2024

program
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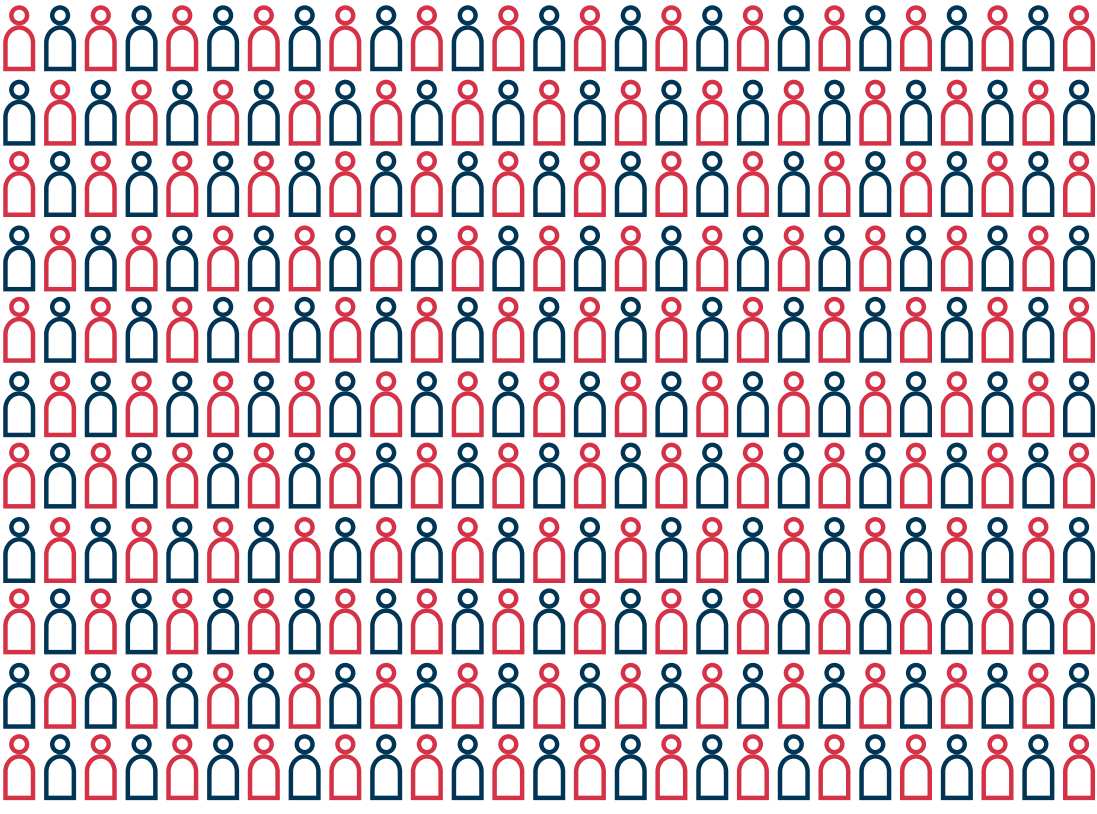
programme and COLLECTION OF ABSTRACTS

61. STUDENTSKÁ VĚDECKÁ KONFERENCE
STUDENT´S SCIENTIFIC CONFERENCE

Lékařská fakulta v Plzni, Univerzita Karlova
Faculty of Medicine in Pilsen, Charles University



PŘEHLED SEKCÍ
SESSION OVERVIEW

	Hnědá posluchárna Brown lecture hall	Seminární místnost Seminar room U3.16	eminární místnost Seminar room U3.17	Seminární místnost Seminar room U3.18
9:00	Clinical Research - DSP	Preclinical Studies/ Clinical Research - DSP	Theoretical Disciplines MSP	Preclinical Studies/ Clinical Research - MSP
10:40	Clinical Research - DSP	Preclinical Studies/ Clinical Research - DSP	Theoretical Disciplines MSP	Preclinical Studies/ Clinical Research - MSP
12:30	Clinical Research - DSP	Surgery - DSP	Theoretical Disciplines DSP	Preclinical Studies / Clinical Research - MSP
14:10	Clinical Research - DSP	Varia - DSP	Theoretical Disciplines DSP	Clinical Research/ Surgery - MSP

ABSTRAKTY
ABSTRACTS

THE IMPORTANCE OF METHYLATION INVESTIGATION IN CANCER TUMORS

E. Mosaieby (3rd year of DSP)
Department of Pathology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Michael Michal, Ph.D.

Background: Methylation, an epigenetic modification, plays a crucial role in the regulating of gene expression and is becoming more widely acknowledged as a key player in cancer development and progression. Gaining knowledge of the methylation patterns in cancer cells can provide valuable insights into the molecular mechanisms driving tumorigenesis.

Methods: In brain tumors, methylation profiling has emerged as a powerful tool for subclassification, offering more precise diagnoses and prognoses. This presentation explores the significance of methylation investigation in brain tumors through two cases where methylation made a significant difference in diagnosis, and as a result in treatment approach.

Results: Case 1 involved a 7-year-old boy with a tumor in the posterior fossa (cerebellum) characterized by H3 wildtype and IDH wildtype statuses. While Fluorescence In Situ Hybridization (FISH) and Next Generation Sequencing (NGS) analyses classified this tumor into groups A and B, methylation profiling revealed it to be a diffuse pediatric-type high-grade glioma. Case 2 featured a 34-year-old male initially diagnosed with high grade glioma based on microscopic analyses but methylome analyses could identify it precisely as a glioblastoma.

Conclusion: In conclusion, these cases highlight the importance of methylation investigation in cancer tumors, particularly in brain tumors, holds great promise for improving our understanding of tumor biology and guiding personalized treatment strategies. It is necessary to conduct more study in this field to clarify the role of methylation in cancer and to apply the findings to therapeutic settings.

THE STUDY OF DIFFERENTIALLY EXPRESSED GENES IN METACHRONOUS COLORECTAL LIVER METASTASIS SAMPLES USING RNA SEQUENCING

B.H. Rao (3rd year of DSP), K. Seborova, V. Liška, O. Vyčítal, O. Fiala, P. Souček
Department of Experimental Surgergy, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): V. Hlaváč, Ph.D.

Background: Colorectal cancer (CRC) holds the ninth position for the highest incidence in the Czech Republic. Approximately 25-30% of CRC patients experience liver metastasis during the disease allowing surgical resection and/or systemic chemotherapy as treatment options. The existence of a reliable biomarker enables the prediction of optimal treatment after liver metastasis resection could significantly improve patient life.

The project aims to study the prognostic and therapeutic targets by analyzing the differentially expressed (DE) genes in the tumor and non-tumor adjacent liver tissue and in patients stratified by early relapse (ER) of metachronous colorectal liver metastasis using RNA sequencing technology.

Methodology: The total RNA from 43 pairs (tumor and liver) of fresh frozen metachronous colorectal liver metastasis (mCLM) samples collected at University Hospital Pilsen, Czech Republic was isolated using TRIzol reagent. Lexogen QuantSeq 3'mRNA-seq library prep kit protocol was employed for RNA library preparation, followed by sequencing using the NextSeq 500 platform. The DE analysis of tumor vs. non-tumor and ER with cut off 6 months was performed using the DESeq2 package in R and the clusterProfiler package was employed to perform GeneSet enrichment analysis (GSEA).

Results: The DE analysis of tumor vs. non-tumor showed around 3,000 significantly dysregulated genes. Out of which genes HOXA1, MUC2, COL17A1, MISP, and AGR2 were 7-fold significantly upregulated whereas, genes CFB, LGI1, MT1B, SULT1E1 were 3-fold significantly downregulated. The genes LINC02141, CELA2B, and QRSL1P3 were found significantly upregulated in patients with ER. The GSEA showed significance in various processes like extracellular matrix organization, structure organization, skeletal development, integrin binding, glycosaminoglycan binding, and cellular components like collagen-containing extracellular matrix, basement membrane, apical part of cell, and plasma membrane. Upregulation of genes MUC2, COL17A1, and AGR2 indicates the possible role in promoting tumor invasion and metastasis whereas HOXA1 and MISP play an important role in cell proliferation and migration.

Conclusion: The DE analysis indicates the complex alterations in various biological processes like cell proliferation, invasion, immune responses, and metabolism. Novel in mCLM etiology genes, CELA2B and QRSL1P3, and the non-coding RNA LINC02141 will be further investigated. The overall GSEA indicates that the extracellular matrix (ECM) organization, structure, and cellular components are important for the interplay between the tumor and its microenvironment. Further validation on a larger cohort and functional analysis by in vitro experiments may be required to understand the exact molecular mechanism for developing novel therapeutic targets and finally improve the clinical management strategies for patients with mCLM.

This work was funded by the European Union's Horizon 2020 Research and Innovation Programme, under grant agreement No 856620; the Grant Agency of Charles University program Cooperatio – Surgical Disciplines, no. 207043; the Czech Health Research Council grant no. NU21-07-00247.

THE PROGNOSTIC ROLE AND CLINICAL ASSOCIATION OF CD20+B CELLS IN COLORECTAL CANCER

W. Ye (2nd year of DSP) E. Ali, S. Pavlov, F. Ambrozkiewicz, L. C. Červenková
Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisors: prof Kari Hemminki MD. Ph.D, MUDr. Andriy Trailin Ph.D

Background: Immune response to tumors can control tumor growth or contribute to an immunosuppressive environment that promotes tumor progression. The Immunoscore® method based on direct quantification of CD3+ and CD8+ cell densities in tumor center (CT) and its invasive margin first proposed in 2012, In comparison with other available prognostic parameters, including TNM, the immunoscore showed the highest independent predictive power for colon cancer. The prognostic importance of infiltrating subsets of T lymphocytes in CRC has been widely accepted, and subsequently led to be criteria of the Immunoscore. The role of infiltrating B lymphocytes remains controversial and in matters of prognostic effect has to be explored. We want to find out the association between CD20+ cells and clinicopathology parameters, and their prognostic impact on patients.

Methods: FFPE tissue samples from pCRC and CRC liver metastasis (LM) of 99 patients were stained immunohistochemically for CD20, the densities of CD20+ cells was assessed in tumor center (TC), inner margin (IM), outer margin (OM) and peritumor (PT) from pCRC and synchronous and metachronous liver metastasis by using QuPath software. These were evaluated for association and correlation with histopathologic features by the Fridman test and Spearman correlation analysis. And prognostic significance of immune cells, for time to recurrence (TTR), disease-free survival (DFS), and overall survival (OS) was evaluated using Kaplan-Meier and Cox regression analyses.

Results: High cell density of CD20+ B cells in PT of pCRC (HR=0.45 (0.22-0.93, p=0.031)), in IM (HR=0.49 (0.24-0.99, p=0.047)) and in PT (HR=0.42 (0.22-0.81, p=0.009)) of liver metastasis were associated with a longer OS of patients in the synchronous group. High density of CD 20+ immune cells in PT of LM correlated with a longer DFS of patients in the metachronous group (HR=0.35 (0.13-0.89, p=0.027)). Good response to treatment is associated with longer DFS (HR=2.98 (1.38-6.43, p=0.005)) of patients in the metachronous group after liver metastasis resection. The patients without nodal invasion in the metachronous group had longer TTR (HR=2.29 (1.14-4.60, p=0.020)) after liver surgery.

Conclusion: Our results suggested that high cell density of CD20+ in PT of pCRC and IM and PT of LM indicated better outcomes of patients. Response to treatment and lower nodal stage have positive prognostic impact on CRC patients,

PREDICTIVE SIGNIFICANCE OF COMBINED PLASMATIC DETECTION OF BRAF MUTATIONS AND S100B TUMOR MARKER IN EARLY-STAGE MALIGNANT MELANOMA

M. Sharif (4th year of DSP) (1), J. Polivka (1), M. A. Gouda (2), M. Pesta (3), H. Huang (2), I. Treskova (4), V. Woznica (4), J. Windrichova (5), K. Houfkova (3), R. Kucera (5,6), T. Fikrle (7), J. Ricar (7), K. Pivovarcikova (8), O. Topolcan (5), F. Janku (2)

(1) Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University
(2) Department of Investigational Cancer Therapeutics, the University of Texas MD Anderson Cancer Center, Houston, USA
(3) Department of Biology, Faculty of Medicine in Pilsen, Charles University
(4) Department of Plastic Surgery, University Hospital Pilsen
(5) Department of Immunochemical Diagnostics, University Hospital Pilsen
(6) Department of Pharmacology, Faculty of Medicine in Pilsen, Charles University
(7) Department of Dermatovenerology, University Hospital Pilsen
(8) Department of Pathology, University Hospital Pilsen

Supervisor(s): Ing. et Ing. Jiří Polívka, Ph.D. (1)

Background: Melanoma is the most aggressive skin cancer with the ability to recurrence also after early-stage tumor surgery. The aim was to identify early-stage melanoma patients at high risk of recurrence using liquid biopsy, estimating of mutated BRAF ctDNA, and the level of tumor marker S100B in plasma.

Methods: 80 patients were enrolled in the study. BRAF V600E mutation was determined in FFPE tissue and plasma samples using ultrasensitive ddPCR with pre-amplification. The level of S100B was determined in plasma.

Results: The best prediction of melanoma recurrence after surgery was observed in patients with combined high level of S100B (S100Bhigh) and ctDNA BRAFV600E (BRAFmut) in preoperative (57.1% vs. 12.5%, P=0.025) as well as postoperative blood samples (83.3% vs. 14.3%, resp., P=0.001) in comparison with low S100B and BRAF wild-type. Similarly, patients with preoperative and postoperative S100Bhigh and BRAFmut experienced worse prognosis (DFI P=0.05, OS P=0.131 and DFI P=0.001, OS=0.001, resp.).

Conclusion: We observed the benefit of the estimation of combination of S100B and ctDNA BRAFmut in peripheral blood for the identification of patients in high risk of recurrence and unfavorable prognosis.

ALTERATION OF LYMPHOCYTE POPULATIONS IN MULTIPLE MYELOMA PATIENTS

T. Dekojová (5th year of DSP), H. Gmucová, D. Macečková, R. Klieber, P. Ostašov, M. Leba, T. Vlas, A. Jungová, V. S. Caputo, M. Čedíková, D. Lysák, P. Jindra, M. Holubová

(1) Department of Haematology and Oncology, Faculty of Medicine in Pilsen Charles University
(2) Department of Histology and Embryology, Faculty of Medicine in Pilsen Charles University
(3) Laboratory of Tumor Biology and Immunotherapy, Biomedical Center, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): Mgr. M. Holubová, Ph.D.

