plasticity driven, among others, by EMT?

Total levels of GSLs were analyzed

in *in vitro* EMT model derived from breast tissue to reveal association of GSLs with the phenotypic status of cells (epithelial vs. mesenchymal). These associations were then investigated in clinical samples of breast non-tumor and tumor tissue using set of commercial antibodies recognizing GSL-related epitopes, lineage and EMT surface markers. Our data indicate that in comparison with stromal-like/mesenchymal cells, breast epithelial cells appeared more positive for surface SSEA1 staining and have higher levels of Gb3. Promising estrogen receptor alpha (ERa)-dependent alterations of surface Gb3 and mainly its glycosylated progeny SSEA3 were observed between epithelial and stromal-like cells of tumor origin when compared with their non-tumor counterparts. Therefore, further analyses are essential to validate more precisely the unique associations we observed between the surface presence of specific GSL-related epitope (e.g. Gb3, SSEA3, SSEA1) and distinct cellular phenotype.

In conclusion, single-cell profiling of surface GSL-related epitopes provides a more detailed description of heterogeneity present in subpopulations of epithelial and stromal-like cells residing in breast tumor tissue microenvironment.

Study was supported by Internal Support of Research Program of the Institute of Biophysics of the CAS (J.P.), by the Czech Science Foundation project GA CR 21-11585S (K.S.) and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU (K.S., J.B.).

Gut microbiome can influence local and distant immune environment and tissue structures: a germ-free animal lesson perspective also for cancer-microbiome relationships

Luca Vannucci[1], Paolo Tenti[1,2], Pavol Lukáč[1,2], Lenka Rajsiglová[1,2], Fabián Caja[1], Dmitry Stakheev[1,3], David Vondrášek[4], Renata Štepanková[5], Tomáš Hudcovic[5], Peter Makovicky[6], Petr Šíma[1], Daniel Smrž[1,3]

[1]Institute of Microbiology of the CAS, Prague, Czech Republic.
[2]Faculty of Science, Charles University, Prague, Czech Republic.
[3]2nd Medical Faculty Motol, Prague, Czech Republic.
[4]Institute of Physiology of the CAS, Prague, Czech Republic.
[5]Institute of Microbiology of the CAS, Nový Hrádek, Czech Republic.
[6]University of Ostrava, Ostrava, Czech Republic

The microbiome is in tight relation to our organism with the possibility to influence tissue structures and functions. The intestinal microbiome can modulate immunity not only locally but also systemically. Evaluating reconstitution of the microbiome in germ-free (GF) animals and comparing the tumor development and immunity in conventional (CV, with regular microbiome) and GF animals, we can evidence differences both in tissue structure remodeling, quick immunological adaptation and even changes in the thymus cytokine expression between the two conditions.

We demonstrated that the "conventionalization" of GF animals induces quick remodeling of the collagen scaffold in the colon mucosa of the GF animals with a "maturation" of the stroma in about one week from the passage from a sterile to a conventionally contaminated environment. This was associated to a cytokine modulation indicating IL-10 as a major player in establishing a regulated not-inflammatory microenvironment at various levels (colon, ileum, thymus). When GF and CV animals were induced to colon carcinogenesis by the same protocol, a lower number of tumors developed in the GF animals than in the CV and the immune downregulation in GF animals was less intense than in the CV animals. While in the animals with microbiome was evaluated the establishment of tumor microenvironment it was evident a remodeling of collagen scaffold with fibrotic evolution was likely the one observed by inducing chronic colitis, with the central contribution of IL-6. Interestingly, inducing colitis in GF animals the structure appeared to "mature" but not to be disorganized.

Taken together, we can consider a new important field of study the evaluation of the microbiome interaction with the tissue during cancer development as a possibly relevant element not only in the establishment but also in the progression of colon tumors with effects that can be extended to the systemic immunity and influence the anticancer response and the therapies.

Acknowledgments: we thank the support by Institutional Grant RVO 61388971 (CZ), GAAV IAA500200917 (CZ), MEYS CR (Large RI Project LM2018129 Czech-BioImaging), ERDF (project No. CZ.02.1.01/0.0/0.0/18_046/0016045) and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union –Next Generation EU, Generali Ceska Pojistovna Foundation (CZ), UniCredit Bank (CZ), SIAD s.r.o. (ITA/CZ), and ARPA Foundation (ITA).

AI in pathology: state of the art and AI-powered image analysis with ImageJ

Kateřina Čížková

Department of Histology and Embryology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic

Image analysis is central to pathology, providing objective and standardised assessment of tissue samples, reducing human subjectivity. The integration of artificial intelligence (AI) has revolutionised the field. AI algorithms excel at pattern recognition and extracting meaningful insights from images, enabling advanced and accurate analysis. These algorithms can be trained to identify specific features, such as abnormal cells or tissue structures, with remarkable accuracy. The adaptive nature of AI ensures continuous improvement as pathology practices evolve. ImageJ, an open source software widely used in research, is particularly notable for its versatility, with the ability to incorporate machine learning plug-ins to further enhance its image analysis capabilities.

New prognostic markers and therapeutic strategies in solid tumors

Lucia Knopfová[1,2], Jarmila Navrátilová[1,2], Říhová Kamila[1,2], Adamová Barbora[1,2], Iva Staniczková Zambo[3], Markéta Hermanová[3], Dagmar Adámková Krákorová[4], Peter Múdry[5], Petr Vlček[6], Michal Hendrych[3], Marek Stiborek[7], Jan Preisler[7], Karel Souček[1,2,8], Petr Benes[1,2]

[1]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic.
[2]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic.
[3]1st Department of Pathology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic.
[4]Masaryk Memorial Cancer Institute and Faculty of Medicine Masaryk University, Brno, Czech Republic.
[5]Pediatric Oncology Department, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic.
[6]1st Department of Surgery, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Brno, Czech Republic.
[7]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic.
[8]Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, v.v.i, Brno, Czech Republic

Therapeutical resistance and metastases formation are associated with high mortality of cancer patients. The aim of our research is the identification of factors that regulate these processes and their subsequent testing/validation as prognostic markers and therapeutical targets to provide benefit for cancer patients. We identified transcription factor c-Myb as a negative metastatic regulator and positive prognostic factor in a subset of breast and colon cancer patients. Experiments with cancer cell lines and preclinical mouse models revealed the immunomodulatory role of c-Myb within primary tumors and during dissemination. On the contrary, high expression of c-Myb is associated with therapeutical resistance, high metastatic activity and negative prognosis in osteosarcoma suggesting its cancer-type/subtype specific role. Other metastasis- and chemoresistance-related genes/proteins were identified using transcriptomic screening and bioinformatic analyses and are currently being investigated. In addition, the sensitivity to preclinically developed drugs or new drug combinations is tested in 3D models of solid cancers including organotypic cultures. Multimodal semi-automatic imaging methods are being developed to analyze drug distribution, cytotoxicity to various cell types within complex tumor microenvironment and markers of cancer cell proliferation and cell death. Using this approach, we identified disulfiram/5-fluorouracil and perifosine/ABT-737 synergism in colon cancer 3D cultures. Other drug combinations as well as the modulatory effect of microbiome are currently being tested in our laboratory.

Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

Single cell molecular pathology with Xenium *in situ*

Agnieszka Ciesielska

10x Genomics

Developing treatments for complex diseases requires building a complete understanding of both disease and treatment-response mechanisms. As we navigate a century where transformative advances in biology will reshape the way we deliver human health, translational and clinical researchers need approaches that provide actionable insights that can, ultimately, be leveraged to improve how diseases are diagnosed and treated.

WEDNESDAY 22ND NOVEMBER, 2023

Chairs: Aleksi Sedo, Jan Bouchal

Glutamine metabolism as a target in therapy resistant prostate cancer

Alicia-Marie K. Beier, Christian Thomas, Holger H.H. Erb

Department of Urology, Technische Universität Dresden, Dresden, Germany

Metabolic reprogramming has been recognized as a hallmark in solid tumors. In advanced and incurable castration-resistant PCa, a therapy-induced metabolic shift towards choline, amino acid, and glycolytic metabolism fuels tumor growth and progression. This metabolic alteration may provide opportunities for novel therapeutic strategies, especially in therapy-resistant PCa stages. Therapy resistance is one of the main challenges of prostate cancer (PCa).

Radiotherapy is one treatment option for localized PCa with curative intent. However, the treatment can be impeded by therapy resistance mechanisms and treatment-related side effects. This resistance rises from a small cell population inside the tumor, so-called PCa stem cells. A study by Mukha et al. demonstrated that radioresistant PCa cells have a high glutamine demand. It could be demonstrated that glutamine's catabolism serves as energy production and the maintenance of the redox state. Consequently, glutamine withdrawal or chemical inhibition of the glutamine metabolism, such as GLS1, results in PCa radiosensitization, whereas non-malignant prostate cells were unaffected.

Next to radiotherapy, chemotherapy with docetaxel (DTX) is one treatment option for hormone-sensitive and castration-resistant metastatic prostate cancer (PCa). Despite initial remission, acquired DTX resistance is inevitable. The mechanisms behind DTX resistance are not deciphered yet. However, a mesenchymal phenotype is associated with DTX resistance. Mesenchymal cancer cells have been associated with high invasion and motility potential and seem to adopt a cancer stem cell (CSC)-like phenotype with increased tumor-forming potential. Therefore, distinct energy requirements are mandatory, resulting in metabolic reprogramming prioritizing energy production. This reflects, for example, in increased oxidative phosphorylation (OXPHOS), glutathione (GSH) production, and ROS scavenging, features associated with glutamine (Gln) metabolism. Metabolic analysis revealed that docetaxel-resistant (DR) cell lines obtain the most ATP production by oxidative phosphorylation, powered substantially by glutamine (Gln). Likewise, Gln is indispensable and known to play an important role in redox homeostasis ROS balance. Targeting the Gln therapy-resistant PCa cells revealed that lower Gln concentrations significantly reduced 2D monolayer and 3D spheroid cell proliferation as well as the clonogenic potential in the selected PCa cell models. Moreover, Gln withdrawal decreased the migratory behavior of these cells. The experiments revealed that the DR cells were susceptible to Gln deprivation. This observation could be confirmed by pharmacological inhibition of the glutamine metabolism by the GLS1 inhibitor Telaglenastat. GLS1 knock-down experiments revealed that GLS1 mainly mediates the observed effects.

Gln availability highly influences the proliferation, clonogenic potential, and DNA-repair ability of PCa cells. Especially cells with cancer stem cell-like phenotype, such as radioresistant and mesenchymal DR cells, are more sensitive to Gln deprivation. Thus, these findings highlight a possible clinical rationale for blocking the Gln metabolism by chemical inhibitors like Telaglenastat as a therapeutic strategy to target these therapy resistant PCa cells, thereby diminishing accelerated tumor progression and metastatic spread.

Spliceosome component SRSF9 is involved in an rs5918762 allele-specific manner in alternative splicing of androgen receptor variant 7

Jasper Van Goubergen[1], Miroslav Peřina[1,2], Florian Handle[1,3], Elisa Morales[1], Oliver Schmidt[4], Marcus V. Cronauer[5], Frédéric R. Santer[1]

[1]Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Innsbruck, Austria.
[2]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[3]Institute of Pathology, Neuropathology & Molecular Pathology, Medical University of Innbruck, Innsbruck, Austria.
[4]Institute for Cell Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria.
[5]Institute of Pathology, Universitätsklinikum Bonn, Bonn, Germany

Prostate cancer (PCa) is a leading cause of cancer-related deaths among men in the European Union. In advanced stages androgen deprivation therapy (ADT) and androgen signaling inhibitors (ARSI), such as enzalutamide and abiraterone acetate, are the mainstay of PCa therapy. However, most patients on ADT eventually progress to a castration-resistant stage (CRPC), which is characterized by reactivation of the AR signaling pathway. One of the main drivers of CRPC is the upregulation of constitutively active androgen-independent AR-variants (AR-Vs), with AR-V7 being the most clinically relevant variant. Compared to the full-length AR (AR-FL), AR-V7 has a distinct and shorter 3' untranslated region (3'UTR) due to the inclusion of cryptic exon 3. Here, we aimed to better understand the mechanisms underlying regulation of AR-V7 mRNA expression and

activity by a better characterization of its 3´UTR.

Polymorphisms within the 3'UTR of AR-V7 can potentially alter its expression and function by influencing the binding of RNA binding proteins (RBPs) and microRNAs (miRNAs). Notably, a common single nucleotide polymorphism (SNP), rs5918762, with a minor allele frequency of 30%, has been identified as the preferred allele for binding the RBP SRSF9. SRSF9 belongs to the conserved SR-protein family, which is involved in alternative splicing. Depletion of SRSF9 leads to decreased levels of AR-V7, indicating its critical role in AR alternative splicing. This interaction between SRSF9 and rs5918762 was confirmed through AR-V7 minigene assays and CLIP-qPCR analysis.

The CLK/SRSF axis, involving CLK2 and SRSF9, is frequently dysregulated in metastatic PCa, with amplification observed in 11.3% and 7.4% of cases, respectively. Targeting this axis can be achieved by using CLK inhibitors like Lorecivivint, which inhibits CLK2 activity, thereby reducing SRSF9 phosphorylation and subsequently decreasing AR-V7 levels. The involvement of CLK2 to SRSF9 was showed through Phos-tag SDS-PAGE.

In summary, this study highlights the impact of rs5918762 on the splicing of AR-V7 by modulating the binding capacity of the spliceosome component SRSF9 to the 3'UTR of AR-V7. These findings emphasize the potential of targeting the SR protein family through CLK inhibitors as a promising strategy to counteract castration-resistant prostate cancer driven by AR-V7.

Molecular basis of cisplatin resistance in testicular germ cell tumors

Laura Matoušková[1], Violeta Bakardjieva-Mihaylova[1], Martina Zwyrtková[1], Kristýna Šoffrová[1], Soňa Křivonosková[2], Jan Stuchlý[1], Blanka Rosová[3], Tomáš Büchler[4], Jan Trka[1], Karolina Škvárová Kramarzová[1], Ludmila Boublíková[1,2]

[1]Dept. of Pediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic.
[2]Dept. of Oncology, 1st Faculty of Medicine, Charles University and Thomayer University Hospita, Prague, Czech Republic.
[3]Dept. of Pathology and Molecular Medicine, 3rd Faculty of Medicine, Charles University and Thomayer University Hospital, Prague, Czech Republic.
[4]Dept. of Oncology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic

Testicular germ cell tumors (TGCT) are the most common solid malignancy in young adult men with steadily increasing incidence. Even though these tumors are highly curable due to their high sensitivity to cisplatin-based chemotherapy, a portion of patients with an advanced disease develops a resistance to cisplatin that usually results in disease progression and patient´s death. The cause of cisplatin resistance has not yet been elucidated.

We study the cisplatin resistance and its potential causes in a unique cohort of refractory TGCT patients and in an experimental cell line model. Patients with acquired cisplatin resistance are enrolled and multiple samples are collected and analyzed including the primary tumor, resected metastasis, cell free DNA (cfDNA) from several time points and germ line DNA as a background control. The samples are analyzed by whole exome sequencing in order to find mutations that could contribute to the development of cisplatin resistance. We identified several candidate genes related to regulation of cell cycle and testis development, e.g. RMBX – homologue of spermatogenesis regulator RBMY, and zinc finger protein gene PRDM9. These genes have not been associated with TGCT before.

The NGS data are highly beneficial, however, they bring only limited information about the functional consequences of the detected molecular variants. Also, because of the tumor heterogeneity and limited number of studied patients and samples due to the rareness of this disease, the process of finding the molecular causes of cisplatin resistance can get even more challenging.

To address this, we prepared resistant TGCT cell lines matched to their original sensitive ones and analyzed them by exome and transcriptome sequencing, which revealed another genes potentially involved in cisplatin resistance, like ATRX - chromatin remodeler. Next, we employed CRISPR-Cas9 gene editing technology to create targeted knock outs of individual genes identified in the previous steps of this project, as well as to carry out CRISPR knock out screen experiments to search for further genes and aberrations with a critical role in cisplatin resistance in TGCT. This may finally allow for the establishment of novel approaches to prevent or overcome the cisplatin resistance in TGCT and improve the dismal prognosis of the refractory patients.

The National Institute for Cancer Research project (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Supported by grants MH CZ - DRO (Thomayer University Hospital – 00064190, University Hospital Motol - 00064203) and GA UK 208423; the research infrastructure was supported by MEYS CZ (LM2015091).

In vivo toxicity and therapeutic efficacy of enhanced blood retention and tumor uptake PSMA-targeting ^{225}Ac-labeled radioconjugates

Zbynek Novy[1], Katarina Hajduova[1], Milos Petrik[1], Katerina Bendova[1], Falco Reissig[2], Kristof Zarschler[2], Klaus Kopka[2], Daniela Kurfurstova[3], Jan Bouchal[3], Constantin Mamat[2], Marian Hajduch[1]

[1]Institute of Molecular and Translational Medicine, Olomouc, Czech Republic.
[2]Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany.
[3]Institute of Clinical and Molecular Pathology, Olomouc, Czech Republic

Introduction: The prostate-specific membrane antigen (PSMA) is overexpressed in prostate cancer tissues at significantly higher levels compared to healthy organs. Therefore, PSMA has emerged as very suitable target for molecular imaging as well as targeted radionuclide therapy of metastatic castration-resistant prostate cancer (mCRPC). In this study we investigated the *in vivo* behavior of two novel macropa-based PSMA inhibitors (namely [225Ac]FR55 and [225Ac]FR94) modified with albumin binding moieties.

Methods: Performed *in vivo* studies involved *ex vivo* biodistribution studies and subsequent immunohistochemical examinations of selected organs from LNCaP-tumor bearing mice. Organs were dissected, weighed, and the accumulated activity was quantified in a gamma-counter at 1, 4, 24, 48, 72, and 120 h post-injection to determine the radiotracer uptake as a percentage injected activity (dose) per gram of the corresponding organ (%ID/g). We have done toxicology study in healthy animals as well. Kidneys, liver and tumor were examined using immunohistochemical staining methods to detect PSMA expression, DNA damage (γH2AX), proliferation status (Ki67) and necrosis (H&E). Finally, therapy study was done in tumor mice.

Results: The highest accumulation of radioactivity was measured in the LNCaP tumors at at 120 h p.i. for [225Ac]FR94 (46.04 ± 7.77 %ID/g). The γH2AX staining revealed significant DNA damage in tumor cells of mice applied with [225Ac]FR55 as well as [225Ac] FR94 compared to untreated controls. Insignificant DNA damage was observed in the kidney tissue compared to the untreated controls in toxicicity study. The therapy study revealed significant therapeutic effect of all studied compounds compared to saline (control) group.

Conclusion: *In vivo* experiments in healthy mice showed low toxicity of tested PSMA inhibitors. Histological examination of the organs confirmed substantial DNA damage in the tumor tissue of mice injected with both studied 225Ac-compounds, on the other hand the same parameter revealed only low DNA harm in the kidneys. The therapeutic efficacy of tested compounds was comparable to gold standard PSMA-617.

A-ring fused heterocyclic derivatives of dihydrotestosterone targeting the androgen receptor in prostate cancer

Miroslav Peřina[1], Márton A. Kiss[2], Gergő Mótyán[2], Eva Szczyrbová[3], Martin Eliáš[3], Vladimír Študent jr.[4], Daniela Kurfurstová[3], Markéta Kovalová[1], Lukáš Mada[1], Jan Bouchal[3], Éva Frank[2], Radek Jorda[1]

[1]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[2]Department of Organic Chemistry, University of Szeged, Szeged, Hungary.
[3]Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Palacký University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic.
[4]Department of Urology, Palacký University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic

High expression of the androgen receptor (AR) and the disruption of its regulation are major contributors to the development of prostate cancer (PCa). Therapeutically relevant non-steroidal or steroidal antiandrogens are able to block the AR action by eliminating AR-mediated transcriptional activity and signalling. Here we report the structure-activity relationship of a broad library of steroidal heterocyclic derivatives of the natural sex hormone 5a-dihydrotestosterone (DHT). A total of 119 compounds containing triazolo[1,5-a]pyrimidines, pyrazolo[1,5-a]pyrimidines, variously substituted pyrazoles, substituted quinolines and pyridines of DHT, synthesised in recent years, were evaluated for their activity towards AR. All compounds were investigated using the AR luciferase reporter cell line in agonist and antagonist mode and their cytotoxic effect was also analysed in panel of PCa cell lines. Triazolo[1,5-a] pyrimidines, pyrazolo[1,5-a] pyrimidines, substituted quinolines and pyridines of DHT displayed only moderate AR-antagonist activity and antiproliferative activity in PCa cell lines. On the other hand, two specific regioisomeric groups of pyrazole derivatives significantly diminished the transcriptional activity of AR and displayed high antiproliferative activity in AR-positive PCa cell lines. The lead compound (3d) generally suppressed AR signalling, moreover, it also led to a sharp decrease in wt-AR protein level, probably caused by proteasomal degradation. We confirmed the antiproliferative activity selective for AR-positive PCa cell lines, the cellular interaction of 3d with AR, and described the binding in the ligand-binding domain by the flexible docking. Moreover, compound 3d was shown to be potent even *ex vivo* in patient-derived tissues, highlighting the therapeutic potential of A-ring-fused pyrazoles.

