Dear students and colleagues,

You are now opening the first page of the 62nd Student Science Conference's collection of abstracts.

Last year, we approached the conference with a mix of excitement and anticipation, eager to see how the new format—with English as the primary language—would be received. We were pleased to discover that the change was met with great enthusiasm. By establishing an English-speaking environment at the Student Science Conference, we aligned ourselves with the broader trend of internationalization within our faculty, reflected both in the growing number of international master's students and in the increasingly diverse composition of research teams, especially at the Biomedical Center.

Therefore, this year, all abstracts are once again written in English. English is the official language of the conference for doctoral students, while for master's students, it is the recommended language—presentations in Czech are allowed at the master's level.

A new and exciting addition to this year's program is a lecture by one of last year's Student Science Conference winners. The winner will share their perspective and the experience they have gained "one year later" in a short talk at the beginning of the conference.

As for the awards, this year we will recognize the three best theses in both the master's and doctoral sections. In addition, the Prof. Vladislav Třeška Prize will be awarded for the best work in the field of surgery.

Finally, I would like to extend my sincere thanks to the thesis supervisors, members of the evaluation committees, and everyone else who has tirelessly contributed to organizing the conference over the years, endowing it with its unique form and atmosphere.

I wish all of us a successful 62nd Student Science Conference!

prof. PharmDr. Radek Kučera, Ph.D.
vice-dean for doctoral study

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08:40 **A lecture by last year's winner Maria Stefania Massaro on Beyond the falcon tube innovations in liver tissue engineering.**
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MSP - Master Study Program
DSP - Doctoral Study Program

Brown lecture hall

08:30 **OPENING**
08:40 Maria Stefania Massaro: Beyond the falcon tube Innovations in liver tissue engineering
16:30 **ANNOUNCEMENT OF RESULTS**

09:00 **DSP CLINICAL RESEARCH**
Chairmen: prof. MUDr. Richard Rokyta, Ph.D.
MUDr. Daniel Rajdl, Ph.D.

10:40 **DSP CLINICAL RESEARCH**
Chairmen: prof. MUDr. Vlasta Merglová, CSc.
MUDr. Petr Pošta, Ph.D.

Brown lecture hall

12:30 DSP CLINICAL RESEARCH

Chairmen: prof. MUDr. Otto Mayer, CSc.
prof. MUDr. Josef Sýkora, Ph.D.

14:10 DSP SURGERY

Chairmen: prof. MUDr. Milan Štengl, Ph.D.
MUDr. Thomas Karvunidis, Ph.D.

Seminar room U3.4

09:00 DSP THEORETICAL DISCIPLINES

Chairmen: prof. MUDr. Ivo Bernat, Ph.D.
Dr. Swaantje Anna Leemburg, Ph.D.

10:40 DSP THEORETICAL DISCIPLINES

Chairmen: prof. MUDr. Martin Matějovič, Ph.D.
MUDr. Jan Horák, Ph.D.

Seminar room U3.4

12:30 DSP THEORETICAL DISCIPLINES

Chairmen: doc. Ing. et Ing. Jiří Polívka, Ph.D.
Dr. Shashank Pandey, Ph.D.

14:10 DSP THEORETICAL DISCIPLINES

Chairmen: MUDr. Jiří Růžička, Ph.D.
MUDr. Vendulka Machartová, Ph.D.

Seminar room U3.15

09:00 MSP PRECLINICAL RESEARCH

Chairmen: prof. MUDr. Hana Rosolová, DrSc.
MUDr. Jana Kuntscherová

10:40 MSP PRECLINICAL RESEARCH

Chairmen: Ing. Petra Chocholatá, Ph.D.
doc. MUDr. Daniel Lysák, Ph.D.

Seminar room U3.15

12:30 DSP PRECLINICAL RESEARCH

Chairmen: prof. MUDr. Zdeněk Rušavý, Ph.D. jr.
doc. Mgr. Yaroslav Kolinko, Ph.D.

14:10 DSP PRECLINICAL RESEARCH

Chairmen: prof. MUDr. Mgr. Zbyněk Tonar, Ph.D.
MUDr. Karel Blahna, Ph.D.

Seminar room U3.16

09:00 MSP CLINICAL RESEARCH

Chairmen: prof. MUDr. Jiří Moláček, Ph.D.
MUDr. Roman Mašek

10:40 MSP PRECLINICAL RESEARCH

Chairmen: prof. MUDr. Jan Beneš, Ph.D.
RNDr. Michaela Kohoutová, Ph.D.

Seminar room U3.16

12:30 MSP THEORETICAL DISCIPLINES

Chairmen: prof. MUDr. Jan Filipovský, CSc.
doc. MUDr. Karel Ježek, Ph.D.

14:10 MSP VARIA

Chairmen: prof. Ing. Jaroslav Hrabák, Ph.D.
MUDr. Jiří Podlipný, Ph.D.

ABSTRACTS NOT INCLUDED

ABSTRACTS

REEVALUATING VITAMIN D SAFETY AND ITS INFLUENCE ON SERUM CALCIUM, MAGNESIUM AND PTH DYNAMICS

Running title: Vitamin D Safety and Mineral Homeostasis

Authors: Hedvika Hatáková (1), Pavlína Černá (1), Michal Jirásko (1)

Supervisor: Radek Kučera (1)

(1) Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University

State-of-the-Art: Vitamin D deficiency is a global issue linked to hypocalcemia, bone disorders, and increased risks of autoimmune, malignant, metabolic, cardiovascular, and infectious diseases. Limited sun exposure, especially above 33° latitude, and low dietary intake contribute to deficiency. Supplementation is widely used for prevention and overall health. However, chronic high doses may cause severe hypercalcemia. The Endocrine Society sets 4000 IU/day as the upper safe limit, while 3800 IU may already induce hypercalcemia in healthy adults. There is still no clear consensus on vitamin D toxicity, and its link to hypercalcemia is under review. This study investigates how vitamin D affects serum calcium, magnesium, and parathyroid hormone (PTH) levels.

Objective: The aim of the study was to reevaluate the safety of vitamin D and its effect on serum calcium, magnesium and PTH levels

Material and Methods: Sixty-five healthy volunteers participated in this study between October 2022 and April 2023. Vitamin D was administered at high doses: 4000 IU daily for two months, followed by a 30-day washout period, and then 8000 IU daily for another two months. Blood samples were collected at baseline, after each supplementation phase, and 30 days post-supplementation. Serum levels of vitamin D, parathyroid hormone (PTH), calcium, and magnesium were analyzed.

Results & Discussion: Statistical analysis revealed a highly significant positive correlation between serum vitamin D and calcium levels ($p < 0{,}0001$). However, this increase was modest, and even at the highest vitamin D concentration observed in this study (208.5 nmol/L), serum calcium levels remained within the physiological range. The study also confirms an inverse relationship between serum vitamin D and magnesium levels.Well known negative correlation ($p < 0{,}0001$) was observed between vitamin D and parathyroid hormone (PTH) levels in the studied cohort, indicating that PTH concentrations declined with increasing vitamin D levels. Model predictions suggest that a PTH value ≤ 1.6 mmol/L would occur only at 285.6 nmol/L, which exceeded the highest vitamin D level recorded.

Conclusion: The study confirmed vitamin D toxicity mechanisms, showing increased calcaemia and decreased parathyroid hormone with higher vitamin D levels. However, both calcaemia and parathyroid hormone remained within physiological limits despite high vitamin D doses. Magnesium decline was modest.

Funding: Supported by "Cooperatio" Program, research area Pharmaceutical Sciences, FNPl, 00669806, BBMRI-CZ: CZ.02.1.01/0.0/0.0/16_013/000167 and LM2015089.

Study program: Doctoral study - Medical Pharmacology | Year of study: 2

ID: 1096

COMPREHENSIVE METHYLATION PROFILING OF MALIGNANT PERIPHERAL NERVE SHEATH TUMORS REVEALS NOVEL EPIGENETIC DYSREGULATION IN KEY ONCOGENIC PATHWAYS

Running title: MAPK Signaling Pathway Key Driver of MPNST

Author: Elaheh Mosaieby (1), Petr Martinek (2)

Supervisor: Michael Michal (1,2)

(1) Department of Pathology, Faculty of Medicine in Pilsen, Charles University and University Hospital, Pilsen (2) Bioptická laboratoř, s.r.o., Pilsen

State-of-the-Art: Malignant Peripheral Nerve Sheath Tumors (MPNST) are extremely aggressive tumors with few treatment options and a poor prognosis. Despite advances in understanding their genetic characteristics, the significance of epigenetic modifications in MPNST development remains unknown. Methylation profiling can help identifying new biomarkers and therapeutic targets by revealing epigenetic dysregulation specific to tumor cells.

Objective: The objective of this study is to conduct a comprehensive methylation profiling of Malignant Peripheral Nerve Sheath Tumors (MPNST) in comparison with normal peripheral nerve tissue. The goal is to identify differentially methylated positions (DMPs) and the associated biological pathways. We aim to explore how these epigenetic alterations may influence the molecular mechanisms and functional pathways that drive MPNST progression.

Material and Methods: A total of 78 MPNST samples and 12 matched normal controls using Infinium Human Methylation BeadChip arrays from public databases were analyzed. Differential methylation analysis was conducted using the LIMMA package, resulting in 168,297 significant DMPs (adj. P.Val < 0.05). Enrichment analysis was performed on genes associated with significant DMPs using Gene Ontology (GO) and KEGG pathway databases. Key pathways were validated using network analysis in Cytoscape.

Results & Discussion: The analysis identified significant hypermethylation in tumor suppressor genes and hypomethylation in oncogenes. The top DMPs included cg19541688 (logFC = 0.61, adj. P.Val = 7.5 × 10^-73), which affects the gene RASSF1, a known tumor suppressor. GO enrichment indicated substantial modifications in small GTPase-mediated signal transduction (p.adjust = 1.09 × 10^-11) and regulation of nervous system development (p.adjust = 7.24 × 10^-11). KEGG pathway analysis revealed dysregulation in the MAPK signaling pathway (p.adjust = 6.1 × 10^-6), which is a key driver of MPNST proliferation and survival. Additional pathways, such as focal adhesion and cellular senescence, were considerably enriched, suggesting a comprehensive epigenetic reprogramming that contributes to tumorigenesis.

Conclusion: With the use of this study's thorough methylation profile of MPNST, significant epigenetic changes affecting important carcinogenic pathways were found. The identified DMPs and associated pathways present potential biomarkers for early diagnosis and novel therapeutic targets. Our findings lay the groundwork for future functional studies to validate these epigenetic changes and their role in MPNST development.

Study program: Doctoral study - Pathology | Year of study: 4

ID: 1080

TESTING BORON DERIVATIVES OF ANTI-ANDROGENIC DRUGS ON PROSTATE CANCER CELL LINES

Running title: Anti-androgenic Effect on Prostate Cancer

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Supervisor: Radek Kučera (1)

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State-of-the-Art: Prostate cancer is one of the most common malignancies in men and its treatment involves androgen deprivation therapy. Current treatments for prostate cancer include hormonal therapy, chemotherapy, and targeted biological therapies. Antiandrogens such as flutamide, bicalutamide, and enzalutamide play a key role in the treatment of advanced disease, but often lead to the development of resistance. Boron derivatives have become the subject of intense research in recent years due to their unique pharmacokinetic properties and ability to selectively interact with target proteins. Studies suggest that modification of antiandrogens with a boron substitution compound could lead to improved affinity for the androgen receptor while reducing toxicity to healthy cells.

Objective: The aim of the study was to test the antiproliferative activity of boron derivatives of nonsteroidal antiandrogens on androgen-dependent and androgen-independent prostate cancer cell lines. And compare the efficacy and toxicity of the new derivatives with commercially available antiandrogens.

Material and Methods: We synthesized a series of boron derivatives of nonsteroidal antiandrogens that were tested on androgen-dependent and androgen-independent prostate cancer cell lines. Non-carcinogenic cell lines were used as controls. In vitro toxicity was assessed using standard WST-1 assays, which allow quantification of cell viability based on metabolic activity. Cells were exposed to test compounds for 24, 48 or 72 hours and IC50 values were determined by comparison with control drugs from the group of nonsteroidal antiandrogens.

Results & Discussion: Several of the most active newly synthesized structural derivatives of nonsteroidal antiandrogens showed higher antiproliferative activity against androgen-dependent prostate cancer lines compared to the standards flutamide and bicalutamide, while at the same time having lower toxicity against non-carcinogenic lines.

Conclusion: Boron derivatives of antiandrogen drugs show promising antiproliferative effects on prostate cancer while exhibiting lower toxicity compared to standard drugs. These results support further research on boron compounds in the field of antiandrogen therapy.

Study program: Doctoral study - Medical Pharmacology | Year of study: 2

ID: 1075

NEOADJUVANT CHEMOTHERAPY IN MUSCLE-INVASIVE UROTHELIAL CARCINOMA OF THE BLADDER PRIOR TO RADICAL CYSTECTOMY: A RETROSPECTIVE EVALUATION

Running title: Bladder Cancer: NAC Prior to Cystectomy

Author: Kseniia Khomenko (1)

Supervisor: Tomáš Pitra (1)

(1) Department of Urology, Faculty of Medicine in Pilsen, Charles University and University Hospital, Pilsen

State-of-the-Art: Urothelial carcinoma of the bladder is the seventh most common cancer globally. Radical cystectomy remains the standard treatment for muscle-invasive bladder cancer (MIBC) and for high- and very high-risk non–muscle-invasive bladder cancer (NMIBC). Neoadjuvant chemotherapy (NAC) is a recommended part of treatment according to European Association of Urology (EAU) guidelines. Studies and meta-analyses have shown a survival benefit with NAC, especially with cisplatin-based regimens. The most commonly used protocols are M-VAC (methotrexate, vinblastine, doxorubicin, and cisplatin) and gemcitabine plus cisplatin (GC), though real-world responses vary with tumor biology, patient selection, and regimen choice.

Objective: To evaluate the pathological response and oncological outcomes of patients with MIBC and high- and very high-risk NMIBC treated with neoadjuvant chemotherapy (M-VAC or gemcitabine/cisplatin) followed by radical cystectomy.

Material and Methods: A retrospective review was conducted on 24 patients with histologically confirmed MIBC or high- and very high-risk NMIBC treated between 2018 and 2023 at our clinic. All patients received neoadjuvant chemotherapy with either M-VAC or gemcitabine plus cisplatin prior to undergoing radical cystectomy. Pathological response was assessed on cystectomy specimens and categorized as complete response (ypT0), partial pathological response (≤ypT1), or no pathological response (≥ypT1-2). Follow-up data were collected to assess recurrence and metastasis rates.

Results & Discussion: Between January 2018 and December 2023, 99 patients underwent radical cystectomy for urothelial bladder carcinoma at our department. Of these, 24 received neoadjuvant chemotherapy with either M-VAC or gemcitabine-cisplatin (GC). Twelve achieved complete pathological response (pT0), two had a partial response (≤pT1), and ten showed no pathological downstaging (≥pT2). During follow-up, three patients developed metastases, while data were unavailable for five. Complete responders had significantly improved disease-free survival, supporting the prognostic value of pathological response. While not statistically significant, pT0 rates were higher with M-VAC. Study limitations include small sample size and retrospective design.

Conclusion: NAC before radical cystectomy in MIBC led to a 50% complete and 8.3% partial pathological response. Response correlated with better outcomes, suggesting that, in select cases, post-NAC surveillance may be feasible. Larger studies are needed to guide treatment choices.

Funding: Charles University Prague, Faculty of Medicine in Pilsen (Cooperatio Program, SURG), Institutional Research of the University Hospital Pilsen (FNPl 00669806).

Study program: Doctoral study - Surgery | Year of study: 2

ID: 1115

THE EFFECT OF SURFACE MODIFICATION OF DENTAL IMPLANTS ON THE ADHESION AND PROLIFERATION OF THE MG-63 CELL LINE

Running title: Titanium Surface Modification Affects MG-63 Cells

Authors: Anna Nekleionova (1), Jana Kolaja Dobrá (1), Jana Dvořáková (1), Vlastimil Kulda (1), Václav Babuška (1)

Supervisor: Václav Babuška (1)

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State-of-the-Art: Anodization is a widely employed electrochemical surface treatment technique that enhances the properties of metal surfaces, including corrosion resistance, biocompatibility, and wear resistance. In recent years, anodization has gained increasing attention in the field of biomedical implants, particularly titanium-based implants, due to its ability to modify surface characteristics in a controlled manner, thereby creating oxide textures and porosity.

Objective: This study aims to compare the cell adhesion and proliferation of MG-63 cells on titanium surfaces anodized under different voltage range.

Material and Methods: Six distinct types of titanium Grade 4 samples were prepared, each differing in surface treatment. The groups included: titanium anodized at 40 V, at 40 V followed by thermal treatment, at 60 V, at 60 V followed by thermal treatment, subjected to surface etching followed by anodization at 60 V, and samples with an etched surface only, which served as the control. The surface morphology of the samples was characterized using scanning electron microscopy. Surface roughness was quantitatively assessed based on the arithmetical mean roughness value. Surface wettability was evaluated by measuring the contact angle of a sessile drop of distilled water. Cell adhesion and proliferation were assessed using the Cell Counting Kit-8 assay at two time points: 2 hours and 48 hours post-seeding.

Results & Discussion: Significant differences in wettability and roughness were observed among the samples; however, these differences did not lead to significant changes in cell behaviour. The etched and anodized surfaces at 60V demonstrated better initial adhesion compared to the control on the plastic surface. No significant differences in proliferation were observed among the materials.

Conclusion: The studied surfaces exhibited variations in roughness and wettability. The morphology of MG-63 cells remained consistent across the various surfaces, suggesting that all studied materials are suitable for further investigations, such as in vivo osseointegration.

Study program: Doctoral study - Medical Biology and Genetics | Year of study: 4

ID: 1098

THE ROLE OF DENTAL CBCT SCANS IN DETECTING CAROTID ARTERY CALCIFICATIONS AS AN INCIDENTAL FINDING

Running title: Carotid Artery Atheroma on CBCT

Author: Walla Samara (1,2)

Supervisor: Omid Moztarzadeh (1, 2)

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State-of-the-Art: Dental CBCT scans, while primarily intended for oral and maxillofacial assessment, can incidentally reveal carotid artery calcifications (CAC), which may signal an increased risk of carotid artery stenosis and stroke. CBCT's high-resolution, three-dimensional imaging enables better visualization of calcified structures near the cervical spine, often capturing the carotid bifurcation. Studies report a CAC prevalence of 4–15% in dental scans. Although not a substitute for vascular imaging, CBCT can act as an effective screening tool. Greater awareness among dental professionals and interdisciplinary referrals can facilitate early detection and timely medical intervention, ultimately improving patient outcomes.

Objective: This study aimed to analyze the prevalence and severity of Carotid artery calcifications in CBCT images, in correlation to gender and age differences.

Material and Methods: The maxillofacial CBCTs of 400 patients, from June 2023 to June 2024 were collected at the Department of Stomatology, University Hospital in Pilsen, Czech Republic .

Age was >50 years old, these patients were evaluated and divided into groups based on the presence of incidental carotid artery calcification, group 1: subjects with no calcified atheroma, group 2: subjects with calcified atheroma.

Results & Discussion: Out of 400 patients, 6 patients who had CBCT were found to have calcified atheroma on their CT scans. These patients will be referred for sonography to assess the severity of the calcifications and determine if further follow-up is needed.

Conclusion: Incidentally detected calcified atheromas are common in CBCT scans and should prompt early referral to a medical specialist. To enhance detection, it is recommended to lower the scanning field by approximately 3 cm to include the carotid bifurcation—a frequent site of calcifications.

Study program: Doctoral study - Stomatology | Year of study: 4

ID: 1118

ORAL MICROBIOTA AS A POTENTIAL THERAPEUTIC TARGET IN PROGRESSION AND MALIGNANT TRANSFORMATION OF PROLIFERATIVE VERRUCOUS LEUKOPLAKIA

Running title: The Role of Rral Microbiota in PVL

Authors: Lucie Nechutná (1,2) Jan Liška (1)

Supervisor: Jaroslav Hrabák (2)

(1) Department of Stomatology, Faculty of Medicine in Pilsen, Charles University and University Hospital, Pilsen (2) Department of Microbiology, Faculty of Medicine in Pilsen, Charles University

State-of-the-Art: Proliferative verrucous leukoplakia (PVL) is a rare and aggressive form of oral leukoplakia characterized by multifocal lesions that frequently affect alveolar mucosa and gingiva. PVL tends to recur despite treatment and, in 40%, leads to oral squamous cell carcinoma. Recent research suggests that the oral microbiota may play a significant role in the pathogenesis and progression of PVL. A study from 2023 demonstrated dysbiosis and loss of diversity of oral microbiota in biopsies of PVL compared to homogenous leukoplakia and healthy mucosa. In our department, we have already examined the presence of 12 aggressive periodontal pathogens with a commercial test. The results show a significant increase of detected bacteria in PVL patients compared to less dangerous mucosal affections.

Objective: As PVL is an idiopathic disease, we aim to map the oral microbiota of 41 PVL patients compared to a control group. This research will provide insights into its pathogenesis, aiming to reduce the risk of malignant transformation, and enhance treatment guidelines and prognosis for PVL patients.

Material and Methods: We collected samples from 41 patients with PVL and a control group after establishing criteria. Samples were taken using sterilized paper points inserted into periodontal pockets. After the DNA is isolated, a specific V3-V4 region of 16 S rRNA will be amplified for taxonomic profiling. We also plan to establish complete metagenomic profiling, including detecting a virome with a special focus on potential oncogenic viruses (e.g., Human Papilloma Virus, Epstein-Barr virus). A bioinformatic analysis using R will be performed after sequencing using the Illumina platform (MiSeq) and Pacific Biosciences long-read sequencer (Vega).