Background: Multiple myeloma (MM) is characterised by the clonal proliferation of plasma cells with the presence of monoclonal immuno-globulin in serum and urine and remains incurable. One of the main features is a weakened immune system that allows MM cells to survive. The emerging approach to the MM treatment is to reinforce the weakened immune system through immunotherapy. Therefore, the current research is focused on the study of immune system imbalance in MM to find the most effective immunotherapy strategies. The aim of this work is to obtain a comprehensive picture of alterations in the circulating lymphocytes in MM patients througout the course of the disease, which could potentially allow creation of a tailored treatment approaches.

Methods: Cryopreserved MNCs were thawed and stained with a panel of antibodies for the detection of lymphocyte populations. The panel for NK cells contained CD45-BV510, CD56-PECy7, NKG2D-PE, NKp46-APC, SLAMF7-BV421, CD38-APCCy7, CD16-FITC and CD3-PerCP. T cells and iNKTs were characterised using CD45-BV510, TCR Va24-JaQ-BV421, CD8-PE, and CD4-PECy7. CD1d-APC was added to the T cell panel to estimate CD1d expression on monocytes. B cells were analysed using DuraClone IM B cells tube (Beckman Coulter). Cells stained with the panels of antibodies were incubated for 15min at room temperature followed by washing with PBS (350g/5min) and then immediately measured on the FACS ARIA Fusion cell sorter.

Results: Our analysis revealed that MM patients exhibited immune alterations in all studied immune subsets. Compared to HI (healthy individuals), MM patients had a significantly lower proportion of CD4+ T cells (17.65% vs 40.85%; $p < 0.001$) and iNKT cells (0.03% vs 0.08%; $p = 0.049$), within B cells an increased proportion of CD21LCD38L subset (5.1% vs 0.4%; $p < 0.001$) and decreased level of memory cells (unswitched 7.5% vs 14.7%; $p<0.01$ and switched 5.6% vs 11.2%; NS), NK cells displaying signs of activation and exhaustion characterised by a more than 2-fold increase in SLAMF7 MFI ($p < 0.001$), decreased expression of NKG2D (MFI) and NKp46 (%) on CD16+56+ and CD16+56- subset respectively ($p < 0.001$).

Conclusion: The immune status is meaningfully impaired in MM patients. Understanding of such alterations is essential for developing sufficiently effective therapy. To overcome the complexity of immune deficiencies, monitoring of immune cells should be part of routine practice to increase the understanding of therapy failure.

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USE OF URINE CYTOLOGY IN SETTING OF UPPER URINARY TRACT UROTHELIAL CARCINOMA: ONE CENTER EXPERIENCE

J. Skopal (2nd year of DSP; 1,3), K. Khomenko (2), A. Straková-Peteříková (1,3), M. Michal (1,3), M. Hora (2), T. Pitra (2), K. Pivovarčíková (1,3)

(1) Department of Pathology, Faculty of Medicine in Pilsen Charles University
(2) Department of Urology, Faculty of Medicine in Pilsen Charles University
(3) Department of Pathology, Biopticka Laboratory Ltd., Pilsen

Supervisor(s): doc. MUDr. K. Pivovarčíková Ph.D.

Background: Urine cytology (UC) is a non-invasive examination, primarily used to detect high grade urothelial carcinoma in a urine sample. There is a relatively small number of publications regarding the use of UC in setting of upper urinary tract urothelial carcinoma (UUTUC), i.e. urothelial carcinoma of the renal pelvis and ureter. Comparing urine cytology detection of upper urinary tract urothelial carcinoma in voided urine samples and washing specimes and general data regarding more prevalent urothelial carcinoma of lower urinary tract.

Methods: Patients treated at the Urological clinic of Pilsen University hospital (in the period 2017 – 2022) with histologically diagnosed UUTUC (diagnosis based on biopsy, resection) and performed UC were retrospectively searched. The results of the cytological examination were compared with their final histologic counterparts.

Results: A total of 68 patients were included in the study (34 with voided urine samples, 16 patients with washing samples, of which 5 patients had concurrently performed voided UC available. Finally, 18 patients with no available information's regarding the sample collection method). In 33 patients, a low-grade urothelial carcinoma (LGUC) was diagnosed from the biopsy, and in 35 cases a diagnosis of high-grade urothelial carcinoma (HGUC) was made (according to WHO 2022). UUTUC was detected by urine cytology in 34 patients (i.e. UC evaluated as atypical urothelial cells/AUC, suspicious for high grade UC/SHGUC, high grade UC/HGUC according to the Paris system for reporting urinary cytology). Urinary cytology specifically detected a total of 12 low grade and 22 high grade UUTUC. In 21 patients with LG UUTUC, the UC was evaluated negatively (meaning negative for high grade UC/NHGUC), the result cannot be considered incorrect due to the current concept of The Paris System classification. However, in 13 patients with HG UUTUC, UC gave a definite false-negative result of NHGUC category.

Conclusion: Urine cytology correctly detected a total of 22/35 cases of high grade UUTUC (63%) and 12/33 cases (36%) of low grade UUTCC. The amount of cytologically detected UC in setting of the upper urinary tract are consistent with the available literature data.

RELAPSE RISK ANALYSIS AMONG RAPE-TREATED AND METASTASIS-AFFLICTED PROSTATE CANCER PATIENTS

V. Rudnev (4th year of DSP), J. Ferda
Department of Imaging Methods, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): prof. MUDr. J. Ferda, Ph.D.

Background: Prostate cancer (Pc) is globally ranked as the fifth highest cause of cancer-associated mortality. Tumor relapse is a predominately reported complication among Pc-patients. However, the recurrence is yet to be determined amongst metastatic patients following the RAPE (Radial Prostectomy) treatment. My study endeavored to assess the relapse risk in RAPE and non-RAPE Pc-patients and to examine the metastasis-associated relapse.

Methods: My study employed a retrospective design to analyze 104 patients with biochemical relapse within 24 months. The information was retrieved from the Plzen patient database of Lochotin Teaching hospital. The patients were divided into two groups: RAPE (postTx) and non-RAPE (preTx), and their PSA (Prostate-specific antigen), ProPSA (premature PSA), PHI (Prostate Health Index) levels, and the F/T ratio (free/total PSA) were calculated before and after the procedure. PSA levels were monitored and screened for local and distant metastasis using PET/CT and PET/MRI.

Results: Out of 129 Pc-patients (M=64 yrs), 41 underwent the RAPE procedure, while 87 represented the non-RAPE cohort. Amongst Pc-patients, treatment-associated relapse was insignificant (RAPE:32.03% (41/129), non-RAPE: 67.44% (87.129), P:0.08). Metastasis-associated relapse was mainly observed in lymph nodes (68.21%; 88/129, P:0.02) and bones (34.10%; 44/129, P:0.03), followed by local relapse (21.70%; 28/129, P:0.805) and lungs (8.3%: 8/129, P:0.553). PSA levels were significantly different before (preTx; M=134.8) and after (postTx; M=2.1) the RAPE intervention (P:0.027). The mean ProPSA levels, PHI levels, and F/T ratio were 24.5, 225.8, and 8.9, respectively, significantly impacting ($P<0.05$) the preTx levels.

Conclusion: Patients with lymph node metastasis and bone metastasis exhibited peaked recurrences. Due to the limited sample size of RAPE patients, treatment-associated relapse is still unclear and may require additive investigation.

EFFECT OF CHRONIC PHARMACOTHERAPY ON SERUM LEVELS OF PROSTATE BIOMARKERS, DERIVED INDEXES AND GLYCOSYLATION PATTERNS OF FREE FORM OF PROSTATE SPECIFIC ANTIGEN

M. Jirásko (3rd year of DSP)
(1) Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University
(2) Glycanostics, Ltd., Kudlakova 7, Bratislava

Supervisor(s): prof. PharmDr. R. Kučera, Ph.D. (1), Ing. Tomáš Bertók, Ph.D., MBA (2), Ing. Jan Tkáč, Ph.D. (2)

Background: More than one million cases of prostate cancer (PCa) are diagnosed worldwide each year and the mortality rate is over 300,000 deaths yearly. In the Czech Republic in 2015, the incidence rate was 70 new cases per 100,000 persons and the mortality rate was over 12 deaths per 100,000 persons. Prostate-specific antigen (PSA) is a glycoprotein whose serum levels and glycated forms change significantly with the development and progression of prostate cancer. To increase the specificity and sensitivity of prostate cancer detection, other derived markers such as the free form of PSA fPSA, [-2]proPSA and the derived fPSA/PSA ratio and prostate health index (PHI) have come into use in clinical practice. Chronic pharmacotherapy of benign prostatic hyperplasia (BPH), which includes alpha1 receptor antagonists, 5-alpha reductase inhibitors and spasmolytics, may significantly affect these biomarkers and make subsequent diagnosis algorithm of PCa more difficult. To monitor changes in PSA, free form PSA (fPSA), [-2]proPSA, derived markers and fPSA glycan patterns induced by chronic BPH pharmacotherapy.

Methods: 564 serum samples from men aged between 39 and 93 years (mean age 68) were used. Information on drug therapy was retrieved from medical records. Serum PSA, fPSA and [-2]proPSA levels were analyzed using ACCESS chemiluminescence kits. From these values, fPSA/tPSA ratio and PHI indexes were calculated. Due to the nature of these biomarkers, the levels were adjusted for age. Changes in glycan composition were detected using Wisteria floribunda lectin (WFL), Phaseolus vulgaris lectins E and L (PHA-E, PHA-L) and Maackia Amurensis lectin (MAL). Specific glycoprophylation was performed using magnetic particle (MP) modified antibodies that selectively enriched fPSA in human serum samples. Subsequently, proteins attached to the MC antibodies were incubated on lectin-modified ELISA plates and the recieved signal was standardized using glycoprotein standard.

Results: Statistically significant reductions in proPSA, fPSA and PHI levels were observed in patients taking 5-alpha reductase inhibitors compared to patients without established pharmacotherapy. Higher fPSA levels, fPSA/PSA ratio and lower PHI levels were observed in patients medicated with alpha-blockers. With the exception of glycan MAL, which was statistically significantly higher in patients taking spasmolytics, glycans remained unaffected by pharmacotherapy.

Conclusion: The study highlights the need for knowledge of chronic pharmacotherapy in BPH patients, as it can significantly influence the levels of biomarkers, both long-used in clinical practices as well as new ones in the form of glycated forms of fPSA. The use glycated forms of fPSA appears advantageous, as it is less susceptible to the influence of long-term medication use, thereby reducing the likelihood of false-negative outcomes and underestimation of prostate cancer risk.

VALIDATION AND CLINICAL RELEVANCE OF TP53 AND KRAS MUTATIONS IN OVARIAN CANCER: IMPLICATIONS FOR PROGNOSIS AND THERAPEUTIC STRATEGIES, AND THE UTILITY OF CRISPR TECHNOLOGY

M. Al Obeed Allah (4th year of DSP; 1), E. Alli (1), I. Krus (2), P. Holý (1,2), V. Haničinec (1), F. Ambrozkiewicz (1), K. – A. Dickson (8), L. Rob (3), M. Hruda (3), M. Mrhalová (4), K. Kopečková (5), A. Bartáková (6), J. Bouda (6), O. Fiala (7), D. J Marsh (8), P. Souček (1,2), and R. Václavíková (1,2)

(1) Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
(2) Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic
(3) Department of Gynecology and Obstetrics, Third Faculty of Medicine, Charles University and University Hospital Královské Vinohrady, Prague, Czech Republic
(4) Department of Pathology and Molecular Medicine, Second Faculty of Medicine and Motol University Hospital, Charles University, Prague, Czech Republic
(5) Department of Oncology and Molecular Medicine, Second Faculty of Medicine and Motol University Hospital, Charles University, Prague, Czech Republic
(6) Department of Gynecology and Obstetrics, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic
(7) Department of Oncology and Radiotherapeutics, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic
(8) Translational Oncology Group, School of Life Sciences, Faculty of Science, University of Technology Sydney, Australia

Supervisor(s): RNDr. Radka Václavíková, Ph.D. (1,2)

Background: Ovarian carcinoma is often diagnosed at advanced stages with poor prognosis. Understanding the genetic mutations driving this cancer, such as those in TP53 and KRAS, is crucial for developing effective treatments. Concerning the dismal prognosis of chemoresistant patients with epithelial ovarian carcinoma (EOC), we aimed to validate the findings of a previous whole exome sequencing study on 50 patients using an orthogonal method on the same patients and a separate set of 127 EOC patients (N=177). We focused on TP53 as a frequently mutated gene relevant for chemosensitivity, included KRAS as an additional therapeutically relevant target, and complemented somatic mutation status with transcript levels of both genes.

Methods: We sequenced tumor DNA with the Sanger method, assessed transcript levels in cDNA transcribed from tumor RNA using quantitative real-time PCR, and compared results with clinical parameters. Direct sequencing confirmed all variants in TP53 and KRAS detected by exome sequencing.

Results: KRAS mutated patients had significantly more frequently FIGO stages I or II ($p=0.007$) and rare (other than high-grade serous (HGSC)) tumor subtypes ($p<0.001$), which was connected with lower KRAS transcript levels ($p=0.004$). KRAS mutation status was not associated with other clinical parameters, including survival. Patients with non-HGSC subtypes harboring TP53 missense variants disrupting the DNA binding loop had significantly poorer platinum-free intervals than the rest ($p=0.011$). KRAS transcript level did not significantly associate with the KRAS mutation status, whereas we observed a significantly lower TP53 transcript level in tumors bearing nonsense, frameshift, or splice site TP53 variants compared to wild-type ($p<0.001$). On the other hand, tumors with missense TP53 variants

had significantly higher transcript levels than wild-type ($p<0.001$). The normalized intratumoral TP53 and KRAS transcript levels were correlated, and three patients with both genes co-mutated had extremely poor survival. Taken together, our study points to KRAS as a target for future therapy of non-HGSC EOCs and reveals the prognostic value of TP53 variants in the DNA binding loop.

Conclusion: Following the functional role of crucial TP53 mutations, CRISPR knockout and base-editing techniques were used to create TP53 isogenic ovarian cancer cell line panels with wild-type (WT), null, and gain-of-function (GOF) mutations, including hotspot GOF mutations like Y220C and R248Q. These lines were studied for functional changes, such as failure to activate p53 target genes, increased clonogenic potential, and resistance to platinum-based chemotherapy. Through two independent approaches, namely drug screening of a TP53 isogenic panel against an epigenetic drug library containing 160 compounds, and in silico analysis, mutant p53 targeting compounds have been identified. The use of CRISPR-engineered isogenic panels shows great promise for advancing cancer drug discovery.

