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- XLVI. Congress of Czech Pathologists with International Participation
- XVI. Days of Molecular Pathology
- XXIV. Congress of the Czech Society of Histotechnologists
- XVI. Diagnostic, Predictive and Experimental Oncology Days

ABSTRACT BOOK

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The Czech Society of Pathologists

The Czech Society for Histochemistry and Cytochemistry

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PROGRAM

ČTVRTEK / THURSDAY - 25. LISTOPADU 2021 / 25TH NOVEMBER, 2021

11:00 — 13:00 **REGISTRATION**

Novel drugs and therapies / 13:00 — 15:00 / Madrid Room

Chairs: Petr Džubák, Milan Urban

13:00 – 13:15	Welcome speech **Marián Hajdúch**
13:15 - 13:30	Biological evaluation of 225Ac-PSMA derivatives for alpha therapy in prostate cancer **Zbyněk Nový**
13:30 - 13:45	Anti-SARS-CoV-2 activity of one existing anti-inflammatory drug **Ermin Schadich**
13:45 - 14:00	Determination of biological activity of compounds from IMTM Proprietary Library based on their cytotoxicity profiling in HTS facility **Soňa Gurská**
14:00 - 14:15	Highly active anticancer drug, PNH173, mechanism of action **Petr Džubák**
14:15 - 14:30	Synthesis of fluorinated derivatives 2-phenyl-3-hydroxy-4(1H)-quinolinone and study of their anticancer activity **Jiří Řehulka**
14:30 - 14:45	Bioisosteric approach in the improvement of selectivity in cytotoxic analogues of betulinic acid **Milan Urban**
14:45 - 15:00	68Ga-Ornibactin for positron emission tomography imaging of bacteria from Burkholderia cepacia complex **Kateřina Bendová**

15:00 — 15:30 **COFFEE BREAK**

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Olomoucký kraj

Biomarkers in personalized medicine / 15:30 — 18:00 / Madrid Room

Chairs: Juan Bautista De Sanctis, Lukáš Najdekr

15:30 - 15:45	Metabolite and lipid profiling of lung and breast cancer **Aleš Kvasnička**
15:45 - 16:00	Colon Cancer and Perturbations of the Sphingolipid and Glycosphingolipid Metabolism **Miroslav Machala**
16:00 - 16:15	Comprehensive LC-MS approach for diagnosis of monogenic disorders **Barbora Piskláková**

ČTVRTEK / THURSDAY - 25. LISTOPADU 2021 / 25TH NOVEMBER, 2021

16:15 - 16:30	Proteomic analysis of exhaled breath condensate could be used for non-invasive lung cancer diagnostics and distinguish it from COPD **Jana Václavková**
16:30 - 16:45	Could be the biomarkers of gynecological diseases detected from cervical mucus? **Tomáš Oždián**
16:45 - 17:00	Novel UHPLC-MS method for detailed analysis of lipid species using a scheduled MS/MS acquisition approach for improved metabolite annotation **Lukáš Najdekr**
17:00 - 17:15	Targeted metabolomic and lipidomic study of SHR24 rat models with tauopathy **Dana Dobešová**
17:15 - 17:30	Therapeutic targeting of repurposed anticancer drugs for protein aggregation: Example of tau aggregates **Narendran Annadurai**
17:30 - 17:45	Supplementation with polyunsaturated and saturated fatty acids enhance NK cyto toxic response in Sprague Dawley-Rats. Effect of gender and age **Juan Bautista De Sanctis**
17:45 - 18:00	Age prediction through detection of DNA methylation **Lucie Kotková**

Interdisciplinary research in pathology / 13:00 — 15:00 / Olomouc Room

Chairs: Jiří Ehrmann, Jan Bouchal

13:00 – 13:15	Welcome speech **Jiří Ehrmann**
13:15 - 13:30	Multiple myeloma 2000+ **Jiří Minařík**
13:30 - 13:45	Current approach to typing systemic and localised amyloidosis **Patrik Flodr**
13:45 - 14:00	A drug repurposing strategy for overcoming human multiple myeloma resistance to standard-of-care treatment **Katarína Chromá**
14:00 - 14:15	Cannabidiol-induced expression of metallothioneins attenuate the anticancer effect of disulfiram **Tereza Buchtová**
14:15 - 14:30	Iodine Contrast Medium Affects Urine Cytology Assessement - A Prospective Single Blinded Study And Its Impact For Urological Practice **Milan Král**
14:30 - 14:45	Cell surface antigen signature and its plasticity in breast and prostate cancer **Karel Souček**
14:45 - 15:00	Inhibition of aryl hydrocarbon receptor (AhR) expression in colon cancer cells disrupts cell proliferation, energy metabolism and expression of enzymes involved in fatty acid synthesis **Jan Vondráček**
15:00 — 15:30	**COFFEE BREAK**

PROGRAM

ČTVRTEK / THURSDAY - 25. LISTOPADU 2021 / 25TH NOVEMBER, 2021

Clinical and molecular pathology / 15:30 — 17:40 / Olomouc Room

Chairs: Gabriela Dostálová, Zdeněk Kolář

15:30 - 15:45 Role imunohistochemie v diagnostice atypické endometriózy
Jiří Lenz

15:45 - 16:00 Teamwork in Thyroid Diseases Diagnostics and Treatment. Total Thyroidectomy Can Still Remain the Method of Choice in Some Bethesda III FNAB Cases
Jaroslava Dušková

16:00 - 16:15 Patofyziologie a patologie M. Gaucher a M. Fabry
Helena Hůlková

16:15 - 16:30 Soubor biopsií ledvin u M. Fabry. Provedené nefrologické screeningy v ČR
Eva Honsová

16:30 - 16:40 Další histopatologická vyšetření u M. Gaucher a M. Fabry
Helena Hůlková

16:40 - 16:55 Bank of Clinical Specimens: Bridging the gap in translational research
Roman Hrstka

16:55 - 17:10 Cytokeratin 7 expression as a predictor of an unfavorable prognosis in colorectal carcinoma
Jan Hrudka

17:10 - 17:25 Morphological study of HNSCC focused on perinerual invasion - a single institutional study with five year follow up
Pavel Hurník

17:25 - 17:40 Identification of candidate genes underlying soft tissue sarcoma progression
Jiří Hatina

PROGRAM

PÁTEK / FRIDAY - 26. LISTOPADU 2021 / 26TH NOVEMBER, 2021

The session of the Czech Society of Pathologists / 08:15 — 10:00 / Evropa Room

Chairs: Pavel Dundr, Zdeněk Kolář, Jiří Ehrmann

8:15 - 8:25	Welcome speech **Zdeněk Kolář, Pavel Dundr, Jiří Ehrmann, Marián Hajdúch, Jana Vaculová**
8:25 - 8:45	Hlava's institute of pathology, professor Jaroslav Hlava and his successors **Pavel Dundr**
8:45 - 9:00	Comparison of 5 different scoring methods in the evaluation of inflammatory infiltration (tumor infiltrating lymphocytes - TILs) in superficial spreading and nodular melanoma (*Hlava's Award 2019*) **Kristýna Němejcová**
9:00 - 9:15	Extracellular Amyloid Deposits in Alzheimer's and Creutzfeldt–Jakob Disease: Similar Behavior of Different Proteins? (*Lambl's Award 2020*) **Nikol Jankovská**
9:15 - 9:30	Central Pathology Review in SENTIX, A Prospective Observational International Study on Sentinel Lymph Node Biopsy in Patients with Early-Stage Cervical Cancer (ENGOT-CX2) (*Hlava's Award 2020*) **Kristýna Němejcová**
9:30 - 10:00	**INVITED LECTURE:** Androgen receptor coregulatory proteins and endocrine and chemotherapies for prostate cancer **Zoran Culig, Innsbruck Medical University**
10:00 — 10:30	**COFFEE BREAK**

Comprehensive genomic tumor profiling powered by GENETICA & ILLUMINA 10:30 — 12:30 / Evropa Room

Chairs: Pavel Dundr, Jiří Drábek, Jeroen Adema, Ondřej Slabý

10:30 - 11:00	Comprehensive genomic tumor profiling **Jeroen Adema**
11:00 - 11:30	Higher degree of personalization of cancer treatment (precision oncology) using comprehensive genomic profiling: experience from two molecular tumor boards **Ondřej Slabý**
11:30 - 11:45	The informatic road to clinical report from NGS somatic testing **Ondřej Brzoň**
11:45 - 12:00	Clonal somatic variants in hematopoietic cells in relation to age and stroke **Rastislav Slavkovský**
12:00 - 12:30	The Value of Comprehensive Genomic Profiling of Metastatic Cancer Using NGS **Brigitte Maess**

WORKSHOP PROJECT BY A-C-G-T: Analysis of Czech Genomes for Theranostics (CZ.02.1.01/0.0/0.0/16_026/0008448)

WORKSHOP PROJECT BY ENIGMA: Etalon of National Interpreted Genome Maps of the Czech Republic (CZ.02.1.01/0.0/0.0/16_026/0008448)

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12:30 — 13:30 **LUNCH**

PROGRAM

PÁTEK / FRIDAY - 26. LISTOPADU 2021 / 26TH NOVEMBER, 2021

COVID-19: Nemoc plná překvapení / 13:30 — 15:50 / Evropa Room

Chairs: Roman Prymula, Marián Hajdúch

13:30 - 13:50	Diagnostika infekce virem SARS-CoV-2 **Pavel Dřevínek**
13:50 - 14:10	Patologie onemocnění COVID-19 **Radoslav Matěj**
14:10 - 14:30	COVID-19 — když data a informace nejsou totéž **Ladislav Dušek**
14:30 - 14:50	Účinnost a reaktogenita vakcín proti COVID-19, máme jinou alternativu jak zastavit pandemii? **Roman Prymula**
14:50 - 15:10	Farmakologická prevence a léčba pacientů **Petr Smejkal**
15:10 - 15:30	Pandemie: čím jsme si prošli a kam směřujeme **Marián Hajdúch**
15:30 - 15:50	Moderovaná diskuze

XXIV. Congress of the Czech Society of Histotechnologists / 15:30 — 17:15 Olomouc Room

Chairs: Jana Vaculová, Daniela Indrová

15:30 - 15:45	Využití a výhody histotopogramu v histologické diagnostice **Kateřina Gospošová**
15:45 - 16:00	Využití histologického zpracování modelů k detekci závažnosti postižení tlustého střeva ulcerózní kolitidou **Jozef Škarda**
16:00 - 16:15	Duální nepřímá imunofluorescence na histologických řezech **Alena Poláková**
16:15 - 16:30	Multiplexní imunofluorescence v histologii **Tereza Hulínová**
16:30 - 16:45	Zpracování tělních tekutin formou cytobloku **Lucie Hocková**
16:45 - 17:00	Informace k přípravě kompetenčních modelů **Miroslava Lamplotová**
17:00 - 17:15	Informace z výboru ČSHL **Jana Vaculová, Pavla Krystková**

15:50 - 17:00 **POSTER SECTION EVROPA 3 ROOM**

Poster presenters are required to be ready for discussions

PROGRAM

SOBOTA / SATURDAY - 27. LISTOPADU 2021 / 27TH NOVEMBER, 2021

Multiplex imunohistochemistry and data management / 09:00 — 11:30
Madrid Room
Chairs: Jiří Ehrmann, Jan Bouchal

9:00 - 9:30	**INVITED LECTURE:** Spatial Phenotyping of Immune Cell Subsets in Patients with Lethal COVID-19 **Paul Murray, University of Birmingham, University of Limerick**
9:30 - 9:45	Awakening of digital pathology in Covid 19 pandemic – time to come to real life **Jiří Ehrmann**
9:45 - 10:10	Contribution of Digital Pathology in Multidisciplinary Tumor Board Approach Precising Medicine **Patrik Flodr**
10:10 - 10:25	Artificial Intelligence and Multiplex Bioimaging in Translational Cancer Research **Mariam Gachechiladze**
10:25 - 10:45	Ultra high content imaging using MICS technology on the MACSima™ Imaging Platform **Bernd Müller-Zülow**
10:45 - 11:00	Introduction to infrastructures for medical data **Petr Holub**
11:00 - 11:15	ELIXIR Czech Republic – Large Infrastructure for biological data pursuits steps towards personalized medicine **Jiří Vondrášek**
11:15 - 11:30	Software tools for data stewardship in personalized and translational medicine **Marián Hajdúch**
11:30 — 11:45	**COFFEE BREAK**

Prognostic, predictive and immune response biomarkers / 11:45 — 13:00
Madrid Room
Chairs: Markéta Kolečková, Jozef Škarda

11:45 - 12:00	Negative prognostic impact of PD-L1 expression in tumor cells of undifferentiated (anaplastic) carcinoma with osteoclast-like giant cells of the pancreas: study of 13 cases comparing ductal pancreatic carcinoma and review of the literature **Jan Hrudka**
12:00 - 12:15	Antitumor immune response - significance and recommended procedures for assessing the intensity of tumor-infiltrating lymphocytes / plasma cells (TILs) in solid tumors **Markéta Kolečková**

PROGRAM

SOBOTA / SATURDAY - 27. LISTOPADU 2021 / 27TH NOVEMBER, 2021

12:15 - 12:30	Prognostic value of tumor-infiltrating lymphocytes (TILs) and their association with PD-L1 expression and DNA repair protein RAD51 in patients with resected non-small cell lung carcinoma **Jozef Škarda**
12:30 - 12:45	Predictive relevance of microRNA in patienta with NSCLC undergoing palliative chemotherapy **Kateřina Houfková**
12:45 - 13:00	LC3A positive “stone like structures” are differentially associated with survival outcomes and CD68 macrophage infiltration in patients with lung adenocarcinoma and squamous cell carcinoma **Jozef Škarda**
13:00 — 14:00	**LUNCH**

Molecular pathology / 14:00 — 15:15 / Madrid Room

Chairs: Jana Steigerová, Josef Srovnal

14:00 - 14:15	Circulating Tumor Cells Detection in Solid Tumors using the CytoTrack instrument **Pavel Stejskal**
14:15 - 14:30	Key genes and pathways associated with Skp2 and Slug in prostate cancer by bioinformatics analysis **Gvantsa Kharaishvili**
14:30 - 14:45	The cell painting assay **Alžběta Srovnalová**
14:45 - 15:00	Transcriptomic Profiling Identified Lnc-GOLGA6A-1 as a Negative Prognostic Biomarker for Meningioma Recurrence **Hanuš Slavík**
15:00 - 15:15	Ceremonial closing and awards for the best oral and poster presentation **Sponsored by Cancer Research Czech Republic**

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Novel drugs and therapies / 13:00 — 15:00

Chairs: Petr Džubák, Milan Urban

Čtvrtek / Thursday - 25. listopadu 2021 / 25th November, 2021

MADRID ROOM

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

Zbyněk Nový[1], Miloš Petřík[1], Marián Hajdúch[1]

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

Introduction

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

Materials/methods

The compounds (FR54, FR55, FR94 and FR96) were tested in mice model using human prostate cell line LNCaP. Ex vivo biodistribution was evaluated in various time points (1, 4, 24, 48, 72 and 120 h p.i.) by dissecting the animals and measuring radioactivity in 12 different organs. The liver, kidneys and tumors were then examined by means of histology (H&E staining, PSMA, gamma H2AX and Ki67).

Results and conclusions

The principal organs accumulating tested PSMA ligands were tumor and kidneys with vastly higher uptake compared to other evaluated organs (up to 120 %ID/g). Tumor-to-blood ratio was 1 490 in case of FR94. Histology showed necrotic lesions in the tumors, high PSMA expression in tumor tissue, DNA damage in FR94/96 treated tumors and higher cellular proliferation in untreated tumors.

Results and conclusions

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

Anti-SARS-CoV-2 activity of one existing anti-inflammatory drug

Ermin Schadich[1,2], Juan Bautista De Sanctis[2], Tomáš Oždian[2], Hana Jaworek[2], Petr Džubák[2], Marián Hajdúch[2]

[1]Olomuc, Olomuc, Czech Republic. [2]Institute of Molecular and Translational Medicine, Palacky University, Olomuc, Czech Republic

Current therapeutic measures against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 2019 (COVID-19) are limited. Because there is no effective antiviral drug in the place, both novel compounds and existing drugs have been tested *in vitro* for activity against SARS-CoV-2. In this study, one existing anti-inflammatory drug was tested for anti-SARS-CoV-2 activity and effects on ceramide profiles of SARS-CoV-2 infected Vero cells. In antiviral assay, Vero cells were infected by SARS-CoV2 at M.O.I of 0.02 and treated by the anti-inflammatory drug and reference drug remdesivir for 72 h. Infected and non-infected control cells were treated by corresponding concentrations of DMSO. The level of viral infection was monitored by measuring cytopathic effects and viral load using MTT assay and qRT-PCR, respectively. In separate assays, Vero cells were infected by SARS-Cov2 and treated by the anti-inflammatory drug. Their total lipids were extracted and used for mass-spectrometry analysis of ceramide profiles. The ceramide profiles of treated cells were compared with those of infected and non-infected control cells. The existing anti-inflammatory drug inhibited the SARS-Cov2 infection of Vero cells. Its C50 against SARSCov2 virus was 4.50 μM. It also modulated the distinct ceramide profiles of the SARS-Cov2 infected Vero cells. The existing anti-inflammatory drug has the marked property to inhibit SARS-Cov2 in infected Vero cells. It also has property to modulate the ceramide profiles of the SARS-Cov2 infected Vero cells, and this is intriguing as such property might preclude the overrated inflammatory responses.

Determination of biological activity of compounds from IMTM Proprietary Library based on their cytotoxicity profiling in HTS facility

Soňa Gurská[1], Lenka Lachnitová[1], Renata Buriánová1, Petr Džubák[1], Marián Hajdúch[1]

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

Introduction

Cytotoxicity profiling is widely used in fundamental research as well as in drug discovery. It is a convenient, phenotypic and predictive mean of characterizing the toxic potential of new chemical entities. The implementation of *in vitro* cytotoxicity testing into high-throughput screening (HTS) platform allows to evaluate the cytotoxic activity of thousands of individual small molecules in a short time.

Materials/methods

The MTS assay as a cytotoxicity test is routinely used in our HTS laboratory. This test was validated on 10 cell lines (8 cancer cell lines and 2 non-cancer cell lines) in 384 and 1536 well plate format. In the primary screen, all compounds are tested at one concentration (50 μM) and the PI (percentage of inhibition) value is calculated. To calculate IC50 values for selected active compounds (PI > 50%), a secondary (dose-response) screen is performed. Data are analyzed by Dotmatics software. To quantify the suitability of cytotoxic assay in HTS, the Z-factor is determined for each plate and cell line.

Results and conclusions

Some results obtained in the cytotoxicity testing will be presented and discussed. The compounds with interesting results will be compared with properties of conventional drugs.

Study was supported by grants: This study was supported by the the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133, and CZ-OPENSCREEN, LM2018130), and the IGA_LF_2021_038 (Palacky University in Olomouc).

Highly active anticancer drug, PNH173, mechanism of action

Džubák Petr[1], Lenka Hrubá[1], Kateřina Ječmeňová[1], Jana Kotulová[1], Jana Václavková[1], Dušan Holub[1], Patricie Zizkovicova[1], Jana Vrbková[1], Barbora Lišková[1], Miroslav Popper[1], Michal Hocek[2], Michal Tichý[2], Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, UPOL, Olomouc, Czech Republic. 2IOCB CAS, Prague, Czech Republic

PNH173 is a nucleoside-based compound and highly active anticancer drug. The nanomolar drug activity was demonstrated on the tumour cell lines *in vitro* and *in vivo* as well. As the potential target was identified adenosine receptor A3. The aim of this study was the analysis of signalling downstream pathways responding to the treatment by PNH173. Particularly, we have focused on PI3K/Akt signalling pathway, especially on ribosomal protein S6 (rpS6). rpS6 is an indispensable component of the mammalian 40S small ribosomal subunit and is subject to phosphorylation in response to multiple physiological, pathological and pharmacological stimuli. In parallel, we have prepared resistant cell lines to study mechanisms of resistance. The results of the genomics and proteomics studies will be presented as well.

This study was supported by the European Regional Development Fund (Project ENOCH No. CZ.02.1.01/0.0/0.0/16_019/0000868), the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN, LM2018130), the Czech Science Foundation (GACR 19-08124S), internal grant of Palacky University (IGA_LF_2021_038) and Technology Agency of the Czech Republic (Project TN01000013).

Synthesis of fluorinated derivatives 2-phenyl-3-hydroxy-4(1H)-quinolinone and study of their anticancer activity

Jiří Řehulka[1], Kristýna Vychodilová[2], Petr Krejčí[2], Soňa Gurská[1], Pavel Hradil[2], Marián Hajdúch[1], Petr Džubák[1], Jan Hlaváč[2]

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

Quinolinones, which are often reported as inhibitors of bacterial topoisomerase or gyrase, inspired the synthesis of novel 2-phenyl-3-hydroxy-4(1H)-quinolinone derivatives. The cytotoxic activity *in vitro* was tested against human cancer cell lines. The most active derivative displayed selective cytotoxicity against the panel of human cancer cell lines. In addition, it exerted also an antiproliferative effect and induced mitotic arrest in acute lymphoblastic leukemia cell line CCRF-CEM. A detailed study of the compound revealed modulation of microtubule dynamics and inhibition of tubulin assembly *in vitro* as well as in cancer cell lines.

This work was supported by the Czech Science Foundation (reg. No. 18-26557Y), by grants from the Czech Ministry of Education, Youth and Sports (EATRIS-CZ, LM2018133, CZ-OPENSCREEN, LM2018130 and Czech-BioImaging, LM2018129), by the Euro-

pean Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and IGA_LF_2021_038 (Palacky University in Olomouc).

Bioisosteric approach in the improvement of selectivity in cytotoxic analogues of betulinic acid

Milan Urban[1], Ivo Frydrych[1], Barbora Lišková[1], Soňa Gurská[1], Marián Hajdúch[1], Petr Džubák[1], Jan Pokorný[2]

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

30-Oxobetulinic acid is a semisynthetic pentacyclic triterpenoid with high cytotoxicity in cancer cells. Unfortunately, the presence of a strong Michael acceptor in the molecule causes its insufficient selectivity towards cancer cells. First of all, a set of analogous molecules where the toxicophore was replaced by far less toxic azine moiety was prepared in that the reactive C=O bond was replaced to isosteric C=N bond, and indeed, the resulting compounds remained cytotoxic against CCRF-CEM cell line (2-4 μM) while in all other tested cell lines, the IC50 was in high micromolar concentrations. The main drawback of azines, however, was their low stability in water at room temperature. Therefore the final set of compounds was designed with another isosteric replacement - instead of C=O or C=N bond, we used C=C bond. And as we expected, the resulting dienes were stable and selective. Synthesis, cytotoxicity, mechanism of action and pharmacological properties will be discussed as well as both advantages and disadvantages among both new sets of compounds.