Results & Discussion: In our pilot study, we monitored 12 aggressive perio-pathogens in 38 cases of PVL, 38 OLP with desquamative gingivitis, and 38 with chronic periodontitis. All pathogens were relatively quantified by qPCR Taqman probe. PVL had a threefold higher number of these bacteria compared to control groups. The difference was statistically significant with p=0,0009. Our research underscored the significant association of aggressive periodontal pathogens with PVL. Understanding the interactions between the oral microbiota and host tissues can be crucial for identifying potential therapeutic targets. The results of this subsequent study will reveal the differences in the full spectrum of bacteria and viruses.

Conclusion: The results highlight an essential cofactor in PVL pathogenesis and a part of future therapy guidelines. Using antibacterial therapy and agents that limit bacterial products should improve the treatment and prognosis of PVL, as conventional therapy is mostly unsuccessful.

Funding: This work was supported by the project Nr. LX22NPO5103 „National Institute of virology and bacteriology" supported by the National Recovery Plan (EXCELES Programme).

Study program: Doctoral study - Medical Microbiology | Year of study: 3

ID: 1131

ENHANCING MRONJ PREVENTION: INTRODUCING A VERY HIGH RISK CATEGORY FOR PATIENTS WITH IMUNOSUPRESSED MEDICAL PROFILES

Running title: New MRONJ Category for Imunosuppresed Patients

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State-of-the-Art: Medication-related osteonecrosis of the jaw (MRONJ) is a serious adverse event, presented with exposed necrotic bone in patients undergoing antiresorptive or antiangiogenic medications for conditions like osteoporosis and cancer. Current prevention approaches classify patients into low- and high-risk categories on the basis of drug duration, and immune state However, patients with complicated medical conditions, such as those with several commodities, polypharmacy, or advanced stages of the disease, may not fall into these categories, leading to potential gaps in prevention strategies. Current guidelines do not specifically address this group, which may benefit from a more refined classification to better tailor prevention measures.

Objective: To evaluate whether the third risk category for patients with complex health conditions enhances patient classification and ensures more targeted preventive measures for patients who are more susceptible to medication-related osteonecrosis of the jaw.

Material and Methods: Medical records of 127 patients with risk of MRONJ treated at the Faculty Hospital of Pilsen (2021–2024) were retrospectively analyzed. Collected data included number and location of extracted teeth (maxilla/mandible), detailed medical history, medication type and duration, and comorbidities such as diabetes mellitus, renal dysfunction, chemotherapy, radiotherapy, hormone therapy, and biological therapy. Patients were classified into low- and high-risk levels based on the American Association of Oral and Maxillofacial Surgeons (AAOMS) guidelines, with an additional third group of very high-risk for those with severely compromised health, introduced as part of our study. The distribution of patients and preventive measures were evaluated using descriptive statistics.

Results & Discussion: Analysis revealed that a significant proportion of patients had severely compromised immune systems, which placed them outside the existing low- and high-risk categories. These patients require additional preventive protocols that go beyond current classification guidelines. By introducing a third classification, we were able to identify this distinct group and tailor risk assessments more accurately. This adjustment highlighted the limitations of current protocols in addressing patients with severe immunosuppression, suggesting the need for more targeted and specialized preventive measures. Further validation of this new classification is essential to optimize prevention strategies for these high-risk patients.

Conclusion: Introducing a third risk category for patients with severely compromised immune systems offers a more nuanced MRONJ risk assessment. This study suggests that refined classifications could improve clinical decision-making and patient outcomes, particularly for those with multiple comorbidities.

Study program: Doctoral study - Stomatology | Year of study: 4

ID: 1101

PROGNOSTIC VALUE OF CD169+ MACROPHAGES IN PRIMARY COLORECTAL CANCER AND SYNCHRONOUS OR METACHRONOUS LIVER METASTASIS

Running title: Prognistic Value of Macrophages in CRC

Authors: Wenjing Ye (1), Andriy Trailin (1), Sergii Pavlov (1), Filip Ambrozkiewicz (1), Esraa Ali (1), Lenka Cervenkova (1), Václav Liška (1,2)

Supervisor: Kari Hemminki (1,3)

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State-of-the-Art: Macrophages in the tumor microenvironment can regulate the progression of tumors. CD169+ macrophages are a unique type of macrophage subset that differ from M1 and M2 macrophages and are primarily expressed in secondary lymphoid organs. CD169+ macrophages have been reported to play an important role in anti-tumor immunity. Colorectal cancer (CRC) is the third most common type of cancer worldwide and the second leading cause of cancer-related deaths. The presence of distant metastases significantly reduces survival rates in CRC. The role of CD169+ macrophages in microenvironment of primary colorectal cancer and its synchronous and metachronous liver metastasis (LM) remains unclear.

Objective: We aimed at investigating distribution and prognostic value of local CD169+ macrophages between primary colorectal cancer (pCRC) and its paired synchronous and metachronous LM.

Material and Methods: Formalin-fixed paraffin-embedded tissue sections from pCRC and synchronous (N=55) or metachronous (N=44) LM were stained by immunoperoxidase method using anti-CD169 antibodies. Densities of CD169+ macrophages were assessed in tumor center (TC), inner margin (IM), outer margin (OM) and peritumor area (PT) of pCRC and LM by using the QuPath image analysis software and assessed as prognostic variables for overall survival (OS) and disease-free survival (DFS).

Results & Discussion: The only significant difference between LM and pCRC was greater cell density in PT region of LM in synchronous group. OM and PT region showed greater CD169+ cell densities than TC and IM in pCRC and LM in both groups. Patients with synchronous LM had greater CD169+ cell densities in IM of pCRC and TC and OM of LM compared to metachronous group. High densities of CD169+ cells in the TC of pCRC in synchronous group were associated with longer OS, and their high densities in IM and PT region of pCRC in metachronous group were associated with longer DFS. Contrariwise, high densities in IM of LM in metachronous group were negatively associated with OS. Our findings highlighted unique features of tumor microenvironment between pCRC and LM and between synchronous and metachronous LM.

Conclusion: CD169+ macrophages in pCRC confer prognostic benefit for CRC patients with synchronous and metachronous LM. Contrariwise, in metachronous LM CD169+ macrophages associate with a shorter OS. We suggest CD169+ macrophages may serve as a useful prognostic marker in CRC.

Funding: AZV NW24-03-00521.

Study program: Doctoral study - Experimetnal Surgery |Year of study: 3

ID: 1091

MOLECULAR INSIGHTS INTO METACHRONOUS COLORECTAL LIVER METASTASIS: TRANSCRIPTOME AND MIRNOME PERSPECTIVES

Running title: Transcriptome and miRNome in mCLM

Authors: Bhavana Hemantha Rao (1), Veronika Boušková (1,3), Lucie Heczko (1), Petr Holý (1,3), Karolína Šeborová (1,3), Václav Liška (1,2), Ondřej Vyčítal (1,2), Ondřej Fiala (1,4), Pavel Souček (1,3), Viktor Hlaváč (1,3)

Supervisor: Viktor Hlaváč (1,2)

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State-of-the-Art: Colorectal cancer ranks as the third most prevalent malignancy globally, with liver metastasis occurring in up to 70% of cases. Metachronous colorectal liver metastasis (mCLM), developing six months after the primary tumor's resection, presents unique clinical challenges due to its distinct molecular profile and complex underlying mechanism. Recent advancements in transcriptomics and miRNomics have explored potential biomarkers and pathways that play a vital role in mCLM development. These studies provide insights into tumor progression, metastatic behavior, and prognostic implications.

Objective: This study aimed to investigate the transcriptome and miRNome profiles of mCLM, identifying key deregulated genes, micro RNAs (miRNAs), pathways, and their clinical relevance.

Material and Methods: Differential expression analysis was performed on 36 mCLM and adjacent non-malignant liver tissue sample pairs from RNAseq to identify deregulated genes and miRNAs. Gene set enrichment analysis and consensus molecular subtypes (CMS) classification were used to explore pathways and classify tumors. miRNA-mRNA interactions were investigated through correlation analysis, with prognostic relevance assessed by survival analysis. Validation of key interactions was performed using multiMiR database.

Results & Discussion: The transcriptomic analysis identified 1809 upregulated and 1639 downregulated genes in mCLM compared to the adjacent non-malignant liver, along with 108 upregulated and 92 downregulated miRNAs in miRNome analysis. Upregulated genes were associated to EMT, G2M checkpoints, and E2F targets, including PMEPA1, ITGA2, CDK1, CCND2, MKI67, SOX4, and S100A6. CMS classification showed that 47% of the samples were CMS2 (canonical pathway) and 22% were CMS4 (mesenchymal subtype), with distinct mutations (e.g. BRAF, KRAS, APC, TP53) and copy number variations. Key miRNA-mRNA interactions were identified, including PEA15 with hsa-miR-320b/c, TEX2/CTSO with hsa-miR-103a-3p, and PHLDA3 with hsa-miR-1304, highlighting potential regulatory mechanism in mCLM progression.

Conclusion: This study revealed holistic molecular insights into the mechanisms driving mCLM and novel miRNA-mRNA interactions with potential prognostic implications.

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Study program: Doctoral study - Experimetnal Surgery | Year of study: 3

ID: 1095

SMALL NUCLEOLAR RNA EXPRESSION AND THEIR PROGNOSTIC VALUES IN NON-VIRAL HEPATOCELLULAR CARCINOMA

Running title: snoRNA Expression in non-viral HCC

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State-of-the-Art: The absence of accurate prognostic indicators contributes to the ongoing challenge of hepatocellular carcinoma (HCC), resulting in high mortality rates and poor prediction of the recurrence and survival. There has been limited research exploring the relationship between small nucleolar RNAs (snoRNAs) and HCC. Studies have previously demonstrated that the expression of both SNORA47 and SNORD126 has been altered or dysregulated in liver cancer tissue. In HCC, siRNA transfection suppresses SNORA47, decreasing cell invasion, proliferation, and metastasis by modifying an epithelial-mesenchymal transition. Conversely, increased SNORD126 levels have been associated with HCC, contributing to increased cell division.

Objective: Our aim of study is to provide a better understanding of how snoRNA expression affects patients' outcomes in non-viral HCC.

Material and Methods: We analyzed HCC and non-tumor adjacent tissue from 35 patients who had undergone resection in Pilsen University hospital between 1997 and 2019. Using q-PCR, we assessed the expression levels of specific snoRNAs, namely SNORA47 and SNORD126. Patients were categorized into low and high expression groups based on the median expression of SNORA47 and SNORD126. We then conducted Kaplan-Meier analysis to assess the association of SNORA47 and SNORD126 expression levels with patient outcomes: time to recurrence (TTR), disease-free survival (DFS), and overall survival (OS).

Results & Discussion: SNORA47 expression was higher in tumor tissue than in non-tumor adjacent tissue. In contrast, SNORD126 expression was lower in tumor tissues compared to non-tumor adjacent tissue. Low expression of SNORA47 was associated with longer TTR ($p = 0.03$) and DFS ($p = 0.04$). In univariate analysis, high expression of snoRA47 significantly correlated with shorter DFS (HR: 2.55, 95% CI: 1.02–6.38, $p = 0.05$) and shorter TTR (HR: 3.85, 95% CI: 1.02–14.5, $p = 0.05$). Whereas low expression of SNORD126 was associated with longer TTR ($p = 0.05$) but not DFS. The combination of SNORA47-SNORD126 low expression was linked to longer TTR and DFS ($p = 0.01$ and 0.02, respectively). Furthermore, no association was seen between expression and OS. No correlations with clinical data were observed.

Conclusion: The findings suggest that SNORA47 and SNORD126 show potential as a promising prognostic marker in non-viral HCC. The analysis revealed that the combination analysis provides better prediction than alone for assessing the prognosis of non-viral HCC patients.

Funding: This research has received funding from the grants, and by the project National Institute for Cancer Research—NICR (Programme EXCELES, ID Project No. LX22NPO5102), funded by the European Union—Next Generation EU.

Study program: Doctoral study - Experimetnal Surgery | Year of study: 3

ID: 1078

WHOLE-EXOME SEQUENCING OF PRIMARY COLORECTAL CANCER AND ITS PAIRED SYNCHRONOUS AND METACHRONOUS LIVER METASTASES

Running title: Whole-exome Sequencing of Colorectal Cancer

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State-of-the-Art: In spite of the fact that colorectal cancer is the second most deadly cancer worldwide, only limited number of studies focused on analysis of mutations detected in primary and metastatic tissue from the same individuals. Moreover, information about differences between tumors with synchronous metastases (which are diagnosed at the same time as primary tumor) or metachronous metastases (which are diagnosed after primary tumor removal) are even more scarce.

Objective: We aimed to investigate the differences in mutational changes associated with synchronous and metachronous course of the disease using paired patient samples of primary and metastatic cancer.

Material and Methods: We performed whole-exome sequencing of 210 FFPE patient samples of both primary colorectal cancer and liver metastases. Subsequent analyses included differential mutations, genetic interactions, copy number variation and tumor mutation burden.

Results & Discussion: APC and TP53, well known driver genes, were the most commonly mutated genes in our data set. Mutations in MPDZ gene were only detected in primary tumors of patients with metachronous metastatic disease, whereas VCAN, MTCL1, MDN1, SHROOM2, SPEG and GLI2 were significantly more prevalent in primary tumors of the synchronous group. Furthermore, different genetic interactions were present in synchronous and metachronous patients. Whilst in primary tumors of patients with synchronous metastases TP53 mutations associate with APC, in metachronous group it is mostly mutated together with NBPF11 and PRAMEF15. Paired analysis of tumor mutation burden showed positive correlation between tumor mutation burden of primary and metastatic tissue in synchronous group only.

Conclusion: Our results support the hypothesis that distinct molecular pathways underlie different time course of the disease (eg. MPDZ, VCAN, MTCL1 and other genes). However, large scale studies are required to further investigate this topic and validate our results.

Funding: This research has received funding from the Grant Agency of the Czech Republic - project number 23-05609S.

Study program: Doctoral study - Experimetnal Surgery | Year of study: 4

ID: 1114

INVESTIGATION OF PLACE FIELD STABILITY ACROSS TWO DISTINCT CONTEXTS IN THE DORSAL CA1 REGION OF THE HIPPOCAMPUS USING ELECTROPHYSIOLOGY AND MICRO-ENDOSCOPY IN FREELY BEHAVING TRANSGENIC MICE

Running title: Stability of Place Fields in Murine Hippocampus

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State-of-the-Art: Hippocampal neurons encode key dimensions of experience, including space, time, and contextual information, through distinct activity patterns that underlie cognitive representations. These dynamics are inherently non-linear and critically influence memory retrieval, with dysfunction potentially contributing to pathological states such as hallucinations or delusions. This study seeks to optimize and validate the formation of stable place maps using a transgenic mouse model expressing calcium-sensitive indicator GCaMP6f, to better understand the neural dynamics of hippocampal encoding during context-specific spatial memory activation. This novel method has the benefit of spatial resolution of cells as well as acquiring data of a larger population of cells in the targeted region of interest.

Objective: To study the kinetics of memory trace activation across large populations of cells in the dCA1 region of the hippocampus, across two distinct contexts, using one-photon calcium imaging and multi-unit electrophysiology in freely behaving mice.

Material and Methods: GCaMP6f-expressing C57BL/6 transgenic mice from the GENIE Project were identified using hot-start PCR and gel electrophoresis of tail-derived DNA. A craniotomy above dCA1 of the hippocampus was followed by either cortical aspiration and GRIN lens implantation or multi-unit tetrode implantation, both secured with dental cement. Mice underwent a 6-day training protocol, transitioning from a double to a single maze. During experimentation, neural activity was recorded using the nVista miniscope or Axona system, while locomotion was tracked via overhead camera. Calcium data were processed in IDPS (motion correction, SNR enhancement, PCA-ICA), and electrophysiological signals were clustered using Klusta-Kwik. Neural and positional data were aligned using MATLAB scripts to compute ratemaps.

Results & Discussion: Combined analyses from Ca-imaging and electrophysiology revealed impaired stability of hippocampal spatial representations across distinct contextual environments. Ca-imaging, despite capturing large cell populations, showed low consistency of place fields across trials. These findings suggest limitations in context-specific map formation under the current experimental conditions. Continuous chronic recording during training may clarify when and how spatial representations emerge. Enhancing trajectory tracking with tools like DeepLabCut or AnimalMotionViz could improve spatial resolution. Varying analysis parameters or using alternate software (e.g., CNMFe) may refine cell detection, while MATLAB scripts require further optimization for accurate data integration.

Conclusion: Further refinement of behavioral training protocol, analytical methodologies-including data assimilation, accuracy, signal processing-will be necessary to extract and enhance the spatial encoding in mice in order to further experiment on physiological and pathophysiological conditions like psychosis.

Funding: Funded by Cooperatio NEUR, and by Grant Agency of The Czech Republic Grant No. 22-16717S.

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 4
ID: 1136

ROBOTIC LASER TISSUE SOLDERING FOR ATRAUMATIC SOFT TISSUE FUSION GUIDED BY FLUORESCENT NANOTHERMOMETRY

Running title: Laser Soldering for Atraumatic Tissue Fision

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State-of-the-Art: Minimally invasive surgery (MIS) has revolutionized patient care by reducing trauma and recovery time. However, suturing soft tissues in MIS remains challenging due to limited haptic feedback and the fulcrum effect. Laser tissue soldering offers a promising alternative, enabling atraumatic, seamless tissue fusion. This study introduces fluorescent nanothermometry-guided laser soldering, integrating temperature-sensitive nanoparticles into protein-based solders for real-time thermal feedback. The technique ensures precise temperature control (60–80°C) to avoid tissue damage while achieving strong, watertight bonds.

Objective: This study aims to develop a feedback-controlled laser tissue soldering system using fluorescent nanothermometry for precise, atraumatic soft tissue fusion in minimally invasive and robotic surgery, overcoming limitations of sutures and adhesives.

Material and Methods: The solder paste comprised albumin, gelatin-methacrylate (GelMA), TiN nanoparticles (photothermal agents), and $BiVO_4$:Nd^{3+} nanoparticles (fluorescent nanothermometers). A 750 nm laser heated the paste, while real-time temperature monitoring was achieved via fluorescence intensity ratio (FIR) analysis. A PID controller modulated laser power to maintain optimal temperatures (60–80°C). For thermal imaging, a convolutional neural network (CNN) upscaled low-resolution data from fiber bundles. The system was tested ex vivo (porcine liver, intestine) and in vivo (porcine model) using robotic and laparoscopic setups. Mechanical testing compared soldered and sutured liver samples for tensile strength.

Results & Discussion: The feedback-controlled system achieved precise temperature regulation (<10 s response, no overshooting) and robust tissue bonding. Automated soldering outperformed manual methods, producing uniform seams without thermal damage. CNN-based thermal imaging accurately reconstructed temperature distributions ($\Delta T_{max} = 2.6 \pm 1.0$°C). In vivo experiments demonstrated seamless integration with laparoscopic tools, with histology confirming strong adhesion and no tissue damage. Tensile strength tests showed soldered liver outperformed sutures due to uniform stress distribution. The technology's adaptability to robotic systems (e.g., KUKA, Dexter robots) highlights its potential for clinical translation, particularly in fragile tissues like intestines or blood vessels.

Conclusion: Fluorescent nanothermometry-guided laser soldering enables precise, atraumatic tissue fusion in MIS. With automated feedback and robotic compatibility, this method surpasses sutures in bond strength and safety, offering a transformative approach for minimally invasive wound closure.

Study program: Doctoral study - Surgery | Year of study: 3

ID: 1130

PROGNOSTIC FACTORS IN SURGICAL TREATMENT OF HIGH GRADE GLIOMAS

Running title: Prognostic factors in surgical treatment of HGG

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State-of-the-Art: High grade gliomas are incurable tumors with fatal prognosis. Number of clinical prognostic factors have been known for many years. The focus has shifted to molecular biomarkers in recent years, which is reflected in the new WHO tumor classification 2021. New molecular methods, such as NGS offer the option to create a personalized molecular tumor profile. Liquid biopsy from blood and cerebrospinal fluid seems to be another promising method, which could be used as one of the modalities for monitoring the course of the disease in the future. In our study we present two groups of patients.

Objective: 1. A retrospective analysis of a group of patients and evaluation of factors impacting the surgical treatment. 2. A prospective analysis group of patients and evaluation of prognostic factors, including methods such as NGS, liquid biopsy and volume measurment.

Material and Methods: The retrospective group consists of 143 patients operated in years 2015-2021. 68 of these patients were lost to followup and thus were not included in the final analysis, which was discussed last year. The prospective group consists of 40 patients operated in years 2022-2024. The analysis evaluates prognostic factors and the effect of these factors on the outcome. Pre- and postoperative volume measurement was also performed. Liquid biopsy from blood and cerebrospinal fluid and evaluation of specified miRNAs is currently in progress.

Results & Discussion: The tumor volume ranged from 1,28ml to 83,08ml, averaging 29,3ml. A radical ressection (>90 % tumoral volume) was achieved in 88,9 % of cases, with an average volume ressection rate of 96,7 % (57,5 – 100 %). Average OS was 364 days. PFS was 210 days. Radical resection (> 90 %) was coupled with higher OS compared to non-radical resection (412 vs 277 days). Average OS with MGMT methylated tumor was higher (449 days), as well as OS following chemotherapy of MGMT met. tumor (701 days). OS was similar in EGFR amplified tumors (366 days). TERT mutated tumors presented with lower OS (311 days). Chromosome 7 and 10 aneuploidy was coupled with lower OS (294 days). Lastly, patients presenting with more than 1 negative molecular marker had an average OS of 264 days.

Conclusion: The surgical results and demographics are consistent with literature. The presence of negative molecular markers is coupled with a lower overall survival times. The last part, liquid biopsy of specified miRNAs encountered in blood and cerebrospinal fluid is currently in progress.