This study was supported by the Czech Health Research Council grant no. NU20-09-00174, the Grant Agency of Charles University, project no. 307123 and COOPERATIO Surgical Disciplines no. 207035, “Maternal and Childhood Care”.

TWO DE NOVO UBR1 VARIANTS IN TRANS AS A CAUSE OF JOHANSON-BLIZZARD SYNDROME

L. Strych (3rd year year of DSP; 1), P. Komrsková (1), T. Vaneček (2,3), T. Zavoral (1)

(1) Department of Medical Genetics, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
(2) Sikl's Department of Pathology, University Hospital Pilsen,
(3) Biopticka laboratory Ltd., Pilsen

Supervisor(s): MUDr. I. Šubrt, Ph.D. (1)

Background: Johanson-Blizzard syndrome (JBS) is a rare autosomal recessive disease caused by pathogenic, mostly inherited, variants in the UBR1 gene. JBS is usually suspected/diagnosed based on characteristic anomalies, but only genetic testing provides a definitive diagnosis.

Objective: We aim to identify the causal variants in a Czech proband with clinically suspected JBS and provide segregation analysis.

Methods: The proband with clinically suspected JBS underwent clinical exome sequencing (CES) using a virtual panel for UBR1 gen. Sanger sequencing was used for validation, characterization, and segregation of variants in the family. The variants were also characterised using quantitative Real-Time PCR (qPCR) and in-silico analysis.

Results: Using CES in the proband, we identified two novel causal variants in the UBR1 gene, c.3482A>C and c.3509+6T>C. Although variants were found in trans, neither was detected in the parents. Sanger sequencing of the cDNA revealed that the novel variant c.3509+6T>C caused activation of cryptic donor splice site, which removed a shorter non-canonical GC-AG intron. The inclusion of 70 bp of the intronic sequence generated frameshift and premature termination codon leading to nonsense-mediated decay, which was detected by qPCR. The novel missense variant c.3482A>C in Zn-stabilized domain RING-H2 altered a highly conserved histidine interacting with the Zn2+ atom by proline.

Conclusion: To our best knowledge, we report the first molecular confirmation of JBS in the Czech Republic and the first case of identification of two de novo variants in two alleles. Our findings expanded the spectrum of pathogenic variants in the UBR1 gene and demonstrated the weakness of some prediction tools in the prediction of non-canonical splicing, the importance of functional studies, and the benefits of protein modelling.

Study was supported by LM2023067 and SVV 260654.

NANOFAT GRAFTING OF CHRONIC WOUNDS

O. Troup (4th year of DSP)
Department of Plastic Surgery, University Hospital in Pilsen, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. I. Třešková Ph.D.

Background: Nanofat is fatty tissue that is mechanically or enzymatically emulsified to form tiny droplets. As a standard, the fat for the preparation of nanofat is obtained by the liposuction method. Nanofat shows a high concentration of cell progenitors (adipocyte derived stem cells, fibroblasts, preadipocytes etc.) and growth factors (vascular endothelial growt factor), and for its regenerative and rejuvenating effects, it has gained significant popularity in the field of plastic surgery in recent years. Regenerative effects of nanofat could also be used in the treatment of chronic wounds, which are often causally unsolvable and have a minimal or zero tendency to heal spontaneously. Studies on animal models have been carried out showing a positive effect on chronic wound healing, although there is lack of clinical studies concerning this topic. The aim of this study is to find out whether nanofat grafting is a feasible and suitable adjunct to the care of chronic wounds.

Methods: A total of 5 patients with a chronic wound older than 3 months of different etiologies are included in the study. Fat tissue is harvested by liposuction from the abdominal area and then processed into nanofat. Nanofat is injected superficially into the edge and base of half or part of the defect so that the remaining part serves as a control. Photo documentation, measurement of the wound and evaluation of the healing process are carried out at weekly intervals. Patient are followed up for 5 weeks.

Results: Following the procedure, there was no development of complications or worsening of local findings in any of the patients. From the follow-up to date, there is an obvious improvement of the local findings in at least 2 patients.

Conclusion: Nanofat grafting is relatively easy, economically available method, where one's own tissue is used with a minimal risk of complications. The limitation of this study is the small number of patients and the limited means of objective evaluation of this method. In the future, it would be convenient to collect larger group of patients and diversify patients into groups upon the initial cause of their chronic wound.

SHOCK INDEX FOR EARLY DETECTION OF LOW PLASMA FIBRINOGEN IN TRAUMA – FINAL RESULTS

M. Horáková (4th year of DSP)

(1) Department of Anaesthesiology, Resuscitation and Intensive Care, University Hospital Pilsen
(2) Department of Anaesthesiology, Perioperative Medicine and Intensive Care, J. E. Purkinje University, Masaryk Hospital in Usti nad Labem

Supervisor(s): prof. MUDr. Jan Beneš, Ph.D., MUDr. Josef Škola, Ph.D.

Background: Bleeding stands out as the primary cause of death among severe trauma patients. Trauma-induced coagulopathy manifests as a multifaceted disorder involving endotheliopathy, platelet dysfunction, diminished clotting factors activity, sympathoadrenal activation, and hyperfibrinolysis. Among all coagulation factors, fibrinogen typically declines earliest in bleeding trauma cases. Hypofibrinogenemia correlates with heightened blood loss, increased transfusions, and poorer outcomes in injured patients. Prompt identification of hypofibrinogenemia is crucial to initiate fibrinogen replacement therapy. Although early fibrinogen administration holds promise as a treatment approach, there's currently no sufficiently specific indicator of hypofibrinogenemia in prehospital settings.

The shock index (the ratio between heart rate and systolic blood pressure) serves as a predictor of transfusion requirements and the necessity for haemostatic resuscitation in severe trauma cases. Our objective was to assess whether prehospital and admission shock index values could predict low plasma fibrinogen levels in trauma patients. To validate the hypothesis that prehospital shock index measurements in severe trauma patients could aid in identifying hypofibrinogenemia upon hospital admission.

Methods: We conducted a prospective observational cohort pilot study at two major trauma centers in Ústí nad Labem and Plzeň, Czech Republic. Trauma patients aged over 18 years, transported by helicopter emergency medical service to these centers, were prospectively evaluated for demographic, laboratory, trauma-related variables, and shock index at the scene, during transport, and upon admission to the emergency department. Hypofibrinogenemia, defined as a fibrinogen plasma level of 1.5 g·L–1, served as the cut-off for further analysis. Out of 322 screened patients, 264 (83%) were included for further analysis.

Results: Prehospital shock index and admission shock index were predictive of hypofibrinogenemia, with areas under the receiver operating characteristics curve (AUROCs) of 0.79 (95% CI 0.64–0.91) and 0.79 (95% CI 0.66–0.91), respectively. A prehospital shock index ≥ 1 had a sensitivity of 0.5 (95% CI 0.19–0.81), specificity of 0.88 (95% CI 0.83–0.92), and negative predictive value of 0.98 (95% CI 0.96–0.99) for predicting hypofibrinogenemia. The shock index could potentially identify trauma patients at risk of hypofibrinogenemia early in the prehospital phase.

Conclusion: The shock index emerges as a straightforward clinical tool capable of identifying adult trauma patients at risk of hypofibrinogenemia from the outset. Shock index values < 1 exhibited a 97% predictive value in excluding severe hypofibrinogenemia. This implies that the risk of severe hypofibrinogenemia was minimal when systolic blood pressure exceeded heart rate.

CAROTID ARTERY STENOSIS ON PANORAMIC RADIOGRAPHS AND CT SCANS IN PATIENTS WITH MALIGNANCIES AFTER RADIOTHERAPYŮ

W. Samara (3rd year of DSP), N. Pour
Department of Stomatology, University Hospital in Pilsen
Supervisor(s): MUDr. Omid Moztarzadeh, Ph.D., doc. MUDr. et MUDr. Lukas Hauer, Ph.D.

Background: This study aimed to identify the progression of carotid artery stenosis (CAS) in patients with of carotid artery stenosis (CAS) in patients with head and neck cancer following radiation therapy (RT) by characterizing associated risk factors.

Methods: Panoramic radiographs (OPG), computed tomography (CT) scans, cone-beam CT (CBCT) scans, and ultrasonography (US) of 69 patients with head and neck tumors were selected and analyzed to identify the presence of CAS. Data on tumor location, smoking status, hypertension (HTN), hyperlipidemia (HLD), diabetes mellitus (DM), and treatment were collected from the patients' medical records. Patients who received chemotherapy or no treatment were excluded from the study. The differential diagnosis of other radiopacities and anatomical landmarks were excluded. Patients were divided into two groups: those with CAS (group1) and those without CAS (group 2) and their clinical information was compared.

Results: The overall prevalence of CAS on the panoramic radiographs was 16%. Of the 69 patients,44 underwent radiography before and after radiotherapy, only seven had mild CAS on radiographs after radiotherapy, and no significant difference in CAS was identified before and after radiotherapy. There were also no differences between the groups regarding age, sex, smoking, hypertension, diabetes mellitus, hyperlipidemia, tumor location, and RT dose before and after radiation ($p>0.05$).

Conclusion: Radiotherapy does not seem to affect the prevalence of CAS, although it has been identified in some patients after radiotherapy completion.

A CASE STUDY OF VITAMIN D SUPPLEMENTATION THERAPY AND ACUTE RESPIRATORY TRACT INFECTION

E. Dědečková (4th year of DSP), V. Šimánek, Kučera Radek, Markéta Králová, O. Topolčan
Department of Pharmacology and Toxicology, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. V. Šimánek, Ph.D., prof. PharmDr. Kučera Radek, Ph.D.

Background: Low serum 25-hydroxyvitamin D concentrations are associated with a higher susceptibility to acute respiratory tract infections (ARTIs). The aim of this case study is to present the association between vitamin D levels, supplementation, and the incidence of ARTIs.

Methods: Determination of total vitamin D levels was performed with the chemiluminescence kit ACCESS 25(OH) Vitamin D on a Unicel® DxI 800 instrument.

Results: A 23-year-old female patient with vitamin D deficiency successfully increased her vitamin D level from 45.60 nmol/l to 85.91 nmol/l (reference range 75-200 nmol/l) by supplementation. However, surprisingly, in the following period, instead of the expected level of 120 nmol/l, a decrease in vitamin D level to 70.04 nmol/l was observed, although the patient continued taking supplementation. Further investigations revealed that the patient had been suffering from common symptoms of acute respiratory tract infection during the time of supplementation.

Conclusion: This case study demonstrates the complex relationship between vitamin D levels, proper supplementation, and ARTI. The observed decline in vitamin D levels during supplementation and ongoing acute respiratory tract infection suggests that respiratory infections may significantly affect vitamin D metabolism.

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COMPARISON OF COMMERCIAL MICROBIAL DNA ISOLATION KITS FOR THE STUDY OF THE GUT MICROBIOME

H. Bartošková (2nd year of DSP)
(1) Department of Public Health and Preventive Medicine, (2) Department of Microbiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Ing. A. Zavaďáková, Ph.D. (1), prof. Ing. J. Hrabák, Ph.D. (2)

Background: Recent years have brought a global breakthrough in the treatment of obesity with newly approved medicinal products based on incretins. It is known that changes in body weight are closely related to changes in the composition of the gut microbiota. The primary aim of the study is to compare the composition of the intestinal microbiome in obese patients treated vs. untreated by medicinal products based on incretins. The sequencing of the V4 hypervariable region of microbial 16S rRNA is a commonly used method to study the intestinal microbiome from stool samples. The first step for this analysis is the isolation of DNA from stool samples using commercially available kits. This work aimed to compare two commercial kits that are suitable for this purpose. The first kit was the QIAamp PowerFecal Pro DNA Kit (Qiagen), which is intended for the isolation of microbial DNA from stool and intestinal samples, the second one was the NucleoSpin® Tissue Kit (Macherey-Nagel), which is intended for the isolation of DNA from any tissue, cells, bacteria, yeast, samples of serum, plasma, or other body fluids. The comparison criteria were yield, price, user-friendliness, and time-consuming.

Methods: Three stool samples were included in the analysis. DNA was isolated from each sample with both commercial kits. Genes encoding the V4 region of 16S rRNA were amplified using PCR and appropriate primers. The presence of amplicons of the desired size was verified by gel electrophoresis. The DNA was further purified using magnetic beads and its concentration was measured fluorometrically.

Results: The main advantage of the QIAamp PowerFecal Pro DNA Kit is that the kit contains tubes with beads facilitating stool homogenization with lysis buffer (bead beating), which the NucleoSpin® Tissue kit does not contain. Working with the NucleoSpin® Tissue kit is more time-consuming due to the 1 - 3-hour incubation of the stool sample in proteinase K/SDS solution at the beginning of the isolation process. However, its advantage is its low price (CZK 5,200 / 50 samples) compared to the QIAamp PowerFecal Pro DNA Kit (CZK 8,600 / 50 samples).

Conclusions: The selection of a suitable commercial kit for DNA isolation is an integral part of sequencing the V4 region of microbial 16S rRNA from stool samples. We have verified that for the purposes of our study, the use of the QIAamp PowerFecal Pro DNA Kit (Qiagen) is more suitable in terms of yield, ease-of-use and time demands.

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MASS SPECTROMETRIC ANALYSIS OF MICROBIAL POLYSACCHARIDES: MISSION IMPOSSIBLE OR A POWERFUL TOOL IN ROUTINE DIAGNOSTICS?

L. Dadovska (2nd year of DSP), J. Hrabak
Department of Microbiology, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): prof. Ing. J. Hrabak, PhD.

Background: The use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) revolutionary advanced taxonomical identification, as well as identification of antibiotic resistance mechanisms in diagnostic microbiology. Nevertheless, for bacterial epidemiology as golden standard is still employed whole genomic sequencing which is cost and time-consuming and bioinformatically challenging. This void in proteomic epidemiology could be filled with analysis of microbial surface polysaccharides with advantage of direct analysis of microbes from clinical specimens. Detection of polysaccharides by MALDI-TOF MS is yet demanding due to a poor ionization ability thus in clinical routine practically impossible. The hypothesis of this study was to show whether an innovative approach to polysaccharide ionization for analysis of bacterial lipopolysaccharides and other external structures of the cell wall can facilitate bacterial identification and typing by MALDI-TOF MS.