WEDNESDAY 22ND NOVEMBER, 2023

Chairs: Milada Šírová, Marek Kovář

Antitumor activity of HPMA copolymer conjugates bearing taxanes and platinum-based drug for the treatment of head and neck cancer

Katerina Behalova[1], Daniil Starenko[1], Marek Kovar[1], Libor Kostka[2], Vladimir Subr[2], Tomas Etrych[2]

[1]Laboratory of Tumor Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic. [2]Department of Biomedicinal Polymers, Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Prague, Czech Republic

Head and neck cancers (HNC) account for 4 % of all malignancies worldwide. Their incidence is increasing, however, development of new chemotherapeutics in this area is stagnating for decades. Moreover, the chemotherapeutics that are currently used to treat HNC cause severe side effects which significantly lower the quality of life of the patients. One promising approach to reduce the number of side effects and increase the efficacy of the treatment appears to be the use of HPMA copolymer conjugates bearing the conventional cytostatic drugs bound via pH-sensitive hydrazone bond. Such conjugates possess significantly improved pharmacological features in comparison to the free low-molecular-weight drugs used routinely in the clinical practice including significantly prolonged half-life in the circulation, passive accumulation of the drug in the tumors due to the EPR effect, solubilization of water insoluble cancerostatics and lower side toxicities. Taxanes and platines or their combination are commonly used for chemotherapy of HNC. We investigated the anticancer activity of HPMA copolymer-based conjugates bearing picoplatin derivative with 2-oxobutylpyridine linker and docetaxel derivatized with 5-methyl-4-oxohexanoic and levulinic acid both *in vitro* and *in vivo*. We report the data showing high anticancer activity of these compounds in FaDu, SCC7, 4T1, CT26 and LL2 cell lines using 3H-thymidine incorporation assay (cytostatic activity), MTT assay (cytotoxic activity) and Annexin V assay (apoptosis induction) *in vitro*. After these initial experiments, we studied their toxicity and anticancer activity *in vivo* in BALB/c mice bearing CT26 or 4T1 tumors and RAG2-/- mice bearing FaDu tumors. Conjugate bearing docetaxel derivatized with levulinic acid showed strong effect on inhibition of CT26 tumors. Treatment of RAG2-/- mice bearing FaDu tumors led to complete regression of all experimental mice. Thus, this conjugate represents a potentially promising tool for the treatment of HNC as well as other taxane-sensitive tumors.

Acknowledgement: This work was supported by the project National Institute for Cancer Research (Programme Exceles, ID Project No. LX22NPO5102) – Founded by the European Union – Next Generation EU) and by the Czech Health Research Council (project no. NU 21-03-00273).

Stimuli-responsive polymer nanoprobes intended for fluorescence-guided surgery

Tomáš Etrych[1], Robert Pola[1], Eliška Grosmanová[1], Jan Pankrác[2], Maxime Henry[3], Martin Kaňa[4], Jan Bouček[4], Jean-Luc Coll[3]

[1]Institute of macromolecular chemistry CAS, Prague, Czech Republic. [2]First Faculty of Medicine, Charles University, Prague, Czech Republic. [3]University Grenoble Alpes, Grenoble, France. 4University Hospital Motol, Prague, Czech Republic

Nano-sized carriers are widely studied as suitable candidates for the advanced delivery of various bioactive molecules such as drugs and diagnostics. Treatment of malignant tumors usually requires a multi-modality approach including surgery, chemotherapy, and radiation therapy. Indeed, surgical removal still represents the primary treatment modality for most solid tumors. Most tumor cells should be removed during surgery as any remaining malignant cells could cause disease relapse but it is desirable to spare healthy tissues and to remove only the necessary margin from the immediate vicinity of the tumor. Therefore, it is essential to accurately determine the boundary between healthy and tumor tissue to enable precise and safe resection. This could be achieved by using a fluorescent contrast agent that accumulates in the tumor to a greater extent than in the healthy tissue, thus increasing the signal-to-noise ratio between malignant and healthy tissue.

Herein, the development of long-circulating stimuli-responsive polymer nanoprobes tailored for the fluorescently-guided surgery of solid tumors is reported. Nanoprobes are designed as long-circulating nanosystems preferably accumulated in solid tumors due to the Enhanced permeability and retention effect, so they act as a tumor microenvironment-sensitive activatable diagnostic. This study designs polymer probes differing in the structure of the spacer between the polymer carrier and Cy7 by employing pH-sensitive spacers, oligopeptide spacers susceptible to cathepsin B-catalyzed enzymatic hydrolysis, and non-degradable control spacer. Increased accumulation of the nanoprobes in the tumor tissue coupled with stimuli-sensitive release behavior and subsequent activation of the fluorescent signal upon dye release facilitated favorable tumor-to-

background ratio, a key feature for fluorescence-guided surgery. The probes show excellent diagnostic potential for the surgical removal of intraperitoneal metastasis and orthotopic head and neck tumors with very high efficacy and accuracy. In addition, the combination of macroscopic resection followed by fluorescence-guided surgery using developed probes enable the identification and resection of most of the CAL33 intraperitoneal metastases with total tumor burden reduced to 97.2%.

Acknowledgement: This work was supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) – Funded by the European Union – Next Generation EU and by the Ministry of Health of Czech Republic project (NU21-08-00280).

Hydrophilic polymer carriers for tumor targeted delivery of photosensitiser precursors

Kevin Kotalík[1], Vladimír Šubr[1], Marcela Filipová[1], Tereza Běláková[1], Tomáš Křížek[2], Alice Šimonová[2], Jun Fang[3], Tomáš Etrych[1]

[1]Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic. [2]Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic.
[3]Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan

Photodynamic therapy (PDT) is one of the modern approaches suitable for cancer treatment. The therapeutic agent, called photosensitizer (PS), is used for generation of singlet oxygen causing tumor cell apoptosis after light irradiation. One of the commonly used photosensitisers is protoporphyrin IX, which can be delivered as such or as a precursor, 5-aminolevulinic acid (5-ALA), for its biosynthesis. Unfortunately, common photosensitisers still lack tumor targeting properties and may be toxic even at dark. Polymer drug carriers can overcome these disadvantages of PSs due to enhanced permeability and retention (EPR) effect, which is responsible for preferential accumulation of nanosized drug delivery systems in tumor due to its leaky vasculature. Moreover, nanomedicines are retained in the tumor tissue because of the absence of lymphatic drainage leading to higher efficacy and lower system toxicity of polymer-conjugated anticancer agents.

Herein, we present new water-soluble biocompatible nanotherapeutics based on poly(N-2-hydroxypropyl methacrylamide) (PHPMA) with conjugated 5-ALA via a pH sensitive hydrazone bond. The controlled synthesis, physico-chemical properties, drug release profiles as well as in vitro and *in vivo* evaluation of the novel nanomedicines will be shown and discussed. We were able to synthetize nanotherapeutics with high content of 5-ALA and narrow molar mass distribution which were not cytotoxic at dark, however, they showed significant toxicity upon irradiation. Moreover, charge properties and drug release profiles of nanomedicines with various spacers between polymer carrier and the hydrazone bond were studied.

Advanced glycopolymers as potent inhibitors of galectin-induced tumor progression

Petr Chytil[1], Marina R. Tavares[1], Marcela Filipová[1], David Vrbata[2], Milada Šírová[2], Pavla Bojarová[2], Tomáš Etrych[1]

[1]Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

The overexpression of galectins is known to be associated with several life-threatening disorders, namely neoplastic diseases. In tumorigenesis, galectins participate in cellular adhesion, invasion, angiogenesis, and metastatic processes. Most studies focus on the two most common galectin-1 and galectin-3, prospective targets for therapeutical interventions. Human galectin-1 and galectin-3 have shown affinity to ligands based on N-acetyllactosamine. Moreover, the multivalent presentation of these ligands often increases the affinity by several orders of magnitude via the cluster glycoside effect.[1]

This study presents the synthesis and characterization of various glycopolymers based on water-soluble N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, which are known for their biocompatibility, lack of any toxicity or immunogenicity. [2] Glycopolymers, prepared using controlled radical reversible addition−fragmentation chain-transfer (RAFT) polymerization, differed in the structure and content of carbohydrate ligands, namely N-acetylated lactosamines or their glycomimetics. We evaluated the structure-activity relationship of glycopolymers concerning their binding affinity to galectins. By tuning the glycopolymers' structure, namely ligands' type, content, and presentation on the polymer carrier, we could selectively target and inhibit galectin and, additionally, discriminate between galectin-1 and -3. Prepared glycopolymers showed significant inhibition of Gal-3-induced angiogenesis and T lymphocyte apoptosis *in vitro*. Preliminary *in vivo* experiments are undergoing. Thus, HPMA-based glycopolymers are attractive as effective drug-free nanomedicines supporting targeted tumor treatment.[3-5]

Acknowledgments

We gratefully acknowledge the support of the Czech Science Foundation GAČR (project 22-00262S).

References

[1] Laaf D. et al., Trends Biotechnol., 2019, 37, 402−415.

[2] Chytil P. et al., Macromol. Biosci. 2018, 18 (1), 1700209.

[3] Tavares M.R. et al., Biomacromolecules, 2020, 21 (2),

641-652.

[4] Filipová M. et al., Biomacromolecules, 2020, 21 (8), 3122–3133.

[5] Vrbata D. et al., J. Med. Chem., 2022, 65 (5), 3866–3878.

Powerful increase of gemcitabine *in vivo* antitumor activity through drug delivery system based on biocompatible HPMA copolymers: drug release kinetics is crucial

Kateřina Běhalová[1], Natálie Klusová[1], Robert Pola[2], Milada Šírová[1], Tomáš Etrych[2], Marek Kovář[1]

[1]Laboratory of Tumor Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Department of Biomedicinal Chemistry, Institute of Macromolecular Chemistry of the Czech Academy of Sciences, Prague, Czech Republic

Gemcitabine (Gem) is the nucleoside analog (2‘, 2‘-difluoro 2‘deoxycytidine) and anticancer drug with potent cytostatic activity causing inhibition of DNA synthesis through so called masked chain termination. Gem is used alone or in combination with paclitaxel bound to albumin nanoparticles (Abraxane) for the first-line treatment of pancreatic carcinoma. It is also used for the treatment of metastatic or inoperable breast carcinoma (in combination with paclitaxel), advanced or metastatic non-small cell lung carcinoma (in combination with cisplatin), ovarian carcinoma (in combination with carboplatin) and advanced or metastatic bladder cancer (in combination with cisplatin).

The most unfavourable pharmacological features of Gem are very extremely short half-life and possible metabolic inactivation via cytidine and deoxycytidine deaminases. Thus, we have recently designed, synthesized and characterized a set of linear HPMA copolymer conjugates (Mw ~ 40-45 kDa, Đ <1.1, HD ~ 8 nm) bearing Gem (12-15 wt%) covalently linked through amide bond using primary amine group of Gem. Such conjugation should dramatically increase the half-life of Gem in circulation and protect it from cytidine and deoxycytidine deaminases during the transport within the body. Four conjugates with different spacers between the HPMA copolymer backbone and Gem were prepared with the aim to study the effect of Gem release kinetics on the in vitro and *in vivo* antitumor activities of these conjugates. P-Gem1 (β-Ala spacer), P-Gem2 (Gly-Phe-Leu-Gly spacer), P-Gem3 (aminocaproic acid spacer) and P-Gem4 (valeric acid spacer) showed different rate of Gem release in buffer pH 7.4: P-Gem2 > P-Gem1 > P-Gem4 > P-Gem3. The release rate of Gem was somewhat faster in the serum upon incubation *ex vivo* though the order of the conjugates was the same. We determined in vitro cytostatic activities of these conjugates in mouse breast carcinoma 4T1, mouse lung carcinoma LL2, mouse pancreatic carcinoma Panc02 and human pancreatic carcinomas MiaPaca2, Panc-1 and BxPC-3. The in vitro cytostatic activities perfectly matched the release rate of Gem since the faster Gem release the higher cytostatic activity of the conjugate was observed in all four cell lines.

We evaluated the maximum tolerated dose (MTD) of our conjugates in BALB/c mice bearing 4T1 tumors in the next step. We found that MTDs of the conjugates (i.e. toxicity) corresponded to their *in vitro* cytostatic activities and thereby to the Gem release kinetics. Thus, the higher rate of Gem release, the higher in vitro cytostatic activity but also higher toxicity *in vivo*. *In vivo* antitumor activity of the conjugates was studied in the final set of experiments. All four conjugates showed more potent inhibition of 4T1 tumor growth in comparison to Gem when all conjugates and Gem were i.v. administered at 90 % of MTD as a single bolus. P-Gem3 and P-Gem4 (i.e. the conjugates with rather slow Gem release) were particularly effective and completely cured 3 and 4 out of 8 mice, respectively. These two conjugates also demonstrated remarkably higher anticancer activity in comparison to Gem in immunocompromised RAG2-/- mice bearing human MiaPaca2 tumors. Thus, we have demonstrated that the therapeutic performance of Gem could be dramatically improved via its conjugation to water-soluble biocompatible polymeric carrier and that the kinetics of the Gem release is crucial for the therapeutic activity with slow Gem release being superior to the fast one.

Targeted drug delivery using polymer carrier with P-gp overcoming capacity for treatment of chemoresistant tumors

Milada Šírová[1], Martin Kaňa[1], Alena Braunová[2], Markéta Frejková[2], Tomáš Etrych[2], Blanka Říhová[1], Marek Kovář[1]

[1]Inst. of Microbiology CAS, Prague, Czech Republic.
[2]Inst. of Macromolecular Chemistry CAS, Prague, Czech Republic

Success of cancer chemotherapeutic regimens is often hampered by either inherent or acquired resistance of cancer cells. One of the mechanisms developed during the treatment is an increased drug clearance by the transporter-facilitated efflux, resulting in (multi)drug resistance (MDR) of cancer cells. It can be conferred by overexpression of transporters, such as adenosine triphosphate binding cassette (ABC) pumps, from which P-gp is probably the most prominent one. Some recently developed nanodrug delivery systems may represent a significant approach for overcoming this resistance. The polymer carriers of the drugs mediate the transport of the drug to the cells without possible participation of the transporters. Moreover, polymers with their own

biological activity can be used for the construction of the delivery systems.

Here, we used an amphiphilic diblock polymer nanotherapeutics containing a hydrophilic block based on the *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymer and a hydrophobic poly(propylene oxide) block (PPO). The amphiphilic character of the diblock polymer ensures self-assembly into micelles with hydrodynamic radius Rh ~15 nm in aqueous solutions. The carrier serves simultaneously as a drug delivery system and an inhibitor of MDR. Doxorubicin (Dox) was bound to the diblock polymer through a pH-sensitive hydrazone bond, enabling prolonged circulation in blood, tumor-specific delivery of Dox and subsequent stimuli-sensitive controlled release within the tumor mass at a decreased pH. The presence of PPO in the polymer carrier leads to inhibition of P-gp, depolarization of mitochondrial membrane potential, and ATP loss in the target cells. *In vivo*, the diblock polymer system proved an excellent EPR-driven therapeutic activity. Importantly, significant therapeutic outcome was seen also in chemoresistant tumors characterized by inherently elevated P-gp expression.

Notes

Notes

Impaired HSC fitness and accelerated leukemogenesis in a mouse model of chronic inflammation

Srdjan Grusanovic[1], Monika Burocziova[1], Petr Danek[1], Maria Kuzmina[1,2], Miroslava Kari Adamcova[1], Romana Mikyskova[1], Karolina Vanickova[1], Sladjana Kosanovic[1], Jana Pokorna[1], Milan Reinis[1], Tomas Brdicka[1], Meritxell Alberich-Jorda[1,3]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Faculty of Science, Charles University, Prague, Czech Republic.
[3]Childhood Leukaemia Investigation Prague, Department of Pediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University in Prague, University Hospital Motol, Prague, Czech Republic

Background

Chronic inflammation (CI) is a hallmark of autoinflammatory disorders and is characterized by excessive production of cytokines. CI has been proposed as a factor that promotes solid tumor progression and metastasis. Accordingly, population-based studies identified a history of CI diseases as a risk factor for leukemia development. In order to investigate how CI affects hematopoietic stem cells (HSCs), as well as how it promotes leukemia development, we employed a mouse model suffering from chronic multifocal osteomyelitis (CMO). CMO mice exhibit a progressive autoinflammatory disorder that resembles human chronic recurrent multifocal osteomyelitis. CMO mice are asymptomatic until 7 weeks old, and after develop swollen paws, tail kinks, increased bone marrow (BM) cellularity, number of granulocytes, and production of cytokines.

Aims

To investigate (1) how sterile CI affects HSCs, (2) identify the mechanisms that are mediating these effects, and (3) determine whether the inflammatory environment can accelerate AML development.

Methods

Flow cytometry, generation of murine chimeras, limiting dilution transplantation assays, competitive BM transplantations, RNA sequencing, intraperitoneal injection of IL-6-, IL-6R-blocking antibody or Stat3 inhibitor, phospho-flow analysis, survival assays, MLL-AF9 retroviral infection, TP53 deficient mouse model

Results

Our data showed that CMO HSCs are expanded in asymptomatic mice, and that this expansion continued as the mice grew older. Limiting dilution transplantation assays indicated that CMO HSCs are functionally impaired. Interestingly, MyD88-deficient CMO mice did not develop the inflammatory phenotype, however their HSC population was still expanded and functionally impaired. Next, we generated chimeras to investigate the effect of the CMO immune compartment and the CMO BM niche on HSCs. Our results showed that both CMO compartments affect HSC functionality. Furthermore, RNAseq analysis suggested that the loss of HSC function is in part mediated by the IL-6/Jak/Stat3 signaling pathway. Indeed, we detected increased levels of IL-6 in CMO serum, BM, as well as increased pStat3 levels in CMO HSCs. Treatment of CMO mice with IL-6- or IL-6R-blocking antibody significantly prevented the HSC expansion, while targeting pStat3 prevented their expansion and functional impairment. Remarkably, MLL-AF9 leukemic cells demonstrated a faster leukemic onset in CMO mice than in WT mice, and they exhibited enhanced growth in vitro when IL-6 was added to the cultures. Next, we investigated whether CI promotes cancer progression in a TP53 deficient mouse model. We crossed CMO mice to TP53-deficient mice, and monitored their survival. Remarkably, CMO/Tp53+/- double mutants showed significantly accelerated tumor development and succumbed to disease faster than Tp53+/- non-inflammatory mice. TP53 deficient mice do not exhibit myeloid transformation, however, we observed AML development in one CMO/Tp53+/- mouse, suggesting that CI might induce AML transformation in some genetically predisposed murine models.

Summary - Conclusion

Altogether, our data indicate that CI has a detrimental effect on HSCs and highlight the possibility of adding clinically available Stat3 inhibitors to the current treatment in order to preserve stem cell functions. Further, our results suggest that CI acts as an additional factor in the development of AML, providing additional understanding of the mechanisms of transformation.

The work was partially funded by the National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102)-Funded by the European Union-Next Generation EU.

Targeting Plectin in hepatocellular carcinoma

Zuzana Outlá[1], Kateřina Korelová[1], Magdalena Přechová[1], Gizem Oyman-Eyrilmez[1], Jan Kosla[1], Samuel M Meier[2], Gerhard Wiche[3]

[1]Laboratory of Integrative Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Department of Analytical Chemistry, University of Vienna, Vienna, Austria.
[3]Department of Biochemistry and Cell Biology, Max Perutz Labs, University of Vienna, Vienna, Austria

Recently, it has been demonstrated that cytoskeleton-based mechanical homeostasis plays a critical role in carcinogenesis. The interplay of cytoskeletal components is ensured by cytoskeletal linker proteins (cytolinkers) of the plakin protein family. Plectin, a prototypical ubiquitously expressed cytolinker, physically crosslinks cytoskeletal networks and anchors them at junctional complexes, thus maintaining cell integrity. Over the past years, plectin was found to be upregulated in various tumor

types. Consistently, our results show plectin upregulation in hepatocellular carcinoma (HCC) patients and in HCC-derived cell lines, where its expression correlates with cell motility and invasivity. Therefore, we decided to employ plectin targeting to 1) study the role of plectin-mediated cytoskeletal crosstalk in the migratory and tumorigenic potential of HCC cells and 2) validate plectin as a potential target for HCC treatment.