Study program: Doctoral study - Surgery | Year of study: 4
ID: 1129

DECELLULARIZED BILE DUCT AND ITS PARTIAL RECELLULARIZATION BY MESENCHYMAL STEM CELLS IN VITRO – IS IT SUITABLE FOR BILIARY TRACT SURGERY?

Running title: Bile Duct Reconstruction by Decellularized Tissue

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State-of-the-Art: A bile duct reconstruction after injury or resection is a staple of hepatopancreatobiliary surgery. Research to find suitable bile duct substitute for anatomical reconstruction has been conducted over the last 100 years without an optimal result. New methods and discoveries in Tissue Engineering field offers potential answers to this issue, with potential aplication for grafts made from decellularized tissue with or without cellular reseeding modification.

Objective: The goal of this study was to verify the possibility of decellularization of allogeneic porcine extrahepatic bile ducts (eBD), their recellularization by human mesenchymal stem cells (hMSC), and their usage for common bile duct reconstruction in experimental settings.

Material and Methods: Porcine eBDs were harvested and cryopreserved at -80°. Bile ducts were decellularized by perfusion with detergents in 4 1-hour cycles. Scaffolds were evaluated for the presence of residual nuclei. For 24 hours, static seeding with hMSC was done in the incubator, followed by dynamic perfusion seeding in an in-house-developed bioreactor. Live-dead assay and immunofluorescence staining were used to evaluate the presence of living cells on the decellularized bile duct scaffold. An experimental group of six domestic pigs underwent a common bile duct resection with reconstruction by decellularized allogeneic eBD, surviving for 4 weeks to expose complications and show biointegration in the recipient tissues, compared to a control group with common bile duct reconstructed by direct anastomosis.

Results & Discussion: The decellularization process yielded great results in the complete decellularization of eBD without any nuclei present at the end control and complete preservation of scaffold architecture. Static seeding of hMSC has shown lower presence of living cells with their shape rounded as a result of limited adhesion to scaffold, perfusion seeding has shown improved results with more living cells, alligned with the direction of the flow, with star-shape conformation resulting from adhesion to the scaffold.In experimental reconstruction of porcine common bile duct all pigs survived the observation period with one pig developing a bile duct stenosis, similar to the control group. Histological evaluation has shown partial reepithelialization and biointegration into recipients tissues at 4 weeks.

Conclusion: Decellularization and recellularization of porcine eBD with hMSC has been succesfull, demonstrating crutial role of bioreactors and mechanical stimulation in recellularization process. Theese results showed promissing outcomes as a step towards future in vivo testing of eBD recellularized with hMSC.

Funding: The study was supported by the Charles University, project Grant Agency of Charles University No. 434522 and Cooperation project "Surgical disciplines" (COOPERATIO-207043, Charles University).

Study program: Doctoral study –Surgery | Year of study: 2

ID: 1132

IMMUNOHISTOCHEMICAL AND STEREOLOGICAL ANALYSIS OF IMMUNE CELL INFILTRATION IN METASTATIC MELANOMA

Running title: Tumor Infiltrating Immune Cells in Melanoma

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State-of-the-Art: Melanoma is a highly aggressive skin cancer characterized by significant immune interactions within its tumor microenvironment. Despite significant advances in immunotherapy, the treatment of metastatic melanoma remains challenging. While effectiveness varies among patients, immune checkpoint inhibitors have improved survival rates. Immune cells that infiltrate tumors are essential for the immune response against melanoma. Among these, tumor-infiltrating lymphocytes (TILs) play a crucial role in disease progression and treatment response. Most published studies evaluate only a limited number of parameters, and their results are inconsistent regarding the predictive value of different subtypes of TILs and other immune cells.

Objective: This study aimed to evaluate the infiltration of immunohistochemically positive (IHC+) cells in metastatic melanoma and their potential associations with metastatic site (lymph node vs. skin), immune-related toxic reactions, and different responses to immunotherapy.

Material and Methods: The study included 28 patients diagnosed with metastatic melanoma who received immunotherapy. Various immune cell types infiltrating melanoma metastases were histologically quantified using multilevel sampling and stereological techniques. Immunohistochemical analysis was used to evaluate the expression of the markers CD1a, CD1d, CD3, CD4, CD8, CD20, CD56, CD68, FOXP3, LAG3, PD1, and PD-L1.

Results & Discussion: We demonstrated that the area fraction of all immune cell indicators differed between skin and lymph node metastases. There were variations in CD3 and PD-L1 markers between patients with and without immunotherapy-related toxic effects. In patients with varying responses to immunotherapy, we identified three markers (CD1a, CD20, and PD-L1) that may serve as prognostic indicators. When analyzing a more homogeneous subset of lymph node metastases, CD1a, CD8, and PD-L1 played a similar role.

Conclusion: Our study identified biomarkers that may help predict immunotherapy-related toxicity and treatment efficacy. Tumor-infiltrating immune cells have a significant role in forming the tumor microenvironment and influencing treatment efficacy.

Funding: The grant SVV 260 773 and the Cooperatio program area MED/DIAG and ONCO supported the study.

Study program: Doctoral study - Anatomy, Histology and Embryology | Year of study: 4

ID: 1079

PROGNOSTIC VALUE OF T CELLS IN PRIMARY COLORECTAL CANCER AND ADJACENT NORMAL MUCOSA IN PATIENTS WITH LIVER METASTASES

Running title: T Cells Impact Prognosis of Colorectar Cancer

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Supervisors: Kari Hemminki (1), Andriy Trailin (1)

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State-of-the-Art: 25%-50% of early-stage Colorectal cancer (CRC) patients progress to either synchronous or metachronous metastases, which predominantly affect the liver and drastically worsen survival. The prognosis for CRC with liver metastasis (LM) remains poor.

Prognostic performance of T cells was demonstrated in CRC with LM. However, integral assessment of different T cells subsets was rarely performed in a single cohort. Additionally, creating prognostic models on data available from the primary tumor and the normal colonic mucosa (NM) was less considered.

Our hypothesis posits that quantification and comparative analysis of different T cells in and between primary CRC and adjacent NM might have a prognostic value in CRC patients with respect to the presence of synchronous or metachronous LM.

Objective: To determine if analysis of T cell subsets in tumor center of primary colorectal cancer (CRC), adjacent normal mucosa and ratio between those sites can improve prognostic accuracy in CRC patients with the respect to presence of synchronous and metachronous liver metastasis (LM).

Material and Methods: Paired formalin-fixed paraffin-embedded specimens of primary colorectal cancer and tumor-adjacent normal mucosa (NM) were collected from patients, who either already had LM (synchronous, n= 55) or developed them thereafter (metachronous, n=44). After immunohistochemical staining, several subsets of T cells (CD3+, CD8+, CD45RO+, CD4+, and Foxp3+) were quantified in NM and tumor center of pCRC. Immune cell densities in NM and TC as well as their ratios (TC/NM) were correlated with clinical and pathological variables and tested as prognostic variables for overall survival (OS).

Results & Discussion: In both synchronous and metachronous groups, NM exhibited higher densities of CD3+, CD8+, CD45RO+, and CD4+ T cells compared to TC. Conversely, Foxp3+ cells were significantly more abundant in TC than in NM. CD45RO+ cells showed positive correlation with both CD8+ and Foxp3+ cells in TC of patients with synchronous LM. Densities of no cell type in the metachronous group were associated with OS. In synchronous group, patients with high densities of Foxp3+ T cells in NM and TC had longer OS in Cox and Kaplan-Meier analyses. Additionally, high ratio of CD45RO+ T cells between TC and NM was associated with longer OS in both types of analyses. The findings support parallel evaluation of immune cells in pCRC and adjacent NM for informed prognostication and warrant further exploration.

Conclusion: Our study revealed significant differences in the T cell landscape between pCRC and adjacent NM with Foxp3+ cells predominating in TC of pCRC and other cell types predominating in NM. Favorable prognostic associations of Foxp3+ and CD45RO+ T cells were shown only in synchronous group.

Funding: This research was funded by the grant AZV NU21-03-00506

Study program: Doctoral study - Experimetnal Surgery | Year of study: 4

ID: 1076

ATRA INCREASES INKT CELL-MEDIATED TARGETING IN HEMATOLOGICAL MALIGNANCIES BY INDUCING CD1D EXPRESSION

Running title: Using ATRA to Imporve iNKT Killing

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State-of-the-Art: Invariant natural killer T cells (iNKTs) are a rare heterogeneous lymphocyte subpopulation that are at the border of innate and adaptive immunity. iNKTs are activated by lipid antigens presented via the CD1d molecule which is expressed on many cell types including tumor cells. During tumor progression, malignant cells tend to decrease levels of CD1d to escape immune surveillance. All-trans retinoid acid (ATRA), a commonly used chemotherapeutic agent, has the ability to increase CD1d expression on the cell surface, making cancer cells more susceptible to iNKT cell-mediated targeting. However, CD1d axis alone is sometimes insufficient without lipid antigen bound. Therefore, the strategies to improve iNKT killing are being investigated.

Objective: Here we investigated the use of ATRA to regulate CD1d expression in various hematological cell lines and monitored its effect on iNKT-mediated cytotoxicity.

Material and Methods: iNKT cells (n=5) were isolated from mononuclear cells and cultured in presence of irradiated (25 Gy) C1R cells engineered to express CD1d, artificial antigen α-galactosylceramide (α-GalCer) (1 μg/ml) and IL-15 (150 IU/ml). Cell lines of hematological malignancies: RPMI-8226, H929, K562, RAJI, MOLM-13 and KG-1a were cultured according to the manufacturer's recommendations. ATRA treatment was applied to the cancer cells at 0, 1, 2.5, 5 and 10 μM concentration and CD1d expression was measured at 48 h and 72 h by flow cytometry. iNKT cells were co-cultured with cell lines pretreated with ATRA, α-GalCer or with ATRA in combination with α-GalCer. After 24 h of co-culture the percentage of dead cancer cells was measured by flow cytometry.

Results & Discussion: ATRA increased CD1d expression with the highest induction at 5 μM after 72 h in MOLM-13 (6 846 control vs. 162 397 treated median of fluorescence intensity (MFI)), RPMI-8226 (4 070 control vs. 23 869 treated MFI) and H929 (5 196 control vs. 120 946 treated MFI). In MOLM-13 the pre-treatment with ATRA only slightly increased the iNKTs killing ability (mean control 6%, mean treated 14.4%, $p \leq 0.05$) but, the addition of α-GalCer enhanced it significantly (mean dead cells 58.9%, $p \leq 0.01$). In RPMI-8226 and H929, the ATRA pre-treatment alone significantly improved the iNKT killing activity (RPMI-8226: mean control 12.3%, mean treated 68.3%; H929: mean control 33.7%, mean treated 66.1%; $p \leq 0.01$), and combination with α-GalCer improved it further (by 12% in RMPI-8226 and by 22% in H929; $p \leq 0.05$).

Conclusion: Our findings demonstrate that ATRA can upregulate CD1d expression in acute myeloid leukemia (MOLM-13) and multiple myeloma cell lines (RPMI-8226 and H929), which improves the cytotoxic activity of iNKTs but depends on the presence of lipid antigen.

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Study program: Doctoral study - Anatomy, Histology and Embryology | Year of study: 2

ID: 1082

TUMOR-STROMA INTERACTIONS PROMOTE STEMNESS IN UROTHELIAL CARCINOMA: INSIGHTS FROM SIDE POPULATION ANALYSIS

Running title: Tumor-Stroma Crosstalk in Urothelial Cancer

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State-of-the-Art: Cancer stem cells are heterogenous population of cells, which are thought to be particularly responsible for insufficient treatment response, metastasis of tumours and evasion of the immune response. Although they can differ even within a single tumour, they can be defined by some common features. Using the flow cytometry method, we can demonstrate the expression of surface antigens common to the immature cells, along with tumour specific markers. Flow cytometry also allows the detection of cells with a hight efflux transporter activity. These cells not only secrete chemotherapeutics, but also DNA-binding dyes, which can be used in the "Side population analysis", where cells with active transporters form a population with a lower fluorescent signal, the so-called side population.

Objective: The aim of this study was to conduct a deeper analysis of the influence of tumour stroma on the stem phenotype of urothelial carcinoma using a methodology that I learned during my postgraduate internship in Innsbruck.

Material and Methods: To mimic the natural interaction between cancer and the stromal cells, we used a proven co-culture system of the urothelial cancer cell line RT112, and cancer associate fibroblast cell line BC44Fibr. The RT112 cancer cell line was seeded onto culture flasks containing settled fibroblasts to achieve the best possible cell-to-cell contact. After six days of co-culture, the proportion of side population cells were analysed using Vybrant™ DyeCycle™ Violet staining (Thermo Fisher Scientific, USA) and to distinguish between the two types of cells we used CD 90 antibody. Cell suspension was subsequent analysed by flow cytometry (BD, USA). Obtained data were compared to the result from RT112 monoculture.

Results & Discussion: We established an optimal seeding density for both cell types to maximize cell interaction. Our 2D co-culture model not only allows the exchange of signalling molecules between cells but also provides the possibility of communication via cell-to-cell contact. To distinguish between the two cell types, the CD90 antibody was used, which specifically binds to fibroblasts and not to RT112. The results of our pilot co-culture study suggest that the presence of stromal fibroblasts can have a dramatic effect on the representation of cancer stem cells (CSCs). The higher representation of cells with an active efflux pump in the tumour cell population in direct contact with the stroma is consistent with our previously presented results in this cell line.

Conclusion: We successfully optimized a 2D co-culture model of the fibroblast line BC44Fibr and urothelial carcinoma tumour cells. The effect of stromal cells, in accordance with our previous results, increased the proportion of cells capable of actively secreting DNA-binding dye, i.e., cancer stem cells.

Funding: SVV-2024-260654, SVV-2025-260773, Academic Minigrant 4EU+ MA/25/F1/0/018, The League Against Cancer Prague, The research internship was supported by the Erasmus.

Study program: Doctoral study - Medical Biology and Genetics | Year of study: 4

ID: 1142

A NOVEL METHOD FOR DERIVATIZATION AND MASS SPECTROMETRIC ANALYSIS (LC/MS AND LDI-TOF) OF SACCHARIDES WITH A SPECIAL FOCUS ON BACTERIA

Running Title: Mass Spectrometric Microbial Serotyping

Author: Lucia Ďaďovská (1), Veronika Pašková (1), Jaroslav Hrabák (1)

Supervisor: Jaroslav Hrabák

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State-of-the-Art: MALDI-TOF MS shortened the turn-around time of taxonomic identification of microbes and detection of antibiotic resistance in routine diagnostic medical microbiology. However, there is no application of the technique for epidemiological typing that would allow a deeper comparison of identified bacterial isolates (serotyping) or identification of the microbe directly from clinical sample. This void could be filled with analysis of microbial cell surface structures like lipopolysaccharides and lipoteichoic acid as those molecules are abundant in the bacterial cell and stable in the body fluids. MS analysis of polysaccharides is currently complicated due to their poor ionization ability, complexity, and molecule size. Thus, polysaccharide detection in diagnostics laboratory is impossible.

Objective: The hypothesis of the study was to demonstrate whether an innovative approach to polysaccharide ionization for analyzing bacterial lipopolysaccharides and other external structures of the cell wall can facilitate bacterial identification and typing by MALDI-TOF MS.

Material and Methods: One milligram of various saccharides (e.g. D-glucose, isotopic D-glucose-1,2-13C2, glucose-6-phosphate, lactose, starch), purified lipopolysaccharides, and bacterial cultures was dissolved in 10 µL concentrated formic acid or its isotopic variant (13C) and incubated at 98 °C for 10 minutes in the thermocycler for digestion. This digestion procedure also yielded esters of saccharide's hydroxyl groups. The reaction was filtered using Amicon® Ultra Centrifugal Filter, 3 kDa MWCO. Filtrate was derivatized with concentrated HD ligand (250 mmol/L) solution at pH 4.0. After incubation at 50 °C for 20 minutes, the derivatized saccharides were visualized using a timsTOF Pro 2 spectrometer (Bruker Daltonics, Bremen, Germany) and rapiflex MALDI-TOF MS (Bruker Daltonics, Bremen, Germany).

Results & Discussion: We developed a novel method for analyzing saccharides using mass spectrometry that is translated into detecting microbial lipopolysaccharides in LDI-TOF MS for bacterial serotyping. The first step of the method consists of acid hydrolysis of glycosidic bonds of microbial polysaccharides. In addition, hydroxyl groups of saccharides are esterified, further facilitating their identification in spectra. In the second step, aldehyde groups of digested reducing sugars react with a novel self-ionizable HD reagent we designed, synthesized, and patented. HD reagent possesses perfect ionization ability that no matrix is needed. This derivatization allows the obtaining of unique spectra for each bacterial species and purified lipopolysaccharides representing specific lipopolysaccharide fingerprints.

Conclusion: We discovered an innovative glycomic MS method for analyzing microbial polysaccharides and bacterial serotyping. The procedure can be used in diagnostics laboratories. It is also a promising tool for analyzing microbes directly from clinical specimens.

Funding: The study was financed by the Czech Health Research Council grant Nr. NW24-09-00464, the GA UK project Nr. 550225 and the NIVB (Programme EXCELES, ID Project Nr. LX22NPO5103)—funded by the EU—Next Generation EU. The method was patented (PV 2024-48), and PCT application has been submitted.

Study program: Doctoral study - Medical Microbiology | Year of Study: 3

ID: 1144

OUTBREAK INVESTIGATION OF EXTENSIVELY DRUG-RESISTANT ACINETOBACTER BAUMANNII OF ST2 IN THE INTENSIVE CARE UNIT OF A LEBANESE HOSPITAL

Running title: Extensively Drug-Resistant A. baumannii of ST2

Authors: Tsolaire Sourenian (1), Marc Finianos (1), Jaroslav Hrabak (1), Abdallah Medlij (2), Ibrahim Bitar (1)

Supervisor: Ibrahim Bitar (1)

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State-of-the-Art: Acinetobacter baumannii is a leading multidrug-resistant (MDR) pathogen responsible for nosocomial infections, particularly in intensive care units (ICUs). This incidence has been driven by unregulated antibiotic use, posing significant concerns and high mortality rate due to its extensive drug-resistance (XDR).

Objective: The aim of the study is to investigate an XDR A. baumannii outbreak in the ICU of a tertiary hospital in Lebanon.

Material and Methods: A total of 47 swabs were sampled from one of the ICU rooms (n=40; environmental surfaces and n=7; clinical A. baumannii swabs from outpatients). The strains were selected by Muller Hinton (MH) agars containing 4 µl/mL of meropenem. Species identification and antibiotic susceptibility profiles were detected using MALDI-TOF MS and broth microdilution assays respectively. Both short and long reads (Illumina and Sequel I/Minion) whole genome sequencing (WGS) were performed on the positive swabs (n=21). Clonal relatedness was assessed by multilocus sequence typing (MLST) and genomes were compared through SNP-based phylogeny.

Results & Discussion: Nineteen isolates were XDR A. baumannii, of which 12 were from environmental swabs (including side rails, monitors, air mattresses and toilet chairs). WGS revealed the presence of blaOXA-23 gene in all isolates, and it was located on the chromosome. MLST analysis showed that all strains belong to ST2 international clone. The SNP-based phylogeny suggested that the outbreak originated from multiple ST2 isolates which clustered into two main clades (A and B). Clade A confined 10 strains having only 0 to 1 SNP when compared to each other. Whereas, clade B had 2 subclades: one containing 6 strains that differed by 0 to 21 SNPs among each other, and the other one had 2 strains with 102 SNPs difference.

Conclusion: We reported a nosocomial ongoing four-year outbreak of XDR A. baumannii in a Lebanese ICU, intensified by the war casualties, reveals critical gaps in screening and disinfection protocols. Urgent intervention at both national and hospital levels is necessary to halt future outbreaks.

Study program: Doctoral study - Medical Microbiology | Year of study: 4

ID: 1083

NEUROPEPTIDES B AND W AS NOVEL TARGETS IN RESEARCH ON GASTROINTESTINAL DYSFUNCTION IN DIABETES MELLITUS

Running title: Neuropeptides B/W in Diabetic Gut Dysfunction

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State-of-the-Art: Neuropeptides B (NPB) and W (NPW) and their receptor (NPBWR1) are involved in the regulation of energy homeostasis, stress response, and neuroendocrine functions. Although they were identified more than two decades ago, their role in gastrointestinal physiology remains poorly understood. It has been confirmed that NPW influences gastrointestinal motility by reducing antral contractions and slowing gastric emptying. However, NPB/W function in other parts of the gastrointestinal tract has not yet been studied. In the context of diabetes mellitus (DM), it is well known that gastrointestinal motility is disrupted due to autonomic neuropathy and alterations in neuropeptide signaling. The potential NPB and NPW role in the pathophysiology of gastrointestinal dysfunction remains unclear.

Objective: Our goal was to confirm the gene expression of NPB, NPW and NPBWR1 in the cardia, corpus, pylorus, duodenum, and colon and assess the impact of type 1 and type 2 DM on the expression of these genes. Subsequently, we analyzed the effect of NPB and NPW on colonic contraction force in vitro.

Material and Methods: The study involved 12-week-old Zucker Diabetic Fatty (ZDF) rats (type 2 DM; n = 7), lean rats with streptozotocin-induced DM (type 1 DM; n = 7), and lean control rats (n = 7). Tissue samples from the cardia, corpus, pylorus, duodenum, and colon were dissected, washed, and frozen. Later, they were homogenized. The total RNA was isolated and converted into cDNA, which was analyzed by qPCR to determine the relative gene expression of the target genes (NPB, NPW, NPBWR1). For the in vitro measurements, we used a ring of colonic tissue placed into a chamber perfused with oxygenated Tyrode´s solution (37°C). Acetylcholine-induced contractions were recorded first in the control solution and then in the solution containing NPB or NPW (1 nM, 10 nM, and 100 nM gradually).