Methods: All analyses were performed by diluting glucose, sucrose, lactose, purified lipopolysaccharides, and overnight bacterial cultures of various bacterial species in tartaric acid or different amylases, following optional previous bacterial culture incubation with lauroyl sarcosinate or ethyl acetate. In the next step, a newly designed self-ionizable ligand was added. Digested polysaccharides were visualized using a timsTOF Pro spectrometer (Bruker Daltonics, Bremen, Germany). MALDI-TOF MS measurements were made by Sirius MALDI-TOF Mass Spectrometer (Bruker Daltonics, Bremen, Germany).

Results: We developed a novel method for acid and/or enzymatic hydrolysis of glycosidic bond of microbial polysaccharides to obtain unique spectra for each bacterial species in the m/z window between 500 and 2000. Yielded aldehyde groups of reducing sugars react with novel self-ionizable ligand we designed and synthesized. That molecule contains a reactive amine that with structures containing an aldehyde group creates a stable bond. Additional vanillin-derived structure enhances ionization of the complex. We demonstrated its excellent ability to ionize mono- and disaccharides showing a signal at m/z 586 (glucose) and m/z 748 (lactose). In bacteria as well as purified lipopolysaccharides, we obtained unique spectra representing specific LPS fingerprint.

Conclusions: We developed a novel assay for mass spectrometric analysis of aldehyde-containing molecules, including saccharides, that can be used for the detection of microbial polysaccharides. The self-ionizable ligand allows ionization using MALDI-TOF mass spectrometer without the use of matrix. The assay allows specific ionization of molecules of interest and suppressing of the other signal and opens a possibility for the detection of microbes directly from clinical specimens.

The study was financed by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU. Patent application describing the procedure has been submitted.

OCCURRENCE OF B-LACTONE SYNTHESIS AMONG OXA-48-LIKE CARBAPENEMASE-PRODUCING BACTERIA COLLECTED IN THE CZECH REPUBLIC BETWEEN JANUARY AND JUNE 2023.

V. Heringová (4th year of DSP), L. Ďaďovská, J. Hrabák
Department of Microbiology, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): prof. Ing. Jaroslav Hrabák, Ph.D. (1,2)

Background: Production of carbapenemases represents a global threat leading to degradation of carbapenem antibiotics. Most of carbapenemases degrade β-lactam antibiotics by hydrolytic cleavage of the β-lactam amide bond. Some OXA-48-like-producing bacteria also show a different mechanism of antibiotic degradation with no hydrolytic cleavage but a rearrangement of the antibiotic molecule to form a β-lactone. This degradation mechanism is responsible for lower sensitivity of direct methods used for carbapenemase detection (e.g., pH-based, MALDI-TOF MS-based). The hypothesis of this study was to show whether β-lactone formation depends on the OXA-48-like carbapenemase variant.

Methods: Carbapenemase-producing bacteria recovered from the Czech Republic between January and June 2023 were included in this study. Carbapenemase production was confirmed by MALDITOF MS-based meropenem hydrolysis assay allowing detection of β-lactone formation (specific signal at m/z 362.5). Carbapenemase genes were detected by multiplex PCR and the presence of blaOXA-48-like was confirmed by PCR followed by amplicon sequencing to identify specific variant.

Results: Out of 689 carbapenemase-producing bacteria belonging to Enterobacterales collected during the study period, 105 isolates produced OXA-48-like carbapenemases, of which seven isolates expressed blaOXA-181 variant, four isolates blaOXA-232, 26 isolates blaOXA-244. The rest of isolates (n=68) carried blaOXA-48. When screened by MALDI-TOF MS for β-lactone presence, samples in the two most abundant gene groups (blaOXA-48 and blaOXA-244) showed considerable trends toward β-lactone synthesis. β-Lactone was detected in seven-fold more samples in group blaOXA-244 (23 positive) and three-fold (50 positive) in group of blaOXA-48. Therefore, a statistical analysis confirmed a significant relationship between β-lactone positivity and OXA-48 sensu stricto presence in the sample. Interestingly, all 36 but one Escherichia coli isolates synthesized β-lactone. Among Klebsiella pneumoniae isolates, 41 out of 58 were β-lactone positive. β-Lactone was not identified in any other carbapenemase producer.

Conclusions: In this study, we demonstrated that the occurrence of β-lactone in the spectra of MALDI-TOF MS-based meropenem hydrolysis assay has a high positive predictive value for OXA-48-like carbapenemases. However, it was not possible to demonstrate the association of β-lactone production with the type of blaOXA-48-like gene.

The study was supported by Charles University Grant Agency (GA UK), project Nr. 280323, and by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU

FIRST REPORT OF NDM-1 PRODUCING PSEUDOMONAS AERUGINOSA IN THE CZECH REPUBLIC; A CLONAL OUTBREAK IN A LOCAL HOSPITAL

T. Sourenian (3rd year of DSP), I. Bitar
Department of Microbiology, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Dr. I. Bitar, Ph.D.

Background: The incidence of carbapenem-resistant Pseudomonas aeruginosa represents a significant challenge in clinical settings, posing substantial obstacles to effective antimicrobial therapy for patients afflicted with infections caused by this pathogen. For the first time in the Czech Republic, a notable prevalence of New Delhi metallo-β-lactamase-1 (NDM-1) in P. aeruginosa has been observed. The aim of this study is to genomically characterize NDM-1-producing P. aeruginosa detected in a nosocomial outbreak in Plzen, originated from a hospitalized patient with a travel history.

Methods: During 2022, twenty clinical P. aeruginosa isolates were recovered from 3 different hospitals (18 being from the same hospital) in the Czech Republic. Antimicrobial susceptibility testing and carbapenemase genes were performed by broth microdilution method and PCR respectively. Isolates were subjected to whole-genome sequencing (WGS) using NovaSeqX Plus Illumina and PacBio Sequel I. Both the resistome and the virulome were analyzed in silico using online databases: ResFinder and VirulenceFinder (VFDB). Biofilm formation assay and serum bactericidal assay (SBA) were performed on all isolates to evaluate virulent characteristics. The clonality of the circulating isolates was examined by detecting single nucleotide polymorphisms (SNPs) using Snippy v4.6.0. SNPs-based phylogeny was generated using 215 ST773 genomes retrieved from the EnteroBase repository.

Results: All isolates were resistant to carbapenems. WGS data confirmed that P. aeruginosa belonged to the ST773 lineage. It revealed the presence of blaNDM-1 in all isolates (n=20) localized on a genomic cassette array (22, 344 bp). All isolates were able to form Biofilm and were resistant to serum bactericidal activity except for one isolate (not related to the outbreak in Plzen). SNP analysis performed on the collected isolates revealed that the isolates of the same hospital were closely related (1 to 11 SNPs) whereas others were diverse. In addition, phylogenetic analysis clustered our outbreak associated isolates within the same subclade.

Conclusion: This study described the emergence and clonal dissemination of the NDM-1-producing P. aeruginosa ST773 isolates causing nosocomial outbreak in the Czech Republic. Index case patient repatriated with a travel history from areas reported to host NDM-1-producing P. aeruginosa strains can inadvertently instigate an outbreak within a hospital environment.

MEGA PIPELINE: AN AUTOMATED PIPELINE FOR EASY MICROBIAL WHOLE GENOME SEQUENCING ANALYSIS

M. Finianos (4th year year of DSP), I. Bitar
Department of Medical Microbiology,Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Ibrahim Bitar, Ph.D

Background: Whole genome sequencing (WGS) has become a standard approach for detailed analysis of microbial isolates. Several bioinformatics tools are manually implemented to perform the required analysis, starting from quality control (qc), assembly, polishing, assemblyqc, uploading to: ResFinder, PlasmidFinder, and pubmlst, and VFAnalyzer; and subsequent annotation.

Accordingly, we developed an automated analysis pipeline containing all the tools necessary for preliminary analysis of microbial WGS.

Methods: the pipeline is separated into 6 stages, starting with the reads' qc, generating a comprehensive report for all reads tested. Then, the assembly, including the trimming of the adaptors, followed by de novo assembly. A polishing step, using the alignment of reads to the assembly. Furthermore, the quality of the assembly is tested. In the final steps, the assembly files are then: typed using MLST, and scanned for resistance genes, Plasmid replicon types, and for Virulence genes. Results are then summarised in a common output table for all isolates. Moreover, isolate specific detailed report are saved in the output, and all generated polished assemblies can be found in a single directory for easy access. This Pipeline is automated by the combination of snakemake and python.

Results: we present a fast and user-friendly microbial WGS analysis pipeline, that requires minimal user input, merely specifying input and output. With a single command, a large number of samples (hundreds up to thousands) can be run in batch. Moreover, the input files can vary from fastq reads to already assembled genomes (downloaded from public databases) which come in handy when doing comparative analysis of large set of samples. The summarized results are saved in a clear and user-friendly format. In addition, the pipeline contains checkpoints, if an output was missing, the failed step is repeated with different commands, accommodating with its user-friendliness.

Conclusion: The pipeline was tested on large batches of reads and was proven to be effective and quick in generating assemblies and results with minimal user attention. Furthermore, the error checking helps the bioinformatics unfamiliar users in generating the results and troubleshooting.

THE STATUS OF THE ADAPTIVE IMMUNITY IN NORMAL MUCOSA OF COLORECTAL CANCER, SIGN FOR CANCER PROGRESS?

E. S. A. Mahmoud (4th year of DSP)
Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): prof. Kari Jussi Hemminki, MD, Ph.D

Background: Little is known about the association of immune cells in normal mucosa (NM) adjacent to colorectal cancer (CRC) with tumor characteristics and prognosis. To better understand the contribution of immune cells in NM in the progress of CRC, we evaluated and quantified the distribution of adaptive immune cells from adjacent normal mucosa to colorectal tumor tissue and correlated it with patient outcomes and different pathological variables.

Methods: patients with liver metastasis of CRC (N=99), who underwent resection of the primary tumor in Pilsen University Hospital between 1999 and 2021 were included in the study. CD3+, CD8+, and CD45RO+ T cells, FOXp3+ regulatory T cells, and CD20+ B cells were stained immunohistochemically on resected specimens of primary CRC and adjacent NM. The density of immune cells was estimated automatically using image analysis software in pairs of NM and pCRC from the same patients. Associations between densities of immune cells in NM and pathological variables (such as tumor stage, tumor grade, and left and right tumor localization) were performed. We compared cell densities between NM and the tumor center (TC) of CRC. Patients were classified as having increased or decreased cell density in TC compared to NM. We performed survival analysis to investigate the association of immune cells in NM and changes in their densities from NM to TC with time to recurrence (TTR), disease overall survival (DFS), and overall survival (OS) after colon surgery.

Results: NM showed significantly greater densities of CD3+, CD8+, and CD45RO+ T cells and CD20+ B cells compared to the tumor center of tumor specimens. The densities of FOXp3+ cells were greater in TC. Increased densities of CD3 in TC vs NM (number of patients =74) have a longer TTR, DFS, and OS compared to the decreased densities (number of patients =22) (hazard ratios < 0.52 and P value < 0.022). T4 stage of CRC was associated with greater densities of normal mucosal CD3+, CD8+, and CD45RO+ T cells and CD20+ B cells compared to T1-3 stages, P. value > 0.042. The tumor grade 3 was associated with smaller densities of CD3+ T cells (median=468) in adjacent normal mucosa compared to grade 1 (median= 972) P value= 0.026 and to grade 2 (median = 993) P value= 0.034.

Conclusion: the observed decrease of quantities of CD3+, CD8+, and CD45RO+ T and CD20+ B cells and the increase of protumor FOXp3+ T cells in normal vs tumor tissue reflect the alterations of the immune milieu and the tumor immunosuppressive environment. The increased densities of CD3+ T cells in TC vs NM are associated with better outcomes, which may reflect the impact of CD3+ T to gain a proper host immune response against CRC. The greater densities of immune cells in normal mucosa of higher T stages may be related to the immune reaction of adjacent normal tissue toward the larger size of the tumor. More studies are needed to investigate the immune reaction of the immune cells in NM vs tumor tissues.

This study was supported by the Ministry of Health of the Czech Republic, grant AZV NU21-03-00506.

MODULATION OF BACTERIAL RESISTANCE AND VIRULENCE USING ANTISENSE OLIGONUCLEOTIDES INTERFERING WITH LIPOPOLYSACCHARIDE SYNTHESIS – A PRELIMINARY STUDY

J. Müller (2nd year of DSP; 1,2), L. Nalos (2,4), J. Horák (1,2), V. Tégl (5), P. Vintrych (6)
(1) First Department of Internal Medicine, Faculty of Medicine in Pilsen, Charles Unviersity,
(2) Biomedical Center, Faculty of Medicine in Pilsen, Charles University,
(3) Department of Microbiology, Faculty of Medicine in Pilsen, Charles University,
(4) Department of Physiology, Faculty of Medicine in Pilsen, Charles University,
(5) Department of Anesthesiology and Intensive Care Medicine, Faculty of Medicine in Pilsen, Charles University,
(6) Department of Cardiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Prof. MUDr. M. Matějovič, Ph.D. (1,2), prof. Ing. J. Hrabák, Ph.D. (2,3)

Background: Multidrug-resistant bacteria belong among the biggest public health threats of the 21st century. On the top of the WHO list of priority pathogens are carbapenem-resistant gram-negative bacteria. The cornerstone of their outermost membrane is lipopolysaccharide (LPS), representing the most potent antigenic structure. The key enzyme in the synthetic pathway of lipid A, an essential component of LPS, is KDO-transferase. Inhibition of this enzyme leads to the attenuation of bacterial virulence and resistance. One of the tools available for this gene-based interventions are antisense oligonucleotides (ASO), which are already approved for clinical use in various diseases, suggesting their favorable safety profile. However, before translation to the clinical setting of intensive care unit and sepsis, a thorough investigation in a large animal model is necessary. The goal of our research is a new gene-based method development based on antisense oligonucleotides targeting essential enzymes of LPS synthesis. In this preclinical study we aimed to assess the safety, optimal dosage, administration, and detection of our ASO construct in the porcine model of sepsis.

Methods: ASO was synthesized as a double-stranded DNA containing a strong bacterial promoter derived from ISEcp1 insertion sequence and reverse complement of a partial Escherichia coli KDO transferase (kdtA gene). The construct was labeled by vitamin B12 and coupled with a hair-pin structure to increase the penetration into bacterial cells and its stability. To further investigate the properties of this molecule, we conducted a preliminary animal study with a 24-hour septic model. Twelve pigs were anesthetized and instrumented (arterial, pulmonary and central venous line catheter). After a recovery period of six hours, animals were assigned to one of four groups based on the administration of bacteria (sepsis vs. control) and dosage of ASO (low vs. high dose). The bacteremia was induced by infusion of E. coli ST131. ASO were administered in a short bolus followed by a continuous infusion until the end of the experiment. Two dosing schemes (200 and 400mcg vs. 300 and 600mcg) were selected, based on the interim PCR data. Extensive laboratory and hemodynamic parameters were collected every 6 hours. Tracheal aspirate, arterial blood, and urine samples from time-points 2-4, as well as tissue samples from the end of the experiment were analysed by PCR and real-time quantitative PCR.