To this end, we generated plectin-deficient (KO) HCC cell lines using CRISPR/Cas9 approach, together with plectin functional mutants lacking the IF-binding domain. In parallel, we addressed the effect of plectin pharmacological targeting using plecstatin, a high-affinity plectin ligand. Mass spectrometry-based shotgun proteomics identified response signatures of both genetic and pharmacological plectin targeting in HCC cells, showing the effect on signaling pathways crucial for cytoskeletal architecture, cell adhesion, polarity, and motility. Next, we observed aberrant actin stress fiber organization and collapsed vimentin IF networks using immunofluorescence microscopy. In line with observed phenotypes, traction force microscopy determined altered contractility in plectin-disabled cells. These changes were reflected in the 2D and 3D migration of HCC cells upon plectin inactivation. Our findings were confirmed *in vivo* using a lung colonization assay. To further explore the effect of plectin targeting on tumor progression, we studied the tumor growth of HCC-derived xenografts in immunocompromised mice. Next, to test the therapeutic potential of plecstatin in HCC, we employed the hydrodynamic tail vein injection to induce HCC. Together, these results demonstrate the importance of plectin in HCC cell migration, invasion, and tumorigenicity.

Acknowledgement: This work was supported by the Grant Agency of the Czech Republic (GA21-21736S) and Ministry of Education, Youth and Sports (ID Project No. LX22NPO5102 - Funded by the European Union - Next Generation EU).

Cytoskeletal Disruption Drives DNA Damage and Carcinogenesis

Petra Novotná, Miloslava Maninová, Martin Gregor

Laboratory of Integrative Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Colorectal carcinoma is the most frequent cancer with a higher propensity among inflammatory bowel disease (IBD) patients. One of the hallmarks of IBD-associated colorectal cancers (CRC) is chromosomal instability. The possible link between cancer progression and mechanical stress has not yet been fully established. Here, we use the inactivation of cytolinker protein plectin to target intestinal epithelia's mechanical homeostasis to study cytoskeletal disruption's effects on carcinogenesis.

Plectin, a large cytolinker from the plakin protein family, binds intermediate filaments, interlinks them with other cytoskeletal components, and tethers crosslinked networks to junctional structures at the cell periphery. Also, plectin anchors the cytoskeleton to the nuclear envelope. Such configuration allows cytoarchitecture to absorb mechanical forces and protect the cell nucleus with the genome. To target intestinal mechanical homeostasis, we generated a mouse model with plectin ablation in the intestinal epithelium (PleΔIEC). Further, for more detailed in vitro studies, we generated plectin-deficient cell lines using CRISPR/Cas9 technology. We show that PleΔIEC mice spontaneously develop colorectal cancer characterized by the higher frequency of p-gH2AX-positive foci. *In vitro*, plectin deletion causes the aberrant organization of intermediate filaments associated with delayed adaptive reconfiguration in response to mechanical stress. These results correlate with higher deformability and increased DNA damage in plectin-deficient cells and subsequent chromosomal instability within the cell population. Finally, we show that mechanically-induced DNA damage increases the tumorigenic potential of plectin-deficient cells.

Taken together, we demonstrate that plectin-mediated mechanical homeostasis protects epithelia against DNA damage and carcinogenesis.

Funding: This work was supported by the Grant Agency of the Czech Republic (GA21-21736S) and the Ministry of Education, Youth, and Sports (ID Project No. LX22NPO5102 - Funded by the European Union - Next Generation EU).

Inhibition of P-glycoprotein mediated multidrug resistance and STAT3 signaling pathway by polymeric conjugates bearing HIV protease inhibitor derivatives

Daniil Starenko[1], Kateřina Běhalová[1], Libor Kostka[2], Vladimír Šubr[2], Tomáš Etrych[2], Marek Kovář[1]

[1]Institute of microbiology of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Institute of macromolecular chemistry of the Czech Academy of Sciences, Prague, Czech Republic

Multidrug resistance (MDR) is one of the key problems that can appear during the chemotherapy of malignant diseases. There are several molecular mechanisms responsible for the acquirement of MDR by tumors. The most common one is overexpression of certain ABC-transporters and particularly P-glycoprotein. This transporter enables tumor cells to efficiently efflux broad spectrum of cytostatic agents. Constitutive activation of the STAT3 signaling pathway, which leads to increased expression of antiapoptotic factors, i.e. Bcl-2, could be another factor of chemoresistance. Some compounds known as chemosensitizers can inhibit P-glycoprotein or STAT3 pathway and thus overcome MDR. In this study we evaluated the potential

of HIV protease inhibitors (lopinavir, ritonavir, saquinavir, nelfinavir, atazanavir and indinavir), their derivatives prepared by esterification with 5-methyl-4-oxohexanoic acid and polymeric conjugate bearing the most potent derivative (lopinavir derivative) to inhibit P-glycoprotein and STAT3 pathway. The ability of tested compounds to sensitize tumor cell lines towards the action of conventional cytostatic drugs and their polymeric conjugates was also tested and confirmed. Finally, we evaluated the toxicity and antitumor activity of combination of the polymeric conjugates bearing doxorubicin and lopinavir derivative *in vivo*. We observed that such therapy had low toxicity and lead to considerable reduction of P388/MDR tumor growth.

Effect of acetylsalicylic acid nanopolymer on tumour antigen expression in human cell lines

Jenny Valentina Garmendia[1], Juan Bautista De Sanctis[1], Eva Koziolová[2], Markéta Frejková[3], Ivo Frydrych[1], Tomáš Etrych[2]

[1]Institute of Molecular and Translational Medicine. Faculty of Medicine and Dentistry. Palacky University. Olomouc., Olomouc, Czech Republic.
[2]Institute of Macromolecular Chemistry, Czech Academy of Sciences. Prague., Prague, Czech Republic.
[3]Institute of Macromolecular Chemistry, Czech Academy of Sciences. Prague., Olomouc, Czech Republic

Natural Killer (NK) cells and cytotoxic T CD8 cells are predominantly involved in tumour cell lysis. Both cell types share a non-major histocompatibility complex recognition of tumour antigens defined by specific activating receptors NKG2C or NKG2D or inhibitory receptors. The expression of ligands of NKG2C/NKG2D receptors renders the tumour cells susceptible to cytotoxic lysis. The study aimed to determine the effect of a nanopolymer containing acetylsalicylic acid (ASA) on the expression of tumour antigens MIC A/B and ULBP1 and ULBP2-5 (ligands of NKG2C/NKG2D receptors). The incubation was performed for 24 hr in DMED-0.1 % serum, polymer, 2 and 5 mg/ml. The ASA polymer induced a significant two and three-fold expression of MICA/B antigen on the HCT116 colon carcinoma cell line and A549 lung epithelial cell line, not affecting mutated HCT116 (p 53 KO) or the leukemic cells K562, CMT3. A significant twofold increase in ULBP1 expression was observed only in HCT116 cells. The rise in both tumour antigens resulted in increased lysis of this treated tumour cell line by cytotoxic immune cells compared to non-treated cells. The A549 cell lines were partially susceptible to lysis. No difference was observed with the mutated HCT116 or the leukemic cell lines. Conclusion: The ASA nanopolymer can induce antigen expression in HCT116 and A549 cell lines, not affecting normal cells or other cell lines.

Discrimination of resected glioma tissues using surface enhanced Raman spectroscopy and Au@ZrO_2 plasmonic nanosensor

Václav Ranc[1], Ondrej Pavlacka[2], Ondrej Kalita[3], Miroslav Vaverka[3]

[1]Institute of Molecular and Translational Medicine, Olomouc, Czech Republic.
[2]Department of mathematical analysis, Faculty of science, Palacký University Olomouc, Olomouc, Czech Republic.
[3]Department of Neurosurgery, Faculty Hospital Olomouc, Olomouc, Czech Republic

Gliomas present one of the most prevalent malignant tumors related to the central nervous system. Diagnosis and follow-up classification of gliomas mostly rely on tumor histopathologic properties, which provide limited information on the response to a follow-up therapy or different patient prognosis. A surgical extraction is still a preferred route for glioma treatment. Nonetheless, neurosurgeons still have a considerable challenge to intraoperatively and correctly detect actual margins of the targeted glioma because of its great infiltrate nature. The residues of the original tumor or existing tiny satellites being left aside are one of the main reasons leading to recurrences of the disease and a poor prognosis for the respective patients. Here we evaluated the possibility of using surface-enhanced Raman spectroscopy to analyze freshly resected brain tissues. The developed method is based on the application of Au@ZrO_2 nanocomposite, which dramatically lowers the fluorescence present in the Raman data, and thus considerably improves the quality of the measured signal. The developed method allows for rapid discriminating between the glioma's periphery and central parts, which could serve as a stepping stone toward high-precise neurosurgery.

Characterization of drug release rate: an approach using Surface plasmon resonance, capillary electrophoresis, diffusion-ordered NMR spectroscopy

Alena Libánská[1], Tomáš Špringer[2], Lucie Peštová[2], Kevin Kotalík[1], Rafal Konefal[1], Alice Tomnikova[3], Tomáš Křížek[3], Jiří Homola[2], Eva Randárová[1], Tomáš Etrych[1]

[1]Institute of Macromolecular Chemistry CAS, Prague, Czech Republic.
[2]Institute of Photonics and Electronics CAS, Prague, Czech Republic.
[3]Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic

Polymer nanocarriers with controlled drug release are considered as advanced therapeutics with enhanced therapeutic effects and minimal side effects. Precise determination of the drug release kinetic is crucial in the development of safe and effective nanomedicines. Herein, we introduce a novel application of analytical techniques,

namely surface plasmon resonance biosensor technology (SPR), capillary electrophoresis, and 1H diffusion-ordered nuclear magnetic resonance, which were innovatively applied for drug release determination. The methods were innovatively employed to quantify the pH-triggered release of three structurally different drugs (dexamethasone, docetaxel and hexyl ester of aminolevulinic acid) from a polymer carrier. Various parameters, including applicability to diverse samples, biological relevance of the experimental setup, method complexity, and analysis outcome, were used to evaluate and compare the suitability of these methods for drug release characterization. Additionally, the performance of these methods was compared to the widely-used „gold standard" high-performance liquid chromatography method. The SPR method emerged as the most versatile approach for evaluating diverse drug molecule releases. It allowed continuous observation in a flow-through setup and required only a small sample volume. However, it exhibited limited sensitivity for measuring low release rates (< several per cent) and prolonged release observation beyond 4 hours of incubation.

Age prediction from semen samples through the detection of DNA methylation

Lucie Kotková, Helena Jurtíková, Rastislav Slavkovský, Jana Vrbková, Barbora Blumová, Jiří Drábek

IMTM, Faculty of Medicine and Dentistry, Palacky Univeristy Olomouc, Olomouc, Czech Republic

Prediction of different phenotypic traits of an unknown donor from DNA can be an important intelligence tool, helping to narrow the police investigation. Probably the most rapidly developing phenotyping tool is age prediction.

Due to methylation tissue specificity, our prediction model for blood samples cannot be used for other tissues. Thus, we aimed to develop an age prediction model for semen samples, suitable for sexual assault cases.

We tested 41 CpGs in 11 genes previously published as connected to chronological age in sperm cells. These positions were tested in 5 multiplex PCR reactions. Tested DNA was extracted from whole semen, so we worked with mixed methylation profiles of sperm and epithelial cells.

We obtained methylation data for individual CpGs using bisulphite conversion and amplicon methyl-specific sequencing on Illumina MiSeq and NovaSeq.

We selected the 5 most informative CpGs and built a prediction model able to correctly predict 82,6 % of samples with a maximum error of 4 years. Here we present the parameters of the prediction model, together with a comparison of its performance in both sequencing platforms. This set of samples and sequencing results will be also used to explore the differences in age prediction for DNA from whole semen samples, separated sperm cells and epithelial cells.

Acknowledgements: LM2023033, CZ.02.1.01/0.0/0.0/16_019/0000868, CZ.02.1.01/0.0/0.0/16_026/0008448, EF16_013/0001674, LX22NPO5102, and IGA LF UP 2023_006

Differential roles of ARF GTPase-activating proteins GIT1 and GIT2 in regulating microtubule nucleation in glioblastoma cells

Vadym Sulimenko[1], Eduarda Dráberová[1], Vladimíra Sládková[1], Taťána Sulimenko[1], Věra Vosecká[1], Omar Skalli[2], Pavel Dráber[1]

[1]Department of Biology of Cytoskeleton, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Department of Biological Sciences, University of Memphis, Memphis, USA

Glioblastoma multiforme (GBM) is the most malignant form of glioma in adults, for which there is no effective treatment. Microtubules (MTs) play a critical role in cell division and migration, impacting the viability and invasiveness of malignant tumors. Reorganization of highly dynamic MTs is central to these processes. γ-Tubulin ring complexes (γ-TuRCs), composed of γ-tubulin and γ-tubulin complex proteins (GCPs), act as key regulators of MT nucleation and organization. Here, we report that GIT1 and GIT2 form complexes with γ-TuRC proteins and associate with centrosomes in U373 human glioblastoma cells. Furthermore, we found that their centrosomal association depends on Ser/Thr phosphorylation. Depletion of GITs in U373 cells revealed the distinct roles of GIT1 and GIT2 in centrosomal MT nucleation as well as cell morphology and migration. Collectively, these results suggest that GIT family isoforms play important and distinct roles in regulating MT organization and physiology of glioblastoma cells.

Supported by Ministry of Education, Youth and Sports of the Czech Republic (Program EXCELES, Project LX22NPO5102) and by the Czech Science Foundation (grant 21-30281S).

CD147 – a promising target in head and neck cancer?

Barbora Peltanová[1,2], Hana Holcová Polanská[1,2], Martina Raudenská[2,1], Klára Hánělová[2], Jiří Navrátil[1], Tomáš Vičar[2], David Kalfeřt[3], Jan Plzák[3], Jan Betka[3], Marek Grega[4], Michal Masařík[1,2,5]

[1]Department of Pathological Physiology, Faculty of Medicine, Masaryk University,, Brno, Czech Republic.
[2]Department of Physiology, Faculty of Medicine, Masaryk University,, Brno, Czech Republic.
[3]Department of Otorhinolaryngology and Head and Neck Surgery, First Faculty of Medicine, University Hospital Motol, Charles University, Prague, Czech Republic.
[4]Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.

[5]*BIOCEV, First Faculty of Medicine, Charles University, Vestec, Czech Republic*

CD147 (also known as EMMPRIN or basigin) is a transmembrane glycoprotein enriched on the cancer cell surface. CD147 is capable of interacting with many protein partners, such as VEGFR, SMAD4, integrins, MCTs, CyPA, GLUT1, CAIV, Annexin II, CAV1, and therefore is involved in mediating many cellular processes, such as cancer cell proliferation, apoptosis, adhesion, migration, invasion, metastasis, angiogenesis, tumor immune response, drug resistance and metabolism. The overexpression of CD147 has been observed in many different types of cancer, such as pancreatic, bladder, hepatocellular, ovary, lung, breast, cervical, prostate and head and neck cancer. Our previous study showed that clustering based on the expression of CD147 and MCT1, MCT4, CAV1 and ACTA2 in HNSCC tissue results in the formation of two patient clusters, which were associated with patient survival and the cluster of patients with worse overall survival was characterized by high expression of these genes in tumor tissue. These data suggest CD147 as an attractive candidate for anticancer targeting. Therefore, we aimed to characterize the phenotype of knock-out CD147 HNSCC cell line FaDu. The changes in migration and invasion ability of KO FaDu cancer cells were investigated in 3D; the shift in their metabolic activity, which resulted in changes in the production of lactate and various cytokines was analyzed by the Seahorse assay and lactate/cytokine assay; and the biomechanical properties were assessed by atomic force microscopy and time-lapse monitoring. Our previous study also demonstrated that HNSCC-derived cancer-associated fibroblasts (CAFs) are able to reprogram the cancer cell metabolism. Therefore, we generated heterotypic spheroids to investigate the ability of CAFs to rescue the wild-type phenotype in CD147 KO FaDu cancer cells. The results will be discussed more in depth on the poster.

Supported by Ministry of Health of the Czech Republic, grant nr. NU21-03-00223 and by Czech Science Foundation Grant No. 21-06873S .

CD38 and tumor associated macrophages in prostate cancer immune microenvironment

Nino Vashakidze, Gvantsa Kharaishvili, Aviv Philip Goncharov, Immanule Shaul Carlebach

Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic

Introduction: Prostate cancer remains the second most common cancer in men with diverse course. The lethality of metastatic castrate-resistant prostate cancer is powered by the lack of therapeutic approaches, including immunotherapy. Strategies to relieve immunosuppression mediated by myeloid-derived suppressor cells and tumor associated macrophages (TAMs) might be effective in patients with these tumors. Interestingly, levels of infiltration by TAMs subpopulations were predictive for malignancy grade, tumor size and disease recurrence and associated with extracapsular tumour extension in prostate cancer. Therefore, deep exploration of tumor associated macrophages, study of their context-dependent and spatial heterogeneity may uncover many aspects of this question. Prostate cancer is an age-related disease. Low-grade, chronic, sterile inflammation, named “inflammaging,” is a steadfast phenomenon in elderly patients. Immune changes with aging are known as ,,immunosenescence”. Both events stimulate CD38 expression, which is implicated in tumorigenesis and tumor progression by modulating immune regulation, metabolism, signal transduction, cell adhesion, migration, and especially, in macrophage polarisation.

Aim of the study: our aim is to study tumor associated macrophages spatial heterogeneity and intensity in prostate cancer tissues and their benign counterparts and their association with pathological parameters and CD38.

Material and methods: 47 formalin fixed paraffin embedded prostate cancer samples were stained with CD163, CD68, CD204, CD38, H2AX and p16 antibodies by immunohistochemistry and statistically processed. PRAD TCGA database analysis was done to identify prostate cancer associated macrophage profiles and pathways associated with different CD38 levels.

Results: Immunohistochemical analysis demonstrated that CD38 expression was observed in benign and malignant prostate tissue compartments. We have found positive correlation between CD38 and H2AX positivity in immune cells (Rs=0,501, p=0,011) and stromal fibroblasts (Rs=0,599, p=0,001). In CD38 positive cases, significant enrichment of genes involved in fatty acid metabolism, protein secretion, reactive oxygen species pathway and more was observed. We have found higher density of CD68+ mononuclear phagocytes and CD163+ scavengers in prostate cancer adjacent stroma in comparison to stroma adjacent to benign prostate hyperplasia (both p<0,05). Our laboratory previously showed that the number of CD204+ tumor associated macrophages was also significantly higher in the malignant structure than in benign prostate hyperplasia (p<0,05). CD163+ M2 type macrophages were predominantly seen in tumor core than in tumor margin. Interestingly, CD68+ and CD163+ macrophage number was significantly higher in cases with positive lymph nodes. Analysis of TCGA prostate adenocarcinoma (PRAD) database also confirmed abundance and positive association of M2 type macrophages and negative or no association with macrophages with M1 and M0 phenotype.

Discussion: Our results confirm higher density of macrophage population in prostate cancer and are in concordance with previous findings

that M2 type, CD204+ and CD163+ macrophages are more characteristic for malignant structures. Our results were validated on publicly available PRAD dataset. CD38 positivity in stromal components highlight the role of tumor microenvironment in ageing associated inflammation. Further multiplex immunostainings will help to stratify more immune cell populations and their prognostic/predictive potential in prostate cancer.

Micelle-forming amphiphilic copolymers for the treatment of resistant solid tumors

Markéta Frejková[1], Alena Braunová[1], Martin Kaňa[2], Milada Šírová[3], Tomáš Etrych[1]

[1]Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic.
[2]Department of Otorhinolaryngology and Head and Neck Surgery, Charles University and University Hospital Motol, Prague, Czech Republic.
[3]Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Drug delivery systems (DDS) based on polymer carriers offer many advantages compared to low-molecular-weight drugs. Among others, their utilization reduces adverse effects and provides suitable pharmacokinetics, resulting in much more effective anticancer treatment[1]. However, curing resistant tumors still remains a challenge. Multidrug resistance (MDR) is often caused by the overexpression of ATP-dependent efflux pump P-glycoprotein (P-gp) in tumor cells, resulting in reduced intracellular drug concentration and the low efficacy of the tumor treatment.