Results & Discussion: Gene expression of NPB, NPW and NPBWR1 was detected in all examined tissues. NPBWR1 expression in the corpus and pylorus was at the detection limit and, hence, not quantitatively assessed. In STZ rats, we observed a statistically significant downregulation of NPB expression in the corpus (0.56-fold, $p = 0.028$) and a statistically significant upregulation of NPW expression in the pylorus (2.62-fold, $p = 0.034$), duodenum (1.91-fold, $p = 0.046$), and colon (1.7-fold, $p = 0.046$). In the ZDF rats, we revealed a significantly upregulated NPBWR1 expression in the colon (2.19-fold, $p = 0.038$). The amplitude of colonic contraction significantly decreased after applying NPB and NPW during the in vitro measurements. This effect was more pronounced with increasing concentration of applied neuropeptide.

Conclusion: We observed significant changes in NPB/W signaling system gene expression in various gastrointestinal organs of DM rats. Both neuropeptides showed a relaxing effect on colonic smooth muscle, which, together with an increased NPBWR1 expression, may help to explain gut symptoms in diabetic patients.

Funding: This study was supported by the Charles University Grant Agency, project No. 10524: The effect of diabetes mellitus on the innervation of the gastrointestinal tract.

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 2

ID: 1084

RECONSTITUTION OF INVARIANT NATURAL KILLER T CELLS POST-ALLOGENEIC STEM CELL TRANSPLANT: PILOT DATA FOR PHASE I CLINICAL TRIAL

Running title: iNKT Cells After Allogeneic Stem Cell Transplant

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State-of-the-Art: Invariant natural killer T cells (iNKTs) constitute a distinct subset of T cells, with attributes of T cells and natural killer (NK) cells, bridging innate and adaptive immunity, with profound immunomodulatory potential.

Haematopoietic stem cell transplant (alloSCT) remains the only potentially curative option for several hematological malignancies. Despite advances in optimal donor selection, tailored conditioning regimes, and supportive care, alloSCT is still burdened by high mortality and morbidity. Graft-versus-host disease (GvHD) and relapse are the most frequent and serious post-transplant complications. Based on studies monitoring reconstitution of immune subsets after alloSCT, higher levels of iNKTs positively correlate with lower incidence of GvHD and relapse.

Objective: The study aimed to characterize the kinetics of iNKT cells reconstitution in patients post alloSCT. Results will serve as a comparator for an ensuing phase I clinical trial, investigating the administration of allogeneic, donor-derived, ex-vivo expanded iNKTs to prevent GvHD.

Material and Methods: Peripheral blood (PB) iNKTs were monitored in 59 consecutive patients transplanted at our department. INKTs were enumerated 1, 2 and 3 months after alloSCT. PB samples were labeled with anti-CD45, anti-CD3, anti-TCR Vα24-Jα18, anti-TCR Vβ11 antibodies and analyzed using a BD ARIA Fusion flow cytometer. To determine the absolute number of iNKTs per mililiter of blood, the percentages of iNKTs from total leukocyte counts were integrated with the absolute leukocyte count quantified in our standard clinical laboratory. Mann–Whitney test was used for statistical analyses ($p \leq 0.05$).

The association of GvHD occurrence or relapse with iNKTs levels was tested. The effect of different GvHD prophylaxis drugs on post-transplant iNKT levels reconstitution was evaluated.

Results & Discussion: Patients who developed aGvHD had lower median (m) iNKT cell counts at all 3 time points compared to those without aGvHD. At 1 month, m iNKT counts were 67.16 versus 216.94 ($p=0.17$), at 2 months 80.44 versus 164.70 ($p=0.87$), and at 3 months 103.54 versus 228.80 ($p=0.85$).

Similar trend was observed when comparing relapsed and non-relapsed patients. Median iNKT cell counts were 55.4 vs. 467.48 at 1 month ($p=0.25$), 36.8 vs. 600.7 at 2 months ($p=0.09$), and 19.85 vs. 320.32 ($p=0.01$) at 3 months, where the difference reached statistical signifficance.

Patients receiving PTCy as GvHD prophylaxis had significantly decreased iNKT cells compared to patients treated with other prophylaxis regimens. This difference was observed at all time points.

Conclusion: Consistent with previous findings, we observed an association between higher post-transplant iNKT cell counts and a lower incidence of aGvHD or relapse. We also determined a statistically significant decrease in iNKT cells post-transplant in patients receiving PTCy as GvHD prophylaxis.

Funding: Supported by Ministry of Health of the Czech Republic Conceptual Development of Research Organization (Faculty Hospital in Pilsen - FNPl, 00669806) and the grant of Bone Marrow Foundation.

Study program: Doctoral study - Anatomy, Histology and Embryology | Year of study: 2
ID: 1103

CLINICAL EXOME SEQUENCING IN THE DIAGNOSTICS OF RARE DISEASES

Running title: Exome Sequencing in Rare Diseases

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Supervisor: Ivan Šubrt (1)

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State-of-the-Art: Rare diseases comprise a heterogeneous group of over 8000 diseases, predominantly affecting children. The majority of rare diseases have a genetic origin, and thus, accurate diagnosis is confirmed/ established by genetic testing. Although molecular diagnosis is still challenging, clinical exome sequencing (CES) offers a powerful tool for identifying rare diseases. In some cases, only a candidate variant of unknown significance is identified, which requires further validation of its pathogenicity through additional methods.

Objective: The aim of this study was to identify the genetic cause of rare diseases in pediatric patients and their families with rare diseases of unknown etiology using CES and other methods (if required).

Material and Methods: We performed CES with phenotype-driven analysis of 29 patients and their families with a rare disease of unknown etiology. Twenty-eight pediatric patients underwent singleton CES and one fetus trio CES. Causal and candidate sequence variants were subsequently verified by Sanger sequencing, which was also used for segregation analysis in parents when samples were available. Potential splice site variants were further characterized by RNA analysis (Sanger sequencing and quantitative real-time PCR). Detailed in silico protein analysis was used for candidate missense variants. We also used methylation-sensitive restriction analysis to study the skewed X-chromosome inactivation in X-linked diseases.

Results & Discussion: Causal pathogenic variants were found in 7 patients (24%), consisting of 7 sequence variants in UBR1, L1CAM, KDM5C, SPTBN2, POLG, and two structural variants. Two of the seven sequence variants were splicing variants (novel and previously identified) and five missense variants (three novel and two previously identified). In other two patients (7%), we found two candidate sequence variants in VRK1 and SCN3A genes. These are novel missense variants that cannot be classified as pathogenic (causal) at present.

Conclusion: By combining CES and other methods, we found a genetic cause in 7 patients (24 %). In another two patients (7%), we identified two candidate sequence variants. Identifying a genetic cause enables improvement in genetic counseling for patients and their family members.

Funding: LM2023067, SVV 260773

Study program: Doctoral study - Medical Biology and Genetics | Year of study: 4

ID: 1086

SENESCENT CELLS GENE EXPRESSION IN COLORECTAL CANCER IN RELATION TO DNA REPAIR CAPACITY AND CHEMOTHERAPY RESISTANCE

Running title: Senescent Cells in Colorectal Cancer

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State-of-the-Art: Chemotherapy resistance in colorectal cancer (CRC) is driven by factors like genetic mutations and tumor microenvironment. Recently, the role of senescent cells (SnCs) in chemotherapy resistance has been suggested. These cells exhibit traits such as increased SA-β-gal activity, impaired DNA repaired mechanisms, and resistance to apoptosis, impacting treatment effectiveness. Research indicates SnCs could predict CRC outcomes, aligning with the National Cancer Institute's proposed strategy of inducing and removing senescent cells to improve therapy. Our study hypothesizes that the characteristics of SnCs and their relationship to DNA repair capacity may serve as predictive biomarkers for CRC prognosis and chemotherapy responses.

Objective: (1) To investigate how senescent cells affect CRC progression by analyzing SnCs gene expression in tumor and non-cancerous samples from varied responders.

(2) To monitor senescent cells in PBMCs for non-invasive diagnostics.

(3) To evaluate the influence of dABT-263 (Navitoclax) on 5-FU sensitivity.

Material and Methods: For the first aim of our project, we will measure SASP genes in patients' Paired tissues, using RT-PCR. For the second aim, base excision repair DNA damage will be assessed in 100 paired samples using comet assay. For the third aim, the buffy coat will be isolated from patients' blood using Ficoll, after which the cells will be fixed and incubated with SA-β-gal. Senescence will then be confirmed using flow cytometry. For the in vitro experiments, we will utilize two cell lines: HT29, which is negative for replication error (RER-)colorectal cancer without mismatch repair deficiency (RER-), and HCT116 (Lynch syndrome), which is positive for replication error (RER+). Both cell lines will be employed to assess the impact of (Navitoclax) drug on the sensitivity to 5-FU.

Results & Discussion: Results from previous CRC NGS project shows that several SnCs genes was differentially expressed in tumors vs adjacent healthy mucosa. In addition, we identified two anti-apoptotic genes (BCL9 and BCL2L12) differentially expressed between tumor tissue and control. That might indicate, senescent tumor cells inhibition of apoptosis by upregulating anti-apoptotic BCL2 family members.

In our objective to monitor senescent cells in PBMCs, patient exhibited 85% SA-β-GAL activity, compared to 41% in a healthy control. Interestingly, while some patients showed decreased SA-β-GAL activity after tumor removal, others showed increased activity, indicating variability in responses. BER comet assay results shows significant increase in DNA damage in Tumor in comparison to healthy mucosa.

Conclusion: Our preliminary results suggest that chemotherapy resistance stems from therapy-induced senescence (TIS), which upregulates anti-apoptotic BCL2 pathways that protect cancer cells. Inhibiting these proteins with Navitoclax, a senolytic, may reduce resistance and induce apoptosis in cancer cells.

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Study program: Doctoral study - Experimetnal Surgery | Year of study: 2
ID: 1102

ALPHA-METHYL COA RACEMASE (AMACR) REACTIVITY ACROSS THE SPECTRUM OF CLEAR CELL RENAL CELL NEOPLASMS

Running title: AMACR Stains up to 77.8 % of CCRCCs

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State-of-the-Art: Clear cell morphology can be seen in a wide range of renal epithelial neoplasms including clear cell renal cell carcinoma (CCRCC), clear cell papillary renal cell tumor (CCPRCT), papillary renal cell carcinoma (PRCC), multilocular cystic renal neoplasm of low malignant potential (MCRNLMP), "MiT family" translocation renal cell carcinomas, renal cell carcinomas with fibromyomatous stroma. Except for "MiT family" translocation renal cell carcinoma and renal cell carcinomas with fibromyomatous stroma, most of the above-mentioned tumors can be frequently diagnosed on pure histologic basis. In challenging cases, particularly in limited samples and metastatic settings, a panel of immunohistochemical stains (i.e., PAX-8, CA-IX) can provide adjunctive diagnostic support.

Objective: The aim of this study was to investigate AMACR immunoreactivity within the spectrum of clear cell renal cell neoplasms including low- and high-grade CCRCC, CCRCC with cystic changes, and MCRNLMP.

Material and Methods: The Plzen tumor registry was searched for clear cell renal tumors to aid in assembling the following four cohorts: low grade (LG) CCRCC, high grade (HG) CCRCC, CCRCC with cystic changes, and MCRNLMP. The diagnostic inclusion criteria were: LG CCRCC with predominately (>90 %) solid growth pattern and WHO/ISUP histologic grade 1–2, HG CCRCC with predominately solid growth (>90 %) pattern and WHO/ISUP histologic grade ≥ 3, CCRCC with cystic changes (>50 % of the tumor volume), and MCRNLMP as per the WHO definition . All tumors were reviewed and the diagnosis was confirmed by two urologic pathologists. For each tumor, two representative blocks were stained for AMACR using two different clones. Immunohistochemical staining evaluation was performed independently by three pathologists.

Results & Discussion: Clear cell renal cell carcinoma is a morphologically and immunohistochemically heterogeneous neoplasm. Majority of CCRCCs can solely be diagnosed on H&E basis (or with a limited immunohistochemical panel). However, in diagnostically difficult cases, particularly in limited samples and metastatic settings, immunohistochemistry plays more important role. Data on AMACR reactivity in CCRCC is limited, with variable and conflicting findings. There were at least some AMACR immunoreactivity in 77.8 % CCRCCs . Moderate to strong positivity, or positivity in more than one third of the tumor was detected in 48.9 % of CCRCC using OV-TL12/30 clone. Strong and diffuse AMACR positivity was detected in 8.9 % of all CCRCCs.

AMACR immunoreactivity in MCRNLMP was up to 37.5 %.

Conclusion: We demonstrated relatively high expression rate of AMACR in CCRCC, while very variable in intensity and distribution. This finding may have diagnostic implications especially in limited samples (i.e., core biopsies), as AMACR positivity does not exclude the diagnosis of CCRCC.

Funding: This work was supported by the Cooperatio Program, Research Area SURG, the Institutional Research Fund FN 00669806, and SVV 260652 from the Ministry of Education, Czech Republic.

Study program: Doctoral study - Pathology | Year of study: 3
ID: 1085

PHYSICAL FITNESS OF MEDICAL STUDENTS AT THE FACULTY OF MEDICINE IN PILSEN

Running title: Physical Fitness of Medical Students

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State-of-the-Art: Practical experience with physiological data collection and analysis is an essential component of medical education. It enables students to apply theoretical knowledge in real-world conditions and better understand physiological variability among individuals. Despite the importance of physical fitness in the medical profession, there is limited data on the fitness level of medical students. This study provides current reference data and investigates associations between anthropometric parameters, strength capacities, and resting lactate values in a large sample of students.

Objective: To assess the physical fitness of medical students through selected strength and metabolic parameters, and to analyze the relationships between anthropometric data, muscular performance, and possibly resting lactate values to better understand their overall physiological condition.

Material and Methods: The study included 347 medical students (231 women), aged 21–28, who voluntarily participated during practical training sessions. Basic anthropometric data—age, height, weight, and BMI—were recorded for all participants. Muscular strength was assessed using a handgrip dynamometer and a back-leg-chest dynamometer to evaluate upper and lower body strength. Resting blood lactate levels were measured prior to any physical exertion. The collected data were analyzed using descriptive statistics, correlations, and regression models to identify patterns and relationships among the physiological variables.

Results & Discussion: The average BMI was 22.1 (16.36–34.34) for women and 24.5 (19.8–40.32) for men. Handgrip strength averaged 29.65 kg in women and 47.76 kg in men. Back-leg-chest strength reached 100.5 kg in women and 178.37 kg in men. Resting lactate was 1.04±0.47 mmol/L in women and 1.18±0.75 mmol/L in men. The findings suggest that the students' physical fitness is comparable to that of the general population, with observed gender-based differences. The large sample enables meaningful subgroup comparisons and future trend analysis. Longitudinal monitoring across the course of medical studies would provide valuable insight into potential changes in students' physical fitness over time.

Conclusion: Medical students show physical fitness levels comparable to age-matched population norms. This baseline data may guide further research on trends and correlations in physical fitness throughout medical education.

Funding: This work was supported by the Cooperatio Program, research area Immunity and Infection.

Study program: Doctoral study - Internal Medicine | Year of study: 2

ID: 1143

EFFECT OF PHYSICAL ACTIVITY ON MITOCHONDRIAL RESPIRATION IN PLATELETS

Running title: Exercise Effects on Platelet Respiration

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State-of-the-Art: Mitochondria play a crucial role in cellular energy metabolism, and their function reflects overall cellular health. This study examines the relationship between mitochondrial respiration and physical fitness, focusing on maximal aerobic capacity (VO2max). Findings may improve our understanding of how mitochondrial efficiency, fat metabolism, and metabolic flexibility contribute to the health benefits of physical activity, especially in preventing and managing metabolic and cardiovascular diseases.

Objective: This project explores the potential of using platelets as a less invasive alternative to muscle biopsies for evaluating mitochondrial function.

Material and Methods: A cohort of 14 healthy adult participants was recruited and categorized into sedentary (n=5) and physically active (n=9) groups. During the first visit to the Department of Sports Medicine and Active Health Sciences, participants underwent anthropometric and body composition assessments, dynamometric testing, and spiroergometry. At the second visit, venous blood was drawn for lipid profiling and mitochondrial analysis, and a capillary sample was taken for basal lactate measurement. Mitochondrial respiration in platelets was assessed using the Oroboros O2k device under three conditions: resting, post-anoxia, and after activation with thrombin.

Results & Discussion: Although no statistically significant correlations emerged between VO_2-max and mitochondrial respiration in platelets, a consistent trend toward enhanced respiratory capacity was observed in physically active participants, particularly under stress conditions such as thrombin-induced activation and maximal electron transport system capacity (ETSC). This suggests a more adaptable or resilient mitochondrial phenotype. Moreover, active individuals exhibited a lower metabolic age relative to their chronological age, indicating systemic metabolic benefits of physical activity. These findings highlight the potential of platelet-based assessments as a minimally invasive, repeatable tool to reflect metabolic fitness and support its use in broader research contexts.

Conclusion: While statistical significance was not achieved, observed trends support the hypothesis that platelet mitochondrial respiration reflects differences in metabolic fitness. This pilot study confirms the method's feasibility and supports further research in populations with higher cardiometabolic risk.

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 2

ID: 1105

QUANTIFYING PSYCHIATRIZATION USING COMPUTATIONAL LINGUISTICS

Running title: Quantifying Psychiatrization

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State-of-the-Art: Psychiatrization comprises of a material and cultural aspect, the later of which refers to interpreting mental traits and states previously considered psychiatrically benign as instances of psychopathology. Such cultural aspects of psychiatrization may negatively affect public mental health because perceiving an adverse experience as psychopathological may increase its perceived severity, trigger additional distress and interfere with intuitive coping strategies. Thus, interpreting an experience as a mental health problem may become self-fulfilling. Computational linguistics may be used to track cultural changes, algorithmically quantifying specific cultural dimension from a large corpus of texts.

Objective: The current study expects that ordinary experiences are increasingly viewed through the psychiatric lens. We predict that psychiatric terminology is normalized, being used in non-clinical contexts, and everyday adjectives describing mental states and traits are gaining clinical connotations.

Material and Methods: In an exploratory study utilizing computational linguistics, fragments of texts containing diagnostic (e.g. "ADHD" or "anorexia"), everyday psychological (e.g. "shy" or "sad"), or control keywords (e.g. "large" or "loud") were retrieved from a large (> 4bn words) corpus of Czech journalist texts published offline between 1990 and 2022. A linguistic marker of the cultural aspects of psychiatrization was developed: the clinicalness, calculated as lexical proximity towards the clinical discourse using the wordscores algorithm. The expected correlation between time and clinicalness was measured by Kendall's coefficient for each of 46 keywords.

Results & Discussion: Clinicalness was increasing for everyday adjectives describing human emotions and behaviors (median τ=.07) and less so control adjectives (median τ=.03), but not in diagnostic terms (median τ=.01). These findings support the notion of an increasing psychiatrization. For an individual, such psychiatrization could lead to stigma, self-stigma, additional mental health problems and unnecessary prescriptions. Culturally, psychiatrization could lead to a diversion from the inclusive society due to a tendency to adjust culture to what is seen as normal and change what is seen as pathological. To psychiatry, such psychiatrization may mean a pool of additional patients asking for treatment of matters that would have not otherwise been interpreted as a psychiatric problem or as a problem at all.

Conclusion: Our exploratory, linguistic data are consistent with the notion of increasing psychiatrization of ordinary experiences but not with normalization of mental disorders. Confirmatory research is needed to verify the observed increase in pathologization of everyday adjectives.

Study program: Doctoral study - Neurology and Psychiatry | Year of study: 4

ID: 1072

EVALUATION OF DETRANSITION IN TRANSGENDER PATIENTS

Running title: Detransition in Transgender Patients

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State-of-the-Art: Detransition refers to the process in which an individual who has undergone a gender transition (a change in gender identity, such as hormonal therapy or surgery) decides to return to their original gender identity. Detransition can occur in the following forms: discontinuation of hormonal therapy, reverting to the original name and social role corresponding to the sex assigned at birth, and in some cases, attempts at reverse surgical procedures if they were performed during the transition. The process of detransition is a relatively new and complex phenomenon that is gaining attention from both the professional and general public. The transition process and gender dysphoria have been studied extensively over time, while detransition remains poorly understood.

Objective: The goals of this study are primarily focused on evaluating the number of Czech transgender patients, who opt for detransition. The secondary aim of the study is to assess the reasons leading individuals to detransition, with a particular focus on psychosocial and health-related factors.

Material and Methods: A retrospective study will be conducted initially, focusing on the total number of individuals who have undergone both transition and subsequent detransition. Another method of data collection will involve in-depth semi-structured interviews with participants. These interviews will explore personal experiences with transition and detransition, the motivations and reasons that led to detransition, as well as the psychological, social, and physical aspects associated with the process. In addition to the interviews, participants will also complete questionnaires (WHOQOL-BREF, Beck Depression Inventory, Generalized Anxiety Disorder-7). All obtained results will be statistically evaluated.

Results & Discussion: The objective of this research is to develop a comprehensive understanding of detransition as a phenomenon that remains underexplored within the Czech Republic. Gaining deeper insight into the motivations and factors underlying decisions to detransition may contribute to the refinement of therapeutic approaches and treatment strategies for transgender individuals. The findings of this study may also enhance healthcare providers' and therapists' awareness of the specific needs of transgender patients. Furthermore, the results may support advocacy for legal reforms aimed at improving the living conditions of transgender people.

Conclusion: This study is the first to systematically explore detransition in the Czech Republic, aiming to better understand its causes, process, and impact. The findings will help improve care for transgender individuals and guide those considering detransition.