Results: Intravenous administration of ASO to healthy animals was well tolerated. Presence of the construct was detected in the blood in all subgroups across all time-points demonstrating the stability of our molecule. However, only high-dose group animals exhibited suf-

ficient penetration of ASO to the urine and tracheal aspirate. Quantitative determination in samples is currently under validation.

Conclusion: Although several challenges are associated with their development, ASO-based drugs offer a promising strategy for overcoming antibiotic resistance. Our preliminary study provides a foundation for a large randomised animal trial with translational potential.

INTERACTION OF TUMOUR AND STROMAL CELLS IN COMPLEX BIOLOGY OF THE UROTHELIAL CARCINOMA – BIOLOGICAL MECHANISMS AND POSSIBLE CLINICAL APPLICATIONS

M. Dolejšová (3rd year of DSP; 1), M. Kripnerová (2), B. Vítovcová (2), M. Pešta (2), J. Hatina(2)

(1) Biomedical Center, Faculty of Medicine in Pilsen, Charles University
(2) Department of Biology, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): Michaela Kripnerová (2), Jiří Hatina (2)

Background: A tumour represents a complex ecosystem based on mutual interactions between cellular transformed parenchyma and other stromal, mostly normal, cells. The relative contribution of individual cell types to the development and progression of the disease can substantially differ in various types of cancer. One of the possible cited consequences of this complex action of tumour stromal fibroblasts is the promotion of a special type of tumour cells, called cancer stem cells that are thought to be mainly responsible for continuous tumour growth and also for therapeutic resistance, which could be particularly important in possible treatment. Yes-associated protein (YAP) is a key factor in Hippo pathway important for cell migration, drug resistance and also serving as a cancer stem cell regulator. The tumour stroma, mainly CAFs (carcinoma-associated fibroblasts), creates a specific tumour microenvironment, the so-called niche, which supports the stemness of tumour cells. Our aim was to evaluate the possible role of YAP transcription factor in stem cells monitoring in bladder cancer.

Methods: We used a unique co-culture system of stromal cell line BC44Fibr and two GFP positive reporter bladder cancer cell lines with YAP-TEAD transcription dependent mCherry production (5637 mCherry and RT112 mCherry cell lines). Three basic assays were used to assess changes in the representation of YAP-positive cells – flow cytometer (BD, New Jersey, USA), Incucyte® S3 Live-Cell Analysis (Sartorius) and fluorescence microscopy (Olympus IX 81 with equipment, Japan).

Results: Data obtained from Incucyte analysis and flow cytometer showed, that the number of YAP-positive cancer cells in co-culture system was not significantly higher than in monoculture but the phenotype of cancer stem cells was stabilized for an extended period of time and their differentiation was significantly slowed. Additionally, using fluorescent microscopy we observed YAP positive cells being localized in close proximity to stromal cells which can supply them very efficiently with a variety of chemokines. These findings are consistent with our previous results regarding other possible stemness markers (e.g. cytokeratin 17) in bladder cancer, which were presented by our team at previous occasions.

Conclusion: The published results show that especially in the muscle-invasive type of bladder cancer, the stroma has a fundamental influence. CAFs, as highly secretory cells, are the source of a number of growth factors, which significantly influences tumour growth. Hence, CAFs are probably behind the maintenance of the cancer stem cell phenotype. In many cancers, the deregulation of Hippo pathway is well known and transcription factor YAP could be a useful marker for stem cells monitoring in bladder cancer. Stem cells and undifferentiated cells usually have inactive Hippo pathway, and therefore nuclear transcription factor YAP could be detected as a stemness marker. The results of our research could bring a better understanding and and prediction of the possible clinical behaviour of each tumour and thus better individualize the care of patients with urothelial carcinoma.

KIN-HOUSE PANEL FOR DETECTION OF SOMATIC MUTATIONS IN FREE CIRCULATING DNA OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA USING NEXT GENERATION SEQUENCING

R. Klieber (5th year of DSP) (1,2), P. Ostašov (1), M. Holubová (1,2), D. Macečková (1), D. Lysák (2), P. Jindra (2)
(1) Biomedical Center, Faculty of Medicine in Pilsen, Charles University
(2) Department of Hematology-Oncology, University Hospital Pilsen
Supervisor(s): Mgr. Pavel Ostašov, Ph.D. (1)

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common malignant tumour disease of non-Hodgkin's lymphomas in adults. Free circulating DNA (cfDNA) is characterized by short DNA fragments that are released into the circulation by apoptosis, necrosis or are actively released in extracellular vesicles. cfDNA obtained from blood plasma, represents an easily available biological material for molecular genetic testing of patients in whom tumour cells are not typically released into peripheral blood and body fluids. In lymphomas and other diseases, it has been proven that detection of cfDNA and its concentration correlates with the prognosis and the cancer state. Moreover, it allows determination of the mutational profile in selected genes enabling the monitoring of single-nucleotide and short structural variant dynamics during treatment.

Objective: The work focused on the creation of a panel for next-generation sequencing (NGS) for identification and monitoring of somatic mutations in cfDNA in patients with DLBCL, treated at the Hematology-Oncology Department of the University Hospital Pilsen. The main goal was to create a functional multiplex PCR method for the amplification of specific gene regions, which have a known role in DLBCL, and the subsequent construction of sequencing libraries.

Methods: cfDNA of 14 patients with DLBCL was isolated from whole plasma of non-clotting peripheral blood within two hours from collection. cfDNA was subsequently amplified using 49 primer pairs, targeting the coding regions of 9 genes: CDKN2A (exon 1,2,3), BCL6 (exon 3,5,6,7), MYD88 (exon 1,2,3,4,5), CD79B (1,3,5,6), EZH2 (exon 2,3,4,5,9,11,14,16,19), BCL2 (exon 2), TP53 (exon 4,5,6, 7,8,9,10,11), CARD11 (exon 3,6,20,22,23,25) and MYC (exon 2). Magnetically purified multiplex PCR products were then used for NGS library preparation.

Results: Multiplex PCR reactions were optimized, allowing amplification of loci included in the panel, and sequencing data demonstrated sufficient quality and coverage across all loci (except TP53 exon 5) for subsequent analyses. Examination of the mutational profile in cfDNA revealed a several-fold decrease in variants during treatment. The median number of variants detected at the time of diagnosis was 41 (min = 25, max = 94) out of which only 9 (min = 4, max = 16) were detectable after the first treatment cycle and 6 (min = 3, max = 14) after the second cycle of treatment.

Conclusion: Analysis of cfDNA using NGS represents a promising approach, overcoming the general limitations associated with the collection and processing of tissue biopsies. Tracking the mutational cancer profile from cfDNA using panel sequencing has potential applications in diagnostics, monitoring response to treatment, or monitoring minimal residual disease.

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TRANSCRIPTOME DYNAMICS FOLLOWING NEOADJUVANT CHEMOTHERAPY IN OVARIAN CARCINOMA PATIENTS

T. Tesařová (3rd year of DSP) (1,2), K. Šeborová (1,2), M. Hruda (3), L. Rob (3), P. Souček (1,2) and R. Václavíková (1,2)

(1) Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen
(2) Toxicogenomics Unit, National Institute of Public Health, Prague
(3) Department of Gynecology and Obstetrics, Third Faculty of Medicine, Charles University and University Hospital Královské Vinohrady, Prague.

Supervisor(s): RNDr. Radka Václavíková, Ph.D. (1,2)

Background: Ovarian carcinoma (OvC) is one of the most common gynaecological malignancies. Due to the late diagnosis and development of drug resistance, the overall survival is very low. Therefore, the necessity of understanding its molecular complexity is arising. The study of its transcriptome landscape may culminate in findings of potential therapeutic targets as well as prognostic and predictive biomarkers that would allow oncologists to determine the right treatment for each individual patient.

Aims: This study is focused on identifying deregulations in transcriptome between primary OvC tumor tissues and samples after chemotherapy within the interval debulking surgery (IDS) procedure and serves as the first insight into understanding the changes on transcriptome level triggered by this therapeutic approach.

Methods: Samples obtained from primary biopsies and IDS surgeries were collected and stored at -80°C. RNA extraction was carried out using the Trizol method, the total RNA was quantified and the quality of RNA was evaluated by determining the RNA Integrity Number. For library preparation, the Lexogen QuantSeq 3' mRNASeq Library Prep Kit FWD for Illumina sequencing was selected. The choice between standard or low input protocols was made based on the quantity and quality of the total RNA available. Quality control of the prepared libraries was performed using the High Sensitivity DNA kit, and library quantification was done using Qubit with the dsDNA High Sensitivity Assay kit. The libraries, pooled equimolarly (4 nM), underwent sequencing on the NextSeq 500 platform using the NextSeq 500/550 High Output kit v2.5 (1x75 bp). The sequencing depth was ~10 million reads/sample. The sequencing data underwent quality assessment using the FastQC package and trimming with the Fastp package. The STAR aligner was used to align reads to the genome (GRCh38). Subsequent analyses were carried out using R Bioconductor packages, such as DESeq2, ClusterProfiler, and weight gene co-expression network analysis (WGCNA) to identify alterations in the transcriptome landscape and visualize pathways affected by the deregulation or co-expression of gene networks.

Results: A total of 613 significantly deregulated genes (|log2FC|>1; padj<0,05) were identified in the IDS samples compared to the primary biopsy samples including FOBS, NR4A3, CXCL2, DMBT1, SPAG6, NTS and SST. Notably, these genes are involved in pathways related to the cell cycle, including chromosome segregation, organelle fusion or DNA replication. Additionally, WGCNA revealed 21 modules composed of genes exhibiting similar expression patterns, with 11 of these modules were significantly deregulated between primary and IDS samples.

Conclusion: The transcriptional profile obtained from this study could serve as the foundation for establishing reliable prognostic and predictive biomarkers in ovarian carcinoma, thereby contributing to the advancement of personalized medicine.

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NOVEL TAXANE DERIVATIVES IN IN VITRO AND IN VIVO MODELS OF RESISTANT OVARIAN CANCER CELLS

K. Koucká (3rd year of DSP), A. Spálenková, K. Šeborová, M. Ehrlichová, R. Václavíková, T. Sychra, T. Tesařová, L. Chen, H. Bendale, I. Ojima, P. Souček

(1) Laboratory of Pharmacogenomics, Biomedical Centre, Faculty of Medicine in Pilsen, Charles University

(2) Laboratory of Toxicogenomics, National Institute of Public Health in Prague

Supervisor(s): doc. RNDr. Pavel Souček, CSc.

Background: Taxanes are widely used as anticancer drugs, particularly for treating ovarian carcinomas. One of the main problems in therapy is multidrug resistance to conventional taxanes such as paclitaxel (PTX) and docetaxel. This resistance is a multifactorial process that can be related to drug transport, metabolism or alterations in DNA repair, apoptosis induction, etc. by taxanes. New synthesized experimental Stony Brook taxanes (SB-Ts) – SB-T-1214, SB-T-1216, SB-T-121402, SB-T-121605, SB-T-121606, and their biotinylated conjugates (BLT-PTX or BLT-SB-Ts) seem to be promising candidates for overcoming the multidrug resistance in ovarian cancer cells. The aim of our study was to compare the efficacy of conventional and new experimental taxanes in NCI/ADR-RES ovarian cancer cells, the multidrug-resistant model, and to select the most effective taxane derivatives, which were also tested in vivo on cell-derived xenografts.

Methods: Cell viability in the presence of conventional (PTX) and new experimental (SB-Ts) taxanes was measured by CellTiter-Blue Cell Viability Assay after 72 hours of incubation with tested substances. For in vivo experiments, immunodeficient nude (nu/nu) mice were used for establishment of the NCI/ADR-RES-derived xenografts. After tumors developed, mice were treated with PTX, SB-Ts, or their combination intraperitoneally or intravenously twice a week. Control mice received vehiculum. Tumor volume was measured by caliper after every application.

Results: The efficacy of novel experimental taxanes was up to 50 times higher than PTX with SB-T-121605 and SB-T-121606 being the most effective, hence they were chosen for in vivo experiments. The application of SB-Ts slowed down the tumor growth and the combination of 9 mg/kg PTX + 1 mg/kg SB-T-121606 was the most effective therapy with tolerable toxicity. To decrease the toxicity, biotinylated (BLT) conjugates were tested in vitro and showed lower efficacy than unconjugated taxanes. These conjugates will be tested in vivo in our future experiments.

Conclusion: Stony Brook taxanes effectively overcome the resistance in NCI/ADR-RES cell line model both in vitro and in vivo. Biotinylated-conjugates of these taxanes will be further tested because of their lower toxicity.

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ANALYSIS OF GENE EXPRESSION FOR GFAP IN HEART AND GANGLIA OF DIABETIC RATS

T. Šmrhová (3rd year year of DSP)
(1) Biomedical center, Faculty of Medicine in Pilsen, Charles University
(2) Department of Physiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. M. Chottová Dvořáková, Ph.D.

Background: Diabetes mellitus (DM) is a chronic metabolic disease which is, among others, a primary healthcare challenge of the twenty-first century. Its prevalence, along with its complications, is rising rapidly. One of the most common complications of both types of DM is diabetic neuropathy, caused by persistent hyperglycaemia that interacts with inflammatory pathways at various levels to cause structural and functional damage to the peripheral nervous system. Cardiac autonomic neuropathy is the most clinically important form of diabetic autonomic neuropathy. Glial fibrillary acidic protein (GFAP) is an intermediate filament protein, which was adopted as a marker of satellite glial cells (SGCs), the supportive cells enveloping the somata of peripheral sensory and autonomic neurons. Its elevated expression was recognized as an indicator of gliosis associated with nerve injury and disease.The aim of this research is to investigate the effect of DM on gene expression of GFAP within individual heart compartments and in selected peripheral nervous ganglia in animal model of type 2 DM.

Methods: Zucker diabetic fat (ZDF) rats were sacrificed by decapitation at week 40 of age, heart atria and ventricles, stellate ganglia and upper thoracic dorsal root ganglia (DRG) were dissected. From gathered samples, total RNA was isolated. Obtained RNA was reverse-transcripted and subsequently RT-qPCR analysis was done. Relative expression of mRNA for GFAP was expressed as a ratio of target gene concentration to control gene. Results were considered significantly different when $p<0.05$. Additionally, sections of tissues were examined using the methods of immunofluorescence.