^{68}Ga-Ornibactin for positron emission tomography imaging of bacteria from Burkholderia cepacia complex

Kateřina Bendová[1], Vladislav Raclavský[2], Radko Novotný[2], Zbyněk Nový[1], Marián Hajdúch[1], Miloš Petřík[1]

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Introduction

Bacteria from Burkholderia cepacia complex (BCC) are generally considered non-pathogenic to healthy people, although some of these species are causes of severe nosocomial infections in immunocompromised patients. Due to BCC's broad resistance to antibiotics, it is crucial to rapidly diagnose these infections, so that adequate treatment could be initiated. For this purpose, we present here the use of radiolabelled siderophores for imaging by positron emission tomography (PET). Siderophores are small molecules, that are produced for iron acquisition by various organisms. Iron bound in siderophores might be switched for radioactive gallium-68 and this complex can be detected by PET. This work is focused on radiolabelled ornibactin, siderophore produced by BCC.

Methods

Ornibactin was labelled with gallium-68 and purity of this complex was measured on RP-HPLC. Various *in vitro* characteristic of ^{68}Ga-Ornibactin were tested. Uptake was evaluated in diverse respiratory pathogens and in BCC and it was compared to different siderophores. *Ex vivo* biodistribution studies were performed and *in vivo* imaging by PET was performed both on infected and non-infected mice in muscle infection model.

Results

^{68}Ga-Ornibactin has high radiochemical purity, it shows great stability in human serum, hydrophobic properties and low plasma protein binding values. Compared to various respiratory pathogens, its uptake is highest in BCC. *In vivo* it has rapid pharmacokinetics with renal excretion, and it accumulates in the site of infection.

Conclusion

^{68}Ga-Ornibactin has favorable *in vitro* characteristics, specificity for BCC, optimal pharmacokinetics and can be used for Burkholderia infection imaging.

Biomarkers in personalized medicine / 15:30 — 18:00

Chairs: Juan Bautista De Sanctis, Lukáš Najdekr

Čtvrtek / Thursday - 25. listopadu 2021 / 25th November, 2021

MADRID ROOM

Metabolite and lipid profiling of lung and breast cancer

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Lung and breast cancers represent one of the leading worldwide causes of cancer death. One of the reasons for high mortality and poor prognosis is the unavailability of a reliable early cancer diagnosis. Additionally, the pathobiochemistry of cancer is still not fully understood.

In this work, the lipidomic and metabolomic profiles of the tumor and surrounding normal tissues of 23 patients with non-small cell lung cancer were characterized. In total, 500 molecular species were detected and quantified by a combination of targeted shotgun lipidomic and metabolomic approaches using liquid chromatography-mass spectrometry. Our research revealed significant changes in several biochemical pathways related to the central carbon metabolism, acylcarnitines, dipeptides as well as the disruption in the lipid metabolism observed mainly for glycerophospholipids, sphingolipids, and cholesteryl esters. The findings not only confirm previous studies but point to new potential targets for the treatment of non-small cell lung cancer. The results from comprehensive metabolomic and lipidomic analysis contribute to the stratification of tumor groups and can be helpful with personalization of the treatment.

Additionally, apocrine sweat as an alternative material has been tested, offering the advantage of an easy and non-invasive sampling procedure. For this reason, we have designed a new device for apocrine sweat collection – SLIDE (Sweat sampLIng DevicE). In-depth lipid composition and evaluation of the biological variability in healthy individuals have been carried out. Based on our preliminary results, lipids in apocrine sweat could potentially serve as biomarkers for breast cancer.

This research was funded by AZV CR NU20-08-00367 and project No. 18-12204S sponsored by the Czech Science Foundation.

Colon Cancer and Perturbations of the Sphingolipid and Glycosphingolipid Metabolism

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The development and progression of colorectal cancer is accompanied with alterations of fatty acids, phospholipids and sphingolipids (SLs) in colon tumors, leading to deregulation of tumor cell lipidome. SLs and glycosphingolipids (GSLs) are bioactive, both structural and signaling molecules with a wide range of roles in control of key cellular processes, including cell growth, proliferation, and cell death. They contribute to formation of membrane microdomains, regulation of mebrane-bound proteins and formation of extracellular vesicles; they have also significant roles in cancer development, including colon cancer. Majority of the studies on SL levels and expression of genes of proteins involved in SL metabolism have so far focused on sphingosine-1-phosphate/ceramide ratio as well as on more complex GSLs, which are linked with increased colon cancer cell survival and cancer progression. However, our understanding of molecular basis of tumor development and its links with SL/GSL metabolism is still not well-characterized. We used two basic approaches - investigation of SL/GSL levels and gene/protein expression linked to SL/GSL metabolism in both non-transformed and transformed human cellular models, and determination of the same parameters in human colon cancer tissue. We identified specific alterations of SL and GSL metabolism, in particular significant accumulation of lactosylceramide, and related changes in gene expression, in whole colon cancer samples as well as in isolated tumor epithelial cells. The altered profiles of SLs, GSLs and the genes of SL/GSL metabolism may help to identify novel CRC-specific lipid/gene markers and/or potential therapeutic targets.

[Supported by the Czech Ministry of Health, grant no. AZV-NU21-03-00421.]

Comprehensive LC-MS approach for diagnosis of monogenic disorders

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Urinary organic acid (OA) analysis is an important part of the diagnosis of inherited metabolic disorders (IMD), monitoring of their treatment,

and preventing the development of the metabolic crisis. Clinical manifestations of IMD are usually non-specific, thus laboratory analysis of specific biomarkers is crucial for the correct diagnosis. Routinely, OA analysis is performed by GC-MS, which shows high separation efficiency and selectivity, but also has many shortcomings. These include in particular the time-consuming and laborious extraction and derivatization of analytes from samples and low sensitivity for acylglycines. Thanks to the newly developed LC-MS approach covering a total of 147 metabolites from a range of organic acids, acylglycines, and acylcarnitines it is possible to diagnose more than 90 IMD. Sample preparation is fast (5 min), does not require derivatization, and consists only of diluting the sample to a standard creatinine concentration and the addition of internal standards, while the analysis itself takes 26 min. The evaluation of the results is based on changes in the levels of specific biomarkers, which are assessed using a modified z-score that is linked to the IMD metabolic map in Cytoscape software to simplify the whole diagnostic process. More than 500 urine samples have already been analysed by this platform together with internal and external quality control samples. This personalized approach serves not only for the diagnosis of IMD but also as the second-tier method either for the confirmation or exclusion of positives from the newborn screening and for the monitoring of patients with IMD.

This project is supported by the Czech Science Foundation Grant (NU20-08-00367).

Proteomic analysis of exhaled breath condensate could be used for non-invasive lung cancer diagnostics and distinguish it from COPD

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Nowadays, lung cancer is associated with a bad prognosis because it is usually diagnosed in advanced stages when the tumour spreads further and metastasis are present. However, it could be successfully treated when diagnosed in the early stages. Up to date, no preventive screening exists because the methods currently used for lung cancer diagnosis, such as bronchoscopy, are invasive and associated with a patient´s pain. Thus, there is a need to develop a non-invasive method to monitor the condition of human lungs, which will be suitable for the preventive screening of high-risk groups. We chose an exhaled breath condensate (EBC) as a suitable body fluid collected non-invasively, containing a broad spectrum of analytes from the respiratory tract. Our work was focused on mass spectrometry-based protein analysis. We have implemented our recently published method, which we improved towards high resolution and reproducible in-depth protein identification.

Exhaled breath condensate was collected using Turbo 14 Turbo DECCS System (Medivac, Italy) from both healthy and diseased adult individuals who breathed through a mouthpiece into the collection device for 10 min. Proteins in the sample are solubilized, denatured, reduced, trypsin digested and purified using StageTip technology. Samples are measured by high-resolution mass spectrometry (HPLC-MS/MS-LTQ Orbitrap Elite) in 3 technical replicates. A powerful protein search strategy was developed in Proteome Discoverer software version 2.5 (Thermo Scientific). Data are further statistically evaluated by Statistica and Bioconductor R – package.

We have identified 7694 proteins across a cohort of 296 patients, and 6525 proteins were quantified. The proteins were filtered before further analysis to work only with proteins present in a sufficient number of samples. We applied univariate and multivariate statistical analysis to obtain a lung cancer protein signature in the EBC. The suggested biomarkers could distinguish lung cancer patients from COPD patients and healthy controls. Our lung cancer and COPD biomarker prediction models worked well, seem promising, and will be further studied and validated.

This work was supported by European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868), the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2021_038).

Could be the biomarkers of gynecological diseases detected from cervical mucus?

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There are multiple gynecological diseases and applications, where a good biomarker from a non-invasive source could improve the accuracy of diagnosis and therapeutic prediction. There are several such diseases related to the current development of the civilized world, such as infertility, endometriosis, or cancer of female reproductive organs. In this study, we test the suitability of cervical mucus as a non-invasive source of proteomic biomarkers. Cervical mucus is a viscous fluid produced by cervical glands located in the myometrium of the uterine cervix. During ovulation, cervical mucus starts to be less viscous, which is a good window for non-invasive sampling. Proteomic characterization of cervical mucus was therefore focused on two main goals – to optimize a protocol leading to the best identification score possible and to characterize and localize the source of the identified proteins. The resulting protocol consisted of mucus dissolution, multi-enzyme digestion, and LC-MS proteomic analysis. The proteins identified by this approach were both extra- and intracellular, and those proteins are widely expressed across the female reproductive organs. To our best knowledge, we provide the most extensive proteomic characterization available in current literature.

This work was supported by Czech Health Research Council (NV-18-02-00291), European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and EATRIS plus (871096).

Novel UHPLC-MS method for detailed analysis of lipid species using a scheduled MS/MS acquisition approach for improved metabolite annotation

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Introduction

Lipids are a vast group of biologically important molecules, which are structurally very similar within their classes. Despite the demand for fast UHPLC assays, long separation times decrease ion suppression occurring during the ionisation process, thus increasing the sensitivity and specificity of the analytical assay. This approach allows the discovery of new molecules normally masked or eliminated by ion suppression and co-elution and provides opportunities for the collection of a greater number of MS/MS mass spectra for lipid annotation.

Technological and methodological innovation

We developed a new ultra-high performance liquid chromatography (UHPLC) reversed-phase assay utilizing C_{30} stationary resins for the analysis of lipids present in biofluids and tissues. To maximise the number of compounds with MS/MS data we introduced a 'Scheduled MS/MS acquisition approach', which utilized several *m/z* windows spread over the chromatogram. As a comparison, a 15 minute reversed-phase C_{18} assay was applied with multiple biofluids and mammalian liver samples. Data were processed using Compound Discoverer 3.1 and LipidSearch 4.2.

Results and impact

A 30-minute C_{30} assay showed a 2.4x increase in the number of compounds detected compared to the 15-minute C_{18} assay. Using a single MS/MS file 15,000 compounds were detected and 1100 compounds were annotated applying LipidSearch on average for 30 min C_{30} assay. Using this novel assay will maximize the amount of information gathered from a single injection analysis and reduce the time required to collect these data compared to the commonly applied multiple injections with unique precursor windows [1].

Targeted metabolomic and lipidomic study of SHR24 rat models with tauopathy

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Alzheimer's disease (AD) belongs to a group of tauopathic neurodegenerative diseases, causing extensive neuronal damage leading to the development of dementia, cognitive loss, and many other neurological symptoms. One of the causes of AD is the structural change in the tau protein, which aggregates into neurofibrillary tangles that disrupt the neuronal cytoskeleton. Our study was aimed to describe the biochemical processes in model organisms with tauopathy. For this purpose, transgenic rat models SHR24 (TG) with tauopathy induced by expression of human truncated tau protein and control rat models SHR (CN) at 4, 6, 8, 10, 12, and 14 months of age were used. Four materials (plasma, CSF, brainstem back, and front) were collected from TG and CN rats. Samples were analyzed by targeted metabolomic and lipidomic approaches to characterize alterations in metabolic pathways. The analysis was performed using ultra-performance liquid chromatography coupled with tandem mass spectrometry. Acquired data were subjected to processing and statistical analysis, including univariate and multivariate (non)supervised methods. Both targeted analyses revealed the most

pronounced changes in metabolic and lipid profiles in 14-month-old TG rats compared with same-aged CN rats. For example, significantly elevated levels of the AD biomarker myoinositol, hydroxylated long-chained acylcarnitines, sphingomyelins, phospholipids of choline, serine, glycerol, and lysophospholipids were found in brainstem back samples. These changes can be explained by the development of tauopathy, caused by neuronal loss and membrane breakdown. The results of our study may contribute to the explanation of AD development and possibly to the design of novel diagnostic methods.

This work was supported by the Czech Science Foundation Grant (NU20-08-00367).

Therapeutic targeting of repurposed anticancer drugs for protein aggregation: Example of tau aggregates

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Introduction

Accumulation of amyloid-beta (Aβ) and tau aggregates are two characteristic pathological hallmarks of Alzheimer's disease (AD)[1]. The microtubule-associated protein tau found mainly in neurons, plays a pivotal role in stabilizing neuronal microtubules and in promoting axonal outgrowth. Experimental evidence suggests that the misfolded tau aggregates can spread between neurons in a prion-like manner in AD and other tauopathies by disseminating its associated pathology[2]. Inhibition of tau aggregation and/ or disaggregation of pre-formed tau fibrils along with targeting extracellular prion-like aggregated tau seeds are potential therapeutic strategies for the treatment of tauopathies. Many anticancer drugs have been reported to confer protection against neurodegeneration, supporting the repurposing of approved and experimental or investigational oncology drugs for AD therapy[3].

Materials/methods

Synthetic peptide of the third repeat domain of tau (tau R3) was used for establishing a Thioflavin T-based *in vitro* assay for screening of anti-cancer compounds. The tau R3 peptide has high self-aggregation potential and induces aggregation of the microtubule-binding domain of tau in cells. For the preparation of tau R3 aggregates, tau R3 peptide was incubated with heparin for 48 hours under constant shaking at 37°C in aggregation inducing buffer either in the presence or absence of anticancer compounds and the difference in fluorescence was observed using PerkinElmer EnSpire multimode plate reader by measuring excitation at 450 nm and emission at 485 nm. The formation of tau R3 fibrils and the effect of compounds on inhibiting tau R3 fibril formation was visualized by atomic force microscopy. The effect of compounds on inhibiting tau R3 dimerisation was analysed using non-reducing SDS-PAGE followed by Coomassie gel staining. Interaction of selected anti-cancer compounds with tau R3 was studied in detail with the aid of molecular dynamics study. The seeding effect of tau R3 aggregates either capped or formed in the presence of compounds were transfected to Tau RD P301S FRET biosensor cells and the resulting intracellular tau aggregation was measured using PerkinElmer Operetta high content imaging system.

Results and conclusions

In this study, we found that the anticancer drugs abrogated the formation of prion-like aggregates of tau R3 thus inhibiting the seeding in tau biosensor cells. The drugs used in this study interact with N-terminal hexapeptide VQIVYK or cysteine of C-terminal region of tau R3 thereby preventing R3 dimerization and abolishing prion-like tau R3 aggregates generation. Additionally, R3 aggregates capped with drugs or R3 aggregates formed in the presence of VQIVYK or cysteine-targeting drugs have reduced the seeding effect of R3 aggregates in tau biosensor cells. Results from this study substantiate that tau targeting anti-cancer drugs can be repurposed to prevent tau spreading and AD development.

Acknowledgement

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

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Supplementation with polyunsaturated and saturated fatty acids enhance NK cytotoxic response in Sprague Dawley-Rats. Effect of gender and age

Juan Bautista De Sanctis[1], Catalina Dumut[2], Amanda Centorame[2], Danuta Radzioch[2,1], Marian Hajduch[1]

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Fatty acid supplementation, saturated (ST), and polyunsaturated (PUFA), has been used to assess the mechanism of immune cell metabolism against tumour cells. However, few reports have addressed the impact of gender and age in animal models. Walker-259 mammary carcinoma was used in rats of both sexes, divided into two groups; young (10 weeks) and elder (70 weeks). The animals were fed with standard rat chow, and 300 mg/Kg of either ST or PUFA was administered through gavage daily, for three weeks. In non-tumour bearing, control rats, significant increases ($p<0.01$) in NK cytotoxic response were observed in both genders in the elder group since week 1, with ST and PUFA. In young animals, the significance was observed after the second week for ST treatment ($p<0.01$) and in both groups (ST and PUFA) in the third week ($p<0.001$). When the cytotoxic activity in control rats was compared with that of rats with tumours, the decrease in NK cytotoxic activity was significantly higher ($p<0.01$) in elder rats, independently of the gender and treatment, compared to their younger counterparts. These results suggest that fatty acid metabolism facilitates NK cell killing. However, the marked decrease of NK cytotoxic response in elder rats indicates that the decreased response of NK cells may not be modified extensively by supplementation.

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Age prediction through detection of DNA methylation

Lucie Kotková[1], Rastislav Slavkovský[1], Veronika Holinková[1], Barbora Blumová[1], Helena Jurtíková[1], Jana Stránská[1], Jiří Drábek[1]

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Prediction of different phenotypic traits of an unknown donor (an offender, unknown victim, or witness) from DNA traces can be an important intelligence tool in a forensic setting, helping to narrow down the investigation. Probably the most pursuit and most rapidly developing phenotyping tool all over the world is age prediction.

Currently, there is one commercially available epigenetic typing kit by Qiagen (AgePlex), which is validated for the Polish population. This kit was tested in our institute on a set of 200 blood samples from healthy blood donors, but it did not achieve declared error rates on the Czech population.

We decided to develop and test our age prediction model, suitable for routine forensic practice in Criminalistics Institute in Prague.

We selected 15 CpGs previously published as differentially methylated depending on age and we choose methyl-specific qPCR in duplexes (to lower sample requirements which are always limited in forensic setting) followed by next-generation sequencing using Illumina platform MiSeq or NovaSeq. The statistical model will be developed using data from the same sample set already tested by the AgePlex kit to compare the performance of both prediction models. Here we present our preliminary data.

Acknowledgements

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Interdisciplinary research in pathology / 13:00 — 15:00

Chairs: Jiří Ehrmann, Jan Bouchal

Čtvrtek / Thursday - 25. listopadu 2021 / 25th November, 2021

OLOMOUC ROOM

Multiple myeloma 2000+

Jiri Minarik[1], Tomas Pika[1], Patrik Flodr[2], Petra Krhovska[1], Jaroslav Bacovsky[1]

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In the last 20 years, the diagnostics and treatment of multiple myeloma (MM) experienced an exciting era. The disease is still reckoned incurable, however, the survival measures have improved substantially.

The diagnostics of MM relies on the presence of clonal plasma cells in the bone marrow (≥10%) or histological verification of plasmocytoma in a bioptic sample. Unlike formerly, the presence of monoclonal immunoglobulin is not mandatory for the definition of MM.

Until 2014, only overt MM with the presence of end organ involvement (CRAB: C = hypercalcemia, R = renal impairment, A = anemia, B = bone lesions) was treatment eligible. Due to the introduction of modern drugs with biological mechanism of action even asymptomatic MM including high-risk smoldering MM benefit from early introduction of systemic therapy. The reason for therapy is based on the presence of myeloma defining events (MDE), which include CRAB criteria as well as „the biomarkers of malignancy", i.e. ≥60% of clonal plasma cells in bone marrow, the ratio of involved/uninvolved serum free light chains ≥ 100 or >1 osteolytic lesion (≥5mm) on magnetic resonance imaging.

Modern therapeutic approaches use the combination of novel drugs such as proteasome inhibitors - PIs, immunomodulatory drugs – IMiDs or monoclonal antibodies – MoAbs. The new goal of therapy is the achievement of minimal residual disease negativity. Today, most patients can achieve deep therapeutic responses even in relapsed setting.

The presentation will focus on historical background of MM, the present diagnostic tools, and the progress in therapeutic strategies.

Supported by NV18-03-00500, IGA-LF-2021-001.

Current approach to typing systemic and localised amyloidosis

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Amyloidosis is a heterogeneous acquired or hereditary disease that results from the abnormal and insoluble deposition of beta-sheet fibrillar protein aggregates in various tissues with variable distribution in extracellular space. Nomenclature classification distinguishes 36 amyloidogenic proteins and more are expected (ISA 2018). Our file contains 329 specimens with amyloid deposits diagnosed between the years 2007-2021 in variable tissues and organs stained with Congo red and/or Saturn red with consequent immunohistochemistry analysis (IHC), and part of the file was also analysed by proteomic analysis with the use of laser microdissection-liquid chromatography/mass spectrometry (LMD-LC/MS). Analysis of tissue samples with amyloid deposits over the above mentioned years was improved by consequent application of more accurate special diagnostic staining, with more robust IF a IHC typing with adding LMD-LC/MS as at first experimental equipment and finally diagnostic tool. More complex and multidisciplinary approach in the amyloid diagnostic process opened a new view on pathogenesis of local or systemic amyloidosis with new challenges and difficulties in classification. Analysis of amyloid deposits irrespective of origin and localization is appealing for diagnostic and experimental precising which brings new insights not only particular pathogenesis but also in classification nomenclature, and more non-invasive procedures are being searched.