Study program: Doctoral study - Neurology and Psychiatry | Year of study: 3

ID: 1121

VICTIMIZATION OF THE PATIENTS WITH GENDER IDENTITY DISORDERS

Running title: Victimization of Transgenders

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State-of-the-Art: Data on traumatization in Czech patients with gender identity disorders (F64.0–F64.9) are limited to case reports, lacking comprehensive analysis. This grant aims to provide data on comorbidities (F43.0–F43.9) and childhood abuse. We will collect data from 50 patients at LFP UK (2024–2026), assessing victimization, PTSD, and the causes of under-diagnosis. Diagnoses will be made using M.I.N.I. 5.0.0, SCID, and other tools. Clinician focus groups will explore why these comorbidities are not treated. A free handbook will raise awareness, improving diagnosis and therapeutic interventions. We expect one-third of patients will experience victimization, with 20% developing lifelong PTSD.

Objective: Our goal is to present first data on victimization in patients with gender identity disorder (F64.0–F64.9). Using focus groups, we'll explore why psychiatric effects are not diagnosed or treated. We'll also publish a free online guide to raise awareness and improve care.

Material and Methods: We will collect data from 50 patients treated at the Psychiatric Clinic of LFP UK (2024–2026) for gender identity disorder (F64.0–F64.9, N50). We aim to determine the prevalence of victimization and violent behavior up to the follow-up date and monitor the effects of severe stress (F43.0–F43.9). In the second phase, a focus group with clinicians will explore reasons for underdiagnosis of comorbidities. Diagnoses will be made using M.I.N.I. 5.0.0 and SCID (DSM-IV) for PTSD. Victimization in the past 6 months will be assessed using the MacArthur Interview, recent violence with the MOAS, and childhood trauma (sexual abuse, corporal punishment) via the CECA.Q questionnaire.

Results & Discussion: The aim of our work is to provide initial data on victimization in patients with gender identity disorder (F64.0–F64.9). We will use qualitative methods, specifically focus groups, to explore why psychiatric consequences of victimization are often undiagnosed and untreated. Additionally, we plan to educate care users and the public by creating a free handbook on victimization, available on the LFP UK website. We hope this will increase patients' willingness to discuss these issues with doctors. By improving the diagnosis of comorbid disorders, we aim to design effective therapeutic interventions. We expect victimization in one-third and lifelong PTSD in 20% of patients.

Conclusion: This aims to provide the first data on comorbidities and childhood abuse in Czech patients with gender identity disorders (F64.0–F64.9). We will assess victimization, PTSD, and the reasons for under-diagnosis. A free handbook will raise awareness, improving diagnosis and therapeutic interventions.

Study program: Doctoral study - Neurology and Psychiatry |Year of study: 2

ID: 1140

SALIVARY GLAND ANLAGE TUMOR: A STUDY OF RB1 EXPRESSION AND MOLECULAR GENETIC ANALYSIS OF 8 CASES

Running title: Rb1 Loss in Salivary Gland Anlage Tumors

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State-of-the-Art: Salivary gland anlage tumor (SGAT) is a very rare infantile tumor occurring in the nasopharynx, often presenting with respiratory distress syndrome, stridor, or feeding difficulties in the first few months of life. SGAT is a biphasic tumor combined of epithelial (tubules, ducts and solid or cystic squamous nests) and variably cellular myoepithelial tissue (spindle-shaped cells). The genetic background of this tumor remains poorly understood.

Objective: Eight cases of SGAT were selected from the authors' files. These cases underwent histological and immunohistochemical (IHC) analysis (Rb1, and other IHC markers), as well as molecular genetic analysis.

Material and Methods: For microscopy, the excised tissues were fixed in formalin, processed routinely, embedded in paraffin, cut and stained with hematoxylin & eosin. For immunohistochemistry, sections were cut from paraffin blocks and mounted on positively charged slides. The in-house customized version of Archer FusionPlex Sarcoma kit was used to construct a cDNA library for detecting fusion transcripts and point mutations in 88 and 14 genes respectively. The library was sequenced on an Illumina platform. Illumina TruSight Oncology 500 interrogating 523 genes for single nucleotide variants (SNVs) and indels and TruSight RNA Pan-Cancer Panel assays targeting 1385 genes were performed. For the detection of Rb1 loss the probe ZytoLight® SPEC Rb1/13q12 Dual Color Probe was used.

Results & Discussion: The cohort consisted of 8 patients, including 6 males, 1 female and in 1 case unknown, with an age range from 2 days to 5 years. One case was an incipient stage of SGAT, composed of cystically dilated ducts lined by cylindrical epithelium with a ciliated surface and squamous, partly cystic islands. Another case was a malignant SGAT composed of squamous, partly cystic nests along with a pleomorphic spindle cell component with multiple mitoses. All cases with available tissue blocks showed complete loss of Rb1 staining in tumor cells with positive internal and outer control. None of our cases showed a somatic genetic event; however, a clinically significant RICTOR mutation, potentially germline, was found in one case. FISH and DNA sequencing were negative/non-analyzable for Rb1 mutation.

Conclusion: Despite negative/non-analyzable molecular genetic results of Rb1 gene alterations, Rb1 IHC is an important diagnostic tool in SGAT. Loss of Rb1 immunoexpression suggests an absence or malfunction of Rb1, likely through mechanisms other than direct genetic alterations detectable by FISH or NGS.

Funding: This study was supported by study grant SVV 260652 from the Ministry of Education.

Study program: Master´s degree - General Medicine | Year of study: 6

ID: 1088

SIGNIFICANCE OF PLAG1::LIFR GENE FUSION IN PLEOMORPHIC ADENOMA OF SALIVARY GLANDS: POTENTIALLY SIGNIFICANT PROGNOSTICATOR OF CLINICAL BEHAVIOUR.

Running title: PLAG1:LIFR Fusion in Pleomorphic Adenoma

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State-of-the-Art: Pleomorphic adenoma (PA) is a benign epithelial neoplasm characterized by cytomorphological and architectural diversity, with an admixture of ductal and myoepithelial cells usually embedded in a chondromyxoid or fibrous stromal component. PAs harbor recurrent translocations or intrachromosomal rearrangements resulting in gene fusions involving PLAG1 on 8q12 (> 50%) or HMGA2 on 12q14.3 (10–15%). Seven recurrent fusion partner genes for PLAG1 have been reported (CTNNB1, FGFR1, LIFR, CHCHD7, TCEA1, NFIB, and BOC). Moreover, oncocytic PAs have been shown to contain PLAG1 gene fusions with GEM, CHCHD7, NTF3, FBXO32, and C1orf116.

Objective: Recently, it has been proposed that PLAG1::LIFR fusion might be associated with adverse prognosis of PA.

Material and Methods: A retrospective analysis of 1557 PA cases, retrieved from the Salivary gland tumor registry consultation files, was conducted. A total of 150 cases of PA positive for PLAG1 on immunohistochemistry (IHC) were selected. Fluorescence in situ hybridization (FISH) and/or targeted next generation sequencing (NGS) were performed in order to detect PLAG1 gene rearrangements and/or fusion. Fusions of the PLAG1 gene were identified in 42 PA cases, including PLAG1::LIFR (11/42), PLAG1::CTNNB1 (9/42), and variable other fusions (22/42).

Results & Discussion: PLAG1::LIFR fusions (n=11) were found in the palate (7), parotid (2), submandibular gland (1), and jaw (1). Patients were 82% female (9/11) and 18% male (2/11), with an average age of 54 (range 35–79). The PLAG1::LIFR tumors were benign in 5 cases: PA (3), recurrent PA (1), myoepithelioma (1), while 6 cases were malignant, such as myoepithelial carcinoma (MECa) (3), recurrent MECa ex PA (1), MECa ex PA (2). Lung metastases occurred in 2 cases. PLAG1::CTNNB1 fusions (n=9) were found in the parotid gland (7), submandibular gland (1) and palate (1). Patients were 67% male (6/9) and 33% female (3/9), with an average age of 46 (range 18–68). Tumors were benign in 4 cases: PA (2), atypical PA (2) while 5 cases showed malignant transformation of PA: MECa (4), salivary duct carcinoma ex PA (1).

Conclusion: PLAG1::LIFR and PLAG1::CTNNB1 fusions were both identified in a subset of PLAG1-positive pleomorphic adenomas. PLAG1::LIFR was notably associated with malignant transformation and metastatic behaviour of PA, thus indicating its potential utility as a prognostic marker.

Funding: The work was partly supported by the grant SVV No. 260 773.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1107

SIGNIFICANCE OF MDM2 GENE AMPLIFICATION TO PREDICT AN AGGRESSIVE BEHAVIOUR OF PLEOMORPHIC ADENOMA.

Running title: Does the MDM2 Gene Predict the Behaviour of PA ?

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State-of-the-Art: Pleomorphic adenoma (PA) is a benign neoplasm characterized by cytomorphological and architectural diversity, with an admixture of ductal and myoepithelial cells usually embedded in a chondromyxoid or fibrous stromal component. PAs harbor recurrent translocations or intrachromosomal rearrangements resulting in gene fusions involving PLAG1 or HMGA2 genes. Carcinoma ex pleomorphic adenoma (CXPA) is an aggressive epithelial and/or myoepithelial neoplasm that arises in association with a PA. Its etiopathogenesis remains poorly understood, but it is believed that the development of this tumor is due to the accumulation of genetic, protein, metabolic, and epigenetic alterations in a PA.

Objective: Recently, it has been proposed that the MDM2 gene amplifications could be useful to predict an aggressive course of CXPA.

Material and Methods: A total amount of 14 Atypical PA cases (Group A), 29 Myoepithelial and/or Epithelial-Myoepithelial carcinoma cases (Group B) and 16 CXPA cases (Group C) were retrieved from the Salivary gland tumor registry consultation files. In these cases an immunohistochemical (IHC) detection of MDM2 was conducted. In 12 immunohistochemically positive cases fluorescence in situ hybriditation (FISH) was performed to detect MDM2 gene amplification.

Results & Discussion: In all three groups (Group A,B,C), the distribution of sex (50% male, 50% female patients) and age (average of 67 years) was equal. The immunohistochemical detection of the MDM2 gene was positive in 1/14 atypical PA cases, 9/29 myoepithelial and/or epithelial-myoepithelial carcinoma cases and 2/16 CXPA cases. A positive FISH detection of MDM2 amplification corelated with the IHC findings in Group B and Group C cases and was not found in the one positive atypical PA case.

Conclusion: The MDM2 gene amplification could indeed predict an aggressive outcome of the otherwise benign PA. Although the PA itself expresses this alteration very rarely, the malignant neoplasms arising from PA were significantly more likely to show the MDM2 amplification.

Funding: The work was supported by the grant SVV (No.260773).

Study program: Master´s degree - General Medicine | Year of study: 5

ID: 1099

MAPPING THE JOURNEY OF PATIENTS WITH BREAST CANCER – UNDERSTANDING THE CAUSES, EARLY DETECTION AND DIAGNOSIS OF THE DISEASE

Running title: Journey of Breast Cancer Patients to Diagnosis

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State-of-the-Art: Early detection of breast cancer is a basic prerequisite for improving the prognosis of this oncological disease. Regular self-examination and screening mammography (MMG) from the age of 45 and every 2 years are the main methods for early detection. The trend in patient care and public education is cooperation with patient organizations and improving awareness of recommended procedures within the so-called patient's journey to prevention, early diagnosis and treatment of individual oncological diseases. At the same time, there is little awareness of the perception of the causes of the disease and the insight into the patient's journey from diagnosis to treatment by patients.

Objective: Patient's perception of the cause of the disease, methods of early detection of breast cancer, and the patient's journey from the first symptoms to treatment.

Material and Methods: Questionnaire survey conducted once a week at 8-9:00 on 4.11.-16.12.2024 among randomly selected patients with breast cancer within the Department of Oncology and Radiotherapeutics of the University Hospital Pilsen and Faculty of Medicine in Pilsen. Questions included: the patient's suspicion of a possible risk factor or influence leading to the development of cancer; availability and source of information on regular breast self-examination; performing self-examination; availability and source of information on screening MMG; attitude towards MMG examination; other procedures in prevention and detection; symptom of breast disease and reason for visiting a physician; course and perception of the patient's journey to cancer treatment.

Results & Discussion: 26 patients (24 women, 2 men), median age 51 (33-71), women age 45+ 18/26 (69%). Most frequently perceived risk factors: stress 54%, none 12%, many others 34%. Self-exam awareness and use in 96% and 54%, source of information physician 46% and media 31%. In women 45+ Screening MMG awareness and use in 89% and 83%, source of information physician 70%, MMG omission due to ignorance, lifestyle or resistance 25%. Other screenings: USG 15%, CT 4%. Initial symptom: breast lump 11/26 (42%), MMG and USG detection 27% and 8%, armpit lump 8%. Timeline and pathway perceived without problem in 23/26 (88%), slight mistake or underestimation by the patient or physicians in 12%. While the patient group is heterogeneous, the data reflects a real-world situation and provides a fundamental insight.

Conclusion: We observed strong patient perception of cancer causes and awareness of its early detection, with some patients not adhering to screening MMG. Patients' journeys were positively reviewed by the majority of them. Further research would be beneficial for a more comprehensive view.

Funding: Partially supported by: Faculty of Medicine in Pilsen Cooperatio-ONCO; NEXTIN Pilsen.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1073

DISTRIBUTION AND PROGNOSTIC VALUE OF TUMOR-INFILTRATING MAST CELLS AND NEUTROPHILS IN COLORECTAL CANCER PATIENTS WITH LIVER METASTASES

Running title: Local Mast Cells and Neutrophils in Colorectal Cancer

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State-of-the-Art: Colorectal cancer (CRC) is among the most common and lethal cancers globally. The presence of liver metastasis (LM) is associated with a significantly poorer prognosis, exposing the limitations of current prognostic tools. While immune profiling of T-cells in tumor microenvironment has improved prognostication, the role of innate immune cells in CRC and adjacent normal mucosa (NM) remains underexplored. Both mast cells and neutrophils play crucial roles in the NM of the colon, where they contribute to immune surveillance and tissue homeostasis. In CRC, both mast cells and neutrophils have been shown to play dual roles, either promoting or inhibiting tumor progression.

Objective: We aimed to quantitatively assess the distribution of mast cells and neutrophils in the NM and primary tumor of CRC patients with synchronous LM, and to establish prognostic associations of immune cell densities.

Material and Methods: We enrolled into this retrospective cohort study 55 patients, who underwent resection of both primary CRC (pCRC) and synchronous LM. Formalin-fixed paraffin-embedded tissue sections from NM and pCRC were stained by immunoperoxidase method for mast cells with anti-CD117 antibodies and for neutrophils using anti-CD66b antibodies. Densities of immune cells per mm2 of the tissue were assessed in NM and tumor center (TC), inner margin (IM), outer margin (OM) and peritumor region (PT) of pCRC by using the QuPath image analysis software. Then cell densities were tested as prognostic variables for disease-free survival (DFS) by Cox regression analysis. A two-sided p value <0.05 was considered statistically significant.

Results & Discussion: Mast cell density was significantly greater in NM (median: 212 cells/mm^2) compared to TC (36 cells/mm^2), as well as in OM (76 cells/mm^2) and PT (85 cells/mm^2) of pCRC vs TC and IM (30 cells/mm^2). Neutrophil density was significantly greater in the TC (56 cells/mm^2) compared to NM (12 cells/mm^2). Within pCRC neutrophil density did not differ significantly between TC, IM (58 cells/mm^2), OM (42 cells/mm^2) and PT (36 cells/mm^2).

We observed significant correlation between neutrophils and mast cells in OM (Spearman Rho=0.32) and PT (Rho=0.30) of pCRC. High densities of neutrophils in PT of pCRC were associated with longer DFS (Hazard ratio 0.44, 95% confidence interval 0.22–0.88, p=0.021), but we didn't find associations between mast cell densities in any region with outcomes.

Conclusion: Our study found differential distribution of innate immune cells between NM and CRC with mast cells predominating in NM and neutrophils in TC of pCRC. Neutrophil infiltration in the PT region of pCRC in patients with synchronous LM confers a survival benefit, warranting further investigation.

Funding: AZV NW24-03-00521.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1110

DISTRIBUTION OF IMMATURE AND MATURE DENDRITIC CELLS IN COLORECTAL CANCER PATIENTS WITH LIVER METASTASES

Running title: Dendritic Cells in Colorectal Cancer

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State-of-the-Art: Colorectal cancer (CRC) is a leading cause of cancer-related deaths in Europe, with metastatic disease being the main driver of mortality. The liver is the most common site of metastases from CRC. T-cell infiltration in primary CRC significantly impacts clinical outcomes. Owing to their ability to orchestrate tolerogenic or immunogenic responses to tumor antigens, antigen-presenting dendritic cells (DCs) play a pivotal role in anti-cancer immunity. Multifaceted functions of DCs are acquired through a complex, multistage process called maturation. Immature DCs facilitate tolerance, while mature DCs strongly promote anti-tumor immunity. With impact on infiltration of other leukocytes, tumor associated DCs have been correlated with patient prognosis in CRC.

Objective: We aimed to explore the distribution of immature and mature DCs in primary CRC along with adjacent normal mucosa (NM) and to investigate the prognostic value of DCs in CRC patients with synchronous liver metastases.

Material and Methods: Paired formalin-fixed paraffin-embedded specimens of primary CRC and tumor-adjacent NM were collected from patients who underwent resection of both primary tumor and synchronous liver metastases (n = 55). Immunohistochemical staining followed by computer-assisted image analysis were performed to quantify CD1a+ immature and CD208+ mature DCs in the NM, tumor center, inner margin, outer margin, and peritumor regions of the primary CRC. DC densities across these regions were investigated and tested as prognostic variables for time to recurrence, disease-free survival and overall survival.

Results & Discussion: CD1a+ DCs displayed greater densities in TC compared to the NM (median 6 vs. 0 cells/mm^2, P<0.001), whereas density of CD208+ DCs was non-significantly greater in NM (13 vs 8 cells/mm^2, P=0.06). For CD1a+ DCs, the IM exhibited greater densities than the TC and OM (10 vs. 6 and 5 cells/mm^2 respectively, P<0.05), whereas the PT harbored the lowest densities (1 cell/mm^2). As for CD208+ DCs, the OM displayed significantly greater densities (21 cells/mm^2) compared to other regions of pCRC (TC: 8, IM: 6, PT: 13 cells/mm^2, P<0.05). A higher infiltration of CD1a+ DCs significantly correlated with greater densities of CD208+ DCs in the TC, IM, and OM, highlighting an interplay between these cell types. However, no significant associations were found between survival outcomes and either type of DC

Conclusion: Our study has profiled DCs in normal mucosa and primary CRC tissue showing significant heterogeneity for both immature and mature cell types. Assessing the prognostic value of DCS requires their detailed profiling in liver metastases.

Funding: AZV NW24-03-00521.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1125

LYMPHOCYTE SUBPOPULATIONS IN WOMEN WITH ENDOMETRIOSIS

Running title: Lymphocytes and Endometriosis

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State-of-the-Art: Endometriosis is a chronic, oestrogen-dependent, inflammatory and systemic disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. It affects approximately 10–15% of women of reproductive age and significantly reduces quality of life due to pain and infertility. Despite its benign nature, the exact cause of endometriosis remains unclear. Increasing evidence points to an important role of the immune system in its pathogenesis, with abnormalities observed in both local and systemic immune responses. However, the precise mechanisms of immune involvement in disease development are still not fully understood.

Objective: This study aimed to analyse lymphocyte subpopulations in the peripheral blood of women with ovarian endometriosis vs. healthy controls, focusing on T cell distribution/activation and inhibitory KIR receptor expression on NK cells to reveal immune differences.

Material and Methods: Peripheral blood samples were obtained from 24 women with confirmed ovarian endometriosis and 29 healthy female controls. All participants completed a standardized questionnaire collecting clinical and gynaecological history, including age, weight, height, body mass index, age at menarche, menstrual cycle length, and duration of menstrual bleeding. Peripheral blood mononuclear cells (PBMCs) were isolated and analysed by flow cytometry to determine the representation and activation status of T lymphocyte subpopulations (naive, memory, cytotoxic $CD8^+$, helper $CD4^+$, double-negative CD4 CD8 , and regulatory T cells) and the expression of selected inhibitory KIR receptors (2DL1, 2DL2, 2DL3, 2DL5, 3DL1, 3DL2) on NK cells.

Results & Discussion: No statistically significant differences were found in demographic or gynaecological characteristics between the two groups. The distribution of T lymphocyte subpopulations did not significantly differ; however, the control group showed a higher proportion of activated T cells, suggesting possible functional impairment in the patient group. In contrast, the expression of the inhibitory receptor KIR2DL2 on NK cells was significantly increased in patients with endometriosis, which may contribute to reduced NK cell cytotoxic activity and facilitate the implantation and survival of ectopic endometrial tissue.

Conclusion: These findings suggest that immune alterations in endometriosis may be more related to impaired cell functionality than to absolute cell counts. Reduced T cell activation and increased inhibitory NK receptors may enable persistence of ectopic endometrial tissue.

Funding: This study was supported by the grant of the Ministry of Health of the Czech Republic - Conceptual Development of Research Organization (Faculty Hospital in Pilsen - FNPl, 00669806).

Study program: Master´s degree - General Medicine | Year of study: 3

ID: 1134

IMPORTED INFECTIOUS DISEASES IN A FACULTY HOSPITAL: A 10-YEAR RETROSPECTIVE STUDY

Running title: A Decade of Imported Infectious Diseases

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State-of-the-Art: Imported infectious diseases are a significant part of healthcare systems, especially with increasing global travel. While the COVID-19 pandemic temporarily reduced travel and imported infections, case numbers have risen again post-pandemic. This study analyzes data from a faculty hospital over the past 10 years highlighting notable cases to enhance awareness and preparedness in clinical practice.

Objective: To analyze the occurrence of imported infectious diseases treated at a faculty hospital over a 10-year period (2015–2024) and highlight clinically relevant cases that may serve as educational examples for improving diagnostic vigilance.

Material and Methods: This retrospective study utilized data extracted from the hospital's electronic medical records from January 2015 to December 2024. Diagnoses were identified based on codes associated with imported infectious diseases. For each case, the following parameters were recorded: year, diagnosis, outpatient vs. inpatient care and frequency.