Results: Relative expression of mRNA for GFAP was significantly upregulated in stellate ganglia. On the contrary, we did not observe a significant change of expression within any of the heart chambers or DRG.

Conclusion: Changes of expressions show that GFAP is a valid marker of satellite glial cell reactivity to nerve injury caused by DM.

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KMT2A REARRANGEMENT IN MUCOEPIDERMOID CARCINOMA WITH CRTC1::MAML2 FUSION

B. K. Othman (2nd year of DSP), Alena Skálová
Department of Pathology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): prof. MUDr. Alena Skálová CSc.

Background: Mucoepidermoid carcinoma (MEC) is a malignant salivary gland neoplasm characterized by mucous, intermediate and epidermoid (squamoid) tumor cells forming cystic and solid growth patterns, usually associated with MAML2 rearrangement. MECs are graded into three grades using a variety of grading systems, but the behavior in individual cases is sometimes difficult to predict based on grading alone. In this study, we correlated 27 MECs with MAML2 rearrangement, morphology, AFIP grading, immunohistochemical findings, detection of KMT2A gene, and clinical outcome.

Methods: All tumors were examined histologically, immunohistochemically (p63, CK7, S100, and SOX10), and molecularly using next generation sequencing (NGS) and fluorescence in situ hybridization (FISH). MAML2 gene rearrangement was the inclusion criterion. Excluded cases were MECs from pediatric and young populations (less than 24 years). For FISH assays, dual-color break-apart probes (ZytoVision, Germany) were employed to identify KMT2A and MAML2 rearrangements at 11q23.3 and 11q21, respectively.

Results: We collected a cohort of 27 cases of primary MECs from non-leukemic patients. The tumors were graded according to the AFIP. Gender distribution showed predominance of female patients (67%) and an average age of 56.9 years (range 25 to 78). Majority of the tumors originated from the parotid gland (67%), followed by the palate (15%), the sublingual gland (7%), the tongue (7%), and the retromolar region (4%). In 24 cases, the MECs had a canonical CRTC1::MAML2 fusion, while one of them also had a mutation in HRAS gene. In the 3 other cases, a rearrangement in MAML2 gene was detected by FISH. Rearrangement in KMT2A gene was detected according to cutoff value of nuclei positive for break-apart signal in 13/25 cases (52%, cutoff 10%), 10/25 cases (40%, cutoff 15%), or 5/25 cases (25%, cutoff 30%), respectively.

Conclusion: Regardless of AFIP grading, MECs with KMT2A rearrangement tended to have a more aggressive clinical outcome with recurrences in 5/27 cases (18%) and metastases in 1/27 cases (4%), with cases defined at the 30% cutoff level. Such cases also displayed altered SOX10 expression.

PROGNOSTIC FACTORS IN SURGICAL TREATMENT OF HIGH GRADE GLIOMAS

R. Koleják (3rd year of DSP) (1), M. Švajdler (2), J. Polívka jr., M. Vítovec (3), K. Houfková (4), J. Mraček (1), V. Přibáň (1)

(1) Department of Neurosurgery, University Hospital Pilsen
(2) Department of Pathology, University Hospital Pilsen
(3) Department of Imaging Methods, University Hospital Pilsen
(4) Department of Biology, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): doc. MUDr. V. Přibáň, PhD.

Background: 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. The first part of this study is the analysis of a retrospective group of patients and evaluation of factors impacting the surgical treatment.

The second part is the analysis of a prospective group of patients, evaluation of prognostic factors, including methods such as NGS, liquid biopsy and volumetry.

Methods: 143 patients, operated in Department of Neurosurgery, Pilsen, in years 2015-2021, were analyzed in the retrospective group. 68 patients were lost to followup and thus, not included in the analysis.

31 patients have been added to the prospective group up to this day.

Both groups have been compared.

Results: Demographics: Male patients were more numerous in both groups (57% and 67%, respectively). The average age was similar, 61 years in retrospective group, 60 years in prospective group. Average Karnofsky index at the time of admission was 80 and 75. Average followup was longer in the retrospective group (499 days vs 209 days). Surgery: The average delay of surgical treatment was 15,8 days in years 2015-2021, and 13,5 days from year 2022 and onward. Higher percentage of patients suffered from tumors in eloquent areas in the retrospective group (60% vs 48%), and thus required neurophysiological monitoring more often (56% vs 52%). However, the rate of 5-ALA usage (51% vs 80%) and partial resection (51% vs 38%) favors the prospective group. Adjuvant therapy: The prospective group has received adjuvant therapy in higher percentage compared to retrospective group (93% vs 64% for radiotherapy and 87% vs 48% for chemotherapy), which was connected with a lower rate of recurrence or progression (48% vs 71%).

Histology: A higher number of patients suffered from grade 4 tumor in the prospective group (90% vs 71%). Survival: The overall survival was higher in the retrospective group (345 days vs 276 days), as well as progression free survival (258 days vs 190 days). Meanwhile, survival from progression was longer in the prospective group (162 days vs 88 days)

Conclusion: In comparing the two groups we can see a decrease in partial resection rates coupled with a higher use of 5-ALA in the prospective group. The survival results are logically lower in the prospective group, as followup started in spring of 2022. Overall, the results correspond with the literature. The next step – liquid biopsy and NGS analysis of the prospective group is underway and has not yet been finalised.

NEW WAYS TOWARD LIVER TISSUE ENGINEERING: HOW TO TURN A FALCON TUBE INTO A BIOREACTOR?

M. S. Massaro (5th year of DSP; 1), L. Bolek (2), J. Dejmek (2), L. Červenková (1,3), M. Brousil (1), R. Pálek (1,4), J. Ševčík (1,4), S. Šarčević (1,4), I. Matsviayonak (1), D. Volák (1), V. Liška (1,4) and V. Moulisová (1)

(1) Ústav mikrobiologie LF UK v Plzni a FN Plzeň, (2) Biomedicínské centrum LF UK v Plzni, (3) I.
(1) Biomedical Center, Faculty of Medicine in Pilsen, Charles University
(2) Department of Biophysics, Faculty of Medicine in Pilsen, Charles University
(3) Department of Pathology, 3rd Faculty of Medicine, Charles University
(4) Department of Surgery, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): RNDr. Vladimira Moulisová, Ph.D. (1)

Background: Among the tissue engineering (TE) strategies for organ reconstruction, decellularization has emerged in the last two decades. Detergents, enzymes, physical methods, or a combination of them, are used to completely remove cells and DNA. This leads to the non-immunogenicity and the preservation of the native macro and microstructure. Since the scaffold loses its functionality, TE techniques are necessary to repopulate the matrix with the appropriate cells. After reaching a good decellularization of the entire porcine liver scaffold, our goal is now the functionalization with hepatocytes and endothelial cells.

Methods: The system is composed of a reservoir bottle for the media oxygenation and a distribution line to supply the nutrients in the bioreactor through a peristaltic pump. The chamber prototype was a polypropylene (PP) 50 ml Falcon tube tilted horizontally and drilled to create 4 inlets at the top and one outlet at the bottom. Luer threads inserted in the holes and sealed with rubber o-rings were used to connect the recirculating tubes. The scaffolds lied above a polymeric insert designed in Solidworks™ and 3D printed. It created 4 individual parts inside the chamber to hold 4 scaffolds independently. Our liver scaffolds were then repopulated with human cells and kept in the bioreactor for growing.

Results: The bioreactor was able to sustain the media recirculation up to a week, with the potential to be stable for several days. No damage resulted in the PP prototype after multiple cycles of sterilization with plasma. Both human hepatocyte-like and endothelial cells survived up to a week inside the scaffold, thus demonstrating the efficacy of the system to supply nutrients and oxygen.

Conclusion: The falcon tube chamber is an economic and simple prototype able to guarantee the microenvironment needed to support cells growth on our decellularized scaffold. It could potentially be applied to different decellularized organs or hydrogels structures. The importance to start the repopulation from small pieces lies in the easier reproducibility, but these results can be used for larger pieces and, in the future, the entire organ.

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SURGICAL SEALANT WITH INTEGRATED SHAPE-MORPHING DUAL MODALITY ULTRASOUND AND COMPUTED TOMOGRAPHY SENSORS FOR GASTRIC LEAK DETECTION

S. Šarčević (2nd year of DSP; 1,2), B. Suter (3), I. Herrmann (3), A. Anthis (3), J. Ševčík (1,2), D. Vydra (2), T. Polenda (2), I. Matsviayonak (2)

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

(2) Biomedical Center, Faculty of Medicine in Pilsen, Charles University

(3) Department of Mechanical and Process Engineering, ETH Zurich

Supervisor(s): prof. MUDr. Václav Liška Ph.D. (1,2), MUDr. Jáchym Rosendorf Ph.D. (1,2)

Background: Anastomotic leaks are a serious complication after abdominal surgery. In gastric procedures like gastrectomies and bariatric surgeries leak rates can reach up to 5%. With the growing number of gastric cancer and obesity cases worldwide, more patients are likely to suffer from gastric leaks. Current methods for detecting leaks are limited, often relying on symptoms that appear only after the leak is fully developed. A novel adhesion technology has been introduced that uses tissue-penetrating polymer networks to anchor hydrogel materials, allowing for better sealing even in harsh digestive conditions. It also enables the incorporation of sensing elements for early leak detection. We wanted to develop a surgical sealant with shape-morphing leak-detection sensing elements that can be detected by ultrasound and CT imaging.

Methods: Differently shaped sensing elements were embedded into a layered adhesive hydrogel sealant with a non-adhesive backing. These sensors were made with barium or lanthanum carbonates, which liberate gaseous CO2 upon contact with the acidic gastric fluid. This increases their ultrasound echogenicity and reduces X-ray absorbance. Tantalum oxide was used as a non-reactive reference element. Two pigs were used for proof-of-concept in vivo experiments. Sensing patches were applied to defective and intact stomach tissue sites by a mutually interpenetrating network, followed by UV irradiation of the surgical site. CT images were acquired at distinct time points 0, 3, and 6 hours after surgical implantation.

Results: The anchored hydrogel successfully sealed the stomach defects during the experiment and firmly attached to the tissue serosa, as confirmed by histological analysis. Within 3 hours after surgery, changes in the sensing element shapes were noticeable in the gastric leak scenario. By 6 hours post-surgery, it was easy to see the difference between the reacted and unreacted sensing elements based on CT data. The hydrogel patches not in contact with gastric fluid showed no change throughout the entire investigation period. At the 6-hour mark, ultrasound imaging revealed a distinct bright spot, matching well with the CT signal and standing out from surrounding tissues. Upon opening the abdomen for visual inspection, control sensing patches remained unchanged, while those in contact with gastric fluid showed the carbonate-containing part converted into CO2 bubbles trapped in the hydrogel. The part containing the tantalum oxide remained the same.

Conclusion: We introduce a novel method for detecting gastric leaks after surgery, which are a common and serious complication. Our method uses both CT and ultrasound, allowing for frequent screening and fast leak detection, improving treatment outcomes. Since these imaging methods are already widely used in clinics, our approach can be easily adopted. By embedding these sensors into surgical sealants, we can catch leaks early, before they be-

come life-threatening. We also show how the sensors change shape under leak conditions, making them easy to spot in medical images without specialized equipment.

ANTIMALARIAL RESISTANCE IN IMPORTED INFECTIONS CAUSED BY PLASMODIUM FALCIPARUM

M. Stejskalová (4th year of DSP)
Department of Microbiology, University Hospital in Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. Milan Trojánek, Ph.D.

Background: Tropical malaria caused by Plasmodium falciparum is one of the most frequent imported malaria from high-risk destinations to the Czech Republic. The choice of therapy to treat malaria depends on several factors, including severity of the infection, the geographic location where the infection was acquired and individual patient factors (age, pregnancy status and medical history). A number of drugs are used for the treatment of malaria, especially quinine, chloroquine, sulfadoxine/pyrimethamine, mefloquine or atovaquone/proguanil. However, Artemisinin combination therapy (ACT) or parenteral artemisinins, are generally the firs-line treatment for uncomplicated tropical malaria in this country and worldwide. As is the case with antibiotic treatment, resistance to antimalarials began to emerge as a consequence of the treatment used. A number of mutations have been discovered by using molecular methods, that contribute to the failure of malaria therapy. In P. falciparum, these include mutations associated with the pfcrt gene, the pfmdr1 gene associated with multi-drug resistance, the dhfr gene, the cytochrome B gene, kelch13 gene and many others. Resistance to amtimalarial drugs is a significant problem that poses a serious threat to malaria control. Reduces the effectiveness of antimalarial threatment can lead to treatment failure, increased morbidity and mortality. Whole genome sequencing of P. falciparum and detection of genetic markers of antimalarial resistance from patients with imported infection.

Methods: Detection of hotspot mutations in the plasmodial genome affecting antimalarial therapy failure using bioinformatics analysis of data obtained by whole genome sequencing.

Results: The emergence of antimalarial drug resistance underscores the need for continued investment in research and development of new antimalarial drugs and treatment strategies. Conclusion: Developing drugs with novel mechanisms of action and optimizing drug combinations to combat resistance are ongoing challenges in the field of malaria research.

VERIFICATION OF THE EFFICACY OF TRADITIONAL KARLOVY VARY DRINKING CURE WITH WARM MINERAL SPRINGS IN THE TREATMENT OF NAFLD

A. Huseynli (2nd year of DSP)
Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. L.Špišák, CSc.

Background: Comprehensive spa treatment in Karlovy Vary includes drinking mineral water, diet and exercise. In addition, patients are prescribed therapeutic procedures within the framework of balneotherapy, physiotherapy and medical rehabilitation. It is assumed that spa treatment can be one of the approaches to the treatment of NAFLD, especially for milder degrees, or it can also be seen as a complementary therapy to the main treatment plan in case of more severe disability. The objective of this research is to objectively assess the effectiveness of the traditional drinking therapy using Karlovy Vary mineral springs, focusing on the quantitative measurement of lipid parameters and liver elasticity before and after a 21-day course of the therapy. The study encompasses randomly selected subjects from the Karlovy Vary population, aiming to scientifically validate the health benefits associated with this long-standing therapeutic practice, particularly in terms of metabolic health and liver function.