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A drug repurposing strategy for overcoming human multiple myeloma resistance to standard-of-care treatment

Katarina Chroma[1], Zdenek Skrott[1], Jan Gursky[1], Jaroslav Bacovsky[2], Pavel Moudry[1], Tereza Buchtova[1], Martin Mistrik[1], Jiri Bartek[1,3,4]

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Despite several approved therapeutic modalities, multiple myeloma (MM) remains an incurable blood malignancy and only a small fraction of patients achieves prolonged disease control. The common anti-MM treatment targets

proteasome with specific inhibitors (PI). The resulting interference with protein degradation is particularly toxic to MM cells as they typically accumulate large amounts of toxic proteins. However, MM cells often acquire resistance to PIs through aberrant expression or mutations of proteasome subunits such as PSMB5, resulting in disease recurrence and further treatment failure. Here we propose CuET – a proteasome-like inhibitor agent that is spontaneously formed in-vivo and in-vitro from the approved alcohol-abuse drug disulfiram (DSF), as a readily available treatment effective against diverse resistant forms of MM. We show that CuET efficiently kills also resistant MM cells adapted to proliferate under exposure to common anti-myeloma drugs such as bortezomib and carfilzomib used as the first-line therapy, as well as to other experimental drugs targeting protein degradation upstream of the proteasome. Furthermore, CuET can overcome also the adaptation mechanism based on reduced proteasome load, another clinically relevant form of treatment resistance. Data obtained from experimental treatment-resistant cellular models of human MM are further corroborated using advanced cytotoxicity experiments on myeloma and normal blood cells obtained from fresh patient biopsies including newly diagnosed as well as relapsed and treatment-resistant MM. Overall our findings suggest that disulfiram repurposing particularly if combined with copper supplementation may offer a promising and readily available treatment option for patients suffering from relapsed and/or therapy-resistant multiple myeloma.

Cannabidiol-induced expression of metallothioneins attenuate the anticancer effect of disulfiram

Tereza Buchtová[1], Zdeněk Škrott[1], Katarína Chromá[1], Jiří Řehulka[1], Petr Džubák[1], Marián Hajdúch[1], Dávid Lukáč[1], Stefanos Arampatzis[2], Jiří Bártek[2,1,3]

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Disulfiram (DSF), an established alcohol-aversion drug, and a candidate for repurposing in cancer treatment. DSF's antitumor activity is supported by preclinical studies, case reports, and small clinical trials; however, ongoing clinical trials of advanced-stage cancer patients encounter variable results. Here, we show that one reason for the inconsistent clinical effects of DSF may reflect interference with other drugs. Using a high-throughput screening and automated microscopy, we identify cannabidiol (CBD), an abundant component of the marijuana plant which significantly attenuates DSF's anticancer effects. Importantly, marijuana-derived products containing CBD are popular among cancer patients to mitigate the side effects of chemotherapy. Mechanistically, in cancer cells, cannabidiol triggers the expression of metallothioneins providing protective effects by binding heavy metal-based substances including the bis-diethyldithiocarbamate-copper complex (CuET). CuET is the documented anticancer metabolite of DSF, and we show that the CuET's anticancer toxicity is effectively neutralized by metallothioneins. Overall, this work highlights an example of undesirable interference between cancer therapy and the concomitant usage of marijuana products. In contrast, we report that insufficiency of metallothioneins sensitizes cancer cells toward CuET, suggesting a potential predictive biomarker and/or co-target for DSF repurposing in oncology.

Iodine Contrast Medium Affects Urine Cytology Assessement - A Prospective Single Blinded Study And Its Impact For Urological Practice

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Introduction and Objectives

Urine cytology is one of the most crucial diagnostic methods in assessment of both upper and lower urinary tract tumours. High interobserver and intraobserver variability in cytology specimen evaluation is commonly seen. During endoscopic procedures for suspicious urothelial tumours of upper urinary tract a radiographic imaging using iodine contrast medium is often necessary. Unfortunately, after ureteropyelography we have detected changes in cytology characteristics not correlating with cytology finding in naive urine. The aim of our study was to assess the cytology changes between naive and postcontrast urine.

Materials and Methods

During November 2017 and May 2021 we prospectively assessed urine samples from 89 patients (24 patients with histologically proven urothelial cancer and 65 healthy volunteers). Absence of malignancy was proved usually by CT urography and/or ureteroscopy (both done due to extra-study reasons, e.g. for stone treatment). Study was single-blinded (examined by single expert uropathologist) and Paris classification system for urine cytology assessment was used (1-non-diagnostic, 2-negative for high-grade urothelial cancer, 3-atypical cells, 4-suspect for high-grade urothelial cancer, 5-high-grade urothelial cancer, 6-low-grade urothelial cancer, 7-others: primary and secondary tumours). Furthermore, other cytologic parameters were analyzed (specimen cellularity and clarity, level of cytolysis, cytoplasmatic and

nucleus colour etc.)

Results

Our study proved statistically significant differences in Paris system in naive and postcontrast urine in healthy volunteers (51 % concordance vs. 49 % discordance, p=0,001) versus 79 % concordance in malignant urine specimen. The most important differences were in shift from category 2 (negative) to 1 (non-diagnostic) in 11 % of cases and shift from category 2 (negative) to 3 (atypia), again in 11 %. Other significant changes were for assessment of specimen cellularity (p=0,0003), clarity (p=0,013), level of cytolysis (p=0,001) and cytoplasmic colour (p=0,003) – in all of these the most noticeable changes were seen in the control group.

Conclusion

Our study confirms crucial changes in cytologic assessment of naive and postcontrast urine and is due to its design unique (prospective, patologist blinded). Study conclusions proved the suggestion that postcontrast urine is more often assessed cytologically as abnormal, suspect or on the contrary non-diagnostic). Therefore, before urine cytology sampling, the clinician should avoid applying of iodine contrast to urinary tract.

Cell surface antigen signature and its plasticity in breast and prostate cancer

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The intratumoral heterogeneity, often driven by epithelial-to-mesenchymal transition (EMT), significantly contributes to chemoresistance and disease progression in adenocarcinomas. Therefore our aim is to uncover the molecules that accompany, mirror and regulate cancer plasticity and mechanisms responsible for transcriptional reprogramming of cancer cells and help to identify potential biomarkers and therapies that specifically target tumor- and metastasis-initiating cells and prevent cancer relapse and macrometastatic outgrowth.

We introduced a high-throughput screening platform to identify surface antigens that associate with epithelial-mesenchymal plasticity and chemoresistance in well-defined pairs of cancer cell lines. Using multicolor flow cytometry, we then analyzed the expression of most robustly changed antigens and identified a surface signatures, in breast tumors and docetaxel resistant prostate cancer. We found that surface CD9, CD29, CD49c and Integrin β5 are lost in breast cancer cells that underwent EMT *in vivo*. The tetraspanin family member CD9 was concordantly down-regulated both *in vitro* and *in vivo* and associated with epithelial phenotype and favorable prognosis. We also revealed several antigens directly linked to the resistance to antimicrotubule agents and poor prognosis in prostate cancer patients.

We propose that the overall landscape of the surface fingerprints reflects the epithelial-mesenchymal plasticity in breast cancer and associate with docetaxel resistance in prostate cancer.

Inhibition of aryl hydrocarbon receptor (AhR) expression in colon cancer cells disrupts cell proliferation, energy metabolism and expression of enzymes involved in fatty acid synthesis

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor playing a wide range of physiological roles in processes such as proliferation, migration or control of immune responses. Several studies have indicated that AhR has physiological role in regulation of energy balance and may affect cellular metabolism. Mechanistic links between the AhR and control of tumor cell metabolism thus deserve more attention, as well as the potential of the AhR as a possible target for anticancer therapies, including colon cancer. We observed

that AhR is upregulated directly in colon tumor cells and using wild-type and corresponding AhR knockout (AhR KO) variants of human colon cancer cell lines, we analyzed the functional role of AhR in colon cancer cell proliferation and metabolism, with a focus also on regulation of synthesis of FAs. We observed a decreased proliferation rate in some AhR KO cell lines, accompanied with altered cell cycle progression and a decreased ATP production. We also determined reduced mRNA levels of key enzymes of FA-biosynthetic pathway (such as ACLY, ACACA, FASN or SCD). The loss of AhR was also associated with reduced expression and/or activity of components of the PI3K/Akt pathway and other lipogenic transcriptional regulators, such as SREBP1. Together, our data indicate that disruption of AhR activity in colon tumor cells may limit their proliferation, which is also linked with a suppression of their metabolism. [Supported by the Czech Science Foundation, grant no. 19-00236S, and the Czech Ministry of Health, grant no. NU21-03-00421.]

Clinical and molecular pathology / 15:30 — 17:40

Chairs: Gabriela Dostálová, Zdeněk Kolář

Čtvrtek / Thursday - 25. listopadu 2021 / 25th November, 2021

OLOMOUC ROOM

Role imunohistochemie v diagnostice atypické endometriózy

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Úvod

Atypická endometrióza představuje z klinického i morfologického hlediska problematickou kategorii. Tímto termínem jsou označovány endometrióza s atypiemi žlázového epitelu nebo endometrióza s glandulární hyperplazií, kterou můžou, ale nemusí doprovázet buněčné atypie. Atypická endometrióza je považována za prekurzorovou lézi karcinomů asociovaných s endometriózou.

Materiály a metodika

Retrospektivně jsme revidovali sérii 120 ovariálních endometriomů, ze kterých jsme vybrali 5 případů, které splňovaly histopatologická kritéria pro atypickou endometriózu. Dále jsme vytvořili soubor celkem 40 případů hluboké infiltrující endometriózy (DIE, deep infiltrating endometriosis), který jsme na základě postižení regionálních lymfatických uzlin (LU) rozdělili do dvou skupin: skupina 1 - případy DIE bez postižených LU (n= 28) a skupina 2 - lymfatické uzliny postižené endometriózou (n= 12). S využitím imunohistochemie jsme mezi jednotlivými skupinami porovnali expresi hormonálních receptorů, nádorového supresoru p53 a proliferačního markeru Ki-67.

Výsledky: Nezjistili jsme rozdíly v expresi vyšetřovaných markerů mezi DIE (skupina 1) a endometriózou v LU (skupina 2). Zjištěny však byly statisticky významné rozdíly v expresi hormonálních receptorů a p53 mezi atypickou endometriózou a oběma skupinami. U atypické endometriózy došlo k signifikantnímu poklesu exprese estrogenových i progesteronových receptorů a k nárůstu exprese p53 (množství p53 pozitivních žlázových buněk bylo ve tkáni atypické endometriózy průměrně 26 %, zatímco v DIE a endometrióze v LU byla exprese p53 prakticky negativní).

Závěr

Výsledky naší studie poukazují na možnou roli imunohistochemie v diagnostice atypické endometriózy. Diagnóza atypické endometriózy by měla být zvažována pouze v situacích, kde míra atypií žlázového epitelu dosahuje středního či vysokého stupně a kde zánětlivé změny jsou pouze mírné.

Teamwork in Thyroid Diseases Diagnostics and Treatment. Total Thyroidectomy Can Still Remain the Method of Choice in Some Bethesda III FNAB Cases

Jaroslava Dušková[1], *Barbora Hintnausová*[2], *Vlasta Sýkorová*[3], *Martin Syrůček*[4], *Marek Malý*[5], *Jindřich Lukáš*[6,7]

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BACKGROUND. The latest WHO classification of tumours defines new units of borderline thyroid tumours (BTT). The aim of our study was to evaluate ultrasonographic and cytological features, mutation profile and surgery treatment in rare thyroid tumours. METHODS. An analysis of 8 BTT out of 487 patients, who underwent thyroid surgery between June 2016 and June 2020. The definitive diagnosis was made by histopathological examination. Molecular genetic analysis of genes associated with thyroid oncology (*BRAF*, *HRAS*, *KRAS*, *NRAS*, *TERT*, *TP53*, fused genes) were performed from one FNAB, and 7 formalin-fixed paraffin-embedded (FFPE) samples. RESULTS. BTT were found in a total of 8 patients (1.6 %), with a predominance of men with respect to other operated patients. FNAB samples were classified in the Bethesda system as Bethesda I, Bethesda II and Bethesda III in one, four and three cases, respectively. Hemithyroidectomy and total thyroidectomy were performed equally in four patients. The histopathological diagnosis revealed non-invasive encapsulated follicular neoplasm with papillary-like nuclear features (NIFTP) in three patients, follicular tumour of uncertain malignant potential (FT-UMP) in three patients, well differentiated tumour of uncertain malignant potential (WDT-UMP) in one patient, and hyalinizing trabecular tumour (HTT) in one case. In NIFTP cases mutation in *HRAS* gene in one patient together with probable pathogenic variant in *TP53* gene and in *NRAS* gene in two patients were detected. In HTT patient *PAX8/GLIS3* fusion gene was detected. CONCLUSION: The surgical treatment of BTT is necessarily individual influenced by preoperative clinical, ultrasonographic, cytological and molecular genetic findings, and the presence of other comorbidities.

I. Patofyziologie a patologie M. Gaucher a M. Fabry.

MUDr. Helena Hůlková, Ph.D.

Klinika pediatrie a dědičných metabolických poruch a Ústav patologie 1. LF UK a VFN Praha

Fabryho (FD) a Gaucherova choroba (GD) reprezentují onemocnění ze skupiny lysosomálních enzymopatií, kdy defekt v genu kódujícím určitou lysosomální hydrolázu vede k významnému poklesu její aktivity a ke střádání příslušného nedegradovatelného substrátu v lysosomech různých buněčných typů. Tento mechanismus onemocnění spolu s převažující manifestací ve viscerálních orgánech činí obě jednotky přístupnými enzymové substituční terapii. Screening, diagnostika a léčba pacientů s FD a GD se soustřeďují do specializovaných center a zároveň jsou u obou jednotek dostupné neinvazivní diagnostické postupy (měření aktivity příslušného enzymu v leukocytech periferní krve nebo v suché krevní kapce a následně průkaz konkrétní mutace v genech tyto enzymy kódujících). Následkem tohoto přínosného trendu se primozáchyt probanda patologem stává v současné době velmi vzácným. U FD je renální či endomyokardiální biopsie zvažována v diagnostickém algoritmu až při nálezu varianty nejistého významu sekvenováním genu α-galaktosidázy A (αGal). Existují však podstatné důvody, proč je nutné rozšiřovat a prohlubovat znalosti o změnách na úrovni buněk a tkání u těchto onemocnění. Specializovaná centra poptávají zpětnou vazbu o změnách u dlouhodobě léčených pacientů, což vyžaduje optimalizovat způsob provádění pitev v těchto případech. Dále se u specificky léčených, a tedy déle žijících pacientů mohou manifestovat další změny, které je nutno studovat na všech dostupných úrovních. Konečně velkou výzvu prostupující řadou oborů včetně patologie přináší celogenomové sekvenování u pacientů, kteří nejeví klasický fenotyp lysosomálního střádání a u nichž mohou molekulárně genetické změny v příslušných genech působit poruchy buněk a tkání odlišnými mechanismy.

Fabryho choroba v souboru biopsií ledvin. Nefrologické screeningy v ČR

Eva Honsová

Aeskulab Patologie, Praha

U pacientů s Fabryho chorobou (FC) většina studií dokládá renální manifestaci u prakticky všech mužů a významného počtu žen a dětí.

Problematika hodnocení FC v biopsiích ledvin zahrnuje 3 situace. V první je onemocnění časné a typické, v druhém případě je v biopsii pokročilé onemocnění s rozdílnou morfologií, třetí případ zahrnuje pacienty, kteří mají současně další imunokomplexové onemocnění ledvin.

Renální manifestace FC je plíživá, nebolestivá s klinicky širokou diferenciální diagnózou, což dobře ilustruje fakt, že v našem souboru žádná biopsie nepřišla s klinickou diagnózou FC.

Protože jde o genetické onemocnění, kde odhalení jednoho případu obvykle pomůže několika dalším členům rodiny, má stále význam provádění screeningu v rizikových populacích (v ČR na dialyzačních odděleních) a do budoucna jeho přesunutí mezi screeningy novorozenecké.

II. Další histopatologická vyšetření u M. Gaucher a M. Fabry.

MUDr. Helena Hůlková, Ph.D.

Klinika pediatrie a dědičných metabolických poruch a Ústav patologie 1. LF UK a VFN Praha

Fabryho (FD) a Gaucherova choroba (GD) reprezentují onemocnění ze skupiny lysosomálních enzymopatií, kdy defekt v genu kódujícím určitou lysosomální hydrolázu vede k významnému poklesu její aktivity a ke střádání příslušného nedegradovatelného substrátu v lysosomech různých buněčných typů. Tento mechanismus onemocnění spolu s převažující manifestací ve viscerálních orgánech činí obě jednotky přístupnými enzymové substituční terapii. Screening, diagnostika a léčba pacientů s FD a GD se soustřeďují do specializovaných center a zároveň jsou u obou jednotek dostupné neinvazivní diagnostické postupy (měření aktivity příslušného enzymu v leukocytech periferní krve nebo v suché krevní kapce a následně průkaz konkrétní mutace v genech tyto enzymy kódujících). Následkem tohoto přínosného trendu se primozáchyt probanda patologem stává v současné době velmi vzácným. U FD je renální či endomyokardiální biopsie zvažována v diagnostickém algoritmu až při nálezu varianty nejistého významu sekvenováním genu α-galaktosidázy A (αGal). Existují však podstatné důvody, proč je nutné rozšiřovat a prohlubovat znalosti o změnách na úrovni buněk a tkání u těchto onemocnění. Specializovaná centra poptávají zpětnou vazbu o změnách u dlouhodobě léčených pacientů, což vyžaduje optimalizovat způsob provádění pitev v těchto případech. Dále se u specificky léčených, a tedy déle žijících pacientů mohou manifestovat další změny, které je nutno studovat na všech dostupných úrovních. Konečně velkou výzvu prostupující řadou oborů včetně patologie přináší celogenomové sekvenování u pacientů, kteří nejeví klasický fenotyp lysosomálního střádání a u nichž mohou molekulárně genetické změny v příslušných genech působit poruchy buněk a tkání odlišnými mechanismy.

Bank of Clinical Specimens: Bridging the gap in translational research

Roman Hrstka

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Biobanking becomes an integral part of translational cancer medicine. The Czech national research biobanking infrastructure BBMRI.cz collects and stores human-derived biospecimens and associated data using standardized procedures. These biospecimens are of critical

importance for either existing or future research projects and for patient benefit as well. The constructed system of biobanks at BBMRI.cz consists of two types of storage for patient samples, long-term storage (LTS) repository, and short-term storage (STS) repository. STS repository contains sera only and is iteratively updated at each patient visit to the hospital when the blood specimen is taken for the determination of tumor markers. The LTS repository collects various types of tissues (tumor, metastasis, non-tumor) classified by diagnosis, serum at surgery, genomic DNA and other. The unique design of storing not only the tissue material but also longitudinal strings of sera enables access to patient-derived material during the course of the complex patient treatment. Designed this way, the research Biobanks are becoming truly critical tools to enhance translational cancer research.

In the Czech Republic, BBMRI.cz organizes not only a dedicated set of cancer-oriented biorepositories but also operates a unique set of technologies and knowledge to perform clinical applications of translational research including clinical trials. The user community may take advantage of the expertise of the BBMRI.cz qualified staff and resources archived in biorepositories. BBMRI.cz is a part of the pan-European research infrastructure BBMRI-ERIC (Biobanking and Biomolecular Resources Research Infrastructure) and is connected to the web-based tools BBMRI-ERIC Directory (https://directory.bbmri-eric.eu) and BBMRI-ERIC Negotiator (https://negotiator.bbmri-eric.eu/) representing key services that provide an efficient communication platform for biobankers and researchers. They substantially simplify the communication steps that are necessary to obtain information on the availability of relevant samples/data, particularly if the researchers need to communicate with multiple candidate biobanks.

BBMRI.cz is supported by LM2018125 Large Research, Development and Innovation Infrastructures Project of MEYS Czech Republic.

Cytokeratin 7 expression as a predictor of an unfavorable prognosis in colorectal carcinoma

Jan Hrudka, Hana Fišerová, Karolína Jelínková, Radoslav Matěj, Petr Waldauf

Lékařská Fakulta Univerzity Karlovy, Praha 10, Czech Republic

Colorectal carcinoma (CRC) is associated with significant morbidity and mortality worldwide. Cytokeratins (CKs) are widely expressed in various types of carcinomas, whereas in CRC it is usually CK7 − and CK20 + . A subset of CRCs is CK7 + . This study aims to determine the prevalence of CK7 expression in CRC and its impact on overall survival. We analyzed 300 randomly selected surgically treated CRC cases using paraffin embedded tumor tissue samples and evaluated CK7 and CK20 expression using the tissue microarray method. Tumors with positivity > 10% and > 25% of tumor cells were considered CK7 and CK20 positive, respectively. Expression of both CKs and several clinicalpathological variables (stage, grade, laterality, mismatch-repair/MMR status) were evaluated using patient follow up data (Kaplan–Meier analysis of cancer-specific survival (CSS)). Significant results include shorter CSS (restricted mean 4.98 vs. 7.74 years, P = 0.007) and 5-year survival (29.4% vs. 64.6%, P = 0.0221) in CK7 + tumors compared to CK7 − tumors, respectively; without significant association with grade, stage or right-sided location. These results were significant in a multivariate analysis. CK20 + tumors are more frequently MMR-proficient and left-sided. MMR-deficient tumors are more frequently right-sided and had longer survival. CK7 expression, right-sided location (rmean CSS 6.83 vs. 8.0 years, P = 0.043), MMR-proficiency (rmean CSS 7.41 vs. 9.32 years, P = 0.012), and UICC stages III + IV (rmean CSS 6.03 vs. 8.92 years, P < 0.001) of the tumor correlated with negative prognostic outcomes, whereas the most significant results concern stage and CK7 positivity.

MORPHOLOGICAL STUDY OF HNSCC FOCUSED ON PERINERUAL INVASION - a single institutional study with five year follow up

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Head and neck carcinoma (HNSCC) is a carcinoma with squamous differentiation arising from mucosal epithelium. It affects oral cavity, mobile and fixed tongue and oropharynx. It is the 6th most common cancer in the world with mortality 4-6/100000 people.

We retrospectively analyzed cases of 487 patients with HNSCC who underwent curative surgery with bilateral cervical block dissection in period 2006-2016 in age 29-85 years. We focused on the evaluation of stage, nodal status, PNI, BVI and LVI. Moreover, we added new parameters such as the worst pattern of invasion, tumour budding a lymphocyte infiltration.

Most of our cases exhibited 4th

degree of WPOI (212cases). PNI was present with an increasing frequency of this classification WPOI3: 12,9%, WPOI4: 26,9% WPOI5: 55,6%. Tumour budding (LG – less than 5 buds, IMG – 5-10 buds, HG – more than 10 buds) correlated with incidence of PNI, 85% HNSCC with PNI developed HG budding. Brisk (49,5%) and Non-brisk (42,9%) immune response presented by TIL correlated with these morphological signs. Evaluation of morphology of PNI was also done – type A(1%), type B(58,6%), type C(31%), type D(6%), type E(6%), type F(0%).