In this work, we follow up our previous research based on micelle-forming amphiphilic diblock copolymers containing a hydrophilic copolymer N-(2-hydroxypropyl)methacrylamide (pHPMA) and a hydrophobic P-gp inhibitor polypropylene glycol (PPO), developed for MDR overcoming.[2] Thanks to the exciting results, we focused on improving the polymer system to obtain a system with a) PPO bound via a hydrolyzable linker to a pHPMA carrier, b) easy and reproducible synthesis, and c) the possibility to scale up. We designed, synthesized, and characterized micelle-forming amphiphilic graft copolymers, prepared by grafting of modified PPO onto pHPMA carrier, with a pH-sensitive hydrazone bond between pHPMA and PPO. During one-pot synthesis, an anticancer drug doxorubicin was also bound to the graft copolymer through a pH-triggered hydrazone bond, to prepare a system carrying both the drug and P-gp inhibitor. The physico-chemical properties of the graft copolymers can be tuned by changing/adjusting the number of PPO chains per pHPMA chain. Moreover, the selection of the pH-sensitive linker influences the release rate of the drug and PPO in the target tumor cells. The micelle-forming amphiphilic graft copolymers have shown a significant inhibition effect on P-gp on MDR tumor cell lines in vitro, and *in vivo* experiments are currently underway. We believe the DDS based on the polymer-drug conjugates with intrinsic activity overcoming MDR has great potential in the treatment of resistant tumors.

Acknowledgments

This work was supported by the Czech Academy of Sciences (project no. CNRS-22-01) and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

References

[1] K. Ulbrich, K. Holá, V. Šubr, A. Bakandritsos, J. Tuček, R. Zbořil, Chem. Rev. 2016, 116, 5338–5431.

[2] A. Braunová, L. Kostka, L. Sivák, L. Cuchalová, Z. Hvězdová, R. Laga, S. Filippov, P. Černoch, M. Pechar, O. Janoušková, et al., J. Control. Release 2017, 245, 41–51.

Regulation of enterocyte development in the intestinal epithelium by bmp ligands

Linda Berková[1], Hassan Fazilaty[2], Jan Kubovčiak[1], Monika Šťastná[1], Dušan Hrčkulák[1], Martina Vojtěchová[1], Michael Brügger[2], Vladimír Kořínek[1], Konrad Basler[2], Tomáš Valenta[2,1]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]University of Zurich, Zurich, Switzerland

The absorptive function of the intestinal epithelium is based on the presence of differentiated enterocytes in the intestinal villi. These enterocytes undergo a complex differentiation process controlled by bone morphogenic proteins (BMPs) produced by subepithelial mesenchymal cells. In this study, we investigated the role of individual members of the BMP superfamily in regulating enterocytic programs specific to villus zonation. Our results show that Bmp2 plays a critical role in promoting terminal enterocytic differentiation at the villus tip, whereas activation of Bmp4 promotes lipid metabolism and nutrient uptake. The Bmp2-driven villus tip program is activated by a canonical Smad-dependent mechanism. Furthermore, our study reveals the synergistic interaction between Bmp2 and Wnt5a in activating gene expression in the villus tip. Conventional intestinal organoids lack the majority of differentiated enterocytic subtypes, limiting their representativeness. To better mimic the cellular composition of the intestinal epithelium, we developed an organoid culture system selectively enriched with villus tip enterocytes. Our data suggest that the activity of individual Bmp ligands, in addition to the Bmp gradient, drives specific enterocytic programs. Taken together, these results provide valuable insights into the regulation of enterocyte development by Bmp ligands in the intestinal epithelium. Understanding these mechanisms has the potential

to improve organoid models of the gut and enable more detailed studies of gut physiology and pathology.

Predictive biomarkers testing - from simple tests to complex genomic profiling era

Barbora Kubová[1], Michaela Smolíková[1], Iveta Trombik[1], Kateřina Kunčíková[1], Jarmila Šimová[1], Irena Urbanovská[1,2], David Konvalinka[1], Radek Měch[1], Magdalena Uvírová[1,2]

[1]EUC Laboratory CGB a.s., Ostrava, Czech Republic.
[2]University of Ostrava, Ostrava, Czech Republic

Molecular profiling of tumors is increasingly important as more therapies are indicated in biomarker-defined patient populations. Next-generation sequencing (NGS) is the diagnostic method of choice to assess relevant biomarkers simultaneously from formalin-fixed paraffin-embedded (FFPE) tumor tissues or liquid biopsies, avoiding the need to perform sequential individual tests.

We have started analysis of selected somatic mutations of KRAS and EGFR genes by various molecular biology methods such as real time PCR, primer extension method and reverse hybridization in colorectal and NSCLC cancer patients since 2009. Although these methods have several advantages (as sensitivity, specificity, cost-effectiveness, ultrafast turnaround time etc.), they have also many limitations (as limited multiplexing capability, amount of DNA etc.). Since that time, many new biomarkers were well established in routine diagnostics. From the laboratory diagnostics perspective, it is crucial to adopt a tailored methodology to cover all the clinically relevant gene alterations in the different clinical settings.

NGS technology allows covering several different alterations simultaneously, even starting from low DNA input, and provides information on the fraction of alleles carrying the mutations. From this reason, we included UltraDeep amplicon sequencing (fastGEN technology, BIOVENDOR) in our laboratory into routine use for analysis of somatic mutations within hotspot regions of KRAS, NRAS, BRAF, EGFR and IDH genes.

Currently, complex genomic profiling (changes in DNA and RNA levels) has become increasingly important for therapeutic decision making and is growingly used for treatment selection in patients with advanced cancer. Because of that, we are using comprehensive genomic profiling of tumor sample by TruSight Oncology 500 kit (Illumina) in our lab since 2023 as well.

Interdisciplinary cooperation (molecular tumor board) has become essential in managing the treatment of patients with the approval of new targeted therapies.

Regulated cell death in 3D glioblastoma spheroids induced by near-infrared photothermal therapy using cationic gold nanorods

Monika Žárská[1], Ondřej Novák[2], Tereza Jakubcová[1], Alexandra Urbančoková[1], Filip Novotný[2], Jiří Bártek[1,3,4], Zdeněk Hodný[1]

[1]Department of Genome Integrity; Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Department of Physiology, Second Faculty of Medicine, Charles University, Prague, Czech Republic.
[3]Genome Integrity Unit, Danish Cancer Society Research Center, Copenhagen, Denmark.
[4]Division of Genome Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

Plasmonic photothermal therapy (PPTT) mediated by gold nanorods (GNRs) presents a promising approach for cancer treatment, including the diagnosis of glioblastoma multiforme. GNRs effectively absorb near-infrared light and transform it into kinetic energy leading to the selective heating of the local environment. This effect can be utilized for the precise tumor removal with minimal side effects and allows to eliminate the cancer even in sensitive brain tissue that is difficult to surgery.

Our study investigated the molecular mechanism of cell death involved in GNR-induced PPTT using 3D cell culture models. First, we treated two different glioblastoma cell lines (U87, GL261) with positively charged GNRs and prepared from them uniform-sized cell spheroids. GNRs surface-modified by (16 mercaptohexadecyl) trimethylammonium bromide (MTAB), well known for their extensive cellular uptake, were chosen as photothermal agents. Glioblastoma spheroids were then irradiated with a near-infrared (NIR) laser diode-based system (central wavelength 808 nm) set up to various laser power (10, 15, and 20 W). In a typical scenario, the most common cellular response to photothermal heating is accidental cell death – necrosis. In contrast, we observed that, besides coagulative necrosis, PPTT triggers regulated cell death depending on the laser power and GNRs concentration. During this process, laser-irradiated GNRs activated the translocation of phosphatidylserine to the outer leaflet of the plasma membrane. Moreover, photothermal stress increased the expression of heat shock protein 70 as well as protein cryopyrin (NLRP3), playing a role in programmed necrosis. Already at 5 μM concentration of metallic gold, photothermally-damaged cells could not to recover and completely stopped the proliferation.

To conclude, we found that NIR-irradiation of MTAB-GNRs effectively eliminates glioblastoma cells in 3D structures through the process, which can be modulated to induce an accidental or regulated form of cell death.

Supported by the Ministry of Health of the Czech Republic (grant nr. NV19-08-00523) and by the National Institute for Cancer Research (Programme EXCELES; project no. LX22NPO5102), which was funded by the European Union (Next Generation EU).

Oxysterols in pancreatic cancer: a comparative study of their effects on different cell lines

Alzbeta Spalenkova[1,2], Tereza Tesarova[1,2], Stepan Balatka[1,2], Marie Ehrlichova[1,2], Pavel Soucek[1,2]

[1]National Institute of Public Health, Prague, Czech Republic.
[2]Laboratory of Pharmacogenomics, Biomedical Center, Pilsen, Czech Republic

Oxysterols, 27-carbon derivatives of cholesterol, play a crucial role in cholesterol metabolism and impact various cellular processes, including signaling pathways, membrane protein activity, and membrane fluidity. Several oxysterols have been implicated in pathological conditions, including cancer. It has been observed that different oxysterols can modulate cell proliferation, apoptosis, and migration, e.g. by promoting epithelial-mesenchymal transition in cancer cells. Moreover, various in vitro studies have shown that oxysterols can potentiate or reduce the efficacy of different anticancer drugs, including doxorubicin, 5-fluorouracil, tamoxifen, and platinum derivatives. While most research on oxysterols in cancer has focused on breast carcinoma, little is known about their role in other types of carcinomas, such as pancreatic cancer.

Our new study aims to analyze the impact of nine specific oxysterols (4β-hydroxycholesterol, 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, and cholestane-3β,5α,6β-triol) on pancreatic cancer cells *in vitro*. We included two human pancreatic cell lines, Paca-44 (harboring the G12V KRAS mutation) and BxPC3 (KRAS wild-type), in our study. To examine the effects of different oxysterols, both cell lines were exposed to dilution series of each oxysterol for 72 hours. Subsequently, cell viability was assessed using the CellTiter Blue Cell Viability assay, and the IC50 values for each oxysterol were calculated using GraphPad Prism 6 software.

Our findings revealed that while some of the tested oxysterols exhibited similar effects on both cell lines, resulting in comparable IC_{50} values, others, such as 25-hydroxycholesterol and 5α,6α-epoxycholesterol, yielded different responses in Paca-44 and BxPC3 cell lines, with distinct IC_{50} values (286 μM and 20 μM for 25-hydroxycholesterol, and 103 μM and 207 μM for 5α,6α-epoxycholesterol in Paca-44 and BxPC3 cell lines, respectively). Additionally, two oxysterols, 27-hydroxycholesterol and 4β-hydroxycholesterol, demonstrated minimal or no effect on cell viability in either cell line. In the next phase, we plan to investigate the role of these selected oxysterols in the response to anticancer drugs, such as gemcitabine. This study received support from the Grant Agency of Charles University project no. GAUK 164323, program COOPERATIO "Surgical Disciplines" no. 207043, and the project National Institute for Cancer Research – NICR (Program EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Circulating and salivary DNA-based biomarkers for early diagnosis and recurrence monitoring of oropharyngeal squamous cell carcinomas

Ondrej Bouska[1], Vladimira Koudelakova[1,2], Zuzana Horakova[3], Richard Salzman[3], Marian Hajduch[1,2]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic.
[2]Laboratory of Experimental Medicine, University Hospital Olomouc, Olomouc, Czech Republic.
[3]Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Olomouc, Olomouc, Czech Republic

Background: Oropharyngeal squamous cell carcinoma (OPSCC) incidence has significantly increased over the last decades, despite the fact that overall head and neck cancer incidence decreased. The number of annually diagnosed OPSCCs has more than tripled during the last 30 years in the Czech Republic. In 2021, nearly 800 new cases of OPSCC were diagnosed,, making OPSCC cases more prevalent than cervical cancer cases. Etiologically, there are two distinct groups, including HPV-related OPSCC and non-HPV (HPV-negative) OPSCC, with even their proportional representation also being significantly changed. Currently, HPV-related OPSCCs account for the majority of all newly diagnosed OPSCCs. About 25 % of OPSCC patients develop recurrence within 5 years. Liquid biopsies, such as plasma or salivary samples, are under evaluation for treatment monitoring and recurrence detection by DNA-based biomarkers.

Methods: In this study, newly diagnosed OPSCC patients and patients in remission are enrolled. HPV tumor status was determined by broad-spectrum HPV DNA detection in fresh/FFPE tissue samples and p16 immunohistochemistry. Pre & post-treatment HPV testing in gargle lavage (GL), oropharyngeal swabs (OPS), and plasma samples were performed, followed by regular sampling according to the standard follow-up protocol. In non-HPV OPSCCs, a tumor mutation burden panel was performed to analyse the most frequently mutated genes.

Results: In total, 77 OPSCC patients have been enrolled. HPV-related OPSCC was diagnosed in 88.4 % (61/69) of patients enrolled in the study (8 OPSCCs not evaluated yet). Non-HPV OPSCC patients were more frequently diagnosed with late-stage carcinomas than HPV-related OPSCC patients. Pre-treatment analysis of oral HPV DNA and ctHPV DNA showed 91.7 % sensitivity and 100 % positivity

in newly diagnosed OPSCCs. Predominantly mutated genes in non-HPV OPSCCs were TP53 (75 %) and NSD1 (50 %) genes.

Conclusion: This study aims to validate the collection of liquid biopsies and DNA-based biomarkers detection for early diagnosis and recurrence monitoring in OPSCC patients. Preliminary results of biomarkers analysis showed promising results for early detection of even early-staged OPSCC and its applicability in clinical practice. This study is ongoing, including new patient enrollment.

This study was supported by the Internal Grant Agency of Palacky University (IGA LF UP 2023_006), the European Regional Development Fund-Project ENOCH (CZ.02.1.01/0.0/0.0/16_019/0000868), and the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

Positron emission tomography imaging of Klebsiella pneumoniae infection using gallium-labelled siderophores

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

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.
[2]Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic

Introduction: Klebsiella pneumoniae (KP) is an opportunistic bacterial pathogen that poses threat to immunocompromised patients, especially in the hospital setting. To prevent the spread of the pathogen through the medical facility and to provide an adequate treatment to the patients, it is necessary to quickly diagnose the causative agent. In addition, given the growing threat of antibiotic resistance, it is more than ever essential to have appropriate diagnostic tools available. For this reason, here we explore the possibility of using radiolabelled siderophores, chelators produced by microorganisms for iron scavenging, for positron emission tomography imaging of KP infection. By replacing the iron in siderophores with radioactive gallium-68, it is possible to detect siderophores by positron emission tomography, thereby detecting the pathogen. Based on previous in vitro experiments, we have selected several siderophores that have suitable properties and are therefore applicable candidates for *in vivo* imaging. Materials and Methods: Selected siderophores were radiolabelled with gallium-68. The resulting radiochemical purity of complexes was tested on radio-iTLC. *In vitro* uptake of radiolabelled siderophores was compared in various KP strains. *In vivo* PET/CT imaging was performed on healthy mice, on mice in muscle infection model and on rats in lung infection model.

Results: All siderophores were labelled with high radiochemical purity (>95 %). Uptake in KP cultures varied depending on the KP strain and siderophore. *In vivo*, we observed mainly renal excretion and low activity in blood 90 min p.i. in most siderophores. In addition, some of the selected siderophores showed activity in liver and intestine. We observed signal accumulation in the site of infection in both infection models. Conclusion: We were able to successfully radiolabel various siderophores with high radiochemical purity and demonstrated that the resulting complexes have high in vitro uptake in KP. *In vivo* the siderophores showed quick pharmacokinetics and accumulation at the site of infection both infection models. Funding: Funded by the National Institute of Virology and Bacteriology (Program EXCELES, ID: LX22NPO5103) – financed by European union – Next Generation EU and the Internal Grant Agency of Palacky University (Project number: IGA_LF_2023_006).

Establishment and characterization of preclinical models derived from circulating tumor cells

Zuzana Kahounová[1], Markéta Pícková[1,2,3], Stanislav Drápela[4], Ráchel Víchová[1], Jiřina Procházková[1], Jiří Navrátil[5], Pavel Fabián[6], Karel Souček[1,2,3]

[1]Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic.
[2]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic.
[3]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic.
[4]Department of Molecular Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, USA.
[5]Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, Czech Republic.
[6]Department of Oncological Pathology, Masaryk Memorial Cancer Institute, Brno, Czech Republic

Circulating tumor cells (CTCs) are mediators of tumor dissemination, entering the peripheral circulation from the primary tumor and metastases and being transported to distant sites in the body. Because of their easy availability from the peripheral blood, CTCs have been extensively studied as a possible insight into the metastatic cascade. Preclinical models derived from CTCs, such as xenografts (CDXs) and *in vitro* cell cultures, are urgently needed to understand the biology of CTCs, their role in dissemination and to find potential drugs targeting CTCs.

In this work, we present the establishment and characterization of an *in vivo* CDX model and CDX-derived *in vitro* cell culture of progressive breast cancer. The CTCs-enriched fraction was able to form a tumor (CDX) under the renal capsule 6 months after implantation

and formed CDX was propagated subcutaneously in several passages. Next, a single cell suspension from the formed tumor xenograft was stained for the human CD298 marker and CD298$^+$ cells were sorted using a cell sorter and propagated in 3D spheroids *in vitro*. We further evaluated the stem-like potential of the established cell culture in a clonogenic assay. To investigate the tumorigenic and metastatic potential of the established cell culture, we performed subcutaneous implantation and implantation into the mammary fat pad. Finally, we evaluated several surface markers by spectral flow cytometry to describe epithelial/mesenchymal phenotype of each model.

In summary, we established an *in vivo* and in vitro model of progressive breast cancer derived from CTCs. Characterization of these models may help us understand the plasticity and behavior of CTCs during tumor progression and test potential CTCs-targeted therapies.

This work was supported by grants from the Czech Health Research Council (NU21-08-00023, NU20-03-00201) and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Circulating tumor cell detection and characterization in solid tumors

Pavel Stejskal[1], Josef Srovnal[1], Emil Berta[1,2], Alona Rehulkova[1], Lubomir Vecera[3], Tomas Gabrhelik[3], Filip Haiduk[4], Jan Maca[4], Jan Bruthans[5], Pavel Michalek[5], Pavla Kourilova[1], Marian Hajduch[1]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Czech Advanced Technology and Research Institute, Palacky University and University Hospital in Olomouc, Olomouc, Czech Republic.
[2]Ringerike Hospital, Honefoss, Norway.
[3]Tomas Bata Hospital, Zlin, Czech Republic.
[4]University Hospital Ostrava, Ostrava, Czech Republic.
[5]General University Hospital, Prague, Czech Republic

Introduction: Liquid biopsy (LB) is a novel diagnostic concept based on detecting and analyzing tumors using biomarkers circulating in the body fluids, typically in peripheral blood. Although LB is not a standard tool in the clinic, much progress has been made in the development of the LB methods. CTCs are the primary or metastatic tumor cells released into the bloodstream and are considered precursors of distant metastatic spread and can act as a prognostic and predictive biomarker. Here, we focus on the implementation of CTC detection and characterization in colorectal cancer (CRC), the leading cause of cancer-related deaths worldwide, and glioblastoma multiforme (GBM), the most aggressive subtype of central nervous system malignant tumors.

Material and Methods: The peripheral blood samples were collected in Cell-Free DNA BCT® (Streck, Inc.) tubes. CTCs were identified using CytoTrack CT11™ instrument, a semi-automated immunofluorescence microscopy detecting pan-cytokeratin and EpCAM signals for CRC, and glial fibrillary acidic protein and vimentin signals for GBM CTC detection. The whole genome amplification (WGA) of single cell-derived DNA was optimized using the CCRF-CEM cell line sorted by FACS and three different WGA kits were compared (AMPLI1TM WGA Kit, PicoPLEX® WGA Kit, and MALBAC® WGA Kit). CTCs were analyzed by confocal microscopy to exclude false positivity, subsequently isolated by the CytoPickerTM tool, and subjected to copy number variation (CNV) analysis (CytoScan HD, Affymetrix).

Results and conclusions: We have analyzed the CTC presence in the samples of 150 colorectal cancer and 61 glioblastoma multiforme patients with positivity rates of about 32,4 % and 29 %, respectively. We have compared the WGA methods and selected the PicoPLEX® WGA kit as the most optimal approach for CNV analyses. We have also implemented the CNV analyses of CTCs, revealing cancer-related as well as non-cancer aberrations.

Acknowledgment: This study was supported by the Ministry of Health of the Czech Republic (NV18-03-00470), European Union - Next Generation EU (LX22NPO5102), and Palacky University Olomouc (LF 2023_006).

ThermoFisher SCIENTIFIC

You don't have to be patient anymore

Next-generation sequencing

The wait is over—generate genomic insights in as little as 24 hours with fast and easy NGS

The Ion Torrent™ Genexus™ Dx Integrated Sequencer is now CE-IVD marked. Combining simplicity, flexibility, and speed, the Genexus Dx Integrated Sequencer offers a hands-off automated next-generation sequencing (NGS) workflow with just one user touchpoint and a dual-mode software that enables both diagnostic and research applications. Now every lab can offer nucleic acid to NGS report in a single day.