Methods: The research included a multifaceted approach to health assessment, involving blood tests for liver function markers (serum bilirubin, AST, ALT, GMT, ALP), lipid metabolism indicators (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, atherogenic index), glycemia, and uric acid levels. Furthermore, a FibroScan examination was conducted, employing the Controlled Attenuation Parameter (CAP) elastography method to assess the degree of liver steatosis and potential fibrosis. Conducted from February to May 2023, the research selected participants through a blind, random process facilitated by the Karlovy Vary Information Center, ensuring a non-biased sample from the local population. Out of 500 applicants, 80 were chosen for this exploratory study. The study received approval from the Ethics Committee of the University Hospital in Pilsen, and all participants were fully informed about the study's design.

Results: Preliminary findings revealed that 72% of participants presented with hepatic steatosis, with 26% exhibiting severe, grade 3 steatosis. Remarkably, post-treatment analysis showed significant improvements in FibroScan parameters, highlighting the therapy's potential in mitigating liver steatosis. These results underscore the importance of further research into this traditional therapy, considering the progression of untreated steatosis to more severe liver diseases, such as steatohepatitis, cirrhosis, and liver cancer.

Conclusion: This study provides a first step towards recognizing and harnessing the therapeutic benefits of Karlovy Vary mineral springs, offering promising avenues for the prevention and treatment of liver-related ailments.

ARTIFICIAL INTELLIGENCE CAN GENERATE FRAUDULENT BUT AUTHENTIC-LOOKING SCIENTIFIC MEDICAL ARTICLES

M.Kasal (5th year of DSP), M. Majovský, M. Černý, M. Komarc, D. Netuka
Department of Psychiatry, Faculty of Medicine in Pilsen
Supervisor(s): prof. MUDr. Jan Vevera, PhD.

Background: Artificial intelligence (AI) has advanced substantially in recent years, transforming many industries and improving the way people live and work. In scientific research, AI can enhance the quality and efficiency of data analysis and publication. However, AI has also opened up the possibility of generating high-quality fraudulent papers that are difficult to detect, raising important questions about the integrity of scientific research and the trustworthiness of published papers.

Objective: The aim of this study was to investigate the capabilities of current AI language models in generating high-quality fraudulent medical articles. We hypothesized that modern AI models can create highly convincing fraudulent papers that can easily deceive readers and even experienced researchers.

Methods: This proof-of-concept study used ChatGPT (Chat Generative Pre-trained Transformer) powered by the GPT-3 (Generative Pre-trained Transformer 3) language model to generate a fraudulent scientific article related to neurosurgery. GPT-3 is a large language model developed by OpenAI that uses deep learning algorithms to generate human-like text in response to prompts given by users. The model was trained on a massive corpus of text from the internet and is capable of generating high-quality text in a variety of languages and on various topics. The authors posed questions and prompts to the model and refined them iteratively as the model generated the responses. The goal was to create a completely fabricated article including the abstract, introduction, material and methods, discussion, references, charts, etc. Once the article was generated, it was reviewed for accuracy and coherence by experts in the fields of neurosurgery, psychiatry, and statistics and compared to existing similar articles.

Results: The study found that the AI language model can create a highly convincing fraudulent article that resembled a genuine scientific paper in terms of word usage, sentence structure, and overall composition. The AI-generated article included standard sections such as introduction, material and methods, results, and discussion, as well a data sheet. It consisted of 1992 words and 17 citations, and the whole process of article creation took approximately 1 hour without any special training of the human user. However, there were some concerns and specific mistakes identified in the generated article, specifically in the references.

Conclusions: The study demonstrates the potential of current AI language models to generate completely fabricated scientific articles. Although the papers look sophisticated and seemingly flawless, expert readers may identify semantic inaccuracies and errors upon closer inspection. We highlight the need for increased vigilance and better detection methods to combat the potential misuse of AI in scientific research. At the same time, it is important to recognize the potential benefits of using AI language models in genuine scientific writing and research, such as manuscript preparation and language editing.

ARE WE DEIFYING PSYCHOLOGICAL TRAUMA? PITFALLS OF THE TRAUMA-INFORMED NARRATIVE

V. Pisl (3rd year of DSP)

Psychiatry Clinic, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University

Supervisor(s): prof. MUDr. J. Vevera, PhD.

Background: The popularity of the trauma model of psychopathology is rising, while the notion of trauma is extending into a 'broad trauma', comprising any adverse experience with long-term effects. While problematic aspects of the dominant biomedical model are known, the increasingly influential broad trauma model is lacking critical attention. We examine the discursive practices justifying and promoting the trauma-informed care: a set of assumptions and clinical recommendations based on the broad trauma model, which are presented as universal and uncontested guidelines for mental health practitioners.

Methods: Critical discourse analysis served as a framework for analysis of two major guidelines for trauma-informed care. Building upon previous works analyzing discursive mechanisms asserting dominance of the biomedical model in mental healthcare, we have analyzed the practices justifying and promoting the model underlying the trauma-informed principles.

Results: The trauma-informed care is derived from the notion that care users and providers should all be treated as potentially traumatized. We have identified mechanisms that (1) present the broad trauma model as a neutral, assumption-free description of reality, (2) portray it as superior to other models, and (3) encourage patients to see themselves as traumatized.

Conclusion: Discursive procedures found in the trauma-informed manuals are similar to those documented in biomedical psychiatric literature. Several risks may arise, including iatrogenic harm, politization of mental healthcare, reducing diversity and cost-effectiveness of mental healthcare, establishing trauma as the default cultural reaction to stress, and violating ethical standards. Evaluation of the guidelines from the perspective of safety, cultural validity, ethics, and cost-effectiveness should precede their implementation to clinical practice.

THE IMPACT OF MINORITY STRESS ON TRANSGENDER PATIENTS AND ITS DEVELOPMENT DURING HORMONAL TREATMENT

D. Kestlerová (2nd year of DSP)
Psychiatry Clinic, University Hospital Pilsen
Supervisor(s): doc. MUDr. L. Fiala, Ph.D. MBA

Background: "There is no statistically significant difference in anxiety and depression (as measured by the HAM-A, Beck-II) in transgender individuals due to minority stress (as measured by the HHRDS) before and after starting hormone therapy." Our study aims to investigate the impact of minority stress on transgender individuals and whether it leads to depressive or anxiety symptoms. If so, we also want to investigate whether rates of these symptoms change over time in relation to hormone treatment. Our research will help us understand the impact of possible stigma on transgender patients and whether and how hormone therapy affects symptoms that may accompany minority stress. Such a study has not yet been conducted in the Czech Republic.

Methods: We plan to conduct a study with 60 respondents. All patients who meet the entry criteria will be offered participation in the study. The selected patients will be newly diagnosed transgender individuals, both MtF and FtM, so that the two groups can be compared. Before starting hormone therapy, patients undergo a standard initial sexological and psychological examination, as well as a gynecological examination for women undergoing FtM transition. Basic biochemical blood tests and a blood hormone profile, which includes a set of central and peripheral hormones according to the current sex, will also be taken. Once these initial tests are completed, patients complete the Heterosexist Harassment, Rejection, and Discrimination Scale (HHRDS) with a sexologist. This questionnaire contains 14 items regarding the level of perceived discrimination or stigmatization and other distal minority stressors, including microaggressions, exclusion, and bullying. Furthermore, patients complete the Hamilton Scale of Anxiety-A (HAMA-A) questionnaire with a sexologist. This questionnaire can be used to measure the severity of anxiety symptoms. It consists of 14 items, each defined by a number of symptoms, and measures psychological distress (mental restlessness and psychological distress) and somatic distress (physical distress associated with anxiety). Finally, patients complete the Beck inventory BDI-II with a sexologist to assess depression. It is a self-report depression severity scale that consists of 21 items. These tests will be repeated every three months for at least one year.

Results: A pilot study conducted on 20 transgender patients before the start of treatment confirmed that these patients had an increase in the values of the HAM-A and BECK-II questionnaires.

Conclusion: It is still not clear whether hormonal therapy can have a positive impact on the psychological state of the patients being monitored.

EXPLORING MICROGLIAL CHANGES CEREBELLAR MOLECULAR LAYER IN LURCHER MICE: A QUANTITATIVE STUDY

A. Voinilovych (5th year of MSP), O. Yakushko (1), D. Jelínková (2)
(1) Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University
(2) Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Mgr. Y. Kolinko, Ph.D. (1)

Background: Microglial cells, the resident immune cells of the central nervous system, play a pivotal role in maintaining brain homeostasis and responding to pathological insults. Lurcher mice, exhibiting Purkinje cell degeneration, present an opportunity to explore microglial responses in this context, especially in the molecular layer of the cerebellum. Quantitative assessment of the state of microglia in Lurcher mice promises to advance our understanding of neurodegenerative disorders and guide the development of targeted therapies. In this study, we aimed to quantitatively compare the morphological features of cerebellar molecular layer microglia in healthy 3-month-old wild-type (n=5) and Lurcher mutant mice (n=5) of the same age.

Methods: The tissue samples were processed into 60-μm-thick serial sections and systematic random sampling of the section was provided. Selected slides were immunostained using allograft inflammatory factor 1 (Iba1) antibodies (Jackson Immuno Research, UK) to visualize microglial cells and their processes. A computer-assisted stereology system combined with a Nikon Ti microscope was used to quantify parameters including volume of the cerebellar molecular layers, total number of microglial cells, total length of the cell's processes, and their associated densities.

Results: Similar to the previous study, we observed dramatic reductions in the total volume of the molecular layer in Lurcher mice by 87% ($p=0.01$). Concurrently, the total number of microglial cells was halved. This led to a significant ($p=0.01$) fivefold increase in microglial cell density in Lurcher mice compared to wild-type mice. The total length of microglial processes uniformly decreased in accordance with the reduction in the volume of the molecular layer in Lurcher mice. Therefore, significant changes in the length density of processes were not observed. Nevertheless, when recalculating the average length of processes per cell, it was found that Lurcher mice exhibited only 12% of the average length compared to mice of the wild-type. These findings indicate the activity of microglia in this period of degeneration and contribute to our understanding of microglial involvement in neurodegenerative processes.

Conclusion: In conclusion, our findings underscore the pronounced alterations in cerebellar microglial dynamics accompanying Purkinje cell degeneration in Lurcher mice and may inform future therapeutic strategies targeting neuroinflammation in cerebellar degenerative disorders.

DIFFERENCES IN MICROGLIAL MARKER IN THE CEREBELLUM OF MUTANT MICE LURCHER AND PCD

E. Kosová (4th year of MSP)
Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. Zdeňka Purkartová, Ph.D., Mgr. Štěpán Kápl, doc. MUDr. Jan Cendelín, Ph.D.

Background: Hereditary cerebellar degenerations represent a heterogeneous group of diseases pathogenesis of which is not fully understood. Mutant mice Lurcher and Purkinje cell degeneration (pcd) are models of two of these diseases. Both mutants suffer from an early onset, rapid and almost complete degeneration of Purkinje cells and a reduction of cerebellar cortical interneurons and deep cerebellar nuclei. While Lurcher mice show neuropathology selectively in the olivocerebellar system, in pcd mice, the degeneration also affects the olfactory bulb, thalamus, or retina. Although cerebellar neuropathology as such is relatively similar in both of these mice, some studies suggest differences in local cerebellar niche. We hypothesize presence of differences in microglia activation accompanying massive neuronal degeneration, which might be one of the important local factors responsible for secondary changes. The aim of the study was to compare density of microglial marker Iba1 in individual cerebellar structures in Lurcher and pcd mice.

Methods: We have used three-months-old Lurcher mutant mice and healthy animals of the B6CBA strain and pcd and healthy mice of the B6.BR strain. The mice were euthanized by overdosing with anesthetics and transcardially perfused for tissue fixation. In frozen brain sagittal sections (8–12 per animal), microglia were labelled using anti-Iba 1 primary antibody and fluorescent secondary antibody. In images acquired using a fluorescent microscope, sample zones were randomly assigned in individual cerebellar layers (molecular layer, granular layer, white matter, cerebellar nuclei; 1–2 samples per structure and section). In these zones, intensity of fluorescent signal was measured using ImageJ software as an estimate of relative density of Iba 1.

Results: Both Lurcher mutant and pcd mice showed significantly higher intensity of Iba 1 signal in almost all cerebellar layers than their respective controls. Pcd mice had higher signal density than Lurcher mice throughout the whole cerebellum. We also found strain differences in healthy mice in the granular layer and cerebellar nuclei where B6.BR mice had higher densities than B6CBA mice.

Conclusion: Iba1 signal density is determined by density of microglia and by activity of individual microglial cells. The results showed increased microglial activity in both mutants. Strain differences were more marked in mutant mice. It suggests that neurodegeneration and its type have an impact on microglia. It might be one of the factors influencing local neurogenic potential of the tissue, plasticity, and potentially also response to neurostimulation, cell-based or regenerative therapies.

This study was supported by Cooperatio (research area NEUR).

INFLUENCE OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) AND LEUKEMIA INHIBITORY FACTOR (LIF) ON DIFFERENTIATION OF EMBRYONIC NEURAL STEM CELLS (ENSC) IN VITRO

A. Hudák (3rd year of MSP), V. Přerost
Department of Biology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): RNDr. Z. Houdek, Ph.D., doc. RNDr. M. Pešta, Ph.D.

Background: Given the increasing prevalence of neurodegenerative diseases associated with population aging, our studies aim to investigate the use of neurotrophic factors such as LIF and MIF in the differentiation of embryonic neural stem cells (eNSC) into functional neurons and glia. At optimal concentrations, both MIF and LIF individually show a significant positive effect in directing the differentiation of mouse eNSCs (meNSCs) into neurons and glia, and in enhancing neurite outgrowth, respectively. To statistically prove the viability of using MIF and LIF for differentiation of meNSCs into neurons and glia, at optimal concentrations.

Methods: Multiple trials with varying concentrations and duration of action of MIF were tested, and the optimal settings were determined to be 20 ng/ml. Cell counts (neurons and astrocytes) from trials with other tested concentrations were compared against the 20 ng/ml trial using a paired t-test. Same test was performed on the group treated with LIF with its optimal working concentration of 100 ng/ml against its control. Calculations were made using the predefined MS-Excell function for the t-test.

Results: The result of the t-test for the 20 ng/ml MIF group versus other concentrations shows a statistically significant difference in cell and astrocyte counts, proving it to be the optimal concentration. In the same way, the treatment with LIF at 100 ng/ml was proven to be optimal and statistically significant.

Conclusion: The t-test has proven that the optimal concentrations – 20 ng/ml MIF and 100 ng/ml LIF – allowed for a statistically significant directed differentiation of meNSCs into neurons and glia. This opens an avenue for exploring the effects of MIF and LIF in human NSC, in three-dimensional cell cultures as well as their synergistic effect.