Our study revealed an association between PNI and other analyzed common diagnostic factors as well as newly selected morphological features. Next, we plan to focus on cellular and molecular processes accompanying the initiation of PNI with aim to uncover main cancer characteristics and possible involvement of neuronal chemoattractant.

The study was supported by the Ministry of Health Of Czech Republic, grant nr.AZV NV19-08-00383.

Identification of candidate genes underlying soft tissue sarcoma progression

Jiří Hatina[1], Krzysztof Biernacki[2], Michaela Kripnerová[1], Martin Pešta[1], Dagmara Tymecka[3], Aleksandra Misicka[3], Jiří Šána[4,5], Ondřej Slabý[4,6]

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[4]Molecular Oncology II - Solid Cancer,Central European Institute of Technology, Brno, Czech Republic
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Sarcomas encompass extensive group of mesenchymal tumours, with very heterogeneous biological bases and clinical behaviours. Identification of progression-related genes could have important impact on clinical care for sarcoma patients, serving as both prognostic factors and possible therapeutic targets. We used two well characterized experimental murine sarcoma cell line progression series, a fibrosarcoma- and a liposarcoma–based, and for each, we obtained transcriptomic profile of highly transformed sarcoma cells, whose overlap yielded a mouse sarcoma experimental progression signature, encompassing 88 downregulated and 95 upregulated genes. This has been successively confronted with two large human clinical sarcoma transcriptomic datasets, resulting in 28 downregulated and 27 upregulated genes successfully validated in at least one. Some of these validated genes have been previously brought to attention in rare soft tissue sarcoma types or osteosarcoma (c-jun, Tbx3, Tgfbi, Rab3ip, Alcam, Crabp1, Ecm1, periostin), and our analysis suggests a wider clinical impact, others have been identified in the context of clinical progression in various carcinomas (Snx6, uea transporter Slc14A1, Dpysl3, Ulk2, transient receptor potential calcium channel Trpc1, Steap3, Morc4, Crp2 and Coronin 1C), with our analysis suggesting also a role during sarcomagenesis. The human sarcoma progression genes were finally validated on the TCGA database and possible druggable targets have been selected from both the murine and human transcriptomic profiles based on literature search. Interestingly, TCGA validation resulted in a different combinations of validated progression genes in each the major high-grade sarcoma diagnoses. Among the druggable targets, the most promising validated target is the Cellular Retinoic Acid Binding Protein-1. Interestingly, various semaphorins, especially Sema3A and Sema5A, also seem to play a role in sarcoma progression. This opens an intriguing experimental therapeutic possibility by blocking simultaneously semaphoring signalling and VEGF signalling by therapeutic targeting of their common receptor subunit – Neuropilin 1. Experiments towards this therapeutic approach are in progress.

Supported by the Czech Science Foundation project No. 17-17636S, Charles University specific student research project 260 539/2020, and the 4EU+ University Alliance at Charles University project No 4EU+/21/F1/1.

The session of the Czech Society of Pathologists 08:15 — 10:00

Chairs: Pavel Dundr, Zdeněk Kolář, Jiří Ehrmann

Pátek / Friday - 26. listopadu 2021 / 26th November, 2021

EVROPA ROOM

Hlavův ústav, profesor Jaroslav Hlava a jeho následníci

Pavel Dundr

Ústav patologie 1. LF UK a VFN v Praze, Praha, Czech Republic

V letošním roce si připomínáme 100. výročí otevření Hlavova ústavu, které slavnostně proběhlo 18. dubna 1921. Otevření Hlavova ústavu představovalo vyústění mnohaletých snah profesora Hlavy o zřízení důstojných prostor pro patologii na české univerzitě. Hlava zcela zásadně ovlivnil českou patologii své doby a byl bezpochyby jednou z nejvýznamnějších osobností nejen české a evropské patologie, ale i celé české medicíny daného období. Cílem sdělení je při příležitosti tohoto významného výročí zmínit okolnosti související s otevřením Hlavova ústavu, osobnost profesora Jaroslava Hlavy, přehled jeho výzkumných aktivit a publikačních výstupů. Stručně také budou zmíněni následníci profesora Hlavy na pozici přednostů ústavu patologie.

Comparison of 5 different scoring methods in the evaluation of inflammatory infiltration (tumor infiltrating lymphocytes - TILs) in superficial spreading and nodular melanoma

Němejcová K., Tichá I., Bártů M., Kodet O., Důra M2, Jakša R., Michálková R., Dundr P.

Přednáška se týká naší práce, kde jsme se zabývali problematikou hodnocení tumor infiltrujících lymfocytů (TIL) u kožního melanomu. TIL představují prognosticky významný parametr, který by měl být součástí bioptických nálezů. Problém využití hodnocení TIL však v praxi naráží na nejednotnost v metodice hodnocení a diskrepantním výsledkům reportovaných v předchozích studiích. Pro některé nádorové typy existuje unifikovaná metodika hodnocení, která však v případě maligního melanomu stále chybí. Z hlediska praktického využití je žádoucí používat jednotnou metodiku hodnocení, umožňující srovnání mezi jednotlivými studiemi, ale i posouzení prediktivního významu TIL, zejména s ohledem na imunoterapii.

Proto bylo cílem naší práce porovnat pět různých skórovacích metod s ohledem na jejich prognostický význam. Na skupině 213 případů primárního kožních melanomů jsme hodnotili TIL za využití 5 skórovacích metod: i) hodnocení dle Clarka; ii) hodnocení podle „Melanoma Institute Australia"; iii) hodnocení použité ve studii Saldanha et al.; iv) hodnocení použité ve studii TCGA a modifikované dle Park et al .; v) metodika recentně navržená „International Immuno-Oncology Biomarker Working Group" pro hodnocení TIL ve všech solidních nádorech. Analýzu přežití jsme testovali pro parametry: přežití bez známek nemoci (DFS), přežití bez lokální recidivy (LFS) a přežití bez výskytu vzdálených metastáz (MFS).

Výsledky naší analýzy prokázaly v univariantní analýze prognostický význam TIL pro tři z pěti posuzovaných skórovacích systémů: metodiku hodnocení podle Clarka, metodiku navrženou skupinou MIA a podle systému navrženém „International Immuno-Oncology Biomarker Working Group" – poslední navrženou však pouze při hodnocení stromálních TIL (DFS, LFS a MFS $p<0,05$), přičemž žádná z metod se nezdá být lepší než ostatní. Z hlediska praktického využití je žádoucí používat jednotnou metodiku hodnocení, což by umožnilo srovnání mezi jednotlivými studiemi, ale i posouzení prediktivního významu TIL, zejména s ohledem na imunoterapii.

Pigment Cell Melanoma Res. 2019 May;32(3):412-423. doi: 10.1111/pcmr.12757. (IF 2018/2019 – 4.172)

Extracellular Amyloid Deposits in Alzheimer's and Creutzfeldt–Jakob Disease: Similar Behavior of Different Proteins?

Nikol Jankovska[1], Tomas Olejar[1] and Radoslav Matej[1,2,3]

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[2]Department of Pathology, First Faculty of Medicine, Charles University, and General University Hospital, 128 00 Prague, Czech Republic
[3]Department of Pathology, Third Faculty of Medicine, Charles University, and University Hospital Kralovske Vinohrady, 100 00 Prague, Czech Republic

Neurodegenerative diseases are characterized by the deposition of specific protein aggregates, both intracellularly and/or extracellularly, depending on the type of disease. The extracellular occurrence of tridimensional structures formed by amyloidogenic proteins defines Alzheimer's disease, in which plaques are composed of amyloid β-protein, while in prionoses, the same term "amyloid" refers to the amyloid prion protein. We focused on providing a detailed didactic description and differentiation of diffuse, neuritic, and burnt-out plaques found in Alzheimer's disease and kuru-like, florid, multicentric, and neuritic plaques in human transmissible spongiform encephalopathies, followed by a systematic classification of the morphological similarities and differences between the extracellular amyloid deposits in these disorders. Both conditions are accompanied by the extracellular deposits that share certain signs, including neuritic degeneration, suggesting a particular role for amyloid protein toxicity.

Central Pathology Review in SENTIX, A Prospective Observational International Study on Sentinel Lymph Node Biopsy in Patients with Early-Stage Cervical Cancer

Nemejcova K, Kocian R, Kohler C, Jarkovsky J, Klat J, Berjon A, Pilka R, Sehnal B, Gil-Ibanez B, Lupo E, Petiz A, Sanchez OA, Kascak P, Martinelli F, Buda A, Presl J, Barahona M, Lonkhuijzen LV, Szatkowski W, Minar L, Pakiz M, Havelka P, Zorrero C, Misiek M, Snyman LC, Wydra D, Vergote I, Vinnytska A, Redecha M, Michal M, Tingulstad S, Kipp B, Szewczyk G, Toth R, Garcia FJS, Martin PJC, Poka R, Tamussino K, Luyckx M, Fastrez M, Staringer JC, Germanova A, Plaikner A, Bajsova S, Dundr P, Mallmann-Gottschalk N, Cibula D.

V naší práci jsme se zabývali problematikou hodnocení sentinelových uzlin u karcinomů děložního hrdla. Metastatické postižení lymfatických uzlin je nejdůležitějším prognostickým faktorem u karcinomů hrdla v časném stádiu, ale mezinárodně uznávané postupy na zpracování sentinelových uzlin zatím neexistují. Ukazuje se, že na rozdíl od karcinomů prsu, hraje v případě karcinomů hrdla roli i postižení mikrometastázami. Cílem prospektivní mezinárodní studie SENTIX je zhodnotit, zda může být u pacientek s časným stádiem děložního hrdla nahrazena pánevní lymfadenektomie méně radikální biopsií sentinelových uzlin. Za tímto účelem byly všechny sentinelové uzliny zpracovány ultrastagingovým protokolem (vykrájení celé lymfatické uzliny). Integrální součásti studie je i centrální hodnocení kvality zpracování uzlin patologem (na našem pracovišti).

Z každého z 37 center byl náhodně vybraný příslušný počet vzorků, které byly zaslány k centrálnímu čtení a následně vyhodnoceny na škále: minimální, hlavní, kritické a žádné odchylky od protokolu. Překvapivé bylo, že ačkoli byl protokol zpracování uzlin integrální součástí studie, v první kole byly u 34 % případů nalezeny zásadní odchylky od protokolu (hlavní a kritické), tedy takové, které by mohly vést k selhání při detekci metastatického postižení. Pracoviště s hlavními a kritickými odchylkami pak byla požádána o zaslání vzorků od všech pacientek do druhého kola centrálního čtení a některá centra musela být ze studie vyloučena. V druhém kole pak byly hlavní odchylky od protokolu zastiženy jen v 8 % případů.

Domníváme se, že poměrně vysoké procento závažných odchylek, by mohlo být vysvětleno špatnou komunikací mezi klinikem a patologem, ke kterému se informace o existenci ultrastagingového protokolu nemusela dostat. To reflektuje současný stav s absencí obecně uznávaných guidelines pro zpracování sentinelových uzlin.

Central Pathology Review in SENTIX, A Prospective Observational International Study on Sentinel Lymph Node Biopsy in Patients with Early-Stage Cervical Cancer (ENGOT-CX2).

Androgen receptor coregulatory proteins and endocrine and chemotherapies for prostate cancer

Zoran Culig

Experimental Urology, Department of Urology, Medical University of Innsbruck, Austria

Androgen receptor coactivators p300 and CBP are up-regulated during androgen ablation. Our previous studies indicate that additional therapies such as use of small molecule inhibitors which target these coactivators may provide benefit in advanced prostate cancer. We have investigated the role of p300 in models representing chemotherapy- and endocrine therapy-resistant prostate cancer. Elevated p300 expression was detected in samples from patients who were treated with neodjuvant chemotherapy. Elevated p300 expression was confirmed in publicly available patients´ data. We could demonstrate that docetaxel-resistant prostate cancer cell lines showed increased expression of p300. Short-term docetaxel treatment in prostate cancer cells leads to an increase in p300 expression. We could demonstrate that shRNA down-regulation of p300 resulted in reduced clonogenic potential of docetaxel-resistant cells. Consistently, this treatment impaired cell migration and invasion. The results of these experiments clearly indicate that anti-p300 therapy should be further developed for clinical trials. We also treated enzalutamide-resistant cells with p300 inhibitors. This treatment lead to changes in expression of genes which are related to ribosome and MYC pathways. In therapy-resistant cell lines, ribosomal and MYC protein amplifications were observed. These results have indicated a potential role of the p300-ribosomal protein-MYC pathway in acquisition of enzalutamide resistance. Taken together, the results have indicated that the androgen receptor coactivator is implicated in cellular processes associated with resistance to current prostate cancer therapies.

Comprehensive Genomic Tumour Profiling

powered by

TruSight Oncology 500

Thyroid
ALK, HRAS, NRAS, BRAF, TERF, RET

Breast
PIK3CA, ERBB2, BRCA1, BRCA2 MLH1, MSH2, MSH6, PMS2

Colorectal
BRAF, KRAS, NRAS, ERBB2, MLH1, MSH2, MSH6T, PMS2

Gastrointestinal
KIT, PDGFRA, MSH2, MSH6, PMS2, ERBB2, MLH1

Bladder
FGFR2, FGFR3

Ovarian
BRCA1, BRCA2, PMS2, MLH1, MSH2, MSH6

Melanoma
BRAF, KIT, NRAS, ROS1, ALK

Prostate
BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, PTEN, AR

GeneTiCA & illumina

Comprehensive genomic tumor profiling powered by GENETICA & ILLUMINA / 10:30 — 12:30

Chairs: Chairs: Pavel Dundr, Jiří Drábek, Jeroen Adema, Ondřej Slabý

EVROPA ROOM

Pátek / Friday - 26. listopadu 2021 / 26th November, 2021

Comprehensive genomic tumour profiling

Jeroen Adema

illumina

We would like to introduce you to comprehensive genomic tumour profiling and how this approach is becoming standard of care for biomarker testing. An increasing amount of therapies coming to the market from which patients can benefit from across tumour types. With the increase of therapies there is an increasing demand to test for multiple different biomarkers from a limited amount of tissue. Next generation sequencing is a technology uniquely positioned to be able to address many different biomarkers from a small amount of tissue. Comprehensive genomic profiling of tumours is becoming the standard of care and enables screening across multiple gene >500 genes simultaneously. Comprehensive genomic profiling enables the detection of different variant classes from both DNA and RNA but also more recent complex biomarkers like Tumour mutation burden (TMB), microsatellite instability (MSI) and homologous recombinational deficiency (HRD). Comprehensive genomic profiling enables an hypothesis free approach truly enabling precision medicine.

Higher degree of personalization of cancer treatment (precision oncology) using complex genomic profiling: experience from two molecular-oncological indication commissions

Ondřej Slabý

University Hospital Brno, Brno, Czech Republic

Současný pokrok ve výzkumu zhoubných nádorů a vývoj moderních terapií významně posunul léčebné možnosti těchto onemocnění. Úspěchu bylo dosaženo dokonce u těch malignit, které jsme ještě nedávno považovali za neovlivnitelné systémovou terapií. Prognóza onkologických pacientů se tedy zlepšuje, a to včetně těch s metastatickým onemocněním, přičemž logickým cílem klinického výzkumu je transformace diseminovaného onemocnění z kategorie chorob smrtelných do skupiny onemocnění chronických. Za tímto pokrokem a touto ambicí si kromě protinádorové imunoterapie lze představit především uplatňování poznatků z oblasti molekulární patologie a jejich využití pro individualizované terapeutického plánování. Aplikací těchto poznatků se posouváme od histopatologického hodnocení nádorů na další úroveň, která bere v potaz biologické chování jednotlivých malignit. Umožňuje to vyšší stupeň individualizace léčby nádorových onemocnění, při kterém využíváme technologie umožňující komplexní genomové profilování (sekvenování nové generace, NGS), a který označujeme jako precizní onkologie. Pro precizní onkologii je nezbytný také multidisciplinární přístup v podobě molekulárního tumor boardu (MTB), v českém jazyce lze takovýto mezioborový panel označit jako molekulárně-onkologická indikační komise. Typicky jsou zastoupeny odbornosti jako je klinický onkolog, patolog, molekulární biolog (molekulární patolog), klinický genetik, klinický farmakolog. Rolí této indikační komise je potom nalezení vhodného a vysoce individualizovaného léčebného plánu nad rámec standardní léčby, a to na základě vyhodnocení komplexních genomických analýz. V našem sdělení vás seznámíme s fungováním a dosavadními výsledky dvou molekulárně-onkologických indikačních komisí ve Fakultní nemocnici Brno, komisí pro nádory dětského věku a komisí pro nádory dospělých.

The informatic road to clinical report from NGS somatic testing

Ondřej Brzoň

Institute of Applied Biotechnologies a.s.

Somatic testing that focuses on discovery of low-frequency variants using NGS (next-generation sequencing) poses many challenges in the data processing stage. One of the major obstacles on the road to clinical report is the quantity of data that not only has to be analyzed, but also stored, accessed by different specialist, archived and often reanalyzed as newer and more accurate tools unlock the possibility to discover more. In the lecture, we will show what file sizes are commonly generated by pan-cancer somatic assays and how it can at first sight discourage from their adoption into routine testing which would require a large investment into IT infrastructure. What we will also take a look at is the possible way to deal with these obstacles by employing cloud solutions.

A new cloud platform from Illumina, the Illumina Connected Analytics (ICA) lessens the burden on testing labs by providing the complete informatic solution for somatic testing – from integration with sequencer, management of data, to a suit of ready-to use analytical workflows. The subsequent interpretation of variants is as challenging as the analysis, which is why we'll also introduce leading clinical decision support software, QCI-Interpret from QIAGEN and Clinical Genomics Workspace from PierianDx. Both platforms perform automatic classification of variants based on their pathogenicity and actionability and gather information from numerous sources to provide insights into prognostic outcomes, possible treatments and clinical studies; insight that can radically improve the care of patients.

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

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

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

[2]1st Department of Neurology, St. Anne's University Hospital Brno, Czech Republic

Introduction: It was recently discovered that one of the hallmarks of aging is the accumulation of clonal variants within the cells of the hematopoietic system without the presence of malignant transformation. This phenomenon is also known as clonal hematopoiesis of indeterminate potential (CHIP). Interestingly, CHIP was associated with an increased risk of cardiovascular diseases.

Materials/Methods: In this study, we are detecting somatic mutations of blood cells in 4 cohorts of patients aged >65 years (presence/absence of carotid atherosclerosis or stroke). Samples of patients were compared with a control group of elderly people (>85 years) and healthy donors (<30 years). In 8 patients, DNA was isolated also from carotid plaques. CHIP mutations were identified by the method of massive parallel sequencing using a targeted DNA custom panel (Qiagen) containing 38 CHIP-related genes.

Results and conclusions: It was shown that ~70 % of all patients (n=112) are positive, with mutations observed most often in genes DNMT3A and TET2 (~50 %), as expected. In the control group of elderly people (n=24), 96 % of individuals were positive and no mutations were detected in young donors (n=24). The presence of CHIP mutations was also confirmed in patient samples of plaques. For detecting a statistical difference between 4 patient cohorts, higher numbers of samples have to be analysed in the future.

Acknowledgment: Funded by ENOCH project CZ.02.1.01/0.0/0.0/16_019/0000868 and IGA LF UP 2021_019.

The Value of Comprehensive Genomic Profiling of Metastatic Cancer Using NGS: the Belgian BALLETT Initiative

Dr. Brigitte Maes

Laboratorium voor Moleculaire Diagnostiek, Jessa Ziekenhuis vzw

Brigitte Maes is a medical doctor recognized in Belgium as a medical specialist in clinical biology. She is leading the Laboratory for Molecular Diagnostics (LMD) of the Jessa Hospital, Hasselt, which is a large regional hospital of +/- 1.000 beds in the eastern part of Belgium. The Jessa LMD has grown into a large-size, innovative molecular laboratory that is the reference laboratory for > 10 hospitals in Belgium. The mission of the Jessa LMD is to make precision medicine accessible to all oncology patients by operating in close proximity to the patient and by working closely together with the clinicians. The Jessa LMD is one of the ten ‚next generation sequencing' (NGS) that are recognized by the Belgian government and allowed to perform NGS diagnostic tests in the fields of oncology and haemato-oncology.

Brigitte Maes is a guest-teacher at the University of Hasselt. As an expert in molecular diagnostics and in NGS she is an active member of several governmental committees (of RIZIV, Sciensano, Cancer Centre) and scientific organizations.

COVID-19: Nemoc plná překvapení
13:30 — 15:50

Chairs: Roman Prymula, Marián Hajdúch

Pátek / Friday - 26. listopadu 2021 / 26th November, 2021

EVROPA ROOM

Svět i Česká Republika poslední dva roky bojují s nemocí COVID-19, která bezprecedentně zasáhla do našich životů, běžné praxe, výuky i výzkumu. Lékařská věda prokázala, že díky mezioborové spolupráci je schopná v krátké době vyvinout řešení, která v boji s pandemií potřebujeme. Přesto zůstává toto onemocnění plné překvapení a prokazuje mimořádnou schopnost adaptace na nastavená opatření. Cílem tohoto mezioborového panelu bude prezentovat nejnovější pohledy na infekci člověka virem SARS-CoV-2, analyzovat předchozí neúspěchy i selhání a prezentovat možná řešení, která by pandemii dovedla k endemického výskytu patogena.

Diagnostika infekce virem SARS-CoV-2
Pavel Dřevínek

Patologie onemocnění COVID-19
Radoslav Matěj

COVID-19 — když data a informace nejsou totéž
Ladislav Dušek

Účinnost a reaktogenita vakcín proti COVID-19, máme jinou alternativu jak zastavit pandemii?
Roman Prymula

Farmakologická prevence a léčba pacientu
Petr Smejkal

Pandemie: čím jsme si prošli a kam směřujeme
Marián Hajdúch

XXIV. Congress of the Czech Society of Histotechnologists 15:30 — 17:15

Chairs: Jana Vaculová, Daniela Indrová

Pátek / Friday - 26. listopadu 2021 / 26th November, 2021

OLOMOUC ROOM

Utility and benefits of large-format histology

Kateřina Gospošová, Nikola Machová

Ústav klinické a molekulární patologie a lékařské genetiky, Fakultní nemocnice Ostrava, 17. listopadu 1790/5, 70800, Ostrava-Poruba, Czech Republic

Some tissue specimens are challenging task for the pathologist and they need to be handled with great care. Whole-mount sections are give the pathologist a better overview. It is a technique of processing samples into so-called megablocks, which arise from wide cross-sections of the examined organs and provide a topographically better overview of the location of the examined structures, most often tumors. At our workplace, we use megablocks for the examination of prostate cancer specimen in cooperation with imaging methods (MRI) and we are able to determine the exact location of the tumor and the extent of prostate tissue involvement. We also provide the whole-mount sections of uterus, placenta and colon specimens.