Learn more at **thermofisher.com/genexusdx**

ion torrent

Lupane Derivatives Inhibit Gli1-mediated Transcription in Human Glioblastoma Cell Line via direct interaction with Gli1

Ivo Frydrych[1], Jiří Řehulka[1], Jan Šarek[1], Lucie Borková[2], Jiří Hodoň[2], Martina Medvedíková[1], Hanuš Slavík[1], Jana Vrbková[1], Petr Džubák[1], Marián Hajdúch1, Milan Urban[1]

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

Recently, the evolutionary important Hedgehog (HH) signaling pathway has emerged as an attractive target for anticancer therapy due to its aberrant activation in a number of solid tumors such as basal cell carcinoma, medulloblastoma, rhabdomyosarcoma, and cancers of the pancreas, stomach, lung, and prostate. Constitutive activation of the HH pathway has also been reported in a subset of human gliomas. The Gli family of proteins represents key mediators of the HH pathway. Within this family of transcription factors, Gli1 and Gli2 constitute key transcription effectors regard to tumorigenesis, and constitutive activation of at least one of them is essential for cancer development.

In 2010, Eichenmüller et al. described the inhibitory effect of betulinic acid triterpene on HH signaling in rhabdomyosarcoma. Motivated by this finding, we screened a small library of structurally diverse betulinic acid derivatives as potential antagonists of GLI-mediated transcription, a key step in HH signaling. To address this goal, we developed a cell-based assay using the U-87MG glioblastoma cell line. As a result, we identified two potent inhibitors of GLI-mediated transactivation, even more potent than the known Gli1 inhibitor GANT61. Moreover, these compounds efficiently inhibited the proliferation of U-87MG tumor cells *in vitro* in a dose- and time-dependent manner. Detailed study of the mechanism of action provided genetic evidence for the blockade of the downstream pathway by these compounds via reduced expression of Gli1 transcription targets and Gli1 itself. On the other hand, the effect on ciliary localization of Smo was not affected. The observed effects could be explained as a consequence of the direct interaction of our compounds with Gli1.

Protein engineering of humanized antibody 5D3 designed to prostate cancer therapy

Zora Nováková[1], Arne Skerra[2], Barbora Havlínová[1], Cyril Bařinka[1]

[1]Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic.
[2]Lehrstuhl für Biologische Chemie, Technische Universität München, Freising, Germany

Prostate cancer (PCa) represents one of the most frequent death-related cancers in men especially in countries with high standards of living. The frequency and severity of the disease strongly incites the development of advanced therapeutic modalities. Advanced biomedical tools are mainly directed against prostate-specific membrane antigen (PSMA). PSMA represents a leading biomarker of PCa since its upregulation is linked to formation and progression of prostate tumor.

We have recently developed and engineered a PSMA-specific antibody 5D3 that displays sub-nanomolar affinity and high specificity for native PSMA *in vivo*. To advance 5D3 into preclinical testing, we humanized and subsequently modified CDR regions of parent murine 5D3 to improve its developability by mitigating immunogenicity, sequence liabilities and structural variability. Interestingly, various single point mutations in the CDR sequences led to marked variations in the production yield, stability, and affinity of humanized 5D3 (h5D3). Using bio-layer interferometry (BLI), kinetics of h5D3/PSMA complex formation/dissociation was found to strongly differ among antibody variants and these finding were correlated with cell-based assays. Overall, engineered h5D3 variants can be used for the development of new PSMA-specific theranostics.

Diversity of oral and gut microbioma in adenokarcinoma and squamous cell lung carcinoma

Jozef Škarda[1], Vladislav Raclavský[2], Zdeněk Kolář[3], Hana Bielníková Kryštofová[4], Aneta Brisudova[5], Oldřich Motyka[6,7], Marek Szkorupa[8], Petra Švecová[9], Denisa Rozsivalová[10]

[1]Institute of Molecular and Clinical Pathology and Medical Genetics, University Hospital Ostrava, Ostrava - Poruba, Czech Republic.
[2]Department of Microbiology; Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Czech Republic
[3]Department of Clinical and Molecular Pathology: Faculty of Medicine and Dentistry, Palacky Universi-ty and University Hospital, Olomouc, Czech Republic.
[4]Institute of Molecular and Clinical Pathology and Medical Genetics, University Hospital Ostrava and Faculty of medicine University of Ostrava, Ostrava, Czech Republic.
[5]Department of Clinical and Molecular Pathology: Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic.
[6]Faculty of Mining and Geology, VŠB – Technical University of Ostrava, Ostrava, Czech Republic.
[7]Nanotechnology Centre, CEET, VŠB – Technical University of Ostrava, Ostrava, Czech Republic.
[8]Department of Surgery I, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic.

[9]*Department of Respiratory Diseases, Faculty of Medicine and Dentistry, Palacky University and Uni-versity Hospital Olomouc, Olomouc, Czech Republic.*
[10]*7 Department of Respiratory Diseases, Faculty of Medicine and Dentistry, Palacky University and Univer-sity Hospital Olomouc, Olomouc, Czech Republic*

Lung cancer is the leading cause of cancer incidence and mortality throughout the world. Previous studies state that the intestinal microbiota and microbiota in other locations have an impact on its development. It is generally known that the microbiota has proven to be a key modulator of carcinogenic processes as well as the immune response against various types of cancer cells. Several studies have proven that microbiota influences the effectiveness of immunotherapy. In our study we characterized culturable microorganisms associated with NSCLC that can be recovered from rectal swab and mouth wash. In addition, we also explored differences in the culturable microbiota with two main types of NSCLC – adenocarcinoma (ADC) and squamous cell carcinoma (SCC). With 141 patients included in the study (86 ADC and 55 SCC cases) a significant difference was observed between the two types in seven bacterial species (Collinsela, Corynebacterium, Klebsiella, Lactobacillus, Neisseria, Rothia, and Streptococcus), including the site of origin. The association between microbial dysbiosis and lung cancer is not clearly understood, future studies may elucidate the correlations between gut microbiota and lung cancer development.

Tracing main c-Myc isoforms endogenous expression for targeted anti-cancer therapies

Agáta Kubíčková[1], Soňa Gurská[1], Marián Hajdúch[2]

[1]Institute of Molecular and Translational Medicine- Palacky University Olomouc; Czech Advanced Technology and Research Institute - Institute of Molecular and Translational Medicine - Palacky University Olomouc, Olomouc, Czech Republic.
[2]Institute of Molecular and Translational Medicine- Palacky University Olomouc, Olomouc, Czech Republic

Introduction

c-Myc is one of the most well-studied multifunctional transcription factors involved in several processes, including proliferation, differentiation, apoptosis, ribosome biogenesis, protein translation, angiogenesis, metabolism, DNA repair, immune surveillance and stem cell formation. In addition to selectively targeting more than 15% of the human genome, c-Myc acts indirectly as a transcriptional enhancer. Therefore, its deregulation promotes the malignant transformation that leads to the hallmark features of more than half of human cancers. This project aims to determine the effect of compounds from the LOPAC and Prestwick repurposing libraries on the physiological levels of the two major isoforms of the c-Myc transcription factor. Using two novel technologies (CRISPR/Cas9 and NanoLuc), reporter cell lines were established to monitor physiological levels of the c-Myc transcription factor. The uniqueness of these reporters is that they allow easy quantification of the two major isoforms of c-Myc, whose locus has a rather complex structure. The two isoforms are structurally very similar, although their properties differ. The p64 isoform is referred to as oncogenic, whereas the p67 isoform is associated with an anti-oncogenic activity.

Material and Methods

Validation of the obtained reporter was performed by verifying the molecular weight of the tagged proteins using HiBiT blot. We then proceeded to validate the specificity of the reporters using siRNA targeting c-Myc itself and pathways closely associated with it. The tested pathways are involved in c-Myc stabilization and degradation and transcription factors that directly affect c-Myc expression and c-Myc transcriptional targets.

Results and Discussion

Since we will use high throughput screening (HTS) to determine the activity of compounds in the LOPAC and Prestwick libraries, the NanoLuc assays were optimized to a 384-well plate format, and candidate reference antagonists were tested. The selection was made from compounds interfering with the binding of c-Myc to the promoters of its target genes. In addition, BRDi or MEKi were chosen from compounds reducing c-Myc expression. Potential reference antagonists were tested in a dose-dependent manner for 6 hours.

Conclusion

For HTS alone, 10074-G5 and OTX015 at a concentration of 50 µM were preferred according to their highest activity. HTS of LOPAC and Prestwick libraries will soon be performed using our facility‘s upgraded, fully automated robotic platform.

This work was supported by grants: The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU; the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130), and by the Internal Grant of Palacky University Olomouc (IGA_LF_2023_025).

Expression-methylation profile diversity of long non-coding RNAs in epithelial ovarian cancer patients with different platinum-free interval

Karolina Seborova[1,2], Sunniva Bjørklund[3], Thomas Fleischer[4], Lukas Rob[5], Martin Hruda[5], Jiri Bouda[6], Vendula Smoligova[6], Vessela Kristensen[3], Pavel Soucek[1,2], Radka Vaclavikova[1,2]

[1]Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic. [2]Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen Charles University, Pilsen, Czech Republic. [3]Department of Medical Genetics, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway. [4]Department of Cancer Genetics, Institute of Cancer Research, Norwegian Radium Hospital Oslo University Hospital, Oslo, Norway. [5]Department of Gynecology and Obstetrics, University Hospital Kralovske Vinohrady and Third Faculty of Medicine, Prague, Czech Republic. [6]Department of Gynecology and Obstetrics, University Hospital in Pilsen, Pilsen, Czech Republic

Introduction: Epithelial ovarian carcinoma (EOC) holds the highest mortality rate among gynecological carcinomas. This increased mortality can be attributed to two primary factors: the diagnosis typically occurs at advanced stages, and there is a development of resistance to anticancer therapy regimens that rely on taxanes and platinum derivatives. The development of resistance in cancer cells is a multifactorial process characterized by the complex deregulation of gene expression. Among the various regulatory mechanisms modulating the transcriptome profile, DNA methylation plays a crucial role. In this study, we aimed to explore the association between the global DNA methylation profile and expression profile of long non-coding RNA (lncRNA) and the resistance of EOC patients to adjuvant chemotherapy.

Methods: In this study, a total of 50 epithelial ovarian cancer (EOC) patients were selected, comprising 25 patients with sensitive therapy response and 25 patients with resistant therapy response. The analysis included profiling of the whole transcriptome by RNA sequencing, with focus on lncRNA, in 23 EOC patients and examination of global methylation patterns using DNA microarrays in 50 patients.

Results: Molecular profile of platinum resistant EOC patients differed from sensitive EOC patients in upregulation of five lncRNAs (ADAMTS9-AS1, TCF21-AS1, ARMC3-AS1, LINC-HIST2H3PS2-35, and LINC-BCR-4) and downregulation of three lncRNAs (LINC-IGLL5-5, LINC-TMEM121-12 and CHST6-AS1). Profile further included higher methylation of twelve lncRNAs (HTT-AS1, INTS6-AS1, KCNMB2-AS1, RP3-470B24.5, PLAC4, CALML3-AS1, CTA-407F11.8, lnc-ITGB8-1, RP11-728F11.1, RP11-728F11.3, LOC643339, RP11-114H23.1) and lower methylation of twelve lncRNA (lnc-HMGA1-2, LINC00263, NPHP3-ACAD11, RUSC1-AS1, RP4-535B20.1, RNASEH1-AS1, AC104472.1, RARA-AS1, NPHP3-AS1, SNHG29, SPATA41, XXbac-BPG308K3.6). Higher expression of ADAMTS9-AS1 in platinum-resistant patients was further observed in extended validation cohort of EOC patients (N=126), and also in TCGA database (N=169). During following in vitro studies, we observed significant lower proliferation of multidrug resistant ovarian cancer cells (NCI/ADR-RES cell line) after expression knock-down of ADAMTS9-AS1 by GapmeR technology.

Conclusion: Our study shows a complex network of dysregulated lncRNA expression and DNA methylation profile connected with the platinum resistance status of EOC patients, which should be further characterized. Study was supported by the Czech Health Research Council grant AZV no. NU22-08-00186, Cooperatio program no. 207035, “Maternal and Childhood Care” by 3rd Faculty Medicine, Charles University and the project National Institute for Cancer Research – NICR (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

Exploring the Role of Notch Signaling Pathway in Ovarian Carcinoma: Potential Therapeutic Targets and Biomarkers

Alzbeta Spalenkova[1,2], Karolina Seborova[1,2], Kamila Koucka[1,2], Marie Ehrlichova[1,2], Lukas Rob[3], Martin Hruda[3], Jiri Bouda[4], Alena Bartakova[4], Lei Chen[5], Hersh Bendale[5], Iwao Ojima[5], Pavel Soucek[1,2], Radka Vaclavikova[1,2]

[1]Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic. [2]Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic. [3]Department of Gynecology and Obstetrics, University Hospital Kralovske Vinohrady and Third Faculty of Medicine, Prague, Czech Republic. [4]Department of Gynecology and Obstetrics, University Hospital in Pilsen, Pilsen, Czech Republic. 5Institute of Chemical Biology & Drug Discovery, Stony Brook University - State University of New York, Stony Brook, USA

Ovarian carcinoma has a significant incidence rate, with thousands of new cases diagnosed each year. Unfortunately, mortality rates remain high due to late diagnosis often resulting from the lack of specific symptoms and effective screening methods. Additionally, therapy resistance poses a major challenge in the treatment of ovarian carcinoma, further complicating the management of the disease. Therefore, there is an effort to develop new anticancer therapeutics that aim to overcome such resistance. Among these derivatives are Stony Brook taxanes (SB-Ts). The Notch signaling pathway plays

a crucial role in cancer development and progression. Research findings indicate that the disruption of the Notch signaling pathway plays a role in the development of cancer, the renewal of cancer stem cells, angiogenesis, and resistance to chemotherapy.

In the first phase of our study, we analyzed gene expression levels of nine Notch pathway genes in 152 ovarian carcinoma tissues and 15 non-tumor ovarian tissues by qPCR. As a result, we found that NOTCH3, NOTCH4, and JAG2 genes were upregulated and NOTCH2 was downregulated in tumor tissues when compared to non-malignant ovarian tissues. Moreover, we found significant associations between Notch signaling genes and clinico-pathological characteristics, such as histological subtype, tumor grade, tumor progression, or tumor size.

In the next phase of the study, we analyzed the expression of Notch signaling pathway in CDX models derived from NCI/ADR-RES cell line, a multidrug-resistance model of ovarian carcinoma. Immunodeficient mice with CDX tumors were treated with paclitaxel, experimental SB-Ts (SB-T-121605, SB-T-121606), or their combination. After that, tumors were harvested and the gene expression was evaluated by qPCR. As a result, we found statistically significant downregulation of most of genes from the Notch signaling pathway after all tested therapeutic regimes.

In conclusion, we found new candidate genes of Notch signaling pathway for future studies, possibly making this pathway an attractive target for cancer therapeutics, such as paclitaxel or experimental Stony Brook taxanes. This study received support from the Czech Science Foundation (grant no. 21-14082S), the Czech Ministry of Education, Youth and Sports (INTER-ACTION project no. LUAUS23164), and the National Institutes of Health (NIH), U.S.A. (grant R01 CA103314).

Antiviral Activity of Selected Lamiaceae Essential Oils and Their Monoterpenes Against SARS-Cov-2

Ermin Schadich[1], Sanja Ćavar Zeljković[2,3], Petr Džubák[1,3], Marián Hajdúch[1,3], Petr Tarkowski[2,3]

[1]Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic.
[2]Crop Research Institute (CRI), Palacky University, Olomouc, Czech Republic.
[3]Czech Advanced Technology and Research Institute, Palacky University, Olomouc, Czech Republic

Plant essential oils are known to have the different pharmacological properties including anti-inflammatory and antiviral activity. The antiviral activity of plant essential oils is attributable to their monoterpene and sesquiterpene content and composition. Our study focused on the *in vitro* antiviral property of selected essential oils of Lamiaceae plant species and their monoterpenes against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Nineteen essential oils were extracted from dried material using hydrodistillation procedure and of, and their monoterpene profiles were determined using GC/MS analyses. Both essential oils and their monoterpene components were tested for cytotoxicity to Vero76 cells, and their antiviral activity against SARS-CoV-2 was tested in infected Vero 76 cells. The essential oils of four *Mentha species*, i.e., *M. aquatica L. cv. Veronica, M. pulegium L., M. microphylla K.Koch, and M.* x *villosa Huds.*, but also *Micromeria thymifolia (Scop.) Fritsch* and *Ziziphora clinopodioides Lam.*, and five different monoterpenes, i.e., carvacrol, carvone, 1,8-cineol, menthofuran, and pulegone, inhibited the SARS-CoV-2 replication in the infected cells. However, the differences were also in antiviral activity of essential oils and monoterpenes. The IC50 concentrations of carvone and carvacrol were 80.23 ± 6.07 μM and 86.55 ± 12.73 μM, respectively, and the other monoterpenes were less active (IC50 > 100.00 μM). Structure-activity relations analyses of related monoterpenes showed that the presence of keto and hydroxyl groups is indispensable for activity of carvone and carvacrol, respectively. Furthermore, the carvone-rich essential oil of M. x villosa had the greatest activity among all active essential oils (IC50 =127.00 ± 4.63 ppm) while the other active oils exhibited mild (140 ppm < IC50 < 200 ppm) to weak antiviral activity (IC50 > 200 ppm). Essential oils and their monoterpenes had limited or no cytotoxicity against Vero 76 cells. The results of hierarchical cluster analysis revealed that the differences in the antiviral activity of essential oils were directly associated to the antiviral efficacies of their distinct single monoterpenes. The findings from this study showed the novel antiviral property of plant essential oils and monoterpenes that might be used in the development of different measures against SARS-CoV-2.

This study was published in 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.

Cytoplasmic p21 affects caspase-independent cell death pathway – parthanatos – in colon cancer cells following genotoxic stress

Viswanath Das, Marián Hajdúch, Lenka Kuběnová

Institute of Molecular and Translational Medicine, Olomouc, Czech Republic

The tumour suppressor function of p21 is well-established and has been demonstrated in several studies. However, p21 can also act as an oncogene to promote

cancer progression, migration, and resistance to chemotherapy and radiotherapy under certain conditions. This dual role of p21 depends on its cytoplasmic or nuclear localisation and post-translational modifications. P21 accumulates cytoplasmically following phosphorylation at Thr-57, Ser-130, Thr-145, Ser-146 or Ser-153 by different kinases, prominently Akt/PKB. Phosphorylation at Thr-145 is linked to resistance to apoptosis in HER2/neu-overexpressing cells and paclitaxel in glioblastoma cells. Cytoplasmic p21 has also been linked to decreased response to tamoxifen in MCF-7 cells, NUPR1-mediated chemoresistance to doxorubicin in p53-deficient, triple-negative SUM159 cells, cisplatin resistance in ovarian and testicular embryonic carcinoma cell lines, and resistance to 5-fluorouracil in HCT116 and SW837 cells. In p53-deficient cancer lines, prolonged p21 induction results in a subpopulation of highly proliferating p21-expressing cells featuring increased genomic instability, aggressiveness and resistance to cisplatin, doxorubicin and paclitaxel. The ultimate goal of cancer therapy is cell death, which may occur via different mechanisms. Therefore, it follows that through disrupting these pathways, cytoplasmic p21 likely plays a role in chemoresistance/radioresistance.

In this study, we aimed to determine if chronically overexpressed and cytoplasmically mislocalised p21 alters parthanatos, a distinct form of programmed cell death, following treatment with DNA alkylating agent. To test our hypothesis, we transiently expressed wildtype p21, phospho-mimetic mutant p21 (T145D) and non-phosphorylatable mutant p21 (T145A) in p21-null human colorectal HCT116 cells. These cells were subjected to insulin stimulation to activate Akt/PKB-mediated T145 phosphorylation of p21. Methyl-nitro-nitroso-guanidine (MNNG), a DNA alkylating agent, was used as a genotoxic stressor to induce parthanatos. The induction of parthanatos was monitored by the changes in poly (ADP-ribose) levels and nuclear translocation of apoptosis-inducing factor (AIF), an important event in parthanatos. The interaction of mislocalised p21 with targets associated with parthanatos was analysed by immunoprecipitation of p21 following cellular fractionation of cells.