Statistics (absolute frequencies, trend visualization in line charts) were used to analyze temporal changes. Selected cases of malaria, giardiasis, dengue, and hepatitis E were further described due to their clinical significance.

Results & Discussion: The analysis of the data revealed a noticeable increase in imported infectious diseases in post-COVID era, particularly cases of giardiasis, malaria, dengue, hepatitis E, and enterotoxigenic E. coli infections. Diagnosing imported infectious diseases can be challenging due to their diverse presentations, but it's important for clinicians to be aware of the most common ones seen in our hospital to ensure timely and accurate diagnosis.

Conclusion: The sharp rise in dengue and malaria cases in the last three years corresponds with global post-pandemic travel recovery. Hepatitis E remains the most common imported infection, likely linked to food-related exposure during travel. Our hospital's data aligns with trends seen in other European center.

Study program: Master´s degree - General Medicine | Year of study: 3

ID: 1113

PRIMARY CILIA DEFICIENCY IN A MOUSE MODEL OF SPINOCEREBELLAR ATAXIA TYPE 1

Running title: Primary Cilia Deficit in SCA1 Mice

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State-of-the-Art: Primary cilia are tiny, antenna-like, non-motile sensory organelles found in most vertebrates. Their dysfunction leads to a class of disorders called ciliopathies and also occurs in many neurodegenerative diseases. Spinocerebellar ataxia type 1 (SCA1) is caused by an expanded CAG repeat in the ATXN1 gene; this translates into a polyglutamine (polyQ) ataxin-1 protein. Cellular aggregation of this mutant protein leads to progressive cerebellar degeneration, causing motor deficits such as ataxia and slurred speech. Ciliary dysfunction is observed in neurodegenerative diseases like SCA12 and another polyQ disorder, Huntington's disease. However, the role of primary cilia in the pathogenesis of SCA1 has never been investigated.

Objective: The main objective of this study is to elucidate the role of ataxin-1 in the regulation of ciliogenesis and the role of primary cilia in the pathogenesis of SCA1.

Material and Methods: Primary skin fibroblast cultures of SCA1 and WT mice were established according to the protocol by Bravo et al., 2021. Primary cilia are dynamic organelles, and they assemble maximally during interphase. Hence, to promote ciliogenesis, cells were cultured under serum-free conditions for 24 hours to impede mitosis. The cells were then fixed and immunostained for primary cilia markers, including acetylated α-tubulin and γ-tubulin, along with the mitotic marker PHH3, to accurately quantify cilia in non-dividing cells. In addition to cilia quantification, cell viability was measured using the MTS assay. Mitochondrial function was assessed by citrate synthase assay to analyze enzymatic function and basal respiration was studied using Seahorse extracellular flux analysis.

Results & Discussion: SCA1 fibroblasts showed a significant reduction in the number of primary cilia under both serum-free and serum-containing growth conditions. Alongside this, we observed reduced cell viability in the SCA1 fibroblasts in the MTS assay. The results from DAPI staining during primary cilia quantification revealed no reduction in cell viability, contrary to MTS assay results, indicating possible mitochondrial dysfunction, as mitochondrial dehydrogenase activity is measured in the MTS assay. This is further supported by a decrease in citrate synthase activity and basal mitochondrial respiration. Taken together, our data suggest a defect in either the formation or maintenance of primary cilia in SCA1 fibroblasts, along with mitochondrial dysfunction.

Conclusion: The association between reduced ciliogenesis and mitochondrial dysfunction highlights an unexplored aspect of SCA1 pathology worth investigating. Hence, the above experiments will be replicated in embryonic neural stem cells and neurons for a better understanding of primary cilia in SCA1 pathology.

Funding: Cooperatio Program (research areas NEUR) and GAUK #70124.

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 2

ID: 1122

NOTCH SIGNALING PATHWAY AND ITS ROLE IN OVARIAN CANCER – POSSIBLE BIOMARKERS OF THERAPEUTIC RESPONSE FROM THREE PERSPECTIVES

Running title: Biomarkers in Ovarian Cancer

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State-of-the-Art: Patients with epithelial ovarian cancer (EOC) face high mortality due to late diagnosis, recurrence, metastasis, and drug resistance. The main histological subtype is high grade serous carcinoma, which occurs in up to 80 % cases. One of the most used chemotherapy regimens is paclitaxel combined with platinum-based drugs. Resistance to therapy limits successful treatment and efforts are made to overcome it. NOTCH signaling pathway is significant for the onset and development of cancer. We have studied this pathway in samples of EOC patients and subsequently studied changes in this pathway after treatment with taxanes in in vitro and in vivo models.

Objective: The aim was to analyse and compare the deregulations in the gene expression profile of the NOTCH signaling pathway in samples from patients with EOC and paclitaxel resistant and sensitive SKOV-3 ovarian cancer cell lines in vitro, as well as in in vivo models.

Material and Methods: RNA, DNA, and protein were isolated from tumors of patients with EOC and non-malignant ovarian tissue samples. cDNA was synthesized from RNA and RT-PCR was subsequently performed. Statistical evaluation of gene expression levels stratified by clinical data was done by the SPSS program. For the in vitro study, paclitaxel-sensitive and resistant ovarian cancer cell sublines SKOV-3/sen and SKOV-3/res were used. Expression of NOTCH signaling pathway was analysed after treatment with different concentrations of taxanes – paclitaxel or Stony Brook taxanes (SB-Ts). For in vivo experiments, SKOV-3/sen and SKOV-3/res cell-derived xenografts were established in immunodeficient nude mice. After tumors developed, mice were treated with paclitaxel, SB-Ts, or their combinations i.p. twice a week.

Results & Discussion: In tumors from EOC patients, a notable upregulation of NOTCH1/3/4, and JAG2, along with a downregulation of the NOTCH2 gene, was observed in comparison with control tissues. We found a significant correlation between low NOTCH4 expression and the presence of peritoneal metastasis as well as a shortened platinum-free interval. In the in vitro resistant cell subline model, treatment with experimental SB-Ts led to a significant upregulation of NOTCH3, demonstrating high efficacy against paclitaxel-resistant ovarian tumor cells. Additionally, the administration of SB-Ts resulted in NOTCH3 upregulation in an effective

combination regimen with paclitaxel, compared to paclitaxel alone and untreated controls, in the in vivo cell-derived xenograft mouse model of resistant ovarian cancer.

Conclusion: Based on our results, we suggest the NOTCH3 gene as a potential target for preclinical studies in resistant ovarian cancer. The current study also highlights the NOTCH4 gene as a potential predictive biomarker of therapeutic response in EOC.

Funding: Supported by the Czech Health Research Council grant NU22-08-00186, the Czech Science Foundation grant 21-14082S, the Czech Ministry of Education, Youth and Sports program INTER-EXCELLENCE, sub-program INTER-ACTION, project no. LUAUS23164, and the NIH, U.S.A. grant R01 CA103314.

Study program: Doctoral study - Experimetnal Surgery | Year of study: 4
ID: 1120

CONNECTIONS BETWEEN MILD LIVER FIBROSIS MODEL AND LIVER TISSUE ENGINEERING

Running title: Liver Fibrosis and Liver Tissue Engineering

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State-of-the-Art: Decellularized tissues represent a very promising material for tissue engineering. After all cells are gently removed from the original tissue, the remaining extracellular matrix preserved in its native tissue-specific architecture provides a perfect environment for repopulation with new cells, ideally harvested from individual patients. Hepatic is an important target for such an application, due to the liver being one of the organs with the highest demand for both transplantation and in vitro models, as it is the one in charge of blood detoxication and its disease and failure is the 11th major death cause worldwide.

Objective: The aim of my work is to follow up on my study focused on the design of mild liver fibrosis model, and implement the knowledge, skills and optimized protocols during the development of liver tissue in vitro. Decellularized liver will be used as the biomaterial for tissue engineering applications.

Material and Methods: For liver fibrosis model, the scaffold was generated by crosslinking tyramine-grafted gelatin with HRP with additional crosslinking with glyoxal. Initially, the material properties were tested for pore size (SEM), stiffness (rheology measurements), and swelling capacity. Then, cytocompatibility was tested by culturing both scaffold types with HepG2 cells, and eventually more detailed in vitro study with HUVECs and porcine hepatocytes was conducted to assess the effect of different scaffold stiffness on primary cell behaviour. Here, the methods included immunofluorescence staining of specific protein markers as well as gene expression assays, both being used for quantification of individual protein expression. Cell cultures were tested individually as well as in the co-culture.

Results & Discussion: Gelatin-tyramin (Gel) scaffold as control healthy liver tissue and Gel rehydrated in glyoxal (Gel-GlyO) scaffold mimicking F1 fibrosis were compared. GlyO addition increased the scaffolds' stiffness from 2 kPa to 5 kPa. Pore size reduced in Gel-GlyO scaffolds and swelling ratio was higher for Gel. Cell viability was 86% at day 5 for HepG2 in Gel-GlyO, 90% for HUVECs in both materials, and 60% on day 7 for porcine hepatocytes (pHeps) in Gel-GlyO, while 30% at all timepoints in Gel. Cell functionality showed increased angiogenesis markers (vWF and CD31) for HUVECs in Gel-GlyO at days 1 and 3. Co-culture had enhanced pro-fibrotic proteins activity (VEGFR-2 and CYP2E1) in Gel-GlyO at days 1 to 7. After 7 days, pHeps did not show changes in SLC2A2 gene expression in Gel-GlyO compared to Gel.

Conclusion: A mild fibrosis model was successfully designed and tested having a potential to be used as in vitro diagnostic and/or drug testing platform. Moreover, it provided new knowledge and experimental tools that are utilized in my current TE project, cell repopulation of decellularized liver scaffold.

Funding: The work was funded by the Spanish Ministry of Science and Innovation through PID2022-136433OB-C21 and -C22 grants, the National Institute for Cancer Research (Programme EXCELES, No. LX22NPO5102, Next Generation EU), and the COOPERATIO-207043 project (Surgical disciplines), Charles University.

Study program: Doctoral study - Experimetnal Surgery | Year of study: 1 **ID: 1137**

DIAGNOSTIC ALGORITHM FOR SALIVARY GLAND ONCOCYTIC LESIONS

Running title: Salivary Gland Oncocytic Lesions

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State-of-the-Art: Rendering a supervised machine-learning-based diagnostic algorithm that combines morphological, immunohistochemical, and molecular data into a diagnostic tool is practically useful. Using dimensionality reduction to distil dominant oncocytic architectural phenotypes with statistically validated clustering fidelity and its application to a large, registry-based, real-world dataset improve its robustness.

Objective: Diagnosing salivary gland lesions with oncocytic differentiation is challenging as it encompassing 21 reactive and neoplastic lesions. We propose a diagnostic algorithm to suggest differentials based on analyzing oncocytic detection in the salivary gland lesion in AS' registry (501 out of 6383 SGL).

Material and Methods: We developed a weighted probabilistic scoring algorithm incorporating dimensional reduction and hierarchical clustering analysis of morphological features, immunohistochemical profiles, and molecular labelling that represents the most significant weighted values. If molecular information is missing, the algorithm computes the values corresponding to morphology and immunohistochemical vectors.

Results & Discussion: Salivary gland lesions with oncocytic differentiation comprise malignant neoplasms (e.g., acinic cell carcinoma, salivary duct carcinoma, myoepithelial carcinoma), benign neoplasms (e.g., pleomorphic adenoma, Warthin tumor, oncocytoma), cystic lesions and reactive process (e.g. multifocal oncocytic metaplasia), with different extent of oncocytic differentiation, either extensive, focal, or wall-lining, in which oncocytes are positive for MIA. Principal component analysis revealed three distinct oncocytic architectural patterns with eigenvalues >1.0: extensive (λ_1=2.84), focal (λ_2=1.92), and wall-lining (λ_3=1.37). Hierarchical clustering demonstrated discrete entity groupings with a cophenetic correlation coefficient of 0.89.

Conclusion: The algorithm demonstrates acceptable performance metrics in differential diagnosis generation. Although oncocytic differentiation can theoretically occur in any lesion, the salivary oncocytic lesions were confined to real data with adequate molecular investigation.

Funding: The work was supported by the grant SVV (No. 260 773).

Study program: Doctoral study - Pathology | Year of study: 3

ID: 1094

ALTERED REACTIVITY TO THREATENING STIMULI IN DROSOPHILA MODELS OF PARKINSON'S DISEASE, REVEALED BY A TRIAL-BASED ASSAY

Running title: Altered Reactivity to Threatening Stimuli

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State-of-the-Art: To better understand behavioral manifestations of neurodegenerative pathologies, it is useful to employ simple, low-cost, and easy-to-maintain model organisms such as fruit flies (Drosophila melanogaster). Fruit flies exhibit a rich behavioral repertoire in response to threatening stimuli, including freezing (or stopping) and various escape behaviors such as jumping, slow or fast take-off, and running. These behaviors can be effectively analyzed in models of neurodegenerative diseases that affect locomotor activity, such as Parkinson's disease (PD).

Objective: To assess the effectiveness of a single-animal, trial-based behavioral assay in detecting subtle motor impairments through responses to predator-mimicking visual stimuli in Drosophila model of PD and explore the potential role of dopamine receptors in motor behavior regulation.

Material and Methods: In our study, we used a Drosophila model expressing human mutant alleles of parkin (R275W), α-Synuclein (A53T), and dopamine receptor mutants (Dop1R, Dop1R2, DopEcR). Flies were tested using a behavioral apparatus designed to assess responses to predator-mimicking passing shadows. Natural predator-avoidance behavior served as the motivational context for evaluating their motor impairments.

Results & Discussion: Based on our single-animal, trial-based behavioral assay, we found that parkin flies reacted more slowly to predator-like stimuli compared to controls, while α-Synuclein flies exhibited prolonged stopping durations relative to controls. These findings are consistent with the motor and cognitive deficits observed in humans with PD. Additionally, behavioral similarities between parkin and Dop1R and DopEcR mutant flies were also observed, suggesting a potential connection between dopamine receptors and motor activity.

Conclusion: These results suggest that single-trial behavioral analysis can reveal subtle motor deficits in Drosophila's predator-avoidance behavior and support the idea that dopamine receptors may have specialized and diverse roles in the regulation of motor function.

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 4

ID: 1128

STRUCTURAL REMODELING OF THE RETINA IN SPINOCEREBELLAR ATAXIA TYPE 1 MOUSE MODEL

Running title: Retinal Remodeling in SCA1 Mice

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State-of-the-Art: Spinocerebellar ataxia (SCA) type 1 is one of the varieties of polyglutamine diseases, caused by the expansion of the cytosine-adenine-guanine (CAG) repeat in specific genes.

SCA1 is characterized by olivopontocerebellar atrophy and ophthalmic abnormalities affecting the optic nerve and retina. Ophthalmological findings correlate with disease duration. Despite mouse models being commonly used to study neurodegenerative diseases, no histological assessment of the retina has been reported in SCA1 mice. That is why further study is necessary to help clarify the extent of retinal involvement and determine whether retinal changes are comparable between animal models and humans.

Objective: The objective of our study was to compare the volumes of retinal layers and the number of cells in retinal cell layers between mice with spinocerebellar ataxia type 1 and healthy mice at 6 and 10 months of age, using stereological techniques.

Material and Methods: The eyeballs samples were obtained from 6- and 10-month-old mice (8 from SCA1 mice and 8 from healthy mice in each age group). Two series of sections were created from each paraffin block by selecting every 50th section. One series was stained with hematoxylin and eosin to analyze the general morphology of the retina and its cells. The second series was stained using antibodies to rhodopsin [1D4] to evaluate the number of rod nuclei in the outer nuclear layer. The total retina volume, the volumes of different retinal compartments, and the number of cells in the outer and inner nuclear, ganglionic layers were determined using stereological techniques. The Mann–Whitney U test was performed to test for between-group effects.

Results & Discussion: It was found that the total volumes of the outer and inner segments of photoreceptor cells were reduced in SCA1 mice at 6 months compared to healthy mice. No statistically significant changes were observed in the volumes of different retinal layers between healthy mice and SCA1 mice in both age groups, except the volume of nerve fiber layer, where changes were found in a healthy 10-month-old group. Healthy mice showed a statistically significant decrease in cone numbers with age, whereas SCA1 mice exhibited a trend toward a reduction in cone count from 6 to 10 months. The fraction of cones in both the healthy and SCA1 mice groups reduced at 10 months compared to the corresponding groups at 6 months of age. The ganglionic layer cell count in SCA1 mice decreased by 22% ($p<0.01$) with age.

Conclusion: The observed changes in SCA1 mice retina were manifested as a reduction in the volumes of photoreceptor inner and outer segments at 6 months, fewer cones in the outer nuclear layer, and a decreased number of cells in the ganglionic layer at 10 months of age.

Funding: This study was supported by EMBO, grant number SLG 5433 and by the Cooperatio Program, research area MED/DIAG.

Study program: Doctoral study - Anatomy, Histology and Embryology | Year of study: 3

ID: 1111

DIAZEPAM TREATMENT IN A LURCHER MOUSE MODEL OF CEREBELLAR MOTOR AND COGNITIVE AFFECTIVE SYNDROME.

Running title: Diazepam Treatment in Lurcher Mice

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State-of-the-Art: Various studies have shown the involvement of the cerebellum in non-motor functions, apart from motor coordination. Lurcher mice are one of the best models for studying cerebellar ataxia and behavioral and cognitive impairments resulting from selective olivocerebellar degeneration. Altered behavior of these mice is hypothesized to be caused in part by stress-induced behavioral disinhibition, i.e., inability to inhibit responses to stimuli and to avoid maladaptive behavior in anxiogenic situations. Such behavioral traits may hypothetically influence the performance of Lurcher mice not only in behavioral but also in cognitive and motor tests.

Objective: The study aimed to investigate whether an anxiolytic compound, Diazepam, could reduce signs of behavioral disinhibition and thereby improve pathological behavioral phenotype in Lurcher mice.

Material and Methods: Lurcher and wild-type mice of the B6CBA strain aged 4-5 months were used for the experiments. Wild-type littermates of Lurcher mice served as healthy controls. Mice were treated with GABA agonist Diazepam in two doses (0.5 mg/kg and 1 mg/kg), which should have anxiolytic but not sedative effects in mice according to the literature. Saline (vehicle) was used as a control. The tests were arranged in a three-week protocol that included open-field test, elevated plus maze test, grip strength measurement, Morris water maze test with a hidden and visible goal tasks, and rotarod test. The examination always started 30 minutes after the injection.

Results & Discussion: Performance of control Lurcher mice was worse in the rotarod and grip strength test than in control wild-type mice. Importantly, Diazepam did not affect grip strength, suggesting that muscle function necessary for performance in other tests has not been affected by this unspecific factor. On the other hand, the higher dose of Diazepam worsened the performance of Lurcher mice, but not wild-type mice, in the rotarod test. Diazepam had no significant effect on exploration in the open field and no effect on entering the anxiogenic open arms of the elevated plus maze. In the Morris water maze, the performance of the mice was not changed by Diazepam except for inconsistent reduction of swimming speed.

Conclusion: We have confirmed that low doses of Diazepam had no significant sedative effect on mice. However, Diazepam did not improve the performance of Lurcher mice in the tests. In the rotarod test, Diazepam even exerted a negative effect, although it did not affect muscle strength.

Funding: This work was supported by GAUK project No. 49724 and Cooperatio (NEUR and MED/DIAG research areas).

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 2
ID: 1106

ESTABLISHING CHRONIC HIGH-DENSITY ELECTROPHYSIOLOGY RECORDINGS IN FREELY MOVING RATS USING NEUROPIXELS PROBES.

Running title: Chronic Implantation of Neuropixels Probe on Rat

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State-of-the-Art: The hippocampus plays a key role in spatial navigation and episodic memory through place cells, which generate cognitive maps via spatially selective firing. CA1 and CA3 contribute differently to memory storage and retrieval. Understanding their interaction requires high-yield recordings from large neuronal populations across regions. Chronic implantation of high-density Neuropixels probes enables stable, large-scale neural data acquisition from multiple hippocampal sub-regions in freely moving rats. This study aims to optimize and validate Neuropixels-based recordings targeting CA1 and CA3 in Long-Evans rats to advance our understanding of hippocampal dynamics during memory retrieval.

Objective: To develop and validate surgical implantation, recording protocols, and analysis methods for hippocampal Neuropixels recordings in freely moving rats.

Material and Methods: Neuropixels 1.0 probes were implanted in adult Long-Evans rats using a stereotaxic surgical procedure under anaesthesia. A small craniotomy was performed to access the dorsal hippocampus, and probes were inserted to target both CA1 and CA3 simultaneously. Post-operative care was provided for 3 days, followed by recovery and habituation in the experimental environment. Neural signals were acquired using Open Ephys acquisition software, alongside video tracking with Bonsai to map location-specific neuronal firing. Both spike and local field potential (LFP) data were analysed. Electrode placement was confirmed using histological analysis following data collection, after perfusion and brain extraction.

Results & Discussion: The Neuropixels implantation protocol was successfully optimized for chronic recordings in freely moving rats. Functional targeting was confirmed by the presence of robust hippocampal theta oscillations and sharp wave ripples in local field potentials. High-quality single-unit activity was reliably captured from both CA1 and CA3 pyramidal layers, with well-isolated waveforms and stable spike clusters across sessions. This approach enables simultaneous monitoring across hippocampal depths, offering enhanced spatial resolution compared to traditional tetrode recordings.

Conclusion: Recordings show stable, low-noise signals, suitable for memory and navigation studies. The optimized method enables investigation of hippocampal circuits and network dynamics, providing a reliable foundation for future experiments examining network dynamics under normal and altered cognitive states.

Funding: Funded by Cooperatio NEUR, and by Grant Agency of The Czech Republic Grant No. 22-16717S.

Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 2

ID: 1135

IMPACT OF AUTOFLUORESCENCE ASSISTED SURGERY ON THE LONG TERM TREATMENT SUCCESS IN ORAL SQUAMOUS CELL CARCINOMA (OSCC)

Running title: Autofluorescence - Treatment Success in OSCC

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Supervisor: Petr Pošta (1)

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State-of-the-Art: Lip and oral cavity cancer is the 16th most common worldwide malignancy with relatively high mortality (90 % of these are OSCC). The incidence of OSCC in the Czech Republic is around 680 new cases each year with a rising tendency. Surgery still has the best curative outcome and a clear resection margin is crucial. Several methods have been used to determine healthy and malignant cells and to assist the surgeon to set the appropriate resection margin. The impact of most of those methods on the long term treatment success is unclear. The effect of autofluorescence on evaluating the tumor range seems to be significant ad have been published in several articles (Poh et al., Pošta et al.). We evaluate the effect of autofluorescence on patient survival with OSCC in our study.

Objective: The positive resection margin in OSCC is considered to be a negative prognostic marker. The autofluorescence is proved to be efficient in mucosal margins evaluation. We are assessing the impact of autofluorescence on 3 and 5 years survival rates.

Material and Methods: A total number of 96 patients suffering from OSCC were randomized into a study and a control group without statistically significant differences. Patients in study group were peri- or preoperatively examined with VELscope, which was accompanied by the marking of the range of a loss of fluorescence, and this was followed by surgical resection at least 3 mm behind the markings. In control group the tumor was resected with at least 1 cm macroscopically healthy tissue rim. According to the final stage, the adjuvant treatment was applied following the NCCN guidelines. After the treatment all patients undergo at least 5 year follow-up and the curative results are evaluated. All recorded data were processed with the help of Ing. Petr Hošek, Ph.D. and the programme Statistica.

Results & Discussion: Of the total of 96 patients recruited in this study, both study group and control group counted 48 patients. In study group, 3-year DFS was 61.1 %, in control group it was 63.1 %, 5-year DFS was 56.1 % in study group vs. 56.3 % in control group. Study group had 3-year DSS of 87.7 %, OS of 72.1 % whereas control group 3-year DSS of 71.2 %, OS 68.3 % and 5-year DSS in study group was 87.7 %, OS 67.3 % vs. DSS 63.7 % and OS 57.9 % in control group.

The survival rates do not differ significantly across control and study group. The explanation for this is that autofluorescence examination only influences the quality of mucosal resection margin, but other predictors, mainly deep resection margin, remain the same and may have negative impact on the treatment success.

Conclusion: Resection following autofluorescence defined margins has a positive impact on quality of mucosal resection margin, and therefore has a positive effect on surgical treatment success. The impact of direct autofluorescence on long-term treatment success still remains questionable.

Study program: Master´s degree - General Medicine | Year of study: 3

ID: 1104

MAPPING THE VASCULAR ANATOMY OF THE PRESTICKE BLACK PIED BREED PIG, OUR PILOT RESULT

Running title: Pig Vascular Tree Mapping

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State-of-the-Art: Pigs are widely used in preclinical studies for developing cardiovascular devices due to their anatomical similarities to humans. However, the pig aorta exhibits significant anatomical variability, necessitating individualised preoperative planning.

Objective: The lack of detailed morphometric data on the porcine aorta and its branches can lead to mismatches in device sizing, potentially causing complications or fatalities in experimental settings. For this purpose, we are developing a freely available library of pig aorta data for further use.

Material and Methods: CT-angiography scans of 26 Prestice Black-Pied piglets were acquired - arterial and venous phase images were captured from the animals involved in the biomedical research. The image segmentation was done in the 3D Slicer software (slicer.org). The main visceral branches of the abdominal aorta were visualised and further evaluated - the coeliac artery with its main branches, and the superior mesenteric artery.

Results & Discussion: Acquired data will then be used to train artificial neural networks with the U-Net architecture to develop an automatic pig-specific vessel segmentation method. We also aim to map the Prestice Black Pied breed vascular tree and its variability to gain unique data for training and further research optimisation.

Conclusion: The results so far show significant potential for using the unique database of already obtained data from examined animals in our Biomedical Center. They will be used to train Artificial Neural Networks with the U-Net architecture.

Funding: Charles University Grant Agency (GAUK) and Specific Research (SVV).

Study program: Master´s degree - General Medicine | Year of study: 2

ID: 1141

SCLERAL REACTIONS TO DIFFERENT SUTURE MATERIALS

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State-of-the-Art: In cataract surgery, extracapsular extraction of the lens and implantation of an artificial one into the preserved part of the original lens capsule is the standard method. If this is not possible, a lens requiring fixation is implanted. With advancing surgical techniques, various methods of scleral fixation have been developed. One option is the fixation of the artificial lens with sutures to the sclera. This procedure and the materials used are well established in ophthalmology. Despite its routine use, a detailed comparative analysis of tissue response remains lacking.

Objective: The study aimed to analyze the scleral tissue response to different suture materials used for scleral suspension, comparing them both with each other and over different time intervals.

Material and Methods: A total of 18 adult rabbits were included in the experiment. All underwent the same procedure with scleral suspension, with the second eye serving as the control. Three different materials were used. The rabbits were divided into groups with different survival times after surgery – 1, 3, or 6 months. The integration of sutures into the sclera was assessed mechanically, followed by a histological examination of the samples. After standard histological processing, tissue samples were stained using hematoxylin-eosin for basic evaluation of tissue response, including inflammatory infiltration and the depth of inflammation. For evaluation of collagen formation and composition, picrosirius red staining and polarized microscopy were used. Samples were analyzed using stereological software.

Results & Discussion: Histological analysis confirmed the presence of inflammation in all collected samples. The intensity and depth of inflammation varied both among different suture materials and across different time intervals. Collagen formation increased with prolonged suture retention in ocular tissue.

Conclusion: In the rabbit model, varying levels of inflammation and collagen production were observed across the study groups. Both collagen production and inflammation are crucial clinical factors that may guide the selection of the optimal suture material.

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Study program: Master´s degree - General Medicine | Year of study: 6
ID: 1089

HEMODYNAMIC EVALUATION AND FLUID RESPONSE OBSERVATION THROUGH RESPIRATORY MANEUVERS (HERO)

Running title: Fluid Response through Respiratory Maneuvers

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State-of-the-Art: In managing critically ill patients, initial fluid administration aims to increase stroke volume and cardiac output. Fluid responsiveness is commonly assessed using dynamic indices, with the Passive Leg Raising (PLR) test being a validated, reversible method. However, PLR is contraindicated in patients with unstable spine or pelvic fractures or with trauma brain injury. Respiratory maneuvers, which alter intrathoracic pressure to develop preload changes, offer a potential alternative, especially in spontaneously breathing patients.

Objective: This study aims to evaluate respiratory maneuvers as alternatives to the fluid challenge and PLR test for assessing fluid responsiveness in spontaneously breathing patients when standard methods are contraindicated.

Material and Methods: After informed consent, healthy volunteers underwent non-invasive hemodynamic monitoring (Acumen IQ cuff, HemoSphere monitor (Edwards Lifesciences)). Respiratory maneuvers (maximal inspiration/expiration, Valsalva, Müller) were performed for 20 seconds each, with 2-minute rests in between. Subsequently, a PLR test over 1 minute and a fluid challenge with 500 mL balanced crystalloid over 10 minutes were performed. A positive fluid responsiveness test was defined as a >10% increase in cardiac index (CI) or stroke volume index (SVI). Echocardiography assessing the cardiac function and intravascular volume status complemented baseline and final assessments.

Results & Discussion: Among 11 subjects, 4 responded to the fluid challenge by both CI and SVI (RR), 3 by CI only (R-CI), 1 by SVI only (R-SVI), 3 subjects were non-responders (NR). In RR, maximal inspiration elicited a positive response in CI or SVI in 1 subject, maximal exspiration in 2 subjects, Valsalva in 2, and Müller in 2. In R-CI, only Müller elicited a response in 1 subject. Three RR subjects responded to at least two maneuvers. In NR, Müller and Valsalva were positive in 4 and 1 subjects, on the contrary. These preliminary data suggest that specific respiratory maneuvers—especially Müller and Valsalva—may increase preload with a response, but false positives in NR raise concerns about specificity.

Conclusion: Preliminary findings support the feasibility of using respiratory maneuvers to assess fluid responsiveness in spontaneously breathing patients when standard tests are contraindicated. Multiple maneuvers may be required to enhance diagnostic accuracy. Further data are ongoing.

Funding: The study was supported by the COOPERATIO (Intensive Care Medicine) institutional programme of Charles University.

Study program: Master´s degree - General Medicine | Year of study: 6

ID: 1126

DIAGNOSTIC UTILITY OF PHOSPHORYLATED SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5 IMMUNOSTAINING IN THE DIAGNOSIS OF SECRETORY CARCINOMA OF THE SALIVARY GLAND: A COMPARATIVE STUDY

Running title: Diagnostic Utility of STAT5 Immunohistochemistry

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State-of-the-Art: Salivary secretory carcinoma (SC) is a relatively newly described neoplasm, characterized by ETV6::NTRK3 or ETV6::RET gene fusion. Immunohistochemical (IHC) expression of phosphorylated signal transducer and activator of transcription 5 (STAT5) has been proposed as diagnostic marker specific for SC. We have recently encountered a case of sclerosing polycystic adenoma (SPA) with strong STAT5 immunoexpression.

Objective: In the present study, the STAT5 immunohistochemistry was performed in selected cases of salivary gland tumors in order to clarify its potential differential diagnostic utility.

Material and Methods: A retrospective analysis of total of 44 salivary gland neoplasms retrieved from the salivary gland tumor registry consultation files, including 10 cases formerly diagnosed as acinic cell carcinoma (AciCC), and a spectrum of salivary neoplasms harboring an intercalated duct differentiation, including SPA (10 cases), SC (15 cases) and intraductal carcinoma (IC) (9 cases) was conducted. Formalin fixed, paraffin embedded tissue samples were used for hematoxylin and eosin and IHC staining using STAT5, DOG1, mammaglobin, S100 protein and SOX10 antibodies.

Results & Discussion: SCs harboring an ETV6::NTRK3 gene fusion were all positive for STAT5 in almost 100% of the cells. In 5 cases of SC harboring an ETV6::RET, the STAT5 was diffusely positive in only 3 cases, in one case 10% of cells showed nuclear positivity, and one case was negative. In 9 cases of the IC (intercalated type confirmed by NCOA4::RET fusion), 6 cases showed nuclear positivity in 10-50% of cells, one case was negative, and 2 cases showed only granular cytoplasmic immunoreaction. AciCCs showed nuclear positivity for STAT5 in 10-20% of cells, while 3 cases were completely negative and one case showed only a cytoplasmic reaction. SPA displayed nuclear staining for STAT5 in foci with intercalated type dysplasia in 5 cases, while 4 other showed only focal granular cytoplasmic reaction.

Conclusion: Even though all ETV6::NTRK3 translocated SCs were strongly positive for STAT5, this IHC marker is not entirely specific for SC, as nuclear staining has also been observed in NCOA4::RET translocated IC (intercalated type), in AciCC, and in 5 cases of SPA in up to 10-20% of the cells.

Study program: Master´s degree - General Medicine | Year of study: 6

ID: 1081

EXPANDING ASSAY CONTROLS: USING MULTI-SPECIES PBMC AND WHOLE BLOOD IN THE ENZYME-MODIFIED COMET ASSAY FOLLOWING POTASSIUM BROMATE EXPOSURE

Running title: PBMC as Assay Controls in the Comet Assay

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State-of-the-Art: Background: The enzyme-modified comet assay is a widely used tool to assess oxidative DNA damage. OECD guidelines and consensus statements emphasize the importance of assay controls to ensure experimental validity. A multi-laboratory study identified potassium bromate (KBrO3) as a suitable agent to produce assay controls using monocytic THP-1 cells. However, the high cost of cell cultures and limited resources in handling cultured cells may hinder widespread adoption, especially in hospital-based laboratories. Peripheral blood mononuclear cells (PBMC) and whole blood (WB), commonly used in comet assays, could offer a practical alternative.

Objective: This study evaluates whether cryopreserved PBMC and WB from different species are suitable cells for KBrO3-induced oxidative DNA damage, enabling their use as standardized assay controls in the FPG-modified comet assay.

Material and Methods: WB was collected from humans (venous), pigs (arterial), and rabbits/mice (cardiac) using K2EDTA tubes. PBMC were isolated via density gradient centrifugation, washed, and divided into three experimental sets. Cells were incubated with increasing KBrO3 concentrations (0mM, 3mM, 6mM, 9mM, 12mM) and cryopreserved at –80°C. Within three months, the FPG-modified comet assay was performed, using Ro19-8022-treated HCT116 cells and untreated cells as assay controls. Tail intensity was measured via semi-automated scoring software (Lucia Comet Assay).

Results & Discussion: Linear regression analysis of porcine and rat PBMC showed slopes ranging from 7.9 (±1.0) to 8.5 (±0.4) with $p < 0.004$ and R^2 values between 0.95 to 0.99. Porcine control PBMC (RPMI-1640 only) exhibited mean Fpg-sensitive sites of 0.20 (±0.7) and 63.67 (±0.76) after 12 mM KBrO3 exposure. Rat PBMC from one experiment showed Fpg-sensitive sites of 3.59 in RPMI-1640 and 67.78 after 12mM KBrO3 exposure.

Conclusion: These preliminary findings suggest porcine and rat PBMC are suitable for KBrO3-based assay controls. Further validation, including additional experimental replicates and testing of human and rabbit PBMC, is ongoing to confirm their broader applicability.

Funding: This work was supported by the Cooperation Program, research area Medical Diagnostics and Basic Medical Sciences and by the Ministry of Health of the Czech Republic, grant nr. NU22J-03-00033.

Study program: Master´s degree - General Medicine | Year of study: 6

ID: 1109

EFFECT OF FLUBENDAZOLE ON PANCREATIC CANCER CELLS WITH AN EMPHASIS ON THE MICROTUBULAR CYTOSKELETON.

Running title: Flubendazole Affects Pancreatic Cancer Cells

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State-of-the-Art: Pancreatic adenocarcinoma is a malignant tumor arising from the exocrine part of the pancreas, characterized by a very poor prognosis and limited therapeutic options. The unfavorable prognosis is further exacerbated by the typically late diagnosis due to the tumor's initially asymptomatic growth, as well as by its high degree of chemoresistance. The inadequacy of current treatment strategies highlights the urgent need for innovative approaches. One such possibility is the use of flubendazole (FLU), originally an anthelmintic developed for veterinary use, which has been shown in previous studies to destabilize microtubules and induce apoptosis in various cancer cell types.

Objective: The main aim of this study is to analyze the effect of flubendazole (FLU) on the viability, proliferation, and morphology of pancreatic cancer cells, as well as to observe its impact on the microtubular cytoskeleton of these cells.

Material and Methods: Two established pancreatic cancer cell lines, BxPC3 and MIA PaCa-2, were used in this project. Cell proliferation was monitored in real time using the IncuCyte live-cell imaging system. Morphological changes were also evaluated using phase-contrast microscopy. Changes in the microtubular cytoskeleton were then observed via fluorescence microscopy following immunofluorescent staining of tubulins.

Results & Discussion: FLU effectively inhibited the growth of pancreatic cancer cells, and this effect was observed even after using very low concentrations of the drug. Furthermore, FLU profoundly affected the morphology of the cancer cells, which was particularly evident in BxPC3 cells, where FLU treatment led to the formation of giant multinucleated cells. In addition, FLU significantly disrupted the organization and structure of the microtubular network, with our findings also suggesting an impact on the expression of specific tubulin markers.

Conclusion: Our results indicate FLU as a promising anticancer agent targeting pancreatic tumor cells and their microtubular cytoskeleton even at low concentrations of treatment. Given the poor prognosis of pancreatic cancer and the potential of FLU effect on pancreatic cancer, further investigation is needed.

Study program: Master´s degree - General Medicine | Year of study: 2

ID: 1117

DECIPHERING THE ORIGIN OF CANCER OF UNKNOWN PRIMARY USING EPIGENETIC TOOLS: A VALIDATION STUDY OF THE DNA METHYLATION-BASED HIERARCHICAL TUMOR ARTIFICIAL INTELLIGENCE CLASSIFIER (HITAIC)

Running title: Validation Study of HiTAIC

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State-of-the-Art: Cancer of unknown origin (CUO) represents 1-4% of malignancies of Czech oncological patients and has a high mortality rate due to limited treatment options. Accurately identifying the primary tissue/organ of origin of CUO may significantly improve survival thanks to potentially more tailored therapeutical options. Since DNA methylation pattern of neoplastic cells reflects their tissue of origin as well as changes that occurred during oncogenesis, most tumor types have distinct methylation patterns. HiTAIC, a recently developed web-based application was designed using AI algorithms to trace tissue of origin and tumor type of CUO by utilizing DNA methylation data from 7735 tumors (obtained from publicly available sources, e.g. TCGA) to recognize 27 cancer types from 23 tissue sites.

Objective: We hypothesized that most of the HiTAIC training data were obtained from well differentiated tumors. Therefore, we aimed to perform an independent validation study using highly undifferentiated cases which are more likely to benefit from adjunct diagnostic tools such as HiTAIC in clinical practice.

Material and Methods: 42 samples were divided into two groups. Group 1 consisted of 15 well-differentiated malignancies, whose origin was established based on their morphological and immunohistochemical (IHC) features only. Group 2 consisted of 27 undifferentiated cancers whose primary origin could be correctly established only after incorporation of patient's clinical history or through further investigations (imaging, subsequent biopsy, molecular studies). Formalin-fixed paraffin-embedded tissue blocks from all cases were collected and in selected cases macrodissected to achieve at least 70% of tumor content. The methylation data were obtained using Infinium MethylationEPIC v2.0 platform (Illumina). The raw data files were uploaded to hitaic.herokuapp.com and results were recorded.

Results & Discussion: In Group 1, 14 out of 15 cases (93%) were diagnosed correctly, whereas in Group 2, it was 15 out of 27 samples (56%). HiTAIC achieved an overall accuracy of 69% (29 out of 42). The original study of HiTAIC reported accuracy 93 and 99% in the test and validation sets, respectively (PMID: 37089814). During tumor progression, undifferentiated neoplasms tend to lose their characteristic morphologic and IHC features and the methylome may be slightly altered as well (PMID: 37098294) which probably explains the lower diagnostic accuracy in group 2. While indeed showing excellent performance for well-differentiated neoplasms, these can be usually readily diagnosed in clinical practice using microscopy and immunohistochemistry which are significantly less costly than methylome sequencing.

Conclusion: Although HiTAIC correctly classified only 56% of undifferentiated malignancies, it still represents a useful ancillary diagnostic tool for these challenging cases. However, its users should be aware of its limitations and the results always need to be correlated with other diagnostic modalities.

Study program: Master´s degree - General Medicine | Year of study: 4 | **ID: 1108**

MODELLING EARLY STEPS OF UROTHELIAL CARCINOMA PROGRESSION IN A NOVEL CELL LINE PROGRESSION SERIES

Running title: Modeling Early Urothelial Carcinoma Progression

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State-of-the-Art: Bladder cancer is a common malignancy with high recurrence and progression rates. Its progression from non–muscle-invasive (NMIBC) to muscle-invasive bladder cancer (MIBC) marks a major clinical turning point, often linked to epithelial–mesenchymal transition (EMT) and increased invasiveness, metastasis, and therapy resistance. While models for metastatic progression of MIBC do exist, early progression stages — especially the pTa pT1 transition — are missing. We address this gap using a unique spontaneous progression model based on the RT112 cell line and its more invasive derivative, RT112Heidelberg. This system provides valuable insight into the plasticity underlying early bladder cancer progression and offers a platform for identifying new therapeutic targets.

Objective: Our goal is to unravel the basic biology of early steps of bladder cancer progression and identify crucial biological hubs and potential therapeutic targets relevant for the NMIBC to MIBC transition.

Material and Methods: The model consisted of two human bladder cancer cell lines with the same genetic background: RT112, a widely used urothelial carcinoma cell line representing an early to intermediate disease stage, and its clonogenic, spontaneously progressive subline RT-112Heidelberg. Five methodological approaches were used to assess genetic and phenotypic changes between the cell lines: oxygraphy for metabolic analysis, Incucyte® S3-videomicroscopy for evaluating stromal – cancer cell communication and doxorubicin resistance, conventional and fluorescence microscopy for assessment of morphology, motility and EMT-related traits, flow cytometry for assessment of dynamics of E-cadherin expression, and gene expression analysis of selected EMT- and NRF2-related genes by qPCR.

Results & Discussion: The progressive variant RT112Heidelberg features a highly aneuploid (hypertriploid) karyotype, with numerous structural and numerical aberrations. Its morphology oscilates between epithelial-like is sparse cultures to mesenchymal-like at confluency, with a highly dynamic E-cadherin expression. We didn't see any dramatic change in overall E-cadherin expression level, but a marked increase in vimentin expression, reminiscent of a hybrid epithelial/mesenchymal phenotype. Accordingly, RT112Heidelberg display molitily of the collective type. In keeping with published results showing stabilization of the hybrid E/M phenotype by a constitutively activation of the Nrf2-regulatory circuit, we see both enhanced Nrf2 nuclear expression and increase expression of selected Nrf2 downstream genes.

Conclusion: The clonally related pair of cell lines RT112 – RT112Heidelberg can provide an important experimental platform to model early steps of urothelial carcinoma progression, including the NMIBC to MIBC transition, offering insights into biomarkers and targeted therapeutic strategies.

Funding: This study was supported by SVV-2024-260654, SVV-2025-260773, Academic Minigrant 4EU+ MA/25/F1/0/018 and by the League Against Cancer Prague.