This laboratory technique is more complex and financially demanding. The tissue processing itself requires special materials and technical background. Megablocks are handled according to standardized protocols and histotechnicians have to be trained to cut bigger paraffin blocks. The big advantage of this method provided ability to produce whole-mount sections of tissues with different size. It can be used to study architecture and tissue morphology more clearly.

Využití histologického zpracování modelů k detekci závažnosti postižení tlustého střeva ulcerozní kolitidou

Jozef Skarda[1,2], Alzbeta Krausova[3], Petra Buresova[3], Lenka Sarnova[3], Martin Gregor[3]

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Plectin je vysoce univerzální protein cytokin, poskytuje tkáním mechanickou stabilitu integrací přechodných vláken (IF) s buněčnými spoji. Zde předpokládáme, že cytoarchitektura řízená plectinem je kritickým determinantem funkce střevní bariéry a homeostázy. V našem souboru myši postrádající plectin v střevní epiteliální buňce (IEC; PleΔIEC) spontánně vyvinuly kolitidu, která byla charakterizována rozsáhlým odloučením střevního epitelu od bazální membrány (BM), zvýšenou střevní permeabilitou a zánětlivými lézemi. Exprese plectinu byla navíc v souladu s experimentálními výsledky na daném modelu snížena v tlustém střevě pacientů s ulcerózní kolitidou (UC) a negativně korelovala se závažností kolitidy.

Duální nepřímá imunofluorescence na histologických řezech

Alena Poláková

Ústav patologie a molekulární medicíny 3. LF UK a FTN, Praha, Czech Republic

Stejně jako duální imunohistochemické barvení histologických preparátů se i duální imunofluorescenční barvení používá ke znázornění dvou antigenů či struktur v jednom histologickém řezu. Podstatou imunofluorescenčních metod je vazba diagnostických protilátek s konjugátem značeným flourochromem na antigenní struktury ve tkáních, které lze prokázat v mikroskopu. Pro dosažení kvalitních výsledků vyžaduje duální barvení několik důležitých specifických kroků ve srovnání s klasickým IHC barvením. Stejně jako při běžném IHC se i u duálního IF vyšetření používají speciální mikroskopická podložní skla s adhezivním povrchem s pozitivním elektrostatickým nábojem. Důležitým krokem IF barvení je výběr vhodných primárních protilátek. V případě duálního IF barvení je nutné použít primární protilátky dvou různých druhů/hostitelů, např. myš a králík. Stejně tak i sekundární protilátky, opatřeny vhodným konjugátem, musí odpovídat použitým primárním protilátkám. Samotný postup je v mnoha krocích podobný jako u IHC bavení histologických řezů. Nicméně IF barvení vyžaduje citlivější zpracování s ohledem na to, že se používají fluorochromy, které reagují na běžné světlo. Velký rozdíl oproti klasickému IHC barvení je poslední fáze barvení a montování řezů. Pro dobarvení buněčných jader se používá fluorescenční barvivo DAPI, které se hojně používá ve fluorescenční mikroskopii pro svou silnou schopnost pevně se vázat na DNA a schopnost procházet buněčnou membránou. Pro hodnocení IF barvení je zapotřebí fluorescenční či konfokální mikroskop. Využití duálního fluorescenčního barvení není zatím rutině využíváno v běžné diagnostice. Převážně je duální IF barvení zejména rozšířeno ve výzkumné oblasti. Na našem ústavu se duální barvení používá pro výzkumné účely v oblasti neuropatologie, kde IF využíváme pro znázornění např. plaků beta amyloidu a AT8 u různých neurodegenerací. Dále jsme IF barvení využili při pozorování buněk pankreatu, které produkují různé hormony. V tomto případě, jsme testovali i trojité barvení, kdy jsme prokazovali insulín, glokagon a pankreatický polypeptid na jednom histologickém preparátu.

Multiplex immunofluorescence in histology

Tereza Hulínová[1,2,3], Mária Wozniaková[1,3,2]

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Nowadays, most research in cancer is concerned with monitoring various cellular processes in tumor tissue in order to improve the diagnosis and treatment of the disease. For example, the multi-colour immunofluorescence method, in which up to 4 different proteins can be detected in a single slide, allows a clear representation and a more detailed description of the interrelationships. Multiplex immunofluorescence is one of the new methods used in histology, especially for scientific purposes. The lecture includes an introduction to the problem, description of the methodology, experience with implementation, possible pitfalls and their solutions with examples of the resulting slides. Supported by MH CZ - DRO - FNOs/2020

Zpracování tělních tekutin formou cytobloku

Lucie Hocková[1], Andrea Berkyová[1], Blanka Véghová[1]

[1]Ústav patologie 1. LF UK a VFN Praha, Praha, Czech Republic

Názorný postup pro zhotovení cytobloků z tělních tekutin, vhodné pro zpracování plicních biopsií.

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Multiplex imunohistochemistry and data management 09:00 — 11:30

Chairs: Jiří Ehrmann, Jan Bouchal

Sobota / Saturday - 27. listopadu 2021 / 27th November, 2021

MADRID ROOM

Spatial Phenotyping of Immune Cell Subsets in Patients with Lethal COVID-19

Paul Murray

University of Birmingham, University of Limerick

In this study we report the comprehensive profiling of COVID-19 tissue pathophysiology using cutting-edge molecular histology employing high dimensional multiplexed immunohistochemistry with digital spatial profiling, in situ hybridisation, and bulk tissue transcriptomics combined with immune receptor profiling. As there is considerable uncertainty regarding the specificity of reagents used to detect SARS-CoV-2 infected cells in tissues, we have established the specificity of two antibody reagents and used them simultaneously on the same tissue section to unambiguously identify virus-infected cells at single cell resolution. This has allowed us to assign virus infection to a distinct stage of COVID-19, to specific cell phenotypes and anatomical locations. We have used the simultaneous detection of virus with multi-dimensional IHC and transcriptomics to define the nature of the inflammatory response in early and later stage COVID-19 samples and RNAscope to define the cellular location of cytokines involved in the host response to COVID-19 and highlight pathways that can be targeted for therapeutic intervention. Moreover, we have for the first time defined the TCR repertoire in tissue samples from patients with COVID-19.

Awakening of digital pathology in Covid 19 pandemic – time to come to real life

Jiri Ehrmann[1], Ivo Kaspercik[2], Daniela Galiová[2], Eva Srovnalová[3], Patrik Flodr[4], Ivo Uberall[4]

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[2]Department of Pathology, AGEL Labs, Nový Jičín, Czech Republic

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Corona crisis had many consequences in health care services as well as in education processes. During lockdown, almost all pathology departments had been confronted with lack of qualified pathologist as well as with necessity to process vital biopsies and to regularly teach locked students. Here we describe our approach to use virtual microscopy both in regional department of pathology and in faculty department which is involved also in practical training of histopathology. In this presentation we also comment practical challenges and obstacles which may appear during establishment of daily based digital/virtual pathology like administration and virtual slides processing and archiving, digital home diagnostic including minimal requirement for connectivity and picture resolution, real-time communication between observers (i.e. student and teacher, or colleagues) and appreciation of staff. Discussion is focused on new perspectives of way of working of pathologist and remote/on-line method of teaching and examining students and residents.

Contribution of Digital Pathology in Multidisciplinary Tumor Board Approach Precising Medicine

Patrik Flodr

DCMP FMD UP and FH Olomouc, Olomouc, Czech Republic

Increasing amount of professional tasks in current medicine is a challenge for a building up time saving and more precise workflow with a broad access of medical informations (clinics, labs, imaging) in tumor boards with subsequent record with experts´ conclusion(s). Digitalisation and parameterization of pathological reports and semiautomatic digitalised slides provides practical alternative to traditional light microscopy which enables more flexibility in following managing and interpretation including possibility of implementation of IVD approaches.

Artificial Intelligence and Multiplex Bioimaging in Translational Cancer Research

Mariam Gachechiladze

Molecular Partners AG, Zurich, Switzerland

Translating basic research findings into clinical practice represents the crucial element for the successful development of new treatment modalities and personalized cancer care approaches in both industry and academic settings. Bioimaging plays an important role in this process, together with various cell biology and omics technologies. It enables to visualise single and multiple markers of interest in the context of individual tumor architecture. With the recent success of various immunotherapy approaches in cancer patients the importance of multiplex bioimaging has been significantly increased, and different methods for the detection of multiple biomarkers on a single slide, as well as different image analysis approaches have been developed. Proposed lecture will be focused on the indications of the use of multiplex immunofluorescence in translational immuno-oncology research and relevant artificial intelligence (AI) based image analysis approaches. The automated staining and whole slide imaging approaches from Akoya and AI based image analysis

from Visiopharm will be discussed in detail. In addition, chromogenic multiplex staining, imaging mass cytometry and other AI based image analysis methods will be briefly covered.

Ultra high content imaging using MICS technology on the MACSima™ Imaging Platform

Bernd Müller-Zülow

Miltenyi Biotec Company

The MACSima Imaging System is a fully automated instrument based on fluorescence microscopy. Its MICS (MACSima™ Imaging Cyclic Staining) technology, together with a broad spectrum of recombinant ready-to-use antibodies, allows the analysis of hundreds of markers on a single or multiple samples at a time. Convenient and easy to use, its specially designed sample carriers allow you to examine any kind of fixed specimen, from tissue to single cells, and the powerful and intuitive Qi Tissue Image Analysis Software results in a truly new view onto many samples.

Introduction to infrastructures for medical data

Petr Holub

BBMRI-ERIC, Graz, Austria

Medical research has become dependent on access to high quality data and biological material. Europe has reflected this trend in setting the Research Infrastructures, covering different stages of medical research pipeline from discovery to validation to translational research. The main medical research infrastructures are BBMRI for facilitating access to quality-defined biological material and various types of medically relevant data, ECRIN for support of academically initiated clinical trials, and EATRIS for support of translational research. The presentation discusses how these research infrastructure contribute to data access and processing and how users can benefit from utilizing their services.

ELIXIR Czech Republic – Large Infrastructure for biological data pursuits steps towards personalized medicine

Jiří Vondrášek

Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, and ELIXIR-CZ, Prague, Czech Republic

The focus on precision medicine is a natural extension of major ELIXIR CZ expertise/strategic areas towards future. As it is primarily genomics-driven, there is a very close connection with the genomics area, as well as with structural and chemical biology via drugs used for treatment. And, of course, there is a specific need for data access and management. The implementation of precision medicine approaches is almost always complex and disease specific. Resources are often fragmented and inherently very specific with respect to the kind of disease. Complex solutions are rare.

ELIXIR CZ plans to implement a similar strategy to the most prominent ELIXIR members. ELIXIR CZ is prepared to directly assist medical professionals as an infrastructure with the necessary partial/complex solutions for using a precision medicine approach in their specific medical field. There are few steps needed to be taken without a delay - detailed mapping of the current situation, assessment of stakeholders' needs, implementation of standards and the creation of logistical support in defined medical fields with a special focus on the Czech environment.

Software tools for data stewardship in personalized and translational medicine

Hajdúch M.[1,2], Pavliš P.[1,2], Szotkowski, M.[1,2], Šiška, M.[1,2], Koudeláková, V.[1,2], Džubák P.[1,2]

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[2]EATRIS-CZ: European Infrastructure for Translational Research, the Czech national node.

To effectively manage complex translational and personalized medicine data, we have developed several proprietary tools for data stewardship for *in vitro*, preclinical and clinical data. The tools are available to broad research community and users on daily basis:

Administration module: Provides authorization and authentication of all users on via IMTM/EATRIS-CZ data portal. It is basically central authentication server with single sign on and two phase authentications support (via email or SMS). It also determines detailed roles and privileges of individual users across the portal applications.

ClinData (https://clindata.imtm.cz): Software solution designed for data management of clinical trials, clinical registries, various healthcare or scientific databases. It is electronic case report form based replacement of „Excel" style of storing data. The main programming language is Java 8. We use also String Framework, HTML, JavaScipt, SQL and many others. The ClinData software is server-client application, web based. The only requirement for using it is Internet browser which supports HTML5 standard. The database used for storing data from the ClinData software is Oracle Database (commonly referred to as Oracle RDBMS) It runs on separated server which is firewalled from outside. The operation system is RedHat. The ClinData is currently used for daily management of single/multicentric clinical trials and registries (close to 70.000 patients in more than 50 clinical trials, including the Czech genome/multiome projects). It is interconnected with hospital information systems to avoid unnecessary duplications of clinical data recording.

PreClinData (https://preclindata.imtm.cz): Software solution designed for stewardship of preclinical animal data. Includes most features of

ClinData, but modified for daily management of laboratory animals. Additionally, PreClinData include also simple biostatistcs module to evaluate safety and efficacy of experimental therapies on daily basis (survival, clinical signs, tumor volume, body weights, histopathology, etc.). PreClinData currently hosts above 170 animal studies of multiple users.

MedChemBio Portal (http://medchembio.imtm.cz): Laboratory information and management system for medicinal chemistry, high-throughput screening and chemical biology: It includes compound registration and management, QA, *in vitro* biology, pharmacology, data analysis, storage, export and reporting. The portal is primarily used for analysis of *in vitro* biological activity of small molecules for collaborating chemical groups worldwide. Currently, there are registered above 140.000 biologically active small molecules tested for various biomedical applications.

CovIT (https://portal.imtm.cz): Cloud-based laboratory management and information system CovIT was developed in response to COVID-19 pandemic for laboratories involved in diagnostic PCR testing. The system includes full capabilities of LIMS with automatic reporting of cases to the National Registry of Infection Diseases and also self-reporting of epidemiologically relevant contacts by infected individuals (self-reporting module for contact tracing). Within the CovIT we have reported above 2.5 million results to tested individuals and thus enabled massive testing and tracing of both Czech inhabitants and foreigners.

Prognostic, predictive and immune response biomarkers 11:45 — 13:00

Chairs: Markéta Kolečková, Jozef Škarda

Sobota / Saturday - 27. listopadu 2021 / 27th November, 2021

MADRID ROOM

Negative prognostic impact of PD-L1 expression in tumor cells of undifferentiated (anaplastic) carcinoma with osteoclast-like giant cells of the pancreas: study of 13 cases comparing ductal pancreatic carcinoma and review of the literature

Jan Hrudka[1], Kateřina Lawrie[1], Petr Waldauf[1], Vanda Ciprová[2], Jana Moravcová[1], Radoslav Matěj[1,2]

[1]3. Lékařská Fakulta Univerzity Karlovy, Praha, Czech Republic
[2]1. Lékařská Fakulta Univerzity Karlovy, Praha, Czech Republic

Pancreatic carcinoma remains one of the leading cancer-related causes of death worldwide and is generally characterized by a dismal prognosis and limited potential for oncologic treatment. A rare subvariant of pancreatic cancer, undifferentiated carcinoma with osteoclast-like giant cells (UCOGC), has an unpredictable prognosis according to many previous studies, with unexpectedly long survival in individual cases. In this study, we collected, retrospectively, 13 cases of well-documented UCOGCs and performed immunohistochemistry focused on the expression of the programmed death-ligand 1 (PD-L1) and several other potential therapeutic and predictive markers (i.e. tumor-infiltrating lymphocytes), to explore their correlation with the follow-up of the patients. As a control group, we examined 24 cases of conventional pancreatic ductal adenocarcinoma (PDAC). Significant differences were present in the analysis of PD-L1: UCOGCs were found to express PD-L1 significantly more frequently and have a higher number of tumor-infiltrating lymphocytes than PDAC. The expression of PD-L1 was related to significantly shorter survival in patients with UCOGC and in the entire cohort. Patients with PD-L1 negative UCOGCs displayed surprisingly long survival in comparison to PD-L1 positive UCOGCs and PDACs (both PD-L1+ and PD-L1-). We compared our results with previously published data, and, after statistical analysis, we were able to identify PD-L1 as an effective prognostic marker of UCOGC and suggest a strong need for a clinical trial of immune checkpoint immunotherapy in patients with advanced PD-L1 positive UCOGC.

Protinádorová imunitní odpověď - význam a doporučené postupy hodnocení intenzity tumor - infiltrujících lymfocytů/ plazmocytů (TILs) v solidních nádorech

Markéta Kolečková[1], Zdeněk Kolář[1], Jozef Škarda[2]

[1]Ústav klinické a molekulární patologie, LF UP a FN Olomouc, Olomouc, Czech Republic
[2]Ústav patologie, LF OU a FN Ostrava, Ostrava, Czech Republic

Utlumení imunitního systému s navozením imunitní tolerance má za fyziologických okolností zásadní význam pro ochranu zdravé tkáně před poškozením vlastními imunokompetentními buňkami. Interakci mezi imunitním systémem a nádorovými buňkami shrnuje tzv. teorie imunitního dozoru („cancer imunoediting"), formulovaná poprvé ve 2. polovině 20. století. Stupeň eliminace nádorových buněk je vázán na aktivaci složek jak vrozené, tak získané imunity. Množství tumor-infiltrujících lymfocytů/plazmocytů (TILs) v nádorovém parenchymu/ stromatu či v okolí invazivních partií nádoru včetně přítomnosti terciálních lymfatických struktur je považováno za nezávislý prediktivní ukazatel odpovědi na neoadjuvantní i adjuvantní terapii. Intenzita zánětlivé reakce v nádoru koreluje s až několikanásobně silnější kompletní patologickou odpovědí, delším přežitím pacientů bez relapsu onemocnění i celkovým přežitím. V posledních letech proto vzniklo hned několik návrhů standardizace hodnocení TILs. Do popředí zájmu se rovněž dostává vztah nádorových buněk a TILs k expresi proteinů kontrolních bodů imunitního systému, kterými jsou zejména proteiny pro programovanou buněčnou smrt 1 - PD-1 a jeho ligand - PD-L1. Cílem sdělení je shrnutí nejnovějších poznatků týkajících se imunoterapie solidních nádoru spolu s využitím výsledků hodnocení intenzity TILs v lékařské praxi.

Prognostic value of tumor-infiltrating lymphocytes (TILs) and their association with PD-L1 expression and DNA repair protein RAD51 in patients with resected non-small cell lung carcinoma

Škarda Jozef[1,2,3], Gachechiladze Mariam[4]

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2Medical Faculty Palacky University University, Olomouc, Czech Republic
[3]Medical Faculty, Ostrava, Czech Republic
[4]Inst of Clinical and Molecular PathologyMedical Faculty Palacky University, Olomouc, Czech Republic

Objectives: DNA repair proteins have emerged as potential predictors for immunotherapy response alongside PD-L1 expression, tumor-infiltrating lymphocytes (TILs) and tumor mutational burden. We analyzed expression of PD-L1, TILs count and expression of the homologous recombination (HR) protein RAD51, as potential prognostic factors in patients with resected non-small-

cell lung carcinoma (NSCLC).

Materials and methods: Discovery set included 96 NSCLC patients from the University Hospital Olomouc and a replication set included 1109 NSCLC patients from University Hospital Zurich. Tissue microarrays were stained using the automated staining platform Ventana Benchmark Ultra with antibodies against RAD51,CD3, CD8, CD68 and PD-L1.

Results: Loss of nuclear RAD51 protein was associated with high TILs (r=-0.25, p = 0.01) and PD-L1 status (10.6 vs. 2.4 %, p = 0.012) in patients receiving neoadjuvant chemo-/radiotherapy. In silico analysis from the TCGA data set showed a negative relationship between RAD51 mRNA expression and CD45 (r = –0.422, p < 0.0001), CD68 (r = –0.326, p < 0.001), CD3 (r = –0.266, p < 0.001) and CD8 (r = –0.102, p < 0.001). RAD51 low/PD-L1 high patients were clustered as separate entity in the replication set and in TCGA dataset. High TILs status was significantly associated with improved OS in the replication set (unadjusted HR = 0.57, 95 % CI 0.42-0.76, p < 0.001). Similar results have been seen for CD3, CD8 and CD68.

Conclusions: In conclusion, RAD51 nuclear loss is weakly associated with increased TILs and high PD-L1 at the time of surgery in curatively resected NSCLC and after prior exposure to neoadjuvant chemo- or radiotherapy.

Predictive relevance of microRNA in patients with NSCLC undergoing palliative chemotherapy

Kateřina Houfková[1], Martin Pešta[1], Vlastimil Kulda[2]

[1]Department of Biology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2]Department of Medical Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

Introduction

Lung cancer is the most common type of cancer worldwide. There are two main types of lung cancer: small cell lung cancer and non-small cell lung cancer including two major histological subtypes: squamous cell carcinoma (SCC) and adenocarcinoma. Chemotherapy is an essential modality of palliative treatment for inoperable SCC at advanced stages. The response rate to chemotherapy varies widely from patient to patient; therefore, it is of interest to find biomarkers that predict the effect of cytostatic therapeutics.

Aim of the study

The aim of the study was to evaluate the association of the expression of selected miRNAs with the overall survival (OS) time of patients with advanced SCC receiving palliative care. We selected 17 microRNAs on the basis of previously published literature (miR 15b, miR 21, miR 27a, miR 34a, miR 99a, miR 106a, miR 107, miR 143, miR 150, miR 192, miR 193, miR 211, miR 218, miR 221, miR 224, miR 342 and miR 375).

Materials and Methods

The study group consisted of 81 patients with lung late-stage squamous carcinoma. All patients received palliative chemotherapy with platinum derivatives in combination with paclitaxel or gemcitabine. Total RNA (including miRNA) was extracted from the tissue samples. A quantitative estimation of 17 selected miRNAs was performed by the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method using TaqMan MicroRNA assays. The evaluation of prognostic significance was performed as a univariate analysis of maximum likelihood estimates using the Cox regression hazard model. Statistically significant miRNAs were incorporated into a multivariate analysis and the Kaplan Meier survival distribution functions were generated for combinations of miRNA expression levels.