Our data show that cells expressing phospho-mimetic mutant p21 (T145D) have a decreased nuclear translocation of AIF and PAR activation, which are needed to induce parthanatos following MNNG treatment. Analysis of cells sensitive and resistant to MNNG shows that nuclear translocation of AIF is hampered in resistant cells expressing wildtype or T145D p21. Immunoprecipitation of p21 from MNNG-treated cells shows increased interaction between p21 and AIF in cells expressing wildtype p21 and phospho-mimetic T145D p21 but not in those cells expressing non-phosphorylatable T145A p21. This evidence may shed some light on why cells expressing hyperphosphorylation-mimicking p21 are less sensitive to anticancer drugs, particularly those that induce caspase-independent cell death. In conclusion, our studies reveal that the chronically upregulated cytoplasmic p21, possibly its phosphorylated forms, has multiple cellular targets, including AIF. Sequestration of AIF by p21 might affect how cancer cells respond to chemotherapy and radiotherapy, resulting in cell death by caspase-independent mechanisms, such as parthanatos.

Acknowledgement: This work was supported by the European Union - Next Generation EU (EXCELES, LX22NPO5102 and LX22NPO5107) from the Ministry of Education, Youth and Sports of the Czech Republic (MEYS) and infrastructural projects (CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053).

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

Monika Vidlarova[1], Josef Srovnal[1], Emil Berta[1,2], Lubomir Vecera[3,4], Petr Prasil[5], Tomas Gabrhelik[6], Martin Lovecek[7], Pavel Skalicky[7], Jozef Skarda[8], Pavla Kourilova[1], Marian Hajduch[1]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc, Olomouc, Czech Republic.
[2]Department of Anaesthesia and Intensive Care, Ringerike Hospital, VVHF, Hønefoss, Norway.
[3]Department of Emergency Medicine, The Tomas Bata Regional Hospital in Zlin, Zlin, Czech Republic.
[4]Department of Paediatric Anaesthesiology and Intensive Care Medicine, Medical Faculty of Masaryk University, University Hospital Brno, Brno, Czech Republic.
[5]Department of Anesthesiology and Intensive Medicine, Landesklinikum Amstetten, Amstetten, Austria.
[6]Department of Anaesthesiology, Resuscitation and Intensive Care, The Tomas Bata Regional Hospital in Zlin, Zlin, Czech Republic.
[7]Department of Surgery I, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.
[8]Institute of Molecular and Clinical Pathology and Medical Genetics, Faculty of Medicine, University of Ostrava and University Hospital Ostrava, Ostrava, Czech Republic

Introduction: Pancreatic cancer (PDAC) is one of the most common causes of cancer-related death in the world. In PDAC patients opioids and cannabinoids are often used to treat pain, nausea and side effects of chemotherapy. Additionally, the effects of postoperative analgesia

with morphine and piritramide on survival among pancreatic cancer patients are unknown, as are their interactions with opioid/cannabinoid receptor expression in tumor tissue. In our study, we investigated expression of opioid growth factor receptor (OGFR), opioid receptor mu (OPRM), kappa (OPRK), delta (OPRD) and nociceptin (OPRL) and cannabinoid receptor 1 and 2 (CB1 and CB2) in PDAC patients tumor tissue and we analyzed relationship between their gene expression and patients survival. We also analyzed the effect of the used analgesia (piritramide/morphine) on patients survival.

Methods: Gene expression of 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, ver. 3.5.2. Specific cut-off values for analysed receptors expression were determined using the maxstat() function (maxstat R package, v. 0.7-25). 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 (67.6 %) received morphine analgesia and 23 (32.4 %) received piritramide analgesia in the postoperative period. Patients receiving morphine analgesia had significantly longer cancer specific survival (CSS) than those receiving piritramide analgesia (22.4 months vs. 15 months; p = 0.04). In a multivariate Cox model analysis stratified by disease stage and adjusted for age and sex, piritramide had a negative effect on CSS (HR = 2.904; CI = 1.485 - 5.679; p = 0.002) when compared to morphine. 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).

Acknowledgement: This study was supported by European Union - Next Generation EU (LX22NPO5102) and Palacky University Olomouc (LF 2023_006).

Morphological profiling of 2500 bioactive compounds by high throughput screening using cell painting assay

Alzbeta Srovnalova, Aleksandra Ivanova, Pavel Polishchuk, Petr Dzubak, Marian Hajduch

Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic

INTRODUCTION

Morphological or cytological profiling utilizing the cell painting assay is grounded in the principles of high-content and multiplexed image-based analysis. This method combines the application of fluorescent stains targeting specific cellular organelles (such as the nucleus, endoplasmic reticulum, mitochondria, Golgi apparatus, nucleolus, F-actin, cytoplasmic RNA, and plasma membrane) with automated image analysis. Through this approach, both quantitative and qualitative information concerning cellular organization and morphology can be obtained. A notable advantage of this assay lies in its ability to visualize a wide array of organelles, facilitated by high-content imaging, which in turn enables the derivation of multiple phenotypic parameters at the single cell levels. Consequently, this technique aids in the identification and enhanced comprehension of the effects of chemical compounds. Phenotypic parameters or features encompassing aspects such as shape (width, roundness), threshold compactness, radial distribution, symmetry, texture, among others, are extracted via the image analysis platform. This phenotypic drug discovery approach empowers predictive model systems to evaluate therapeutic candidates and ascertain the mechanisms of action for small molecules, even in the absence of prior knowledge regarding the drug target. Morphological profiling also assumes great significance in the identification of disease-specific phenotypes and in the prediction of the biological impact and toxicity of compounds.

METHODS

In our study, we employed six distinct fluorescent probes to label eight specific cellular organelles. The subsequent imaging was conducted using a high-throughput microscopy system called CellVoyager 8000, which enabled the acquisition of data in four separate channels. The HepG2 cell line was chosen as our experimental model due to its widespread utilization in drug metabolism and hepatotoxicity studies. To induce chemical perturbations, the cells were subjected to treatment with a library comprising 2500 bioactive compounds, each accompanied by annotated modes of action. Throughout the experimental procedure, the cells were processed in four independent replicates. Following chemical exposure, the cells were fixed, stained, and subsequently subjected to analysis using a robotic platform equipped with automation capabilities. Leveraging image analysis software, we extracted over 1500 morphological features from the acquired images. This enabled the generation of detailed profiles, facilitating the identification of subtle phenotypes and enhancing our understanding of cellular responses to chemical perturbations. Bioactive compounds with their known mode of action on different cellular departments were chosen to gain a large dataset of morphological profiles to assess the biological impact of compounds on cells, and group them based on their mode of action. Each extracted

feature value was first scaled by z-score normalization using DMSO outputs as reference. Pearson correlation equation was used to calculate the correlation of those z-scores between the two technical replicates. Compounds were visualized using t-SNE and analyzed using k-nearest neighbor's approach.

RESULTS AND CONCLUSION

The data obtained from the image analysis, encompassing both quantitative and qualitative assessments, yielded the morphological profiles of the 2500 bioactive compounds under investigation. Compounds with similar modes of action displayed highly similar profiles and were subsequently clustered together. These findings validate the utility of morphological profiling in predicting the modes of action for novel compounds. Moving forward, the dataset consisting of 2500 known compounds will serve as a reference to identify the modes of action for newly investigated compounds.

ACKNOWLDEDGEMENT

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

Amplification of androgen receptor and expression of miR-375 as liquid biopsy markers for monitoring of prostate cancer progression and ARTA therapy failure

Eva Szczyrbová[1], Andrea Kopová[2], Jana Stránská[3], Hana Študentová[2], Jan Bouchal[1]

[1]Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic.
[2]Department of Oncology, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic.
[3]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic

Prostate cancer (PC) cells need androgen stimuli provided by androgen receptor (AR) to maintain their growth. The treatment is based on androgen deprivation and further blocking of AR signalling pathways through AR-targeted therapy (ARTA). PC can adapt to the low androgen environment which results in castration-resistant PC. This can happen through point mutations or amplification of the AR gene and by other mechanisms. We used a liquid biopsy ccfDNA and ccfRNA markers to monitor PC progression and ARTA therapy failure. The blood samples were collected from advanced PC patients at Department of Oncology. The samples were than centrifuged (120 g, 20 min) and plasma was frozen. Two ml of baseline plasma (collected before the start of ARTA therapy, n=110) were used for ccfDNA/RNA isolation. The amplification of the androgen receptor in ccfDNA was measured by a highly sensitive droplet digital PCR and levels of miR-375 were analysed by qRT-PCR. Statistical analysis was performed in relation to the clinicopathological characteristics of the patients. The AR gene amplification was present in 13 % of baseline patients (at the start of ARTA, n=110), and the patients were significantly older (p=0.012). Importantly, they had a shorter time between castration and CRPC occurrence (p= 0.054) than patients without AR gene amplification. The patients also had increased levels of PSA, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP; p=0.008, 0.055, and 0.019, respectively) at the start of ARTA. Regarding miRNA-375, its high levels were associated with high levels of hemoglobin, leukocytes, and neutrophils (p=0.033, 0.046, and 0.012, respectively) and low levels of thrombocytes (p=0.046) at the start of ARTA. The baseline patients with high expression of miR-375 benefited from ARTA therapy for a significantly shorter time (p=0.048). The analysis of AR gene amplification and miR-375 from plasma provides useful information for disease monitoring and therapy of the advanced PC patients.

In vitro and *in vivo* efficacy of Stony-brook taxanes in paclitaxel resistant ovarian carcinoma

Marie Ehrlichova[1,2], Karolina Seborova[1,2], Alzbeta Spalenkova[1,2], Tomas Sychra[3,4], Lei Chen[5], Hersh Bendale[5], Iwao Ojima[5], Pavel Soucek[1,2], Radka Vaclavikova[1,2]

[1]Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic.
[2]Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic.
[3]Third Faculty of Medicine, Charles University, Prague, Czech Republic.
[4]Department of Surgery, University Hospital Kralovske Vinohrady, Prague, Czech Republic.
[5]Institute of Chemical Biology & Drug Discovery, Stony Brook University - State University of New York, Stony Brook, NY, USA

Taxanes play an important role in cancer therapy, including ovarian carcinoma. However, the emergence of multidrug resistance poses a significant challenge to their effectiveness. To address this issue, researchers are synthesizing novel taxane derivatives. This study focuses on the selection and analysis of potent compounds from the new taxane derivatives known as Stony Brook taxanes (SB-Ts) in ovarian carcinoma models.

Firstly, the efficacy of second-generation (SB-T-1214, SB-T-1216) and third-generation (SB-T-121402, SB-T-121605, SB-T-121606) SB-Ts was assessed on both paclitaxel-sensitive and paclitaxel-resistant cell

line models *in vitro*. Subsequently, the most promising candidates from the *in vitro* experiments, namely SB-T-121605 and SB-T-121606, were further tested *in vivo* using immunodeficient mouse cell-line derived xenografts (CDX). The results revealed that both novel SB-Ts exhibited greater potency in inhibiting tumor growth in CDX models compared to paclitaxel.

In the latest phase of the project, novel conjugates of SB-Ts were examined, wherein biotin was employed as a tumor-targeting molecule to enhance drug delivery to cancer cells while reducing toxicity. Similarly, *in vitro* experiments were conducted on ovarian carcinoma models to evaluate biotin-linker-taxane conjugates of paclitaxel, SB-T-1216, SB-T-121605, and SB-T-121606, which also exhibited greater potency in reducing cell viability compared to paclitaxel.

Collectively, third-generation SB-Ts demonstrated effectiveness in treating ovarian cancer cells both *in vitro* and *in vivo*. However, concentrations higher than or equal to 5 mg/kg were found to be toxic. Consequently, biotinylated conjugates of these SB-Ts are currently under investigation. This study received support from the Czech Science Foundation (grant no. 21-14082S), the Czech Ministry of Education, Youth and Sports (INTER-ACTION project no. LUAUS23164), and the National Institutes of Health (NIH), U.S.A. (grant R01 CA103314).

The role of the checkpoint kinase 1 (Chk1) inhibition in luminal subtype of breast carcinoma

Veronika Boušková[1,2], Marie Ehrlichová[1,2], Ivona Krus[1], Simona Šůsová[1,2], Viktor Hlaváč[1,2], Renata Koževnikovová[3], Markéta Trnková[4], David Vrána[5], Jiří Gatěk[6], Kateřina Kopečková[7], Marcela Mrhalová[8], Soňa Měšťáková[9], Pavel Souček[1,2]

[1]Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic.
[2]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic.
[3]MEDICON a.s., Prague, Czech Republic.
[4]AeskuLab, k.s., Prague, Czech Republic.
[5]Comprehensive Cancer Center of Hospital AGEL Novy Jicin, Novy Jicin, Czech Republic. [6]EUC Hospital Zlin and Tomas Bata University in Zlin, Zlin, Czech Republic.
[7]Department of Oncology, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic.
[8]Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic.
[9]Department of Surgery, Second Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic

Luminal subtype (estrogen receptor positive, ER+) is the most common and the most heterogeneous type of breast carcinoma in women. MicroRNAs (miRNAs) are short noncoding RNAs with important roles in tumor development, growth, metastasis, and drug resistance. This study focused on search for miRNAs potentially responsible for progression and chemoresistance in luminal breast carcinomas. For this purpose, we determined expression levels of all miRNAs (miRNOMe) in 101 ER+ breast carcinomas and identified 25 miRNAs associated with proliferative markers as tumor grade and Ki-67 index, and/or HER2 amplification. Using different miRNA-mRNA prediction databases, the DAVID tool for enrichment analysis, and the STRING for prediction of protein-protein interactions we identified CHEK1, CDC25A, and CCNE1 as main target genes affecting the proliferation of ER+ carcinomas and miR-195-5p, miR-497-5p and miR-125b-5p as their potential regulators. By determining the expression of these genes in a cohort of 217 patients, we found that high levels of CHEK1 and CDC25A was significantly associated with shorter disease-free survival in patients treated with adjuvant or neoadjuvant chemotherapy, respectively. On the other hand, high levels of miR-195-5p associated with longer disease-free survival in patients treated with neoadjuvant chemotherapy. Using the cell transfection method, we found that miR-195-5p significantly inhibits the expression of all three studied genes (CDC25A, CHEK1, and CCNE1) at the mRNA level in both ER+ (T-47D, MCF-7) and ER- (BT-20) breast cancer cell lines. The influence of miR-195-5p and Chk1 inhibitor Rabusertib on doxorubicin cytotoxicity in these three cell line models has been further evaluated.

Decreased glioblastoma growth and macrophage infiltration in a disintegrin and metalloproteinase domain-containing protein 8 (ADAM8) knockout mice

Nikola Ternerová, Magdaléna Houdová Megová, Tereza Šváblová, Petr Bušek, Aleksi Šedo

Laboratory of Cancer Cell Biology, Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University,, Prague, Czech Republic

Background: Proteases play a crucial role in the progression, invasiveness, and immunomodulation of solid tumors. A disintegrin and metalloproteinase domain-containing protein 8 (ADAM8) is expressed by various immune cell subsets and may affect their migration. Previous

studies have shown increased ADAM8 expression in glioblastoma (GBM) and its association with worse prognosis. The effect of ADAM8 expression on immune cell infiltration in the GBM microenvironment has not been described yet. This study aimed to investigate the impact of ADAM8 expression on tumor growth and macrophage infiltration in a mouse model of GBM.

Method: The Cancer Genome Atlas (TCGA) data from Affymetrix expression arrays platform HG-U133A were utilized for in silico analysis of expression of ADAM8 and CD45 genes in GBM. Data from primary glioblastomas IDH-wildtype were divided into quartiles based on ADAM8 mRNA expression (ADAM8 high, n=90; ADAM8 low, n=90). MCP Counter was used to estimate the abundance of immune cell subpopulations. Syngeneic GI261 cells were orthotopically implanted into ADAM8 wild type (wt) and ADAM8 knock out (ko) mice to investigate tumor growth and macrophage infiltration at the tumor site. Tumor volume analysis was conducted using hematoxylin and eosin staining with the Volumest tool in ImageJ. Infiltration of leukocytes and macrophages into the tumor was examined using immunohistochemical detection of CD45 and F4/80 as markers of leukocytes and macrophages, respectively.

Results: GBM with high ADAM8 expression were more infiltrated with immune cells. Abundance of various immune cell subpopulations including macrophages was higher in GBM with high ADAM8 expression. In an immunocompetent orthotopic glioblastoma mouse model, tumors in ADAM8wt mice were larger than tumors in ADAM8ko mice. Leukocytes did not infiltrate more into ADAM8wt mice. Higher infiltration of macrophages in the tumor tissue was observed in ADAM8wt mice compared to those lacking ADAM8 expression.

Conclusions: Deficiency of ADAM8 in the host leads to decreased glioblastoma growth and lower macrophage infiltration.

Acknowledgment: This work was supported by the Center for Tumor Ecology (CZ.02.1.01/0.0/0.0/16_019/0000785), National Institute for Cancer Research (LX22NPO5102), Cooperatio Program „Oncology and Haematology“.

Fibroblast activation protein expressing mesenchymal cells influence T cell quantity and function in glioblastoma

Magdalena Houdová Megová[1], Nikola Ternerová[2], Tereza Šváblová[2], Barbora Výmolová[2], Eva Balážiová[2], Petr Bušek[2], Aleksi Šedo[2]

[1]Laboratory of Cancer Cell Biology, Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, Prague, Czech Republic, Praha, Czech Republic.
[2]Laboratory of Cancer Cell Biology, Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, Prague, Czech Republic, Prague, Czech Republic

Background: Glioblastomas (GBMs) are malignant brain tumors characterized by highly aggressive growth and ability to induce local, as well as systemic immunosuppression. In epithelial cancers, the presence of mesenchymal cells expressing fibroblast activation protein (FAP) has been associated with affection of T cells functions. Recently, we have described the presence of FAP+ mesenchymal cells in GBMs. This study aimed to investigate their impact on the quantity and functionality of T cells within the microenvironment of GBM.

Method: Immunohistochemistry was employed to detect T cells in paraffin-embedded GBM tissue sections. To assess the co-localization of FAP+ mesenchymal cells and T cells, double labeling IHC was performed on frozen sections. FAP+ mesenchymal cells were derived from human GBMs. T cells were isolated from buffy coats of healthy donors. T cells were activated using anti CD2, CD3 and CD28 antibodies and cultivated in conditioned media from FAP+ mesenchymal cells. T cell proliferation and the expression of activation markers CD69 and CD25 were analyzed using flow cytometry. Concentration of TGF-β1 in conditioned media from FAP+ mesenchymal cells was evaluated by ELISA.

Results: GMBs with a higher quantity of FAP+ mesenchymal cells exhibited higher abundance of CD3+ as well as CD8+ T cells (fold change = 2, p>0.01). Notably, T cells were typically localized in close proximity to FAP+ mesenchymal cells. Upon exposure to conditioned media from FAP+ mesenchymal cells, T cell proliferation and expression of the CD69 activation marker were decreased. 6 out of 10 FAP+ mesenchymal cell cultures produced TGF-β1, which can contribute to affection of T cell functions within the context of GBM.

Conclusions: The presence of FAP+ mesenchymal cells may impact T cell functions by restricting their activation and proliferation.

Acknowledgment: This work was supported by the Center for Tumor Ecology (CZ.02.1.01/0.0/0.0/16_019/0000785), Charles University project GAUK 365022, National Institute for Cancer Research (LX22NPO5102), Cooperatio Program „Oncology and Haematology“.

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

Narendran Annadurai, Agáta Kubíčková, Ivo Frydrych, Marián Hajdúch, Viswanath Das

Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic

Accumulation of aggregated tau protein into neurofibrillary tangles is considered to be the major neuropathological hallmark of several neurodegenerative tauopathies, including Alzheimer's disease (AD), progressive supranuclear

palsy (PSP), and corticobasal degeneration (CBD) [1]. Pre-existing tau aggregates act as "seeds", aiding in the aggregation of soluble tau and propagation of pathological aggregates across cells through the transmission of these proteopathic tau seeds [2]. Additionally, the brain of nontransgenic mice injected with tau extracts from postmortem brains of patients with AD, CBD, and PSP displayed differences in cell-type specificity of tau aggregate transmission [3]. These findings implied that prion-like propagation of unique structural conformations of tau aggregates (prion-like tau strains) accounts for the neuropathological heterogeneity of tauopathies. Ubiquitin-proteasome system and autophagy-lysosome pathway have been reported as the two most important intracellular machinery associated with eliminating tau protein aggregates and misfolded proteins [4].