Study program: Master´s degree - General Medicine | Year of study: 2

ID: 1139

REPEATED FLUBENDAZOLE TREATMENT AND ITS EFFECT ON GLIOBLASTOMA MULTIFORME CELLS

Running title: Flubendazole Affects Microtubules of Glioblastoma

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State-of-the-Art: Glioblastoma multiforme (GBM) is a highly aggressive malignant tumour disease of the central nervous system that is currently treated with maximal radical surgery followed by a combination of radio- and chemotherapy. However, the current treatment protocol is insufficient and, therefore, efforts for its innovation are being made. One of the potentional repurposed drugs is flubendazole (FLU), an anthelmintic used mainly in veterinary medicine, which was proven by previous studies to have an inhibitory effect on various cancer cells. This effect is attributed to the FLU-caused inhibition on the microtubular cytoskeleton.

Objective: Our study aimed to test the effect of repeated FLU administration on GBM tumour cells. We investigated the effect on the viability and overall morphology of GBM cells, but especially on the microtubular cytoskeleton, after administration of FLU in differing quantities at repeating intervals.

Material and Methods: The study was performed on two GBM stabilized cell lines (U87MG and U87MG-IDH1mut) that were separately influenced 1-3x every 24 hours by different concentrations of FLU (200 nM, 500 nM, and 1000 nM). The effect on cell viability and proliferation was determined in real time using the IncuCyte device and a phase contrast microscopy. The effect on the microtubular cytoskeleton was then observed using fluorescent microscopy.

Results & Discussion: Our results showed that FLU effectively reduced the viability of GBM cells – this effect was even greater with multiple FLU administration. Furthermore, FLU inhibited microtubule polymerization, even with a lower dosage of the tested drug (200 nM). In addition, FLU significantly affected the overall morphology of GBM cells, including the structure and organization of microtubular network, while maintaining the effect even after multiple administrations of FLU.

Conclusion: FLU shows a strong effect on GBM tumour cells by reducing their viability and proliferation even at low doses of FLU, with the tested drug also affecting the microtubular cytoskeleton. Our results suggest that FLU is a promising drug with great potential and its effect needs to be further studied.

Study program: Master´s degree - General Medicine | Year of study: 2

ID: 1116

EFFECT OF FLUBENDAZOL ON GLIOBLASTOMA MULTIFORME CELLS WITH FOCUS ON STAT3 MOLECULE

Running title: STAT3 in Glioblastoma after Fubendazole Treatment

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State-of-the-Art: Glioblastoma multiforme (GBM) belongs to the most common and most aggressive tumor diseases, and despite treatment, the average time of survival ranges between 12 and 15 months. The effects of the currently used treatment protocol are limited especially by the low accessibility of the commonly used drug temozolomide in the brain (mainly because of the blood-brain barrier) and the high overall resistance of GBM tumor cells, which is also connected with the increased expression and activation of STAT3 molecule. One of the drugs potentially inhibiting this signal STAT3 molecule is flubendazole (FLU), originally veterinary anthelmintic, which previously showed an inhibitory effect on a wide range of different tumor cells.

Objective: The aim of this work is to investigate the inhibitory effect of FLU on STAT3 molecule in GBM tumor cells, with special focus on the evaluation of FLU effect on tumor cells proliferation and the expression of STAT3 molecule.

Material and Methods: Two stabilized GBM cell lines were used for this project - U87MG and U87MG-STAT3-KO, in which specific STAT3 gene knock–out was created using the CRISPR/Cas9 method. This modification was performed at a collaborating workplace (Department of Medical Biology and Genetics, Faculty of Medicine in Hradec Králové). The proliferation and viability of cells, as well as their morphology, were evaluated in real-time by the Incucyte imaging system and subsequently by the phase-contrast microscope. Changes in STAT3 expression were observed via fluorescent microscopy.

Results & Discussion: FLU decreased the proliferation and viability of GBM cells, and this effect was observed already after administration of very low concentrations. Simultaneously, our results confirmed the inhibitory effect of FLU on the STAT3 molecule. Moreover, the specific knock–out of this molecule reduced the proliferation and also partially affected the morphology of the GBM cells. Additionally, our results showed a decrease in cell proliferation by the effect of FLU on cells with specific STAT3 knock-out, showing possible further affection of cell viability connected to this signal molecule.

Conclusion: FLU is a promising potential antitumor drug, which shows an inhibitory effect on GBM cells proliferation through decreasing the STAT3 expression. It effectively works already at significantly low concentrations, and it could be a promising direction for future research.

Study program: Master´s degree - General Medicine | Year of study: 2

ID: 1133

DENSITOMETRIC ANALYSIS OF GLIA IN THE HIPPOCAMPUS OF A MOUSE MODEL OF SPINO-CEREBELLAR ATAXIA TYPE 1

Running title: Glia in the Hippocampus of a Model of SCA 1

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State-of-the-Art: Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant hereditary neurodegenerative disease affecting the cerebellum and other parts of the central nervous system including the hippocampus. Astrocytes and microglia are activated during various pathological processes in the brain and are involved in pathogenesis of secondary changes in the diseased tissue. Glial cells are important components of the nervous tissue participating in local tissue niche that might have an impact on neural plasticity, residual tissue function as well as its neurogenic potential, and thereby influence response to cell-based and plasticity supporting therapies.

Objective: The aim of the study was to provide densitometric assessment of astrocytes and microglia in the hippocampus of a mouse model of SCA1 in comparison with healthy mice.

Material and Methods: Paraformaldehyde fixed brains of 5 heterozygous SCA1 and 5 strain-matched healthy 6-months-old mice were cryosectioned and processed for immunofluorescent detection of astrocytes and microglia. Astrocytes were labelled with anti-GFAP primary antibody. For identification of microglia, anti-IBA1 primary antibody was used. The sections were photodocumented using a fluorescent microscope. Intensity of fluorescent signal was evaluated in the hippocampi by means of densitometric approach in ImageJ software. Signal density was considered an indirect indicator of combined effect of glial multiplication and activation.

Results & Discussion: Density of fluorescent signals related to the astrocytic marker GFAP in the hippocampi of heterozygous SCA1 mice did not differ from that in healthy animals. Similarly, no significant differences between SCA1 and control mice were found in density of anti-IBA1 immunofluorescence, a microglial marker. Furthermore, the correlation between densities of GFAP and IBA1 was insignificant. SCA1 is a late onset spinocerebellar degeneration and neuropathology is less severe in the hippocampus than in the cerebellum in these mice. Thus, the moderate early primary hippocampal neuropathology does not probably induce glial activation in this mouse model.

Conclusion: The results suggest that hippocampal neuropathology is not accompanied by significant proliferation and/or activation of astrocytes or microglia in SCA1 mice at the age of 6 months.

Funding: This work was supported by Cooperatio (NEUR and MED/DIAG research areas).

Study program: Master´s degree - General Medicine | Year of study: 4
ID: 1097

ASSESSMENT OF IONIZED CALCIUM AND MAGNESIUM MEASUREMENT DURING CVVHD: METHOD COMPARISON AND CLINICAL RELEVANCE

Running title: iCa and iMg in CVVHD: Method Agreement and Interpretation

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State-of-the-Art: Ionized magnesium (iMg^{2+}) is a vital intracellular cation involved in neuromuscular conduction, enzyme activity, and calcium channel regulation. Despite its clinical relevance, it is not routinely measured—unlike ionized calcium (iCa^{2+}). Emerging evidence suggests that iMg^{2+} may provide additional insights into pathophysiological changes during extracorporeal therapies such as continuous veno-venous hemodialysis (CVVHD). Since iMg^{2+} trends may diverge from those of iCa^{2+}, its independent assessment could improve therapy optimization and patient monitoring in intensive care settings.

Objective: To compare ionized calcium measurements between two blood gas analyzers—GEM Premier 5000 (Werfen) and Stat Profile Prime® (NOVA Biomedical)—and to evaluate the correlation and agreement between temporal changes in iCa^{2+} and iMg^{2+} during CVVHD. The study also explores whether iMg^{2+} offers independent clinical value warranting routine monitoring.

Material and Methods: Samples from patients undergoing CVVHD were analyzed. iCa^{2+} and iMg^{2+} concentrations were measured using GEM Premier 5000 and Nova Biomedical analyzers. Method comparison included Bland–Altman analysis (both absolute and percentage differences) to assess agreement, and Passing–Bablok regression to evaluate systematic and proportional bias. Ninety-five percent confidence intervals (CI) were calculated for slope and intercept using robust regression and OLS modeling.

Results & Discussion: iCa^{2+} Comparison (GEM vs Nova): Bland–Altman analysis showed good agreement with a minimal mean difference. Passing–Bablok regression: intercept 0.15 (95% CI: 0.13–0.22), slope 0.87 (95% CI: 0.76–0.89) ΔiCa^{2+} vs ΔiMg^{2+} (Nova): Bland–Altman analysis of absolute differences revealed high variability. Additional Bland–Altman analysis using relative (%) differences showed no significant trend across the range of mean values, indicating no proportional bias. Passing–Bablok regression revealed a weak linear association: intercept 0.71 (95% CI: 0.62-0.81), slope 0.23 (95% CI: 0.11-0.40).

Conclusion: iCa^{2+} measurements showed strong analytical agreement across platforms. While the correlation between ΔiCa^{2+} and ΔiMg^{2+} was weak, the percentage-based Bland–Altman analysis showed no directional trend, suggesting both markers may reflect a shared underlying physiological mechanism during CVVHD. These findings indicate that routine measurement of iMg^{2+} may not provide substantial additional clinical information in this specific setting.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1124

EFFECT OF 24-HYDROXYLASE WITH INCREASING VITAMIN D SUPPLEMENTATION DOSES

Running title: Effect of 24-hydroxylase

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Supervisor: Michal Jirásko (1)

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State-of-the-Art: Vitamin D supplementation is generally well tolerated. Vitamin D can be toxic in adults if administered daily in doses > 10,000 IU for several weeks to months, during which it accumulates in the body and serum concentrations of around 500 nmol/l are reached. Overdose is manifested by loss of appetite, nausea, fatigue, headache, diarrhea, sweating and paresthesias. Laboratory findings include hypercalcemia, hypernatremia and increased urinary calcium and phosphorus excretion. The most serious complication are the calcium phosphate crystals in the kidneys and subsequent renal failure. Our body has a protective mechanism against vitamin D overdose. This protective function is performed by the enzyme 25-hydroxyvitamin D3-24-hydroxylase, an enzyme of the cytochrome P450 family, CYP24A1.

Objective: Our aim was to test the activity and capacity of 25-hydroxyvitamin D3-24-hydroxylase in response to increasing daily doses of cholecalciferol supplementation at 1,000, 2,000, 4,000, and 8,000 IU.

Material and Methods: Participants: Thirty-five volunteers participated in this study. All participants filled out a questionnaire about their health status, medications, and dietary habits during the last six months before being included in the study. Vitamin D was administered in increasing doses of 1000, 2000, 4000, 8000 IU/day, each for two months, and each two-month period of use was followed by 1 month without vitamin D use. Blood samples were collected before, after, and 30 days after each supplementation phase.

Measurements: All samples were measured using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the precise quantification of $25(OH)D_3$ and $24,25(OH)_2D_3$ in human serum (Agilent 6495 Triple Quadrupole LC/MS System, Agilent Technologies, USA).

Results & Discussion: All increases in levels of $25(OH)D_3$ following supplementation and decreases after discontinuation of supplementation were found to be statistically significant. The levels of $24,25(OH)_2D_3$ followed a course of increases and decreases of $25(OH)D_3$. The formation of $24,25(OH)_2D_3$ metabolite was approximately at 10% of $25(OH)D_3$ levels. This ratio did not change from the lowest to the highest measured $25(OH)D_3$ levels.

Conclusion: With the vitamin D supplementation regimen used, 10 % $25(OH)D_3$ was converted to the $24,25(OH)_2D_3$ metabolite. The conversion percentage was stable even at high concentrations of $25(OH)D_3$, demonstrating a sufficiently high capacity of 25-hydroxyvitamin D3-24-hydroxylase for tested supplementation mode

Funding: Supported by the "Cooperatio" Program, research area Pharmaceutical Sciences.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1100

SCREENING OF BIOACTIVE PEPTIDES FROM WILD MUSHROOMS

Running title: Mushroom Bioactive Peptides

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State-of-the-Art: The global rise in antimicrobial resistance (AMR) continues to pose a significant threat to public health, as conventional antibiotics are increasingly losing effectiveness against a wide range of pathogenic bacteria. In this context, mushrooms have emerged as a promising natural reservoir of bioactive compounds, particularly peptides with potent antimicrobial properties. A small but growing body of research has identified several mushroom species as rich sources of antimicrobial peptides. For example, peptides isolated from Tapinella atrotomentosa, Pleurotus ostreatus, and Trametes versicolor have shown promising antibacterial activity against both Gram-positive and Gram-negative bacteria. These findings support the potential of further screening of wild mushroom species to discover novel.

Objective: Objective I: Screening of Bioactive Peptides (BAPs) for their Antimicrobial Activities. Objective II: Optimization of Protocol for Extraction of Bioactive Peptides from Mushroom.

Material and Methods: Screening of Bioactive Peptides (BAPs): Ten different wild mushroom species were collected from forests in the Pilsen region under the supervision of a professional mycologist. The identified species included Trametes versicolor, Pleurotus ostreatus, Flammulina velutipes, Tapinella atrotomentosa, Boletus edulis, Phaeolus schweinitzii, Hypholoma fasciculare, Rubroboletus satanas, Cantharellus cibarius, and Stereum subtomentosum. Bioactive peptides (BAPs) were extracted from each species and subsequently screened for antimicrobial activity. Optimization of Extraction Protocol: A solid-phase extraction (SPE)-based method was developed for the isolation of BAPs from wild mushrooms. To optimize the loading capacity of the SPE column, a synthetic peptide was used as a model compound.

Results & Discussion: Mass spectrometry analysis of BAPs extracted from the ten mushroom species revealed distinct peptide spectra for each species. The analysis confirmed that all detected peptides had molecular weights of less than 3 kDa. Among the tested BAPs, peptides extracted from Rubroboletus satanas exhibited notable antibacterial activity against Staphylococcus aureus, producing a zone of inhibition measuring 1.13 ± 0.21 mm. During the optimization of the solid-phase extraction (SPE) method, the loading capacity of the SPE column was determined to be 500 µg, with a peptide recovery rate of 46%. The optimized method proved effective for the extraction of peptides from various mushroom species. Nevertheless, species-specific variations were observed in both the yield and profile of the extracted BAPs.

Conclusion: In this study, BAPs from ten wild mushroom species were screened. BAPs extracted from Rubroboletus satanas showed antibacterial activity against Staphylococcus aureus. Moreover, SPE method was optimized for effective purification of BAPs.

Study program: Master´s degree - General Medicine | Year of study: 4

ID: 1138

STUDENT-GENERATED INSTAGRAM CONTENT AS A TOOL FOR ENGAGEMENT AND MOTIVATION IN HISTOLOGY AND EMBRYOLOGY CLASSES

Running title: Instagram for Engagement in Histology Education

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State-of-the-Art: Social media is a highly competitive environment for attention. While often suspected of reducing educational value due to accessible and entertaining formats, empirical evidence suggests otherwise. Studies show that academic integration—feeling connected to peers and institution—is a major factor in student success. Constructively designed social media content can support this integration by building motivation, identity, and a sense of belonging. Our project explores this potential through our own content creation for a departmental Instagram account within a medical faculty, contextualized by a comparative analysis of communication strategies used by leading institutions.

Objective: To evaluate the educational and emotional impact of Instagram videos we created as part of medical education, and to compare them with selected posts from some medical faculties worldwide to explore effective communication strategies that support motivation and engagement.

Material and Methods: We created a series of Instagram reels for the Institute of Histology and Embryology at the Faculty of Medicine in Pilsen (@histologie.embryologie), combining visual design, humor, and educational messaging. The content was developed by us with creative autonomy and supportive teachers' input. An English-language version of the account (@histology.embryology) also exists for international students. We analyzed the posts for engagement and educational value. To broaden our perspective, we conducted a content analysis of 70 posts from seven medical education accounts—including our own. These included profiles of Harvard, Yale, Columbia, Stanford medical faculties, the central Charles University account, and @osmosismed (Elsevier). Posts were assessed by format, tone, and engagement.

Results & Discussion: Our Instagram reels—ranging from tutorials to parody—sparked strong engagement (with some reaching over 1200 likes) and real interaction. The most successful was "Do You Want to Pass Histology?", a parody of Who Wants to Be a Millionaire? with a clear educational message. Other formats included tutorials, countdowns, quizzes, polls, and expert interviews. These formats worked because they spoke the students' language—visually, emotionally, and academically. Our comparison with accounts from abroad confirmed: what may seem superficial can be a strategic way to foster engagement, motivation, and academic connection. Still, studies caution that social media—if misused—may increase stress or contribute to maladaptive coping. Effective communication requires balance, clarity, and intent.

Conclusion: Student-generated social media content, when guided by academic purpose, can support motivation, integration, and professional identity. Thoughtfully curated posts may even address aspects of the hidden curriculum—modelling empathy, inclusion, and respectful communication in medical training.

Funding: The work was partially supported by Charles University, Project No. SVV 260 773.

Study program: Master´s degree - General Medicine | Year of study: 3

ID: 1123

ABSTRACTS NOT INCLUDED

THE ROLE OF DIET IN THE DEVELOPMENT OF NON-COMMUNICABLE DISEASES: FOCUS ON THE GUT MICROBIOME

Running title: Dietary Impact on Gut Microbiome and NCDs

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State-of-the-Art: Current research increasingly highlights the gut microbiome as a key factor in the development and prevention of non-communicable diseases (NCDs). Diet is considered the most influential and modifiable factor affecting microbiota composition. Western diets, characterized by high levels of saturated fats, animal proteins, and processed foods, are linked to gut dysbiosis and systemic inflammation. In contrast, plant-based and Mediterranean diets promote microbial diversity and the growth of beneficial bacteria, leading to anti-inflammatory effects. Recent studies also emphasize the role of microbial metabolites such as SCFAs, LPS, and TMAO in the pathogenesis of NCDs, positioning microbiome-targeted dietary interventions as a promising strategy for prevention.

Objective: This article focuses on how various types of diet affect the composition of the gut microbiome and how dietary changes are able to prevent or slow down the development of non-communicable diseases including obesity, type 2 diabetes mellitus, cardiovascular diseases, and low-grade inflammation.

Material and Methods: A review of the topic was conducted in October 2023 employing the MEDLINE database accessed via the free-to-use PubMed interface. Related studies were searched for applying the keywords "gut microbiome", "vegetarian", "plant-based", "vegan", "Mediterranean diet", "Western diet", "gluten-free", "diabetes mellitus", "idiopathic bowel diseases", "cardiovas- cular diseases", "microbiota", "obesity", "protein", "carbohydrates" and "fat". Only publications written in English and published between the year 2000 and the present were considered in the review.

Results & Discussion: The findings confirm that diet significantly influences gut microbiota composition and contributes to the development of NCDs. Western diets, rich in saturated fats, animal proteins, and simple sugars, promote dysbiosis and pro-inflammatory metabolites such as LPS and TMAO, which are linked to obesity, type 2 diabetes, and cardiovascular diseases. In contrast, plant-based and Mediterranean diets increase the abundance of beneficial bacteria (e.g., Bifidobacterium, Lactobacillus) and reduce inflammation. The reviewed studies highlight the importance of dietary interventions in preventing intestinal dysbiosis and reducing the incidence of chronic NCDs.

Conclusion: A balanced diet plays a key role in maintaining gut microbiota homeostasis and preventing chronic NCDs. Promoting fibre-rich and plant-based diets may reduce inflammation and the risk of obesity, diabetes, and cardiovascular diseases through microbiome modulation.

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Study program: Doctoral study - Hygiene, Preventive Medicine and Epidemiology | Year of study: 2

ID: 1074

LASER CAPTURE MICRODISSECTION COUPLED CAPILLARY IMMUNOASSAY TO STUDY THE EXPRESSION OF PCK-2 ON SPACIALLY-RESOLVED ISLETS OF RAT LANGERHANS

Running title: The role of PCK-2 in Pathophysiology of Diabetes

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State-of-the-Art: PCK-2 is the sole isoform responsible for phosphoenolpyruvate (PEP) synthesis in pancreatic β-cells, playing a critical role in gluconeogenesis. While PCK-1 has been extensively studied, the role of PCK-2 in β-cells remains less understood, particularly in the context of diabetes. Long-term type 2 diabetes mellitus may affect the expression of PCK-2 within the β-cells of the Langerhans islets,

potentially influencing insulin secretion and thus contributing to the pathophysiology of type 2 diabetes. Given the irregular distribution of Langerhans islets in pancreatic tissue, laser capture microdissection (LCM) is a method of choice to isolate small samples of these islets.

Objective: The aim of this research is to integrate LCM with capillary-based immunoassay (cap-IA) to quantify PCK-2 levels in microdissected Langerhans islets, providing insights into the relationship between diabetes and β-cell function at the proteomic level.

Material and Methods: Zucker diabetic fat (ZDF) rats were sacrificed by decapitation at week 34 of age and pancreas was dissected. Langerhans islets were isolated using LCM under histological supervision, ensuring selection of β-cell-rich regions. RNA was isolated, followed by reverse transcription and quantitative RT-PCR to assess PCK-2 expression. For protein analysis, Western blotting (traditional and capillary-based) was used, with β-actin serving as a loading control. PCK-2 expression levels were quantified and statistically analyzed to compare differences between diabetic and control groups.

Results & Discussion: In dissected Langerhans islets, a 63 kDa band was detected, confirming PCK-2 expression, which was further validated by RT-qPCR. In a diabetic rat model, Langerhans islets showed significantly higher PCK-2 expression compared to control rats.

Conclusion: The findings demonstrated notable increase in PCK-2 levels in diabetic Langerhans islets compared to healthy controls, highlighting its potential role in the pathophysiology of diabetes.

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Study program: Doctoral study - Physiology and Pathological Physiology | Year of study: 4
ID: 1077

NOTES

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