Results

We found a significant association between the expression levels of three miRNAs (miRNA-34a, -224 and -342), individual or in combination, with overall survival time. Low expression of miR-342 and high expression of miR-34a and miR-224 were associated with shorter OS in the subgroup of smokers. High expression levels of miR 34a were associated with shorter OS time in the subgroup of patients treated with platinum derivate based chemotherapy in combination with gemcitabine. High expression levels of miR 224 were associated with shorter OS time in the subgroup of patients who underwent chemotherapy combined with radiotherapy. Interestingly patients with either high expression of miR-224, miR-342 and miR-34a or low expression of all three miRNAs had significantly shorter OS than those with other combinations of miR-224, miR-342 and miR-34a expression. These results support the so-called ceRNA hypothesis. We hypothesize that the effect of a single miRNA may depend on the level of expression of other members of the miRNA network. To understand the role of one particular miRNA, it is necessary to determine the levels of the other 'co-players'.

LC3A positive „stone like structures" are differentially associated with survival outcomes and CD68 macrophage infiltration in patients with lung adenocarcinoma and squamous cell carcinoma

Škarda Jozef[1,2], Lucia Čierna[1], Mary Gachechiladze[2], Ivo Uberall[2]

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

Aims

The aim of the study was to analyse the prognostic and predictive value of LC3A positive' ,Stone Like

Structures“ (SLSs) in a large cohort of patients with non-small cell lung carcinoma (NSCLC) and to check its relationship with tumor infiltrating lymphocytes (TILs) and PD-L1 expression.

Methods

Tissue microarrays from 1015 patients diagnosed at the Institute of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland, were stained for LC3A, PD-L1, CD3 and CD68 using automated tissue stainer Ventana Benchmark Ultra (Roche). TILs were assessed in matched haematoxylin and eosin stained slides.

Results

LC3A positive SLSs, were significantly associated with worse overall (OS) and disease-free survival (DFS) outcomes in patients with lung adenocarcinoma (LADC) (HR = 2.4, 95 %CI(.994-1.008, p = 0.029) and HR = 3.9, 95 %CI (1.002-1.014), p = 0.002 respectively), whilst it was associated with better OS and DFS in patients with lung squamous cell carcinoma (LUSC), with marginal significance (HR = .99, 95 %CI(.975-1.011),p = 0.042 and HR = .99, 95 %CI (.975-1.008), p = 0.026). Multivariate analysis showed that LC3A SLSs are independent poor prognostic factor only in patients with LADC. In addition, LC3A SLSs, were negatively associated with CD68 count in LADC, whilst there was a positive correlation in LSCC.

Conclusions

LC3A SLSs are differentially associated with the survival outcomes and CD68 count in LADC and LSCC. Further studies are justified for the understanding the underlying biological mechanisms of this phenomenon.

Molecular pathology / 14:00 — 15:15

Chairs: Jana Steigerová, Josef Srovnal

Sobota / Saturday - 27. listopadu 2021 / 27th November, 2021

MADRID ROOM

Circulating Tumor Cells Detection in Solid Tumors using the CytoTrack instrument

Pavel Stejskal, Alona Řehulková, Josef Srovnal, Hanuš Slavík, Marián Hajdúch

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

Introduction

The liquid biopsy (LB) has been introduced as a novel diagnostic concept based on the detection and analysis of biomarkers circulating in the body fluids such as circulating tumor cells (CTCs) in the peripheral blood. Although LB is not a standard tool in a clinical practice, a big progress has been made in LB methods development. CTCs are the primary or metastatic tumor cells released into the bloodstream and are considered as precursors of distant metastatic spread and can act as a prognostic and predictive biomarker. Here, we focus on CTCs detection in colorectal cancer and glioblastoma multiforme patients using pre-enrichment free and immunofluorescence based CytoTrack CT11TM technology.

Material and Methods

To secure the stable conditions for samples delivery, the peripheral blood samples were collected to Cell-Free DNA BCT® (Streck, Inc.). CTCs were identified using CytoTrack CT11TM instrument, a semi-automated immunofluorescence microscopy. The selection markers used were pan-cytokeratin and EpCam for colorectal cancer CTCs detection and glial fibrilar acidic protein and vimentin for glioblastoma CTCs detection.

Results and conclusions

We have optimized the CTCs detection and analyzed samples of 194 colorectal cancer and 27 glioblastoma multiforme patients with positivity rate about 30 % and 20 %, respectively. We assume analysis of larger patient cohort and search for other noninvasive biomarkers together with analysis of the factors influencing CTCs level in the blood.

Key genes and pathways associated with Skp2 and Slug in prostate cancer by bioinformatics analysis

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Introduction

We have previously reported significantly increased expression of Skp2 and Slug (SNAI2) in lymph node positive prostate cancer patients. Skp2 and Slug correlated in patients with high Gleason scores and their coexpression was confirmed in prostate cancer tissues (Mickova et al. 2021). In the present study, we decided to analyze prostate cancer molecular profiling data for gene lists and pathways in relation to different levels of Skp2 and Slug.

Materials and methods

The data for this study originated from publicly available databases. Gene set enrichment analysis and other genome/transcriptome analytic tools were used for pathway annotation.

Results and discussion

Based on the current study, the Volcano plot revealed differentially expressed genes for different levels of SKP2 and SNAI2. GSEA analysis of two datasets showed that high expression of SKP2 was enriched in androgen response, oxidative phosphorylation or inflammatory response pathways. High SNAI2 expression was observed in interferon alfa and gamma response, TGF-beta signaling, EMT and other pathways. The combined increase of both SKP2 and SNAI2 highlighted enrichment of gene sets involved in the regulation of cell polarity, NOTCH signaling, protein secretion or unfolded protein response pathway. Other tools were also used for validation, highlighting the potential relationship of SKP2 and SNAI2 with immune-related pathways in prostate adenocarcinoma.

Conclusions

This is the first study of the potential link of Skp2 and Slug and their combinations with diverse pathways in prostate cancer samples using bioinformatics tools. Gained information could serve as an attractive basis for further research toward anticancer therapeutic strategies.

The cell painting assay

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The cell painting assay is a high-throughput phenotypic profiling assay in which different cellular compartments are labelled with various fluorescence dyes. Visualization variety of organelles using high-content imaging derives multiple phenotypic parameters at the single-cell level leading to the identification and better understanding of the effects of chemical compounds. In our study, six fluorescent bio-probes were used to tag the specific cellular compartments with the subsequent acquisition in five channels. Cells were seeded, treated with perturbated

compounds and subsequent staining, fixation and image acquisition steps were proceeded using high-throughput robotics and automation. More than 1500 morphological features were extracted using image analysis software leading to the production of rich profiles for subtle phenotypes identification. Eight reference 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. The external committee evaluated the results using similarity checks between the resulting morphological profiles and similar profiles generated by EU-OPENSCREEN screening sites using the appropriate software to check how similar the profiles are to each other. Each extracted feature value was first normalized by z-score normalization. Pearson correlation equation was used to calculate the correlation of those z-scores between the two technical replicates. For each reference compound, at least one concentration of each reference compound yielded a correlation factor > 0.95. These multicentric study outputs confirmed the morphological profiles for each tested reference compound.

Transcriptomic Profiling Identified Lnc-GOLGA6A-1 as a Negative Prognostic Biomarker for Meningioma Recurrence

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Background

Meningiomas represent about 20 % of all intracranial tumors. As their growth rates and prognosis cannot be accurately estimated, biomarkers that enable prediction of their biological behavior and identification of their molecular status would be clinically beneficial.

Methods and Patients

Here, we used FFPE tumor samples of 64 meningioma patients with distinct clinical characteristics. Transcriptomic sequencing was performed and biological and functional differences between meningiomas of different types were evaluated by analyzing the differential expression of mRNA and lncRNA. The prognostic value of 11 differentially expressed RNAs was then validated in an independent cohort of 90 patients using RT-qPCR.

Results

Differential expression was observed with respect to recurrence (69 mRNAs and 108 lncRNAs), sex (12 mRNAs and 59 lncRNAs), WHO tumor grade (58 mRNAs and 98 lncRNAs), and tumor histogenesis (79 mRNAs and 76 lncRNAs). Lnc-GOLGA6A-1, ISLR2, and AMH showed high prognostic power for predicting meningioma recurrence, while lnc-GOLGA6A-1 was the most significant factor for recurrence risk estimation (1/HR = 1.31; p = 0.002). Additionally, lnc-MAST4-5 reported valuable prognostic features from the qualitative point of view.

Conclusion

Expression of the lnc-GOLGA61-1 transcript was found to be a more reliable predictor of meningioma recurrence than well-known predictors.

Acknowledgement

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POSTER SECTION

1. Diamond-Blackfan Anemia disease models – development and phenotypization

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Introduction

The ability to generate targeted mutations in the mammalian genome has formed the backbone of genetic research. Here we present CRISPR as a tool in disease model study to understand the fundamentals of diseases by mimicking disease-causing mutations found in patients. Disease models can also provide a platform for identifying therapeutics that reverse disease-causing mutations. The aim of this project was to establish stable cellular models for deeper study of Diamond-Blackfan Anemia phenotype. Diamond-Blackfan Anemia (DBA, OMIM 105650) is a rare macrocytic normochromic anemia characterized by the selective deficiency of erythroid progenitors in the bone marrow. DBA is characterized by autosomal dominant inheritance with incomplete penetrance and variable expressivity even in the same family. The causes of DBA are heterozygous mutations or single-copy deletions in 19 ribosomal proteins. Several ribosomal proteins (RP) have been implicated in the nucleolar stress-mediated p53 responses. Among the many RPs identified, RPL11 and RPL5 are the most important proteins. Indeed, a number of *in vitro* and *in vivo* studies have shown that the p53-mediated cell cycle arrest and apoptosis in DBA models are RPL11 dependent. Apart from its role in p53 regulation, RPL11 is involved in the negative regulation of c-Myc, a transcription factor that plays a crucial role in ribosome biogenesis. RPL11 directly binds to the MB II domain of c-Myc protein and inhibits the recruitment of its coactivator TRRAP to the promoter region of c-Myc target genes, thereby repressing its transcriptional activity. RPL11 also controls c-Myc mRNA levels.

Methods and materials

RPL11 haploinsufficient model generation and tag insertion into the c-Myc locus were done by electroporation of the ribonucleoprotein complexes to U2OS cell line. Monoclonal cell lines were isolated and screened for the presence of HiBiT tag into the c-Myc locus by measurement of luminescence signal. The presence of mutations in START codon of RPL11 gene were assayed by a genetic screen based on restriction profiling and Sanger sequencing. The phenotypic screen of RPL11+/-c-Myc WT/WT and RPL11+/-c-MycWT/HiBiT was based on immunodetection of ribosomal stress-related proteins, RNA interference, and corticosteroid treatment.

Results and conclusions

Firstly, the CRISPR/Cas9 technology was used as a base editing tool to generate an RPL11 haploinsufficient model by changing the START codon of RPL11 gene to STOP. Secondly, the small HiBiT tag was inserted via the CRISPR/Cas9 system to the c-Myc locus of RPL11+/+ and RPL11+/- cell lines. The two cell lines with labeled c-Myc are planned to be used for identification of till now unknown genes which acts as DBA modifiers via screening of whole-genome siRNA library. Moreover, they can be used for screening of compounds with the ability to reduce the level of c-Myc protein and ribosomal stress as well.

2. Colocalization of Skp2 and Slug proteins and their possible interaction in aggressive prostate cancer

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Background

Skp2 is a substrate recruiting component of the E3 ubiquitin-ligase complex, while Slug is a transcriptional repressor involved in epithelial-mesenchymal transition. Skp2 plays an important role in prostate cancer progression, e.g. via recently reported stabilization of EZH2 or Twist1, however, the relationship with Slug needs further elucidation.

Methods

The prostate cancer patients cohort (N=101) was analyzed by immunohistochemistry for the following proteins (Skp2, Slug, AR, Ki-67, and E-cadherin). Colocalization analysis was performed using Perkin Elmer Opal Multiplex kit, Vectra 3.0 imaging system, and confocal microscope Carl Zeiss LSM 780. Prostate cancer PC3 cells were treated with an SCFSkp2 E3 ligase inhibitor MLN4924 and analyzed by western blot.

Results

High Gleason score was significantly associated with higher Skp2 and lower E-cadherin expression ($p<0.001$ and 0.011, respectively). Skp2 was slightly correlated with Slug and AR in the whole cohort (Rs 0.32 and 0.37, respectively), which was enhanced in patients with high Gleason scores (Rs 0.56 and 0.53, respectively) or with metastasis to lymph nodes (Rs 0.56 and 0.37, respectively). Confocal microscopy revealed colocalization of Skp2 and Slug in prostate cancer cells. Chemical inhibition of Skp2 by MLN4924 upregulated p27 and

decreased Slug expression which supports a possible link between Skp2 and Slug proteins.

Conclusion: Immunohistochemistry, colocalization studies, and in-vitro experiments support the association between Skp2 and Slug in aggressive prostate cancer.

Acknowledgment: The study was supported by grants NV15-28628A and DRO: FNOL00098892 from the Czech Ministry of Health.

3. Pros and cons of *in vitro* culture of prostate organoids and tissue fragments

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Current *in vitro* modeling systems does not fully reflect the biologic and clinical diversity of prostate cancer (PCa). Organoids may better recapitulate disease heterogeneity and retain parental tumor characteristics. Short-term ex vivo culture of prostate cancer tissues may also facilitate drug testing in personalized medicine. We aimed to establish both organoid culture and ex vivo tissue culture for future drug testing for patients with castration-resistant prostate cancer.

First, we processed cancer and normal tissue from 50 patients who underwent radical prostatectomy or transurethral resection (TURP). We were able to cultivate organoids from 58% of tumors (29/50) and 69% of the normal tissues (20/29). The average length of cultivation was 21 days however, we were not successful in subsequent passaging and long-term cultivation. The representative case was selected for comparison of organoids and primary prostate tissue. Positivity of pan-cytokeratin confirmed the presence of epithelial cells, and it was in part positive for androgen receptor (AR) and p63. However, overexpression of AMACR and ERG proteins was not recapitulated in tumor organoids. Second, the short-term drug test was performed for ten patients using ex vivo tissue culture. Samples from hormone naïve prostatectomies presented a low level of proliferation as assessed by Ki-67 staining. Another drawback of this approach is inconsistent tissue morphology between separate tissue fragments and treatments. Only one case showed a high proliferation rate for toxicity testing and tumor tissue was present in all tested tissue pieces.

In conclusion, we have established culture of both organoids and tissue fragments from patients with hormone naïve prostate cancer, however, the organoids did not fully recapitulate primary tissue characteristics and heterogeneity between tissue fragments hampered interpretation of the drug testing. Still, these approaches may be promising using tissues from metastatic castration-resistant prostate cancer

4. Level of cytotoxic T cells populations in head and neck squamous cell carcinoma microenvironment as a predictor of patient prognosis

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Head and neck squamous cell carcinomas (HNSCC) belong to a group of diverse tumors, which can be induced by infection of human papillomavirus (HPV) or by tobacco and / or alcohol consumption. The viral etiology of HNSCC relates to the better clinical outcome reflecting the different immune system response. Recently, spatial analysis of the tumor microenvironment has enabled in situ analysis of infiltrating immune cells. Here, we retrospectively analyzed 97 tissue samples of oral and oropharyngeal carcinomas with the known etiology using multispectral fluorescent immunohistochemistry based on Opal™ chemistry (Akoya Biosciences). To evaluate the immune cell infiltration in tumor and stroma compartments we designed 4 panels of 5 – 6 antibodies each. We mainly focused on quantification of CD4+, CD8+, and FOXP3+ T lymphocytes as well as on their subpopulations expressing PD1, CTLA4, or ICOS molecules. The cell counts were compared according to the tumor etiology and univariate and multivariate survival analyses were performed. We confirmed the HPV status as a main predictor of patients' prognosis but the number of PD1+CD8+ T cells, and the number of CD8+ T cells, all T cells and CD8+/FOXP3+ ratio were independent factors influencing the overall and / or disease specific survival.

5. Aktuální možnosti a výzvy v imunoterapii „triple" negativních karcinomů mléčné žlázy

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„Triple" negativní karcinomy mléčné

žlázy (TNBC) představují molekulární podtyp karcinomu prsu s nízkou či nulovou expresí hormonálních receptorů (estrogenový receptor - ER, progesteronový receptor – PR) a HER2/neu/ErbB2. Z morfologického i genetického hlediska zahrnují heterogenní skupinu nádorů, charakterizovanou zpravidla nízkým stupněm diferenciace („high-grade" nádory), vysokou proliferační aktivitou a agresivnějším biologickým chováním s tendencí k zakládání metastáz či relapsem onemocnění v období kratším 5 let. Oproti hormonálně dependentním nádorům je proto systémová terapie (chemoterapie) indikována již v iniciální fázi léčby bez ohledu na výchozí stádium onemocnění. Za normálních okolností jsou imunokompetentní buňky jedince (zejména T – lymfocyty, B - lymfocyty, makrofágy, dendritické buňky) schopny efektivně detekovat nádorové antigeny a iniciovat tak buněčnou i humorální protinádorovou odpověď. Pochopení mechanismu úniku nádorových buněk z imunitního dohledu (teorie imunitního dozoru) se stalo základním pilířem pro zavedení aktuálně se velmi dynamicky rozvíjející metodu onkologické léčby - imunoterapii. Cílem práce je shrnutí molekulárních znaků imunitních buněk (PD-1/PD-L1, CD95/CD95L, CD24, CD44, ALDH1, CD4, CD8, CD163) s možností jejich aktuálního či potenciálně budoucího využití v predikci odpovědi na systémovou terapii a stimulaci protinádorové imunitní odpovědi.

6. The evaluation of the amyloid plaque proteome in amyloidosis

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The amyloidosis is a rare disorder characterized by the deposition of abnormal protein fibrils in the extracellular space of various organs. Over time, the accumulating amyloid damages the tissue microenvironment and causes organ failure. To date, there are 36 known fibril proteins in humans that can cause amyloidosis. Early diagnosis is critical for effective patient management. IHC is the preferred method for routine amyloid subtyping. However, it is an antibody-based method with numerous unspecificities. Therefore, we have introduced proteomic analysis for subtyping of amyloid deposits from FFPE and subcutaneous fat aspirate (SFA) samples.

So far we have obtained 470 FFPE and 102 SFA samples for subtyping of amyloid deposits. In FFPE samples, Congo red positive-stained amyloid deposits were dissected using laser microdissection. The proteins were extracted from dissected materials and digested using trypsin. In the SFA samples, the proteins were solubilized and digested directly with trypsin. All peptide samples were subsequently separated by liquid chromatography, and individual peptides were acquired by tandem mass spectrometry. Acquired spectra were identified and quantified using a MaxQuant. The most abundant amyloid protein determined the amyloid subtype.

The mass spectrometry-based proteomic analysis enables subtyping of different kinds of amyloid proteins (e.g. Ig kappa, Ig lambda, transthyretin). In addition to the already mentioned proteins, we also observed the presence of SAP, ApoE and ApoAI-V. All those proteins are associated with amyloid fibril formation. Mass spectrometry-based proteomic analysis of FFPE and SFA samples offers a powerful tool for correct subtyping of amyloidosis.

Acknowledgement

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7. HILIC-MS/MS as a diagnostic tool for inherited metabolic disorders

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Currently, the diagnosis of inherited metabolic disorders (IMD) is mostly performed using separation methods targeting groups of IMD. The methods are often time consuming with limited sensitivity. For early diagnosis, we have developed a method that allows the determination of a wide range of IMD biomarkers. The method uses a HILIC-MS/MS approach for quantification of a total of 76 urinary biomarkers involved in purine, pyrimidine, BCAA, urea cycle or β-oxidation metabolism. The method is characterized by a short analysis time (10 min) and easy and fast sample preparation (only dilution of urine to a defined creatinine concentration). The patient's general condition plays a major role in the diagnosis of IMD and it is therefore essential to treat each patient individually, just as personalized medicine attempts to do. In our laboratory, to better grasp the results obtained, we are able to evaluate the distribution of urinary analyte concentrations in patients relative to healthy controls using a modified z-score, which is used for subsequent visualization of the

results using metabolic maps in Cytoscape software. This pipeline considerably simplifies the differential diagnostic process which allows monitoring of metabolic imbalances in patients across the metabolisms of interest.

This project is supported by the Czech Science Foundation Grant (NU20-08-00367).

8. Cytotoxicity, mechanism of action and pharmacological parameters of substituted dienes prepared from betulinic acid

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In this work, a set of new substituted dienes were synthesized from betulinic acid by its oxidation to 30-oxobetulinic acid followed by the Wittig reaction. Cytotoxicity of all prepared derivatives was tested *in vitro* in eight cancer cell lines and two non-cancer fibroblasts. Almost all dienes proved higher cytotoxicity than betulinic acid. Moreover, compounds 4.22, 4.30, 4.33, 4.39 had IC_{50} below 5 μmol/L and two of them - 4.22 and 4.39 were selected for more detailed studies of the mechanism of action. Cell cycle analysis revealed an increase in the number of apoptotic cells at 5 x IC_{50} concentration, where activation of irreversible changes leading to cell death can be expected. Both 4.22 and 4.39 treatment led to the accumulation of cells in the G0/G1 phase with partial inhibition of DNA/RNA synthesis at 1 x IC_{50} and almost complete inhibition at 5 x IC_{50}. Interestingly, compound 4.39 at 5 x IC_{50} caused the accumulation of cells in the S phase. Higher concentrations of tested drugs probably inhibit more off-targets than lower concentrations. Mechanisms disrupting cellular metabolism can induce the accumulation of cells in the S phase. Both compounds 4.22 and 4.39 trigger selective apoptosis in cancer cells via mitochondrial pathway, which has been demonstrated by changes in the expression of the crucial apoptosis-related protein. Pharmacological parameters of derivative 4.22 were superior to 4.39, therefore 4.22 was the finally selected candidate for the development of anticancer drug.