R2 and R3 repeat fibrils have distinct aggregation kinetics in vitro and seeding potency in tau biosensor cells [5, 6]. In this study, we hypothesized that distinct tau strains degraded by different protein degradation mechanisms might contribute to the clinicopathologic variety of tauopathies. The difference in the time-dependent intracellular seeding in cells induced with R2 and R3 fibrils was responsible for the difference in autophagy failure. These differences might contribute to neuropathological heterogeneity and disease progression between 3R and 4R tauopathies due to the absence of R2 in 3R tau isoforms. Alterations in p62 and LC3AB-II/I levels that define proteotoxic stress and autophagy failure were observed earlier in R2-induced cells than in R3-induced cells. Conversely, LAMP1 levels remained unaffected, indicating a failure in the fusion of aggregate-containing autophagosomes with lysosomes. The failure in the autophagic degradation might be responsible for increased seed-dependent intracellular aggregation in induced cells. Our study indicates that this difference in the buildup of insoluble tau inclusions might have contributed to the difference in autophagic failure in fibril-induced cells. Next, we tested the effects of autophagy inducers on the clearance of intracellular tau aggregates in fibril-induced cells. EGCG was most effective in autophagy induction and reducing p62 levels, significantly decreasing intracellular seeding. However, EGCG treatment was less effective in clearing intracellular tau aggregates in R2-induced cells than in R3-induced cells. Overall, this study explains the differences in autophagy failure and autophagy induction-mediated clearance of intracellular tau aggregation in cells seeded with exogenous R2 and R3 fibrils.

References: 1. Chung, Dah-eun Chloe, et al. „Cellular and pathological heterogeneity of primary tauopathies." Molecular neurodegeneration 16.1 (2021): 1-20. 2. Frost, Bess, et al. „Propagation of tau misfolding from the outside to the inside of a cell." Journal of Biological Chemistry 284.19 (2009): 12845-12852. 3. Narasimhan, Sneha, et al. „Pathological tau strains from human brains recapitulate the diversity of tauopathies in nontransgenic mouse brain." Journal of Neuroscience 37.47 (2017): 11406-11423. 4. Nixon, Ralph A. „The role of autophagy in neurodegenerative disease." Nature medicine 19.8 (2013): 983-997. 5. Annadurai, Narendran, et al. „Tau R2 and R3 are essential regions for tau aggregation, seeding and propagation." Biochimie 200 (2022): 79-86. 6. Annadurai, Narendran, et al. „Time-and dose-dependent seeding tendency of exogenous tau R2 and R3 aggregates in cells." Biochemical and Biophysical Research Communications 653 (2023): 102-105.

Acknowledgement: This work was supported in parts by the Internal Student Grant Agency of the Palacký University in Olomouc, Czech Republic (IGA_LF_2022_033), 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 Generation EU from the Ministry of Education, Youth and Sports of the Czech Republic (MEYS), and the Grant Agency of the Czech Republic (23-06301J).

Immunomodulatory effect of mesenchymal cells on T cells in a glioblastoma 3D spheroid model

Tereza Šváblová, Nikola Ternerová, Magdalena Houdová Megová, Barbora Výmolová, Eva Balážiová, Petr Bušek, Aleksi Šedo

Laboratory of Cancer Cell Biology, Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, Prague, Czech Republic

Background: Glioblastoma (GBM) is the most malignant primary brain tumor with a poor prognosis. Profound systemic as well as local immune suppression is present in GBM patients. Recently, mesenchymal cells with characteristics of pericytes or cancer-associated fibroblasts were described in GBM. Mesenchymal cells are considered to be immune modulators in GBM microenvironment, but their particular effect on the immune cells is still not fully elucidated. Since studies performed in 2D settings lack the ability to fully recapitulate the complexity of the tumor microenvironment and murine models show various disparities regarding the functioning of the immune system, a broader model portfolio is needed. Thus, to study effects of GBM microenvironment on T cells, we established a 3D spheroid model comprising GBM-derived cancer and mesenchymal cells as well as human T cells.

Materials and methods: Paired glioma stem-like cell and mesenchymal cell cultures were derived from 3 human GBMs. CD4+ T cells were isolated from buffy coats of healthy donors by Ficoll paque gradient separation, followed by negative magnetic isolation. T cells were activated by

anti-CD2, anti-CD3 and anti-CD28 antibodies before co-cultivation in spheroids. Spheroids were prepared by embedding the cells in GeltrexTM. After 5 days of co-culture in spheroids, the expression of T-cell activation markers (CD69, CD25) and immune checkpoint molecules (LAG3, TIM-3 and PD-1) was analyzed by flow cytometry. Immunohistochemistry was used to evaluate the spatial distribution of cells in spheroids to address their possible interactions.

Results: We prepared co-cultures of CD4+ T cells in spheroids comprising glioma stem-like cells with and without mesenchymal cells. Glioblastoma-derived stem-like, mesenchymal cells as well as CD4+ T cells were evenly dispersed within the spheroids. CD4+ T cells showed higher expression of the activation marker CD69 in spheroids with mesenchymal cells. Also, the expression of checkpoint molecule PD-1 was higher on T cells in spheroids with mesenchymal cells, but no differences were seen in TIM3 and LAG3.

Conclusion: We have established a 3D spheroid model to study the role of mesenchymal cells in GBM microenvironment. Mesenchymal cells may contribute to immune modulation in GBM microenvironment by over-activation of CD4+ T cells, which can lead to their exhaustion.

Acknowledgment: This work was supported by the Center for Tumor Ecology (CZ.02.1.01/0.0/0.0/16_019 /0000785), Charles University project GAUK 365022, National Institute for Cancer Research (LX22NPO5102), Cooperatio Program „Oncology and Haematology“.

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

Kateřina Ječmeňová, Agáta Kubíčková, Jana Václavková, Soňa Gurská, Barbora Lišková, Miroslav Popper, Patrícia Žižkovičová, Marián Hajdúch, Petr Džubák

Institute of Molecular and Translational Medicine, Olomouc, Czech Republic

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-171, fluorescent-labeled A3 adenosine receptor antagonist, and the agonist PNH173 confirmed the binding to the orthosteric binding site on A3AR.

The cell lines MIA Paca-2, CCRF-CEM, and K562 were treated with PNH173, followed by transcriptomic profiling, proteomic profiling, and western blotting were performed. Furthermore, β-catenin and c-myc transcriptional activity upon PNH173 treatment were analyzed using a reporter system at various time points. Effects of PNH173 on the cells‘ mitochondrial function were established via Seahorse Agilent technology, where the mechanism of mitochondrial dysfunction was studied. We detected changes in the Wnt, MAPK, and PI3K/Akt signaling pathways, which are the most affected pathways by A3AR.

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

Towards identification and validation of novel DC-SIGN ligands

Martin Ondra[1,2], Klaudia Kvakova[3,4], Petra Menova[5], Petr Cigler[3], Marian Hajduch[1,2]

[1]Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic.
[2]Czech Advanced Technology and Research Institute, Palacky University, Olomouc, Czech Republic.
[3]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic.
[4]Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University, Prague, Czech Republic. [5]University of Chemistry and Technology, Prague, Czech Republic

DC-SIGN (CD209) is a C-type lectin receptor expressed on the surface of both macrophages and dendritic cells. DC-SIGN recognizes both internal mannose-branched structures and terminal di-mannoses. Several pathogens can use the DC-SIGN receptor to facilitate entry into the cell and thus avoid detection by the immune system, including HIV, Ebola virus, and Mycobacterium tuberculosis. The diverse roles attributed to DC-SIGN provide an impetus to identify ligands that could be used to probe its functions. These compounds could also serve as therapeutic drugs. Therefore the selection and preparation of relevant in vitro models are crucial in the identification and functional validation of new DC-SIGN ligands.

Human monocytes (THP1), derived from an acute monocytic leukemia patient, were differentiated into macrophages or dendritic cells expressing DC-SIGN receptor. DC-SIGN ligands identified by Aretz et al. in fragment screening were attached to narrow size-distributed (~200 nm), electron irradiated, and colloidally stable fluorescent nanodiamonds (FNDs). FNDs unique optical properties and biocompatibility allowed us to examine the effect

of individual DC-SIGN ligands by confocal microscopy through FNDs internalization in differentiated macrophages or dendritic cells mediated by the DC-SIGN receptor.

Based on the results obtained, we confirmed in vitro function of newly identified DC-SIGN ligands. We believe new DC-SIGN ligands could be promising compounds in biomedical applications.

This work was supported by European Union – Program EXCELES, ID Project No.LX22NPO5102, the Czech Ministry of Education, Youth and Sports (EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA LF UP 2023_006.).

Aretz, Jonas, et al. „Identification of multiple druggable secondary sites by fragment screening against DC-SIGN.“ Angewandte Chemie International Edition 56.25 (2017): 7292-7296.

How can Single Cell and Spatial Transcriptomics help in cancer research?

Alice Mášová[1], Eva Rohlová[2], Lucie Langerová[1], Martin Mokrejš[1]

[1]Institute of Biotechnology CAS, Vestec, Czech Republic. 2Institute of Biotechnology CAS, Vestec, Denmark

GeneCore is a specialized service provider that focuses on gene expression analysis and its application in various experiments. With our extensive experience in qPCR, digital PCR, and NGS, we excel in designing diverse types of experiments. We have actively contributed to the development of standardized sampling guidelines for biological materials. Our primary research interest lies in the analysis of biomarkers such as cfDNA, CTC (circulating tumor cells), and miRNA in human plasma, specifically for the early detection of cancer.

We prioritize quality control throughout the entire experiment process. In collaboration with the Laboratory of Gene Expression (IBT CAS, Mikael Kubista), we continuously innovate and introduce new techniques, which we incorporate into our offered services. Our range of services includes comprehensive RNA sequencing support, starting from experiment design and library preparation to quality control and data analysis. We also provide miRNA analysis using an ultra-sensitive technique called Two-tailed PCR. Furthermore, we have already implemented methods that enable transcriptome analysis at the single-cell level (scRNA-seq).

We have played an active role in co-organizing various local and international conferences. In 2016, we successfully organized the CTC meeting in Prague, and in 2018, we achieved great success with the Single Cell Europe conference. Additionally, we participated in the organization of Precision Diagnostic Europe, an international online conference held in 2020.

As part of our commitment to knowledge sharing, we regularly deliver lectures in different courses focused on miRNA, RNA, or DNA analysis using quantitative PCR or NGS. Our ultimate goal is to assist researchers and medical doctors in their projects. By utilizing our expertise in rare materials and employing a multidisciplinary approach, we aim to provide valuable insights into various biological questions.

Trastuzumab Induces Cell Death in TNBC Cells: A Step Towards Repurposing the Monoclonal Antibody

Hussein Sabit

MUST, Cairo, Egypt

Breast cancer (BC) is the most common disease malignancy among females worldwide. The alarming rise in breast cancer cases highlights the importance of multifaceted disease management. Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer marked by high levels of intra-tumoral heterogeneity and rapid acquisition of drug resistance. Though it is reported as a targeted therapy for HER2+ BC, trastuzumab (Tz) could also target HER2- BC such as TNBC. In the present study, we evaluated the role of Tz in inducing apoptosis in TNBC (MDA-MB-231 and 4T1) by affecting different signaling pathways. MDA-MB-231, 4T1, MCF-7, and HSF were treated with 20 µg mL-1 of Tz for 24 h. MTT assay was carried out to determine the cytotoxicity of the drug, and the obtained data indicated significant differences between treated and untreated BC cells, but not the normal HSF cells. Apoptosis detection via PI staining revealed that Tz significantly increased cell death compared to untreated BC cells and normal cells. Meanwhile, DNA content also has been affected by Tz treatment, as the TNBC cells were arrested at the G2/M phase indicating the cytotoxicity of the drug compared to MCF-7 (HER2+) and HSF. Analysis of gene expression also indicated that ErBb2, NOTCH1, EGF, PI3K, and PTEN were all upregulated in MDA-MB-231, while 4T1 showed only an elevation in the expression of NOTCH1. Data indicated that Tz could induce cell death in TNBC cells, and this might open a gate for considering repurposing Tz in HER2- BC patients. Though it is promising, these data need further investigation to elucidate the mechanism by which Tz exerts its action in TNBC cells.

Targeted treatment of severe vascular malformations using *PIK3CA* inhibitor alpelisib in children and young adults – single center prospective case series

Martin Sterba[1], Peter Mudry[2], Olga Koskova[3], Renata Faberova[4], Petra Pokorna[5], Ondrej Slaby[6], Jaroslav Sterba[1]

[1]Department od paediatric oncology, Universitiy hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic. [2]Department od paediatric oncology, Universitiy hospital Brno and Faculty of Medicine, Masaryk University and Faculty of Medicine, Masaryk University, Brno, Czech Republic. [3]Department of paediatric surgery, orthopedics and traumatology, University hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic. [4]Department of paediatrics, University hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic. [5]Department of Biology, Faculty of Medicine and Central European Institute of Technology, Masaryk University, Brno, Czech Republic. [6]Department of Biology, Faculty of Medicine and Central European Institute of Technology, Masaryk University, Brno, Czech Republic, Brno, Czech Republic

Introduction: Vascular anomalies occur in about 4% of the population. They are divided into vascular tumors, dominated by hemangiomas, and vascular malformations. The cornerstone of treatment of vascular malformations are still local procedures (dermatology, surgery, interventional radiology), however this approach may not be sufficient for larger or complex lesions involving important anatomical structures. In addition to significant pain and aesthetic problems, severe systemic complications may develop, including chronic systemic coagulopathy, malfunction of vital organs and various body parts. Due to new discoveries in the field of molecular biology, driving mutations of genes playing crucial role in the pathophysiology of vascular malformations have been documented. These mutations in signaling pathways are well known in oncology and thus open new treatment opportunities.

Patients and methods: From 10/2020 to 6/2023, 70 patients with severe vascular malformations underwent molecular genetic testing using either Sanger sequencing, whole exome sequencing (WES), or targeted next generation sequencing (NGS) panel. Tissue diagnosis was performed in 63 of them. Patients with confirmed mutations in *PIK3CA* and TEK were recruited for alpelisib treatment and efficacy analysis. All patients included in our study were Caucasian. Age at the treatment initiation varied from 2 to 35 years of age. Prior to the start and then throughout of alpelisib treatment regular checkups were performed to evaluate the efficacy of the treatment. This consisted of patient's quality of life evaluation, laboratory and clinical examinations, and imaging, mostly MRI to measure volumatic changes of the lesion.

Results: Pathogenic mutation was found in 49 out of 63 tissue biopsies (78%), 4 (6%) of them was germline. After multidisciplinary discussion and consent of patients/parents, targeted systemic experimental treatment using *PIK3CA* inhibitor alpelisib was initiated in 26 of them, blocking the corresponding signaling cascade. Overall, we observed a very good effect of treatment, especially significant improvement in the quality of life, prompt regression of pain within days, normalization of coagulation parameters in a matter of weeks, and in most patients a reduction in the size of the lesion, even in some patients reopening the option local methods of treatment, which were originally unfeasible. Clinically significant toxicity occurred in 3 patients, allergic exanthema in two patients after 8-9 days of TKI administration and hyperglycemia in one patient. Treatment of secondary hyperglycemia/diabetes in these patients requires a specific approach, since both hyperglycaemia and higher insulin levels negate the antineoplastic effect of *PIK3CA* inhibitors, therefore gliflozins should be considered if necessary.

Conclusion: For the comprehensive management of large and complex VM in children, multidisciplinary approach is of vital importance, specialties involved are mostly paediatrics, paediatric oncology, paediatric dermatology, plastic surgery, orthopaedics, interventional radiology, hematology and molecular biology. In patients, where local procedures are ineffective or potentially life threatening, systemic treatment using *PIK3CA* inhibitor alpelisib offers an effective and very well tolerated therapeutic option as part of the multidisciplinary approach. However, numerous questions remain to be answered, such as optimal dose and treatment scheme, treatment duration and long-term side effects.

Regulation of AR-V7 by 3'UTR-binding miRNAs

Elisa Morales[1], Jasper Van Goubergen[1], Miroslav Peřina[2], Frédéric R. Santer[1]

[1]Division of Experimental Urology, Innsbruck, Austria. 2Department of Experimental Biology, Olomuc, Czech Republic

Introduction

Prostate cancer (PCa) is after lung cancer the second most common type of cancer in men worldwide. Male sex hormones, known as androgens, play a crucial role in proliferation as well as in the progression of the tumor. For this reason, one of the standard therapies for the treatment of PCa is androgen deprivation therapy (ADT). Despite castrate androgen levels and initial regression of the tumor, resistance often arises, leading to the development of castration-resistant prostate cancer (CRPC). One explanation for the development of CRPC is the formation of alternative splice variants of the androgen receptor (AR). The clinical most relevant AR isoform is the androgen receptor splice variant

7 (AR-V7), which has transcriptional activity in absence of androgens. While the full-length isoform (AR-FL) is encoded by exons 1-8, AR-V7 encompasses exons 1-3 and cryptic exon 3. Thus, AR-V7 possesses a distinct 3'UTR compared to AR-FL and a differential regulation of both isoforms by the 3'UTR is assumed. Single nucleotide polymorphisms (SNPs) are present in the 3'UTR of AR-V7, which potentially can change its regulatory features. In general, micro-RNAs (miRNAs) post-transcriptionally regulate target-mRNAs. Here, we set out to analyze the effect of SNPs on regulation of AR-V7 mRNA by miRNAs binding in the 3'UTR of AR-V7.

Methods

In previous work, using microSNiPer and miRNASNP-v3, a prediction was made for allele-specific binding of miRNAs. Five miRNAs (hsa-miR-133a-3p, hsa-miR-324-5p, hsa-miR-574-5p, hsa-miR-140-5p, hsa-miR-133b) that indicated to have the highest difference in predicted minimum free energy between reference and alternative allele and are expressed according to the cancer genome Atlas were selected for further research. For analyzing AR-FL and AR-V7 mRNA and protein expression, 22Rv1 and DuCaP cell lines were transfected with the miRNAs of interest and the levels were determined performing qPCR and Western blot.

Dual-Luciferase Reporter assay was used to study whether the miRNAs of interest can bind to the 3'UTR of AR-V7 in an allele-specific manner. For this, the luciferase gene construct pmirGLO-AR-V7, generated by fusing the coding sequence of the firefly luciferase to the 3'UTR of AR-V7 and introducing SNPs by site-directed mutagenesis, was co-transfected with the relevant miRNA in HEK293 cells.

Results

Treatment with hsa-miR-574-5p lowered the expression of AR-V7, and not AR-FL both on mRNA and protein level in 22RV1. Interestingly, treatment with has-miR-324-5p increased AR-V7 mRNA and protein levels in the same cell line. Furthermore Dual-Luciferase Reporter assay potentially attributes allelic effects in terms of miRNA binding to AR-V7 3'UTR.

Conclusion

Taken together, miRNAs has-miR-324-5p, hsa-miR-574-5p alter AR-V7's post-transcriptional regulation, potentially via an allelic-specific dependent manner. Results of this work help to further understand the distinct regulation of expression of AR-V7 compared to AR-FL.

The role of HUS1 and RAD51 in cisplatin-based chemotherapy resistant bladder cancer

Nils van Creij[1], Francisco Roa[1], Frédéric Santer[1], Per Sonne Holm[2], Zoran Culig[1], Renate Pichler[1]

[1]Department of Urology, Medical University of Innsbruck, Innsbruck, Austria.
[2]Department of Oral and Maxillofacial Surgery, Medical University of Innsbruck, Innsbruck, Austria

Background: Bladder cancer is the second most common tumor entity in urology. With an estimated 200.000 yearly worldwide death rate, bladder cancer ranks fourteenth when looking at cancer deaths. Following the European Association of Urology guidelines, radical cystectomy (RC) after neoadjuvant cisplatin-based chemotherapy (NAC) is still the standard treatment for localized MIBC patients. Nevertheless, 65% of bladder cancer patients do not respond to the platinum-based chemotherapy, and those who do respond are likely to develop resistance to the treatment. A previous study from our group has shown a relation between a specific 7p12 chromosomal amplification, and NAC non-responders. This region includes the genes ABCA13, EGFR, FIGNL1, HUS1 and IKZF1. Especially the role of HUS1, which has an important function in DNA repair, seems to be key in cisplatin-based chemotherapy resistance in bladder cancer patients. The aim of this study is to investigate the role of HUS1 and RAD51 as biomarkers and their relation to DNA repair in bladder cancer.

Material and Methods: Using western blot, HUS1 and RAD51 expression was evaluated in two urothelial cancer cell lines, UMUC3 and HT1197, and their cisplatin resistant sublines. To investigate the potential role of HUS1 and RAD51 in cell proliferation, cells were transfected with siRNA or overexpression plasmids of either HUS1 or RAD51. Furthermore, proliferation was analysed either with or without the presence of different concentrations of cisplatin. The silencing and overexpression of either HUS1 or RAD51 was confirmed by western blot.