9. Optimalizace panelové RNAseq analýzy z fixovaných a do parafinu zalitých tkání

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Úvod

Technologie NGS se stále vyvíjejí a RNA NGS (RNAseq) přináší řadu možností pro komplexní hodnocení od genových alterací po expresní profily jednotlivých transkriptů. Pro RNAseq z FFPE vzorků je velmi důležité zvolit a optimalizovat vhodný přístup pro tvorbu sekvenačních knihoven a bioinformatickou analýzu sekvenačních dat. Cílem je zavést v naší laboratoři panelové RNAseq z fixovaných tkání pro rozlišení různých typů lézí na základě analýzy genových alterací, expresních profilů a přítomnosti fúzních, alternativních, aberantních transkriptů.

Materiál a metody

Sekvenační knihovny byly připraveny z 300ng celkové RNA izolované z FFPE (KAPA RNA HyperPrep kit,Roche) a obohaceny s využitím námi navržených biotinylovaných DNA-sond (147 genů/373kbp; HyperCapture,Roche). Sekvenování proběhlo na platformě NextSeq (Illumina). Hrubá sekvenační data byla komplexně analyzována v programu CLC Genomics Workbench (CLC;Qiagen).

Výsledky

Vhodným vstupem pro panelové RNAseq je 100-300ng celkové FFPE RNA, která obsahuje alespoň 50% fragmentů delších než 200bp. Kritickým krokem je fragmentace RNA (i nekvalitní RNA 2min/85°C). Použitá hybrid-capture metoda je efektivní i bez předchozí RNA purifikace (<10% rRNA v analyzovaných datech).

Bioinformatickým postupem v CLC lze takto připravené vzorky analyzovat s celkovým výstupem 10-20 milionu čtení, kde nízko exprimované geny dosahují 10-20 tisíc celkových mapovaných čtení, což je dostatečné pro analýzu jejich genových alterací.

Závěr

Optimalizovaná panelová RNAseq z FFPE s využitím uvedeného software umožňuje analyzovat genové mutace, expresní profily detekovaných transkriptů, alternativní, aberantní či fúzní transkripty. Teprve analýza provedená na rozsáhlém souboru vzorků nám umožní zhodnotit přínos RNAseq pro diferenciální diagnostiku nádorových onemocnění.

Podpořeno MZČR (NV19-03-00007 a RVO64165, VFN v Praze).

10. Exprese transkripčních variant HNF1B u vybraných typů lézí

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Hepatocyte nuclear factor-1-beta (HNF1B) je transkripční faktor a potencionální biomarker některých solidních tumorů. V nedávné době jsme detekovali a popsali řadu dosud neznámých a alternativních sestřihových variant (ASV) HNF1B mRNA, které mohou mít regulační funkci a jejichž změny v expresi mohou potencionálně přispívat k tumorogenezi.

Cílem naší práce bylo kvantifikovat

nejčastěji se vyskytující detekované ASV, porovnat jejich expresi v nádorové a nenádorové tkáni tlustého střeva, prostaty a ledvin pomocí droplet digital PCR (ddPCR) a porovnat výsledky s panelovým RNA-Seq u vybraných vzorků.

Výsledky ddPCR ukázaly, že ASV HNF1B s označením 3p, Δ7, Δ7-8 a Δ8 byly exprimovány napříč všemi analyzovanými tkáněmi s míře 28,2 – 33,5 %; 1,5 – 2%; 0,8 – 1,7% a 2,3 – 6,9 % z celkové exprese HNF1B mRNA a vyskytovaly se individuálně či v kombinaci. Kvantitativní změny v expresi ASV mezi nádorovými vzorky a nenádorovými vzorky byly pozorovány u variant 3p a Δ7-8 v tkáni tlustého střeva, 3p v tkáni prostaty a Δ7 v tkáni ledviny. Dále byla pozorována snížená celková exprese HNF1B mRNA v nádorových tkáních tlustého střeva ($p = 0,019$) a prostaty ($p = 0,047$) v porovnání s příslušnými nenádorovými vzorky. U nádorových vzorků tlustého střeva koreluje snížená mRNA exprese se sníženou proteinovou expresí ($p < 0,001$). Kvalitativní i kvantitativní výsledky u vybraných vzorků popsané pomocí ddPCR odpovídají výsledkům panelového RNA-Seq. Tato shoda potvrzuje vysoký přínos NGS vyšetření při detekci alternativních či aberantních sestřihových variant a zároveň ukazuje možnost komplexního vyhodnocení sestřihových vzorců celé řady genů v rámci jednoho vyšetření.

Práce byla podpořena MZČR NV19-03-00007

11. EGFR resistant mutations pilot testing by ddPCR

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Introduction

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein whose activation leads to DNA synthesis and cell proliferation, migration, adhesion, angiogenesis, and inhibition of apoptosis. A mutation or damage in the EGFR gene causes the EGFR protein to remain in activated state and to drive abnormal cell growth, cancer. EGFR-mutation caused signalling cascade can be interrupted by treatment with tyrosine kinase inhibitors (TKIs). Testing of EGFR somatic mutations is requested before such treatment. Unfortunately, treated cells learn away around this therapy and acquire resistant T790M EGFR mutation, which needs to be tested again. When present, lung cancer should be treated with different drugs, new generation of TKIs.

Droplet Digital PCR technology (ddPCR) is a digital PCR method utilizing a water-oil emulsion droplet microfluidic system as reagents reservoir. Sample (DNA) and reagents are divided into thousands of nanoliter-sized droplets and PCR amplification is carried out within each droplet. The advantage is 1) absolute quantification of target DNA copies per input sample without the need for calibration standards and 2) lower limit of detection of the target compared to qPCR.

Methods and Materials

For pilot testing, DNAs from pleural effusion, FFPE, and plasma samples were isolated. DNA samples were routinely tested by cobas® EGFR Mutation Test /v2 (Roche Diagnostics) and/or fastEGFR kit (IMTM). Retesting by QX200 Droplet Digital PCR System (Bio-Rad) with ddPCR Mutation detection assays (FAM/HEX fluorescent probes) and ddPCR Supermix for Probes no dUTP (Bio-Rad) was performed. Results were analyzed by QuantaSoft Version 1.7.4.0917 (Bio-Rad).

Results and Conclusions

72 plasma, 12 pleural effusion, and 20 FFPE samples were tested by ddPCR for EGFR T790M mutation. As the FFPE samples showed high false positivity probably due to the artefacts from effect of the formaldehyde on the DNA, they were excluded from following experiments.

There were no false negatives samples - all samples with EGFR T790M detected by cobas® EGFR Mutation Test /v2 (3.13%/ -2.5%) or fastEGFR massive parallel sequencing (5%) were positive by ddPCR.

Limit of detection is highly influenced by input amplifiable DNA to the reaction. Low DNA input can be avoided by merging the wells, but in plasma samples sit is till limited to 0.2-1.0% AF.

In conclusion, ddPCR method is suitable for testing of resistant T790M EGFR mutation in plasma samples with higher sensitivity compared to routinely used methods.

Acknowledgements

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12. Biological properties of Betulinic acid analogues with polar groups and BODIPY dye

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Betulinic acid (BA) is a very promising molecule thanks to its versatile biological functions, but its low

solubility in aqueous solutions needs to be overcome by derivatization. Fluorescent labelling became a powerful tool in studies focused on the localization of small molecules in cells. In this work, we synthesized and studied 17 new compounds, including six new derivatives of BA and BT labelled at C-3 and C-28 positions using a small blue-emitting BODIPY dye. We studied their cytotoxicity, which showed the most potent derivatives terminated by amino moiety. Also, the cell-cycle arrest was tested but with no common character within the groups. A fluorescence microscopy study of six BODIPY derivatives revealed that only 2 were detected in living cells. These compounds were colocalized with the endoplasmic reticulum and mitochondria, indicating possible targets in these organelles. Anti-HIV-1 activity study revealed that only completely processed p24 CA was identified in the viruses formed in the presence of 2 tested compounds from the whole group. In the cases of 7 compounds, we identified not fully processed p24 CA and p25 CA-SP1 protein. These findings indicate a similar mechanism of inhibition as described for bevirimat.

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13. USP6-induced soft tissue neoplasms with emphassis on novel EIF5A-USP6 gene fusion

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Background&Aims

The group of so-called USP6-induced neoplasms includes nodular fasciitis, both osseal and extraosseal aneurysmal bone cyst giant cell reparative granuloma of the hands and feet, cellular fibroma of the tendon sheath, osseous pseudotumor of digits and myositis ossificans. The most frequent translocation partner in nodular fascitiis is the MYH9 gene followed by the PPP6R3, RRBP1, CALU, CTNNB1, MIR22HG, SPARC, THBS2, COL6A, SEC31A and COL1A1 genes. Herein, a case of nodular fasciitis with a novel EIF5A-USP6 gene fusion is presented.

Materials and methods

A 41-year-old healthy woman with a painful, rapidly growing subcutaneous mass on the left forearm with a size of 0.8 cm is presented. A soft tissue fragment measuring 1 cm was surgically excised. Due to positive surgical margins, re-excision was performed, yielding another 2 cm fragment. The lesion was extensively histologically investigated. Immunohistochemical and molecular-genetic analysis, namely fluorescence in situ hybridization (FISH), next-generation sequencing (NGS) and reverse transcriptase-polymerase chain reaction (RT-PCR) were also performed

Results

Histology revealed a dermally located, mitotically active myofibroblastic proliferation with myxoid areas, that ulcerated the overlying epidermis. One atypical mitotic figure was also found. The lesion showed positive immunohistochemical staining with smooth muscle actin, while S100 protein and CD34 stains were negative. Using FISH, the USP6 gene rearrangement was detected and subsequent analysis using Archer fusionPlex Sarcoma kit revealed a novel EIF5A-USP6 gene fusion.

Conclusion

In the appropriate clinicopathological context, the detection of USP6 gene rearrangement is extremely useful when diagnosing NF, significantly reducing the risk of misdiagnosis and inappropriate overtreatment.

14. Loss of heterozygosity of the phosphatase and tensin homologue (PTEN) gene is a key molecular event in Skene's gland adenocarcinoma

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Aims/Background

Primary urethral adenocarcinomas are very rare neoplasms accounting for <10% of all urethral carcinomas. Site of their origin is unclear, but they seem to arise from Skene's paraurethral glands, which is the female homologue of the male prostate. The aim of this article is to report the first case of Skene's gland adenocarcinoma in which a molecular genetic profiling was performed.

Methods

The patient was a 73-year-old woman with a polypoid lesion sized 3 × 2 cm located at the interface between the bladder neck and the proximal urethra. Transurethral resection was performed and small tissue fragments with positive margins were obtained.

Results

Histology revealed an epithelial neoplasm consisting of cribriform structures located in the subepithelial connective tissue of the bladder wall and proximal urethra. The lesion showed positive immunohistochemical staining with prostate specific antigen, prostatic acid phosphatase, NKX3.1, and alphamethylacyl-CoA racemase. Using the Illumina TruSight Tumor 170 next-generation sequencing assay, a mutation and loss of heterozygosity of the phosphatase and tensin homologue (PTEN) gene was detected. No fusion in any of the examined genes was found using this assay as well as FusionPlex Solid Tumor Kit and FusionPlex

Sarcoma kit assays from ArcherDX.

Conclusion

Given the rarity of Skene's gland adenocarcinoma, it is uncertain whether the same grading and prognostic criteria that are currently used for prostatic cancer apply here as well. It is also unclear what treatment strategy should be applied, but according to the available literature, it seems that local excision or wide surgical resection could represent sufficient therapeutic modalities.

15. A high throughput screening of MARK inhibitors using MALDI-TOF/TOF mass spectrometry

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MAP/microtubule affinity-regulating kinases (MARKs) were recently identified to be involved in tau protein hyperphosphorylation in Alzheimer's disease brain. Hyperphosphorylated tau protein has decreased affinity to microtubules, impairing their stability. Destabilization of microtubules in neuronal cells leads to neurodegeneration, and unbound tau protein forms neurofibrillary tangles, which are hallmarks of Alzheimer's disease. Many phosphorylation sites of tau protein have been identified, but phosphorylation on Ser262 seems to be the most important for pathological tau hyperphosphorylation. It has been found that Ser262 is phosphorylated by MARK4, which is currently an intensively studied target for the treatment of Alzheimer's disease and other neurodegenerative diseases. In the present study, we developed an assay for the direct detection of MARK enzymatic activity using a MALDI-TOF/TOF mass spectrometer. Our assay was optimized for all four isoforms of MARK protein kinases and used to identify potential inhibitors. The screening included 1280 compounds from LOPAC®1280 International (Library Of Pharmacologically Active Compounds). Six inhibitors with MARK4 IC50 < 1 μM were identified. These compounds were tested for MARK4 selectivity and potential to cross the blood-brain barrier.

16. Intratumor heterogeneity reflected in circulating tumor cells associates with metastatic phenotype of single circulating tumor cell-derived clones

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It is presumed that the main mediators of cancer dissemination are the circulating tumor cells (CTCs) released from primary tumors into blood as a consequence of epithelial to mesenchymal transition (EMT). The EMT process supports the plasticity of CTCs, their adaptation to the new microenvironment and successful colonization of the target organ. In the breast and prostate cancer liquid biopsies were found populations of CTCs with different EMT phenotypes, predicting their metastatic potential and development of drug-resistance. Therefore, CTCs are used as prognostic markers in these types of malignancies in clinics.

Based on that we hypothesised that the EMT heterogeneity of primary tumor cells is reflected in circulating tumor cells and the surface EMT-signature in single CTC-derived clones correlates with their metastatic capacity. To test our hypothesis we prepared a syngeneic model of breast cancer, by injecting murine cancer cell line 4T1 12B into the mammary fat pad. This model is recapitulating whole metastatic cascade including the release of CTCs from the primary tumor into systemic circulation. The single CTC-derived clones were isolated, expanded *in vitro* and re-injected into mammary fat pads for second and third rounds of *in vivo* selection of the most aggressive CTCs-clones. The characterization of EMT-signatures of CTC-clones and their corresponding primary tumors was done simultaneously on a spectral flow cytometer with EMT markers: EpCAM, CD24, CD44, CD49b, CD73 and Sca1. The final analysis of the surface-EMT markers' plasticity was done with a high-dimensional reduction and clustering algorithm (FlowSOME). With this approach we performed complex characterization of surface EMT phenotypes in several single CTCs-clones and their corresponding primary tumors. We identified specific EMT-related signatures of aggressive CTCs-clones enhancing their metastatic ability. This finding can be translated into clinical samples of liquid biopsies and serve as another prognostic indicator of the disease progression towards metastatic spread.

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17. Towards a new high throughput screening tool for the cystic fibrosis drug discovery

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Cystic fibrosis (CF) disease results from mutations in the CFTR gene that encodes for an epithelial membrane protein acting as a chloride ion channel and water transport regulator. The mutations affecting CFTR protein expression result in difficulties in processing, folding, function and/or trafficking to the membrane depending on the type of mutation. Future discoveries of CFTR modulators and correctors rely on the high throughput screening (HTS) of many chemical libraries. To develop a tool suitable for this purpose, a cellular model based on the combination of CRISPR/Cas9 technology and HiBiT [1] tag was prepared.

The human bronchial epithelial cell line (16HBE14o-) endogenously expressing WT-CFTR was used for CRISPR/Cas9 mediated knock-in of HiBiT. Two sets of clones that had different positions of HiBiT in CFTR protein were prepared by limiting dilution and further validated by sequencing, Western blotting, and transepithelial electrophysiology. We identified 32 positive clones for luminesce signal from total 182. From these, 17 heterozygotes and 9 homozygotes were identified by PCR phenotyping and sequenced. Homozygotes containing HiBiT but lacking other mutations were successfully validated using electrophysiological assays.

A novel screening tool that allows the detection of total and cell-surface WT-CFTR expression, driven by its endogenous promoter, has been successfully prepared and will enable screening and testing of a variety of agents that may enhance or impair the function of WT-CFTR, expressed at physiological levels. When F508del and other CF mutations are introduced, it will also provide a platform for HTS of mutation-specific modulators.

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18. Porovnání metodických přístupů pro hodnocení MSI

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Průkaz mikrosatelitové instability (MSI) a deficientní exprese mismatch repair proteinů (dMMR) byl dříve využíván převážně pro identifikaci pacientů s Lynchovým syndromem. V poslední době se stal však průkaz MSI/dMMR vhodným prediktorem k imunoterapii checkpoint inhibitory.

Imunohistochemické stanovení exprese MMR proteinů je stále nejpoužívanějším způsobem k průkazu nedostatečné funkce MMR mechanismů. Pro přímý průkaz MSI, tedy zkrácení či naopak prodloužení mikrosatelitu, se standardně využívá PCR s fragmentační analýzou (PCR-FA).

Hodnotili jsme MSI u 28 solidních nádorů napříč různými diagnózami s využitím NGS námi navrženého panelu (300 genů,944kbp), který zahrnuje 17 vybraných mikrosatelitových markerů, a paralelně byla provedena IHC analýza MMR-proteinů. Multiplexní PCR-FA (BAT-26,BAT-25,NR-21,NR-22,NR-24) byla provedena u 5-ti nejasných případů. Pro hodnocení MSI z NGS dat byl použit software CLC Genomics Workbench (Qiagen). Algoritmus v rámci tohoto softwaru je založen na hodnocení délek čtení mikrosatelitových oblastí a porovnání vůči kontrolám. Vzorek byl hodnocen jako MSI pokud je >25% mikrosatelitů hodnoceno jako nestabilní.

Pomocí analýzy NGS a IHC bylo shodně hodnoceno 23/28 (82%) případů jako mikrosatelitně stabilní (MSS) a 4/28 (14%) jako MSI/dMMR. Tři MSS případy měly dle IHC sníženou expresi MSH6. Jeden případ (POLE-ultramutovaný fenotyp s mutací genu MSH6) vykazoval sníženou exprese MSH6, ale vzorek byl hodnocen jako MSS (NGS a PCR-FA).

Výsledek analýzy MSI pomocí NGS byl shodný u 27/28 (96%) případů s výsledkem IHC MMR proteinů. Vyšetření MSI prostřednictvím námi navrženého NGS panelu, představuje plnohodnotný přístup pro identifikaci pacientů vhodných k léčbě checkpoint inhibitory.

Práce byla podpořena MZČR-projekt RVO64165, Všeobecná fakultní nemocnice v Praze a projekt NV19-03-00007.

19. SARS-CoV-2 detection in pooled primary samples enables a cost-effective strategy for routine testing in non-indicated patients

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Background

The ongoing spread of highly transmissible SARS-CoV-2, the etiological agent of Coronavirus Disease (COVID-19), has resulted in continuously emerging challenges at all levels of containing of the COVID-19 pandemic. One of these challenges is a persisting need of reduction of great cost and time demands. Pooling of the primary samples is promising cost-effective and time saving solution without sacrificing accuracy. Only on condition of lower SARS-CoV-2 prevalence, such as at mass social

events, implementation of the pooling strategy is advantegous for elevating testing capacity and lower number of PCR tests needed.

Methods/Materials

For the validation, 81 SARS-CoV-2 positive samples were used. SARS-CoV-2 detection was performed using two diagnostic PCR detection kits and three pooling strategies were tested with the pooling in the ratio of 1:8 and 1:12. Pooling approach was used during preventive screening in non-indicated patients from 1st August to 12th October. In total, 32,598 patients were tested.

Results

The results of validation study have established applicability of the pooling approach in routine screening, when appropriate isolation techniques and PCR detection systems are used. On the condition of low SARS-CoV-2 prevalence in general population, the pooling in the ratio of 1:12 represents excellent strategy with the great cost and time savings. Validated pooling approach with the pooling in the ratio of 1:12 was implemented for preventive testing. In total, 32598 samples were tested using this approach and overal prevalence in general population was <1 %.

This study was financially supported by grant IGA LF UP 2021_019.

20. Comparison of whole exome sequencing kits

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Introduction

Massively Parallel Sequencing is bunch of methods generating large amount of sequence data faster and cheaper than previously. However, many approaches exist even in subset of these methods used for Whole Exome Sequencing (WES).

Aim of this study is a comparison of 5 different methods of WES and 2 sequencing technologies (ILMN and MGI) in an effort to determine whether these parameters cause differences in the amount and quality of sequencing data.

Material and methods

Five gDNA samples were involved into this study, input amount was 50-400 ng based on specific library preparation (LibPrep) protocol. Libraries were prepared using LibPrep manuals as follows: Agilent SureSelect QXT Target Enrichment + V6 All exons Kit Probes (Agilent Tech.), Twist Human Core Exome EF Multiplex Complete Kit + Twist Exome Probes (Twist Bioscience), KAPA HyperPlus + KAPA HyperExome Probes (Roche), MGIEasy Exome Universal Lib PrepSet (MGI Tech) + Agilent V6 All exons Kit Probes (Agilent Tech), MGIEasy Exome Universal Lib PrepSet + MGI Exome Capture V4 ProbeSet (MGI Tech).

Quality control (QC) of all libraries was performed prior to sequencing using Qubit 2.0 Fluorometer, Agilent 2100 Bioanalyzer, and qPCR. Sequencing was performed on two platforms, ILMN (HiSeq 2500 and NovaSeq 6000) and MGI (MGISEQ 2000RS) with 100 or 150 bp PE read lenght. Bioinformatic analysis was performed using in-house MOLDIMED pipeline. For comparison, the quality parameters of raw .fastq files were assessed using FastQC tool, Qualimap, SAMtools, and BEDTools.

Results and Conclusions

Parameters (Mean Quality Scores, Per Sequence GC content, and General Error Rate) shown that quality of data is satisfactory and comparable for both technologies.

WES panel design was compared with 3 approaches. In 1st, the coverage analysis was done on 3 levels for all 5 methods – with 3 different .bed files (universal uscs-exome+10.bed file; vendor‘s .bed file; intersect .bed file – intersection of identical regions from all vendor‘s designs). Roche Kit had the highest on-target percentage of mapped reads and the highest mean coverage. The lowest duplicate rate was shown in Twist Kit.

The 2nd approach was Per base coverage analysis, that was calculated using vendor´s .bed file at particular thresholds. Besides Agilent probes (both ILMN and MGI sequencers), all 3 methods got above 90% of bases covered at 30x.

For 3rd approach, variant calling analysis of SNVs and INDELs was performed for all methods. Only variants identical for all methods were analysed. More variants were detected using Roche and Twist kits compared with Agilent and MGI kits.

It may be concluded that both sequencing technologies have comparable outputs without significant differences and therefore can be used interchangeably for the same type of analysis. In term of LibPrep method, we observed differences in comparison of 5 investigated approaches, where Roche and Twist Kits reports better parametres than competitive kits for our purposes.

Acknowledgements

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21. Deep amplicon sequencing of predictive markers for effective tumor diagnostics using fastGEN technology

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Tumor DNA testing of KRAS, NRAS and BRAF genes, which are components of the EGFR signaling pathway, is a prerequisite for personalized treatment using anti-EGFR drugs such as panitumumab and cetuximab in metastatic colorectal carcinoma. Also, the information on the mutational status of EGFR is required to indicate proper therapy of metastatic lung tumors with gefitinib, erlotinib or osimertinib, which inhibit tyrosine kinases. Deep amplicon sequencing (DAS) has a potential to be suitable method for simultaneous detection of all somatic mutation within tested regions and with a defined detection limit. At the Institute we have developed and validated a unique fast method for genotyping of hotspot cancer mutations based on DAS using Illumina platform. As this method, also known as fastGEN, is routinely used in tumor DNA diagnostics at our place and showed excellent performance, it was licensed and can be used in other labs as a kit. The commercial partner Biovendor MDx is able to produce the kit in a large quantities, obtain the CE-IVDR certification and distribute it worldwide. The current presentation describes the possible use of the kit for the detection of variants present in low fraction and/or in material with low DNA content. The importance of development of a robust and user friendly bioinformatics pipeline for a successful diagnostic product based on Genovesa platform will be also stressed.

As fastGEN method is universal, we are currently developing further applications for genotyping somatic variants in ABL1, but also hereditary variants in TPMT and CFTR genes.

22. Hodnocení TMB z capture NGS dat. Zkušenosti z rutinní praxe

Romana Michálková, Eva Krkavcová, Nikola Hájková, Jan Hojný, Kristýna Němejcová, Pavel Dundr, Ivana Stružinská

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V prediktivní imunoonkologii se stále více pozornosti upírá na nádorovou mutační nálož (TMB – tumor mutation burden), tedy počet somatických mutací v kódující oblasti nádorového genomu na 1Mb. Ačkoliv byl prognostický potenciál tohoto markeru prokázán v širokém spektru nádorů a v červnu 2020 byl TMB-high (≥10mut/Mb) schválen FDA jako prediktivní marker k imunoterapii solidních nádorů, výpočet stále není standardizován.

V naší laboratoři hodnotíme TMB u vzorků s více než 40% nádorových buněk. Výpočet provádíme z mutačního reportu (NextGENe,Softgenetics), který je výstupem biostatistického zpracování dat capture NGS (300 genů, 944kbp; Nimblegen,Roche). Algoritmus výpočtu TMB spočívá v součtu všech synonymních a nesynonymních variant s frekvencí ≥10%, následné odfiltrování známých variant, včetně zárodečných a „driver" mutací na základě dostupných databází (dbSNP,ExAC, ClinVar,COSMIC) a přepočítání na 1Mb. TMB bylo hodnoceno u 79 neselektovaných solidních nádorů, zahrnující karcinom prsu (12), ženského genitálu (12), kolorekta (9), plic (5), pankreato-biliární nádory (12), nádory neznámého primárního zdroje (13) a další.

TMB-high jsme detekovali u 4/10 případů karcinomů endometria, 1/5 karcinomů plic a u jednoho případu pleomorfního dermálního sarkomu a jedné metastázy karcinomu neznámého primárního zdroje. U karcinomů endometria bylo TMB-high spojeno s přítomností mikrosatelitové instability (2) nebo POLE mutace (2).

Analýza NGS panelů zahrnujících cca 300 genů je vhodná nejen pro detekci klinicky významných genových alterací, ale poskytuje i cenný dataset využitelný pro výpočet TMB. Rutinní NGS analýzou s využitím nekomerčního panelu lze spolehlivě odhalit případy, které by mohly profitovat z cílené terapie na základě prediktoru TMB-high.

Práce byla podpořena MZČR RVO 64165, VFN v Praze.

23. Raman microscopy for cellular investigations

Václav Ranc

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Advances in modern therapeutic approaches require the development of suitable carrier systems for active targeted intracellular drug delivery. And so, analysis of interactions between applied carriers, anchored drugs, and targeted cells/tissues as well as their uptake and intracellular fate are in focus of nowadays research interests. In this paradigm, Raman spectroscopy recently became a strategic analytical technique, due to its non-destructive, chemically selective, and label-free working modus operandi. Here we present an innovative approach towards a spatial analysis of the distribution of selected anti-estrogens in MFC-7 cells. After the spectral characterization of the studied compounds, their internalization in MFC-7 cells was studied using spectral Raman mapping. First, we evaluated the influence of the sample substrate on the quality of the obtained data, where calcium fluoride, glass and silicon were compared. Second, interaction of MFC-7 cells with selected anti-

estrogens was evaluated using multivariate spectral imaging on previously identified characteristic bands. Here developed principles could be afterwards applied in many other scenarios.

24. Functional duality of p21 in cells derived from 3D cultures of cancer cells

Viswanath Das

Institute of Molecular and Translational Medicine, Olomouc, Czech Republic

The cyclin-dependent kinase inhibitor p21 is a key mediator of p53-dependent cell cycle arrest after DNA damage, in addition to p53-independent mechanisms. Emerging studies now show the anti-apoptotic and pro-cell proliferative effects of p21, highlighting the oncogenic role of p21 in cancer. In particular, p21 results in genomic instability and the development of aggressive and chemo-resistant traits in a subset of highly proliferating tumour cells through p53-independent pathways. The cellular localization of p21 has been proposed to be critical either in promoting cell survival or inhibiting cell growth. Here we show that cells derived from multicellular spheroids show an increased induction of p21 despite the reversal from three-dimensional to two-dimensional cultures. The p21-overexpressing, highly proliferative cells are significantly resistant to several standard anti-cancer agents. Further analysis shows that most p21 in this subset of spheroid-derived cells is localized in the cytoplasm and forms a potential ‚anti-apoptosome-like' complex with mitochondrial apoptosis-associated proteins. These findings add to the shifting paradigm on the oncogenic role of p21 due to cellular mislocalization in tumour cells.

25. Fibroleiomyomatous hamartoma – a rare benign lung lesion: a case report

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Background and objectives

Pulmonary hamartomas are common incidental discoveries on chest radiography. The population incidence of pulmonary hamartomas is approximatey 0,25 %. In contrast, pulmonary leiomyomatous hamartoma, is very rare type of lung hamartomas.

Methods

A case report of 41-year-old asymptomatic male patient with abnormal discovery on routine chest X-ray. The imaging test revealed a solid, well circumscribed lesion measuring 6 cm in diameter in upper lobe of the right lung. Thereafter the wedge resection of affected lung parenchyma was performer and bioptic material was processed.

Results

Histopathological examination showed unencapsulated solid lesion with focal polypoid growth pattern, with extensive myxoid degeneration, cellular component of tumor consisted from elongated spindle cells with absence of mitoses and absence of cellular atypias. Peripheral polypoid formations were lined by regular respiratory epithelium. Immunohistochemical examination demonstrated positivity for vimentin, SMA, desmin and bcl-2. The proliferative activity was less than 1 %.

Conclusion

Leiomyomatous hamartoma can not be histologically distinguished from benign metastasing leiomyoma, which generally originates in uterus. Since our patient is a male and he has never had history of smooth muscle neoplasm, pulmonary leiomyomatous hamartoma is considered to be our final diagnosis.

26. Comorbid Alzheimer's and Creutzfeldt–Jakob Disease: Micromorphology of Colocalizing Extracellular Protein Aggregates and Neuronal Dystrophy on 20 Brains

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Alzheimer's disease (AD) and sporadic Creutzfeldt–Jakob disease (sCJD) are both characterized by extracellular pathologically conformed aggregates of amyloid proteins—amyloid β-protein (Aβ) and prion protein (PrP^{Sc}), respectively. To investigate the potential morphological colocalization of Aβ and PrPSc aggregates, we examined the hippocampal regions (archicortex and neocortex) of 20 subjects with confirmed comorbid AD and sCJD using neurohistopathological analyses, immunohistochemical methods, and confocal fluorescent microscopy. Our data showed that extracellular Aβ and PrP^{Sc} aggregates tended to be, in most cases, located separately, and "compound" plaques were relatively rare. We observed PrP^{Sc} plaque-like structures in the periphery of the non-compact parts of

Aβ plaques, as well as in tau protein-positive dystrophic structures. The AD ABC score according to the NIA-Alzheimer's association guidelines, and prion protein subtype with codon 129 methionine–valine (M/V) polymorphisms in sCJD, while representing key characteristics of these diseases, did not correlate with the morphology of the Aβ/PrP^{Sc} co-aggregates. However, our data showed that PrP^{Sc} aggregation could dominate during co-aggregation with non-compact Aβ in the periphery of Aβ plaques.

27. Importance of Evaluation of Bone Invasion Type in Squamous Cell Carcinomas of the Oral Cavity

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The objective of this study was to compare bone invasion type with histopathological, clinical and immunohistochemical prognostic factors. The study included 49 patients who were treated for oral squamous cell carcinoma. Of which, 30 patients with presence of bone invasion on histopathology, were divided according to the type of bone invasion (erosive, infiltrative, mixed). On McNemar's test, statistically significant association was observed between bone invasion types and histopathological grade. In contrast, no significant correlation was observed between bone invasion type and tumour volume or nodal metastases. In tumours with bone invasion of the infiltrative type, higher frequency of locoregional relapses was observed. The 5-year survival since diagnosis was approximately 60 % in the erosive group, 40 % in the mixed group, and merely 15 % in the infiltrative group. Peritumoural microvascular density was not significantly related to bone invasion types. Whereas, a significantly higher intratumoural microvascular density was observed in infiltrative type of the bone invasion, when compared to the erosive and mixed type.

28. Immunohistochemical expression of neuroendocrine markers in a large cohort of primary ovarian tumors

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In the ovary, primary neuroendocrine (NE) tumors are a rare entity of uncertain histogenesis. The main immunohistochemical markers of NE differentiation used to confirm the diagnosis of neuroendocrine tumors include synaptophysin, chromogranin, CD56, and INSM1. However, the data concerning expression of NE markers in ovarian tumors without neuroendocrine morphology is limited and possible prognostic significance of their expression has not yet been investigated. Our aim was to perform a comprehensive evaluation of the expression of NE markers in tumors lacking morphological features of neuroendocrine differentiation, and to assess its prognostic meaning.

Immunohistochemical analysis was performed on 508 primary ovarian tumors, including serous borderline tumors (S-BOT), low grade serous carcinomas (LGSC), high grade serous carcinomas (HGSC), ovarian clear cell carcinomas (OCCC), mucinous borderline tumors (M-BTO) and mucinous carcinomas (MC).

The highest number of positive cases (expression of ≥1 any NE marker) was observed in M-BTO (51/80; 63,8%), followed by MC (19/44, 43,2%), HGSC (35/114; 30,7%), OCCC (15/126; 11,9%), LGSC (12/106; 11,3%), and S-BOT (2/38; 5,3%). The data was statistically processed concerning the extent of NE markers expression, and the possible associations with clinicopathologic data was also investigated. Survival analyses were performed to assess the prognostic meaning of NE markers.

This study represents the first in-depth analysis of the expression of NE markers in primary ovarian tumors of non-neuroendocrine morphology, investigating also its clinicopathologic and prognostic significance.

This work was supported by Ministry o f Health of the Czech Republic (project NV19-03-00007 and RVO GJH 1599-10-180).

29. Comparison of pathomorphological findings in relation to the action of silica dust in an experiment and in the population of miners with clinical image of pneumoconiosis

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In the 1980s, interest in the possible carcinogenic effects of silica dust intensified and a fundamental position had to be taken on this issue. This was done by a working group of the International Agency for Research on Cancer (IARC), which evaluated the carcinogenicity of crystalline silicone dioxide as limited, while amorphous SiO2 was evaluated as

non-carcinogenic. Respirable silica dust and cristobalite were been identified as a group I carcinogen, i.e. as carcinogenic to humans and experimental animals.

The experimental part of our work is focused on histological examination of lung tissue samples in an exposed and control set. In the experiment, model exposure experiments on the animal (Ratus norvegicus) are used. Dust from coal mines with low quartz content and dust with high quartz content is used. Inhalation is carried out in a special dust chamber. Histopathological evaluation is focused on the structure of lung tissue, changes in the epithelium, interstitium, proof of dust deposition in polarization examination, and advancing fibroproliferative changes. From a cytological point of view, epithelial changes in the epithelium, cytological evaluation of nucleoplasmic changes with a focus on dysplastic and neoplastic changes are evaluated. The histopathological analysis of pneumoconioses (anthracosilicoses) was based on a total of 79 autopsy cases of miners in who the contribution of the factors of duration of exposure, smoking, and the presence of a diagnosis of pneumoconiosis to the risk of lung cancer was evaluated.

We proved that hyperplastic and atypical changes in epithelial cells occurred in all groups. Dysplastic and suspected changes were detected on the mucous membrane of both small and larger bronchi. Tumour changes were proved in lung tissue microscopically only. Multifactor analysis in miners has proved that for all malignant tumour diseases, regardless of diagnosis, the length of work and the presence or absence of a diagnosis of pneumoconiosis are statistically highly significant. In the statistical model, smoking appears to be the most serious risk for lung cancer development.

Key words: pneumoconiosis, anthracosilicosis, lung carcinoma, miners, rats, silica dust.

30. Atypical non neural granular cell tumor - an unusual rare entity of the unclear origin (case report)

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Non-neural granular cell (NNGCT) tumour is very rare tumour of uncertain origin, without a proof of neural or Schwannian differentiation. These tumours have been reported in a wide age range (5-83 years) with slight female predominance. We describe a case of a 39-year old woman presented with a 1.1x0.9-cm nodule on her neck with a duration of half a year. The tumour involved the dermis and subcutis, and was composed of sheets and trabeculae of large, monotonous epithelioid cells with eosinophilic, granular cytoplasm with prominent mitotic activity, without necroses and significant cytological atypia with negative expression of S100. We present this case of NNGCT with atypical features to increase the awareness of this uncommon entity with uncertain biological behaviour, with pitfalls in differential diagnostics of this particular lesion.

31. Epiteliální a mezenchymální hamartom nosohltanu

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Hamartom je pseudotumorózní afekce sestávající z místně příslušné zralé tkáně, která je ale chybně zapojena do architektoniky dané tkáně nebo orgánu. V oblasti hlavy a krku se nejčastěji vyskytuje respirační epiteliální adenomatoidní hamartom, seromucinózní hamartom a chondromezenchymální hamartom. Autoři popisují zcela raritní případ „epiteliálního a mezenchymálního hamartomu“ nosohltanu, který nesplňoval diagnostická kritéria ani jednoho z výše uvedených běžnějších typů hamartomů.

45letá žena byla vyšetřena na ORL ambulanci pro zhoršení sluchu a tupé bolesti pravého ucha. Při rinoepifaryngoskopii byl při ústí Eustachovy trubice vpravo nalezen kulovitý útvar krytý intaktní sliznicí. Afekce byla kompletně odstraněna a zaslána k mikroskopickému vyšetření s diagnózou „polypoidní cysta nosohltanu“. V současné době, tj. 2 roky od odstranění léze, je pacientka bez známek lokální recidivy.

Makroskopicky se jednalo o částici velikosti 15 x 7 x 7 mm. Na řezu byla léze solidní struktury a žlutobělavé barvy. Mikroskopicky byl na povrchu částice respirační epitel. Vlastní afekce sestávala ze zralé vazivové, tukové a hladkosvalové tkáně, ve které byly seromucinózní žlázky, krevní cévy a periferní nervy. Tkáně odvozené z endodermu nebyly přítomny. Na základě mikroskopického nálezu byla stanovena diagnóza „epiteliálního a mezenchymálního hamartomu“.

Epiteliální a mezenchymální hamartomy nosohltanu představují extrémně vzácné léze, jejichž etiopatogeneze je nejasná. V rámci diferenciální diagnostiky je nutné vyloučit zejména teratom, který je ale tvořen tkáněmi odvozenými ze všech tří zárodečných listů, a vlasatý polyp, který se však vyskytuje typicky

u novorozenců a je krytý rohovějícím dlaždicobuněčným epitelem, pod kterým jsou pilosebaceózní jednotky.

32. Pure versus comorbid Creutzfeldt–Jakob disease: A study of 215 cases showed a surprisingly low incidence of pure prion disease

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Background

Creutzfeldt–Jakob disease (CJD) is the most common human prion disorder with an incidence of 2 cases per 1,000,000. CJD is often thought to occur as a single neurodegeneration with isolated prion deposits in brain tissue; however, cases with different concomitant neurodegenerative diseases have been described.

AIMS: Our retrospective study sought to correlate clinical, neuropathological, molecular-genetic, immunological, and neuroimaging data for “pure” versus comorbid CJD over the last ten years in the Czech Republic. Cases were diagnosed by the National Reference Laboratory for diagnoses of prion diseases.

PATIENTS AND METHODS: Data from 215 patients diagnosed with definite CJD, including clinical symptomatology, EEG, MRI, and CSF findings, was examined. A detailed neuropathological analysis was performed in all cases, and neuropathological changes were scored using current criteria.

Results

Neuropathological examinations revealed that 11.16% were “pure” CJD, while 62.79% had comorbid tauopathy, 20.47% had Alzheimer’s disease, 3.26% had frontotemporal lobar degeneration, and 2.33% had synucleinopathy. The comorbid subgroup analysis revealed that tauopathy was linked to putaminal hyperintensity on MRIs, and AD mainly impacted the age of onset, hippocampal atrophy on MRIs, and low beta-amyloid levels in the CSF.

Conclusion

Our retrospective data analysis found a surprisingly high proportion of comorbid neuropathologies; only 11% of cases were verified as “pure” CJD, i.e., lacking hallmarks of other neurodegenerations. Comorbid neuropathologies can impact disease manifestation and can complicate the clinical diagnosis of CJD.

33. Composition of tumor infiltrating lymphocytes in various stages of melanoma - student work

Vladimír Židlík[1,2], Patricie Delongová [1,2] Sabina Hencová[2], Nikol Sleková[2], Veronika Smékalová[2], Jozef Škarda[1,2]

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Despite advances in therapy, melanoma remains one of the most aggressive tumors. Accurate prognostic prediction determined histologically is important for selecting the appropriate therapy. Typically monitored prognostic markers include depth of invasion (Breslow, Clark), angioinvasion, and sentinel node examination.

The presence of tumor infiltrating lymphocytes (TILs), which are thought to be the manifestation of the host anti-tumor immune response, seems controversial in some studies. However, it is not clear whether they can serve as an independent predictor of melanoma survival, although many studies suggest a prognostic factor associated with a better prognosis.

The main type of effector T cells are cytotoxic CD8 T cells, representing most of the TILs associated with better prognosis in various types of tumors, including melanoma. In contrast, regulatory CD4 T cells have a different function in modulating the effector immune response. CD4 T cells are important in initiating and subsequently enhancing the cytotoxic response. Studies also show a significant prognostic effect on the presence of CD20 B cells.

The aim of our work was to compare the distribution of individual types of lymphocytes (CD3, CD20, CD4, CD8) in correlation with different stages of melanomas. The results will be part of the poster presentation.

Tento pacient Vás potřebuje k určení správné diagnózy

Nevysvětlitelné kostní příznaky, trombocytopenie nebo splenomegalie?

Klinické příznaky v době diagnózy Gaucherovy choroby často zahrnují hematologické abnormality, zejména trombocytopenii, anémii a vysoké koncentrace ferritinu. Další obvyklé laboratorní abnormality zahrnují prodloužený protrombinový čas, APTT, polyklonální hypergamaglobulinémii.[1]

První typ Gaucherovy choroby se začíná projevovat v různém věku, s rozličnou závažností a progresí příznaků.[1]

HEMATOLOGIE[1]

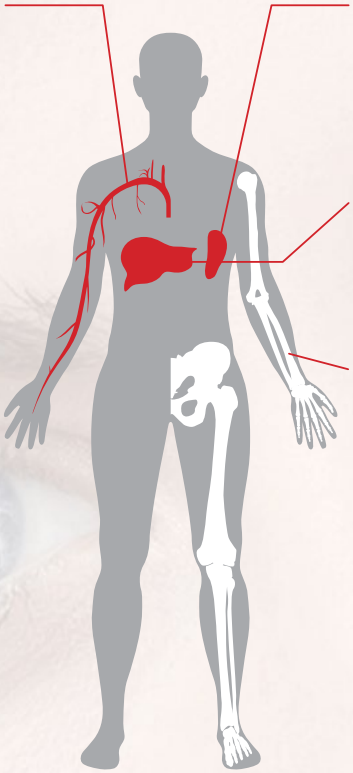
Trombocytopenie
(destičky <100 x 10^9/l)
sklon ke krvácení
a tvorbě hematomů

Anémie
(Hb <135 x g/dl u mužů,
Hb <116 x g/dl u žen)
únava

Leukopenie
(< 4 x 10^9/l)
časté infekce

ANTROPOMETRICKÝ VÝVOJ – DĚTI[2]

- Růstová retardace
- Hmotnostní neprospívání
- Opožděný nástup puberty



ORGÁNY[1]

Slezina
splenomegalie
velikost sleziny může narušovat příjem potravy
infarkt sleziny

Játra
hepatomegalie
poruchy funkce jater (odchylky v proteosyntéze)
fibróza až cirhóza, jícnové varixy při portální hypertenzi

KOSTI[3]

- Chronická bolest kostí nebo kloubů
- Kostní krize – může být doprovázena horečkou
- Osteonekróza, avaskulární nekróza
- Osteopenie, osteoporóza
- Ztenčení kortikální kosti
- Patologické fraktury
- Kompresivní fraktury obratlů
- Aseptické nekrózy hlavic velkých kloubů

Gaucherova choroba

Gaucherova choroba je dědičné lysosomální střádavé onemocnění (LSD) vyvolané deficitem nebo absencí lysosomálního enzymu glukocerebrosidázy.[1]

Typ a závažnost symptomů se u jednotlivých pacientů značně liší od téměř asymptomatického průběhu až po ohrožení života.[1]

GAUCHER

Odhalme jej!

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LÉČEBNÉ CENTRUM

Klinika pediatrie a dědičných poruch metabolismu – Stacionář pro léčbu střádavých onemocnění a národní centrum pro léčbu Gaucherovy choroby
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Takeda

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