Results: We analysed basal HUS1 and RAD51 protein expression in both cell lines, and observed a significantly increased expression of RAD51 in the cisplatin resistant cells, indicating a possible explanation for the reduced chemotherapy toxicity in these cells. Our results furthermore showed that downregulation of HUS1 or RAD51 in combination with cisplatin resulted in an inhibited cell proliferation, indicating that these proteins play a role in regulating cellular viability when cisplatin is present. Surprisingly, in the cisplatin resistant cells, the effect of HUS1 downregulation was not observed. Furthermore, overexpression of one of both proteins could not overcome the toxicity of the added cisplatin. Analysing the corresponding western blot data, we observed a correlation between the two proteins. When silencing or overexpressing HUS1, results showed a decreased or increased RAD51 expression level respectively. When silencing RAD51, also lower HUS1 levels were observed, suggesting that both proteins directly interact with each other.

Conclusions: The relation between HUS1 and RAD51, and their involvement in cisplatin-based chemotherapy resistance in bladder cancer has not yet been described. Our preliminary data might be the first steps to a possible new treatment strategy for platinum-resistant bladder cancer patients. Of

course, further research is needed in unravelling all mechanisms involved, for example by looking at different members of the DNA repair pathway (e.g. CHK1, RAD9, RAD1, yH2AX). Overall, HUS1 and RAD51 might be promising biomarkers to predict chemotherapy response in bladder cancer patients, or might function as interesting targets for newly developed treatment strategies.

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

Barbora Kalousová[1], Rastislav Slavkovský[1], Pavla Kouřilová[1], Patrícia Žižkovičová[1], Kateřina Kubáňová[1,2], Hana Jaworek[1,2], Jiří Drábek[1,2], Marián Hajdúch[1,2], Michal Haršány[3,4], Robert Mikulík[5,4]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic.
[2]University Hospital Olomouc, Olomouc, Czech Republic.
[3]1st Department of Neurology, St. Anne's University Hospital Brno, Brno, Czech Republic. *[4]The International Clinical Research Centre, St. Anne's University Hospital Brno, Brno, Czech Republic.*
[5]Department of Neurology, Tomáš Baťa Regional Hospital, Zlín, Czech Republic

Introduction: Clonal hematopoiesis of indeterminate potential (CHIP) is an age-related condition characterized by the accumulation of somatic mutations in cells of the hematopoietic system. Although CHIP commonly occurs in otherwise healthy individuals aged 60 years and older, it is considered a potential precursor to malignant transformation. Interestingly, CHIP has recently been identified as a vascular risk factor associated with higher cardiovascular mortality. The primary objective of our study was to determine whether CHIP plays a role in 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 carotid 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 observed in cohorts 1 to 4 as follows: 76.1%, 78.3%, 77.7%, and 64.5% (p=0.135), confirming the high prevalence of CHIP in elderly individuals. As consistent with the literature, mutations were most commonly found in genes DNMT3A (230/588; 39%) and TET2 (160/588; 27%). Stroke patients with carotid stenosis had a higher cumulative variant allele fraction (VAF) than the patients with asymptomatic carotid stenosis (5.7%; IQR 2.5-13.4 vs. 3%; IQR 1.2-9.6; p=0.026). Patients who suffered a stroke, regardless of the etiology, 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 serve as a predictor of stroke. Further research is needed to verify and confirm the practical utility of ASXL1 mutations in diagnosis and their association with other factors.

Funded by ENOCH project (Cz.02.1 .01/0.0/0.0/16_019/0000868), IGA LF UP 2023_006 and STROCZECH (LM2023049).

Molecular genetics of extraskeletal myxoid chondrosarcoma focusing on a rare alternative TCF12::NR4A3 gene fusion associated with unusual morphologic features

Jiří Lenz

Department of Pathology, Znojmo, Czech Republic

Background: Extraskeletal myxoid chondrosarcoma (EMC) is a rare malignant soft tissue tumor of uncertain lineage. The molecular genetics of EMC is characterized by nuclear receptor subfamily 4 group A member (*NR4A3*) gene fusions, which are present in more than 90% of cases, with the EWS RNA binding protein 1 (*EWSR1*) gene being the most common fusion partner. Herein, a case of EMC with unusual pathological features and an extremely rare transcription factor 12 *(TCF12)::NR4A3* gene fusion is presented.

Materials and methods: The patient was a 63-year old man with a lobulated, well demarcated lesion sized 50 x 40 mm located in the subcutis of the abdominal wall lateral to the left ilium. Despite extensive histological and immunohistochemical analysis, the accurate diagnosis was rendered only after RNA- sequencing was performed.

Results: Histology revealed hypercellular nodules of spindled to ovoid tumor cells showing mild atypia and low mitotic activity, growing in fascicles, solid sheets or pseudoglandular structures. Large areas of necrosis were easily recognizable, while rhabdoid cells and a small amount of myxoid stroma were present only focally. Immunohistochemistry was noncontributory as the lesion showed positive immunohistochemical staining with CD56 only while S100 protein and INSM1 were negative. Subsequent genetic analysis using the Archer fusionPlex Sarcoma kit revealed *TCF12::NR4A3* gene fusion.

Discussion: According to available

genetic data, *NR4A3* fusions are found in more than 90% of EMC cases and have not been detected in any other sarcoma type. The *NR4A3* gene, which is also called neuron-derived orphan receptor 1 (NOR1), is located on chromozome 9q22. This gene belongs to the steroid-thyroid hormone-retinoid receptor superfamily and acts as an intracellular transcription activator. *NR4A3* plays a crucial role in pathways affecting cell diferentiation and proliferation, apoptosis, metabolism and inflammation. Genetic studies have identified six reciprocal translocations in EMC, namely t(9;22)(q22;q12), t(9;17)(q22;q12), t(9;15)(q22;q21), t(9;3)(q22;q12), t(9;16)(q22;p11) and t(9;9)(q22;p24). The first translocation is the most common, detected in more than 70% of cases, and leads to the fusion of the *NR4A3* gene in 9q22 with the *EWSR1* gene in 22q12. The second translocation, in which *NR4A3* combines with the TATA-box binding protein associated factor 15 (TAF15) gene in 17q12, is observed less frequently (about 15%). The third, fourth, fifth and sixth translocations, leading to the fusion of *NR4A3* with TRK-fused gene (TFG) in 3q11, *NR4A3* with *TCF12* in 15q21, NR4A3 with FUS in 16p11 and NR4A3 with SMARCA2 in 9p24, respectively, have only been reported in isolated cases. Our case is only the third reported *TCF12*-rearranged EMC. The *TCF12* gene encodes a protein which belongs to the basic helix-loop-helix E-protein family. This gene acts as a transcriptional regulator. During embryogenesis, it plays an important role in the initial phase of neuronal differentiation. The spectrum of diseases associated with the *TCF12* gene includes isolated brachycephaly and craniosynostosis. The activity of this gene is functionally related to MAPK/ERK and Wnt signaling pathways. One molecular study revealed a small number of EMCs without any detectable *NR4A3* rearrangements. This finding points to mechanisms other than *NR4A3* gene alterations involved in EMC oncogenesis. An alternative genetic pathway could be related to aberrant co-expression of native *NR4A3* and its transcriptional coactivator, SIX homeobox 3 (SIX3). Our case of EMC was morphologically different from the prototypical EMC. We found hypercellular nodules of relatively uniform tumor cells growing in fascicles, solid sheets or pseudopapillary structures. Epithelioid/rhabdoid features were present only focally. The cells showed mild cytological atypia and low mitotic activity. The abundant myxoid stroma, which is a typical microscopic feature of EMC, was not detected and only very small foci of inconspicuous myxoid change were found. According to the latest edition of the World Health Organization (WHO) classification of soft tissue and bone tumors, hypercelullar EMC with a reduced amount of myxoid stroma have high-grade nuclear cytology and often an epithelioid cell component. In an effort to detect these features, the resected specimen of our case was completely submitted in 10 tissue sections, which were subjected to extensive histopathological assessment. Despite the high cellularity and large areas of tumor necrosis, microscopic features such as high nuclear grade and increased mitotic activity were not present in our case.

Conclusion: We report a unique case of EMC with *NR4A3* rearrangement fused to the non-EWSR1 gene *TCF12*, which despite its high cellularity exhibits mild cytologic atypia and a low mitotic rate. EMC has a broad differential diagnosis and as illustrated in our case, the correct classification may be extremely difficult in highly cellular examples of EMC with minimal myxoid stroma and an extremely rare pseudopapillary growth pattern, which is further compounded by the fact that EMCs do not exhibit a specific immunoprofile. In such cases, the only way to the correct diagnosis is through the application of high-throughput molecular methods such as RNA-seq.

Targeting of tyrosine kinases contributing to KMT2A-r mediated leukemogenesis as a therapeutic approach in mixed-lineage leukemia

Nikola Niederlova[1], Pavla Koralkova[1], Pavla Chaloupkova[1], Iveta Hradilova[1], Jan Gursky[1], Barbora Cudova[2], Helena Urbankova[2], Eva Reznickova[3], Tomas Gucky[3], Denisa Myslivcova[4], Monika Belickova[4], Vladimir Krystof[3], Vladimir Divoky[1]

[1]Department of Biology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Olomouc, Czech Republic.
[2]Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc, Olomouc, Czech Republic.
[3]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic.
[4]Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Mutations in Fms-like tyrosine kinase 3 (FLT3) gene represent the most frequent alterations in acute myeloid leukemia (AML). Overexpression of FLT3, Src family kinases (SFK) and salt-inducible kinases (SIK) has been demonstrated in mixed lineage leukemia-rearranged (KMT2A-r) leukemias. Specific targeting of KMT2A-r/FLT3 signaling represents a great challenge in the field of hematology.

We have recently developed a potent multikinase inhibitor, compound LGR3922, which displays selectivity and potency in in vitro and *in vivo* AML models. Here, we present additional characterization of the compound LGR3922 and demonstrate its potent anti-leukemic activity in KMT2A-r/FLT3 mut cell lines, murine xenografts and ex vivo primary AML samples.

In KMT2A-r/FLT3mut cell lines (MV4-11, MOLM-13, HB11;19), LGR3922 exhibited nanomolar potency against FLT3, SFK, Ca2+/calmodulin-

dependent protein kinase II (CaMKII) and eIF4E (eukaryotic translation initiation factor 4E), which is a direct target of MAP kinase-interacting serine/threonine protein kinase 2 (MNK2). LGR3922 also showed inhibitory activity against salt-inducible kinase 1/2 (SIK1/2) targets, histone deacetylase 4 (HDAC4), and/or transcription factor MEF2C. In addition, LGR3922 demonstrated potent inhibition against serine/arginine-rich (SR) proteins, which are targets of CDC2-like kinase 1 (CLK1). The inhibitory effects of our compound remained in most targets even after a 24-hour washout period.

Effective blocking of KMT2A-r/FLT3-mediated signaling through inhibition of key molecules by LGR3922 was demonstrated in AML xenograft mouse models. In the long-term *in vivo* experiment with repeated doses of our tested compound, LGR3922 showed significant tumor volume regression. Interestingly, no adverse effects on blood count parameters or body weight were observed during LGR3922 administration.

We then evaluated toxicity of LGR3922 on normal hematopoiesis using colony forming unit (CFU) assays with progenitor cells from several healthy donor controls. LGR3922 inhibited colony formation in a dose-dependent manner; maximal tolerated concentration (MTC) for all healthy samples was 100 nM, in some samples the reduced granulocyte-macrophage (CFU-GM) and burst forming unit-erythroid (BFU-E) colony forming ability was not observed up to a concentration of 200 nM.

Then, we performed CFU assays on 4 primary human AML samples with FLT3-ITD mutations (n=2) or FLT3-TKD+KMT2A::MLLT3 (n=2). LGR3922 showed dose-dependent inhibition of the number and cellularity of colonies in all patient samples. Furthermore, LGR3922 at 100 nM significantly reduced KMT2A::MLLT3 and FLT3-ITD mutant cells; FLT3-TKD mutation was not detected.

Taken together, LGR3922 specifically inhibits multiple kinases: FLT3, SFK, CaMKII, MNK2, SIKs and CLK1 *in vitro* and *in vivo*. Simultaneous inhibition of these key targets effectively blocks KMT2A-r/FLT3-mediated signaling resulting in proliferation arrest of KMT2A-r/FLT3 cells. LGR3922 is a unique compound suitable for further development and testing for KMT2A-r/FLT3 AMLs, and, potentially, other types of AMLs.

Funding: Program EXCELES, ID Project No. LX22NPO5102; GACR 23-05462S, UP Young Researcher Grant Competition, ID Project JG_2023_016.

Potential role of CDK13 in neuroblastoma progression

Romana Zatloukalová, Jiří Kohoutek

Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

The CDK13 gene encodes a cyclin-dependent kinase 13 which primary function is associated with phosphorylation of the heptapeptide repeat of the C-terminal domain (CTD) of RNA polymerase II (RNAPII), thus directly affecting transcription and probably some of the phase of splicing. CDK13 may also be involved in the translation of specific mRNAs, such as MYCN, whose expression is often elevated in neuroblastoma patients. The aim of our research was to verify the activity of CDK13 and the level of MYCN protein in the cells. The neuroblastoma cell lines were as the model system. In particular, the SK-N-BE(2) cell line expressing MYCN and SH-SY5Y cell line expressing C-MYC protein were employed. We further studied the effect of CDK13 knockdown on specific proteins that might be part of both the translational and transcriptional pathways regulating MYCN protein levels. The results confirmed the effect of CDK13 activity on the amount of p4E-BP1 protein, which influences MYCN protein translation in neuroblastomas. As part of this thesis, we also investigated the effect of CDK13 knockdown on proliferation, migration and colony forming capacity of cells *in vitro*.

Liquid biopsies in multiple myeloma

Monika Vlachová[1], Tereza Ruzickova[1], Jana Gregorova[1], Lenka Radova[1], Jiri Jarkovsky[1], Martin Stork[2], Ludek Pour[2], Sabina Sevcikova[1]

[1]Masaryk University, Brno, Czech Republic. 2University Hospital Brno, Brno, Czech Republic

Multiple myeloma (MM) is the second most common hematological malignancy characterized by clonal expansion of malignant plasma cells in the bone marrow. The basic diagnostic methods include bone marrow sampling, which is relatively painful and cannot be repeated as often as needed.

Therefore, our research group has focused on so-called liquid biopsies, biopsies from more easily accessible fluids, such as peripheral blood, urine, saliva, etc. These fluids contain so-called non-coding RNA molecules, either bound to proteins or enclosed in exosomes. These non-coding RNA molecules include e.g. microRNAs, perhaps the most studied group of these molecules. These molecules regulate gene expression by binding to the 3‘ untranslated region (UTR) of the target mRNA, leading to its degradation or translational repression. Specific miRNAs may act as tumor suppressors or oncogenes affecting key biological processes, including cell proliferation, immune response and drug resistance. Another group of miRNAs studied are the so-called long non-coding RNAs (lncRNAs). These diverse molecules are incapable of protesynthesis and regulate cellular processes, e.g. by the mechanism of chromatin remodeling. It has been found that non-coding RNA molecules are aberrantly expressed in cancer, which could make them useful as diagnostic, prognostic, or predictive markers and, due to their regulatory role, may represent new targets for precision medicine. Both of these groups are also relatively common in liquid biopsies and are therefore the focus of our research group.

This work was supported by grant

AZV NU21-03-00076 and by the National Cancer Research Institute Project Grant (EXCELES Program, project number: LX22NPO5102) - Funded by the European Union - Next Generation EU.

Casein kinase 1 alpha inhibition in acute myeloid leukemia

Janovská P. [1,3*], *Mikulová A.* [1*], *Prišticová I.1, Plešingerová H.1, Vondálová-Blanářová O.*[1*], *Procházková P.* [1, 2], *Čulen M.* [2], *Buša D.* [2], *Maier L.* [1,3], *Havel Š.* [1,3], *Paruch K.* [1†] *and Bryja V.*[1†]

[†,*] *The authors contributed equally*

[1]*Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*
[2]*Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[3]*CasInvent Pharma, Brno, Czech Republic*

The family of Casein kinase 1 (CK1) serine-threonine kinases has been studied for decades, but the tools for its selective and efficient inhibition have been developed only recently. The CK1 isoforms described in most detail include CK1 delta, epsilon and CK1 alpha. All three play crucial role in developmental signaling pathways, cancer cell signaling and regulation of key processes such as cell cycle, apoptosis, DNA damage, migration and invasion. Not surprisingly, these kinases were also found to be overexpressed in some cancers, including leukemia, and their activity was connected to development/progression of certain cancer indications. Due to their diverse biological functions, they can act as both oncogenes and tumor suppressors depending on the context and therefore their therapeutic targeting is not a simple task.

Inhibition of the CK1 alpha isoform has been the focus of several preclinical studies as a potential form of treatment for acute myeloid leukemia (AML). In principle, AML cells, including the population of leukemic stem cells, depend on the activity of CK1 alpha for their survival. Therefore, targeted inhibition or degradation of the kinase leads to p53-dependent apoptosis of AML cells. The effort to target the kinase efficiently *in vivo* by small molecule inhibitors has been however challenging and the selective readouts for CK1 alpha activity *in vivo* were missing.

Our research led us to development of highly selective and active small molecule inhibitors of CK1 kinases, including the most potent CK1 alpha inhibitors known to this date. In this project, we characterize their effects on AML cells *in vitro* and *in vivo*, showing for the first time the potential of CK1 alpha inhibition on its own, clean of the multi-kinase off-target effects. We aim at description of the sensitivity markers for CK1 alpha inhibition and at selection of patient subgroups that will benefit form the single agent or combined therapies including CK1 inhibition.

The project was supported by National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU.

Notes

Czech Annual Cancer Research Meeting

November 20–22 2023 | Olomouc, CZ

ABSTRACT BOOK CACR MEETING 20.—22.11.2023

Vydává: MedChemBio, klastr medicinální chemie a chemické biologie zájmové sdružení právnických osob
IČ: 72023970, Šlechtitelů 21, Olomouc, PSČ 779 00

Druhý ročník: 2023

ISBN: 978-80-908348-2-8
ISSN: 2788-1695

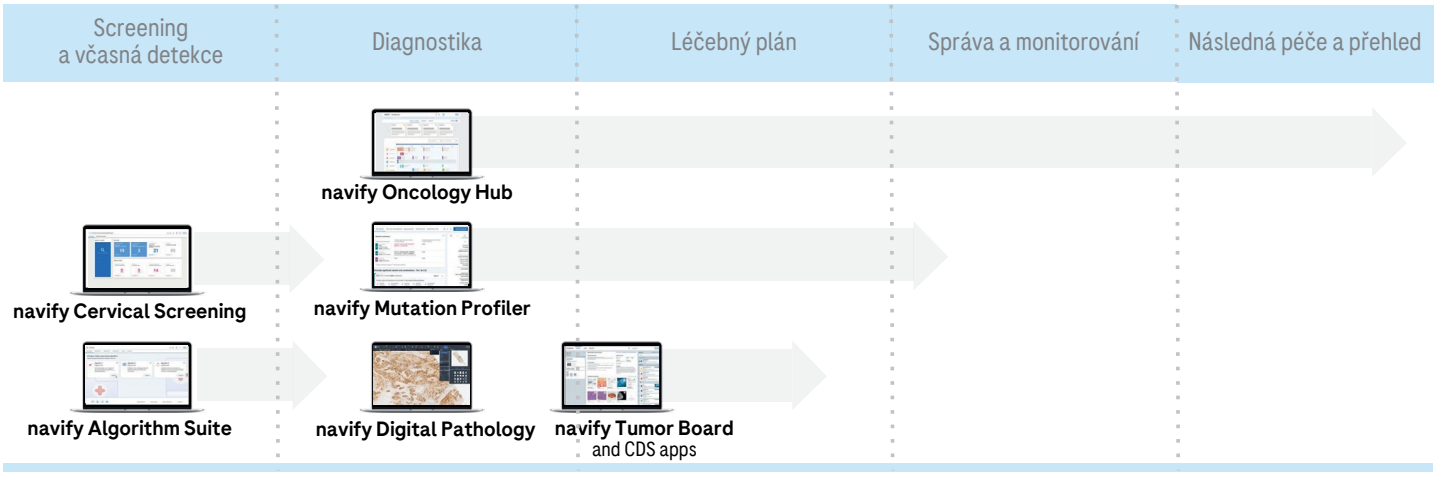
www.cancermeeting.cz

navify

Onkologické portfolio

Cesta onkologického pacienta

Naše řešení



Onkologické portfolio navify otevírá nové možnosti zlepšení onkologické péče. Poskytuje holistické zobrazení dat a vhled do klinických rozhodnutí. Skrze maximalizaci operativní efektivity redukuje náklady.

Pomáháme lékařům v průběhu celé cesty pacienta a především společně pomáháme pacientům.

Roche s.r.o., Diagnostics Division
Na Valentince 3336/4, 150 00 Praha 5 – Smíchov
www.roche-diagnostics.cz

Více informací na: www.NAVIFY.com

MC-CZ-01256