QUANTITATIVE IMMUNOHISTOCHEMICAL ANALYSIS OF ALPHA-SM ACTIN POSITIVE ELEMENTS IN HUMAN INFERIOR VENA CAVA, PORTAL VEIN AND COMMON ILIAC VEIN, A PILOT STUDY

M. Zavřelová (2nd year of MSP), K. Kunčarová

(1) Department of Anatomy, Faculty of Medicine in Pilsen, Charles University (2) Biomedical Center, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): doc. MUDr. Lada Eberlová (1, 2), Ph.D.; Mgr. et Bc. Patrik Mik, Ph.D. (1, 2), MVDr. Lenka Červenková (2)

Background: In our previous study, structure of the porcine portal vein and caudal vena cava was assesed with regard to the allografts and venous reconstructions. In this study, in agreement of the previous study methodology, we aim to compare the structure of the human with the pig abdominal veins to estimate their potential for xenotransplantations. In this pilot phase the amount of contractile proteins in the wall of the human venous counterparts – the supra- and infrahepatic inferior vena cava (IVC), the portal vein (PV), and the common iliac vein (CIV) – were evaluated.

Methods: Tissue samples were obtained in cooperation with the Institute of Forensic Medicine, Medical Faculty in Pilsen, from 15 donors (8 men and 7 women), age 47–85 years. Samples were stitched to cork plates, fixed in 10% buffered formalin, and processed to 3 μm paraffin sections. Contractile elements were visualized using immunohistochemical reaction against alpha-smooth muscle actin. Based on streological principles, area fraction (AA) of the alpha-smooth muscle actin (SMA) was estimated within the intima and media in 4 microphotos captured from each section in a systematic uniform random manner, 20x objective. The point-counting method and the Ellipse software (ViDiTo, Košice, Slovakia) was used for the quantification, the paired t-test, Mann-Whitney U test and bootstrapping for the statistical analysis.

Results: The mean AA of SMA was 34.07%±5.85% (mean±SD) in the infrahepatic IVC, 32.89%±10.48% in the in the suprahepatic IVC, 41.41%±9.08% in the PV, and 41.80%±13.29% in the CIV. The intraindividual difference was statistically significant between the SMA positivity of the intima and media in the infrahepatic IVC and the portal vein ($p = 0.02$). In agreement with the porcine venous counterparts from our previous experiment, the proportion of actin-positive cells was greater in the PV to the IVC grafts. When compared to the porcine IVC (9.34% ± 8.13%), the human IVC had significantly larger AA (SMA) both supra- and infrahepatically ($p < 0.01$).

Conclusion: The different structure of the venous wall in humans is probably due to different hemodynamics compared to quadrupeds. Mapping the representation of different proteins using the method of quantitative histology provides data for cross-species comparisons as well as for the development of biomaterials.

COMPREHENSIVE MOLECULAR CHARACTERIZATION OF SINONASAL ADENOID CYSTIC CARCINOMA: IDENTIFYING NOVEL GENE FUSIONS AND MUTATIONS

I.Kovářová (5th year of MSP)
(1) Department of Patology, Faculty of Medicine in Pilsen, Charles University
(2) Biopticka Laboratory Ltd., Pilsen
Supervisor(s): prof. MUDr. Alena Skálová, CSc. (1,2), RNDr. Radek Šíma, Ph.D. (2)

Background: Sinonasal adenoid cystic carcinoma (AdCC) is an aggressive salivary gland malignancy without effective systemic therapies. Therefore, it is urgent to search for potentially targetable genetic alterations associated with AdCC. AdCC is characterized by alterations in MYB, NFIB or MYBL1 genes with MYB::NFIB or MYBL1::NFIB fusion transcripts in most cases, but these alterations have no prognostic significance and cannot be used to treat patients.

Methods: We have searched the authors' registry, and selected 94 cases AdCCs arising in the sinonasal tract. The tumors were examined histologically, immunohistochemically, and by next generation sequencing (NGS) and/or fluorescence in situ hybridization (FISH), looking for MYB/MYBL1 and/or NFIB gene fusions or any novel gene fusions/mutations.

Results: We collected a cohort of 94 cases of sinonasal AdCC. The group included 44 men and 40 women (in 10 cases gender not known) with an average age of 59,7 (20-89).Tumors were located in nasal cavity and nasopharynx 37 times, in maxillary sinus 30 times, in sphenoid sinus 9 times and in other parts of the sinonasal tract 18 times. AdCC was largely characterized by canonical MYB::NFIB (49 cases) and MYBL1::NFIB (9 cases) fusions. In additional 11 cases of AdCC there were rearrangements in MYB or NFIB genes detected by FISH. Additionally, NGS revealed novel non-canonical EWSR1::MYB, ACTB::MYB, ESRRG::DNM3 and ACTN4::MYB fusion transcripts, each in one case. The following genes with well-known roles in oncogenesis were found to be mutated in 25 tumors, including NOTCH 1,3 (5/25; 20%), BCOR (3/25; 12%), SMARCA4 (2/25; 8%), RIT1 (2/25; 8%), KDM6A (2/25; 8%), SPEN (2/25; 8%), EP300 (2/25; 8%), MGA, RB1, PHF6, PTEN, PBRM1, MTOR, CREBBP, DDX41, CHD2, ROS1, TAF1, CCD1, NF, PALB2, RUNX1, AVCR1B, ARID1A, PPM1D, LZTR1, IDH1, GEN1 and PDGFRA, each of them in one case (1/25; 4%). Additional 24 cases revealed a spectrum of gene mutations of uncertain pathogenetic significance.

Conclusion: There was no morphological difference in AdCC with MYBL1::NFIB compared to those with MYB::NFIB fusions. Interestingly, the presence of mutations was associated with both, a high-grade phenotype of AdCC and worse clinical outcomes of patients.

OOCYTE TFAM INSUFFICIENCY AFFECTS REPRODUCTIVE OUTCOME AND IS TRANSMITTED THROUGH THE FEMALE GERMLINE

M. Mahrouk (3rd year of MSP), M. Shavit
Laboratory of Reproductive Medicine, Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. Ing. Jan Nevoral, Ph.D

Background: The transcription factor A mitochondrial (TFAM), is hypothesized to play a pivotal role in mitochondrial transcription within the oocyte, influencing mitochondrial biogenesis and function. This study investigates whether TFAM deficiency in oocytes affects mitochondrial transcription and whether these effects have implications for intergenerational transmission of mitochondrial function. The objective of the research was to employ quantitative PCR (qPCR) and reverse transcription qPCR (RT-qPCR) to determine the mitochondrial transcription levels in oocytes and somatic tissues of a mouse model with germline TFAM knockout. By doing so, the study aimed to elucidate the role of TFAM in mitochondrial biogenesis within oocytes and its potential effects on post-implantation development and offspring health.

Methods: The study utilized a mouse model, specifically a conditional TFAM knockout (cKO: Zp3-Cre;Tfam^loxP/loxP) to generate Tfam^null oocytes. The cKO females were mated with wild-type males, their reproductive phenotype was characterized, and the Tfam^+/- female F1 offspring were examined for signs of mitochondrial dysfunction in somatic tissues and superovulated oocytes. The methodology centered on PCR techniques to assess mitochondrial transcription. The relative fold expression of mtRNA transcripts normalized to beta actin mRNA was established via RT-qPCR for somatic tissues. Data were analyzed using GraphPad Prism 8.1.1 (GraphPad Software Inc., USA).

Results: The results from qPCR analysis of oocytes revealed a significant reduction of mtDNA copy number in the transcription of mitochondrially encoded mRNA transcripts in Tfam^null Accordingly, the Tfam^null oocytes exhibited decreased levels of mitochondrial transcripts, indicating that TFAM deficiency leads to a reduction in mitochondrial transcription within the oocyte itself. Despite the observed reduction in mitochondrial transcription in Tfam^null oocytes, the transcription of nuclear-encoded mitochondrial factors remained unaffected. Finally, no changes in the mitochondrial mRNA transcription were observed in liver and muscle tissues of F1 offspring.

Conclusion: TFAM deficiency leads to a significant decrease in mtDNA copy number in the oocyte and reduced mitochondrial transcription. Surprisingly, these oocytes can be fertilized, and the somatic mitochondrial capacity of F1 offspring is not affected. Taken together, we assume a compensation effect in animals of the Tfam+/- genotype and, on the other hand, the onset of mitochondrial failure in advanced age

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THE IMPACT OF NOREPINEPHRINE ON MITOCHONDRIAL RESPIRATION IN A SEPTIC PORCINE HEART

S. Basoccu (5th year of MSP), L. Madani, E. Niatsi, J. Steer, J. Jedlicka
Institute of Physiology and Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): prof. MUDr. J. Kuncová, Ph.D.

Background: Mitochondrial respiration is a process of energy conversion of metabolic substrates into ATP. However, mitochondria also function as a source of reactive oxidative species, which might affect them and may lead to mitochondrial dysfunction. Sepsis is a dysregulated host response to infection associated with an excessive production of both pro- and anti-inflammatory cytokines and consecutive multiple organ dysfunction. Norepinephrine is often administered as a vasopressor medication in patients with septic shock, a subset of sepsis characterized by profound hypotension despite adequate fluid resuscitation. Moreover it is a neurotransmitter and hormone released by the sympathoadrenal system playing a role in regulation of the cardiac performance. On the samples dissected from the left ventricular myocardium (LV) of control and septic pigs, we aimed to verify the negative impact of sepsis on the mitochondrial respiratory parameters in a new porcine model of sepsis and to test the hypothesis of putative inhibitory effect of norepinephrine on myocardial oxygen consumption.

Methods: In 10 anesthetized ventilated pigs of both sexes, sepsis was induced by infusion of bacteria resistant to antibiotics. After 24 hours of sepsis, the heart was dissected and homogenized samples of LV were analysed in oxygraph chambers (O2k, OROBOROS, Austria). Leak respiration (state LEAK) was measured with substrates providing electrons to complex I, malate, glutamate and pyruvate. Active respiration was induced by ADP (state OXPHOS I) and further by succinate, a complex II substrate (OXPHOS I+II). Maximum capacity of the electron-transporting system (ETSC I+II) was reached by titration of uncoupler FCCP. After addition of a complex I inhibitor rotenone, state ETSC II was determined. Antimycin A, a complex III inhibitor was used to measure residual oxygen consumption (ROX).

Results: As expected, mitochondrial respiratory parameters in the septic hearts were decreased compared to controls. Interestingly, norepinephrine seemed to inhibit O2 consumption.

Conclusion: The effect of sepsis on mitochondrial respiration in the heart is inhibitory also in this novel porcine model of the disease. The NE inhibitory effect on mitochondrial respiratory parameters cannot be easily explained; however it could be either primary response of mitochondria to the stimulation of adrenergic receptors or it could be secondary to metabolic changes induced by the catecholamine.

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ASSESSMENT OF SUPEROXIDE PRODUCTION AND DNA DAMAGE IN A PORCINE MODEL OF SEPSIS

J. Steer (5th year of MSP), T. Chmelíř, J. Müller

(1) Department of Physiology and Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Czech Republic

(2) Institute of Anesthesiological Pathophysiology and Process Development, University Hospital Ulm, Germany

(3) 1st Department of Internal Medicine, Faculty of Medicine in Pilsen CU

Supervisor(s): Ing. D. Jarkovská, Ph.D. (1), F. Zink, Ph.D. (2), M. Groeger, Ph.D. (2), prof. P. Radermacher, Ph.D. (2), prof. MUDr. M. Štengl, Ph.D. (1), Prof. MUDr. Martin Matějovič, Ph.D. (3)

Background: Sepsis and septic shock represent significant causes of intrahospital mortality in developed nations. Oxidative stress plays a pivotal role in sepsis pathophysiology due to heightened levels of reactive oxygen species (ROS) and compromised antioxidative capacity. Superoxide, a prevalent ROS, induces oxidative damage to DNA, proteins, and lipids. This study aimed to evaluate superoxide production and quantify associated DNA damage in nucleated blood cells in a porcine model of sepsis.

Methods: Sixteen pigs underwent anesthesia, ventilation, and surgical instrumentation in a clinically relevant animal pilot study. Sepsis was induced in eight pigs via intravenous bacterial infusion following a resting phase, while control pigs did not receive bacterial infusion. All pigs received oligonucleotide infusions at concentrations of either 600 µg or 900 µg. Blood was drawn at five time points: before (TP0) and 6, 12, 18, and 24 hours after sepsis induction and at corresponding time points for control animals. Electron paramagnetic resonance spectroscopy (EPR) was utilized to measure superoxide production in whole blood samples and presented in nM/s. The comet assay assessed DNA damage in nucleated blood cells by applying cells in agarose on microscopic slides, followed by lysis, DNA unwinding, and electrophoresis. DNA staining allowed the quantification of intensity and length of migrated DNA which correlates with overall DNA damage.

Results: EPR spectroscopy revealed a wide range of superoxide production in both septic and control pigs across all time points, ranging from 100nM/s to 262nM/s. No significant difference was observed in superoxide production between septic and control animals at any time point.

Comet assay analysis demonstrated increased DNA damage in septic animals after 24 hours compared to controls, whereas differences at 6, 12, and 18 hours were marginal.

Conclusion: Superoxide production exhibits high interindividual variability under septic conditions, with fluctuations also observed in non-septic animals. Although increased DNA damage was evident in septic animals after 24 hours, superoxide may not solely account for this damage. Other ROS or immunological processes during sepsis could contribute to DNA damage. Further investigations are warranted to elucidate the complex mechanisms underlying oxidative stress and DNA damage in sepsis.

HIPPOCAMPAL VOLUME IN WILD-DERIVED AND CLASSICAL LABORATORY MOUSE STRAINS

N. R. Choudhury (1st year of DSP), J. Tuma
Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Jan Cendelín, Ph.D.

Background: Common laboratory mouse strains were derived from Mus musculus domesticus subspecies with admixtures of genes from other subspecies. In contrast, the PWD/Ph mouse strain was wild-derived about 50 years ago and belongs purely to Mus musculus musculus subspecies. Genetic background is known to play an important role and there are significant strain differences in many parameters of laboratory mice including behavior and cognition. It is not surprising that performance in many behavioral, cognitive and even motor tests differs in wild-derived PWD/Ph mice and classical laboratory C57Bl/6 mice. However, physiological substrates of these differences are not completely known. For instance, information about potential differences in morphology of brain structures involved in cognitive and behavioral processes are lacking. The aim of the study was to compare hippocampal volumes in wild-derived PWD/Ph mice and classical laboratory mice of the C57Bl/6 strain.

Methods: Brains of 10 PWD/Ph and 10 C57Bl/6 were sectioned in the coronal plain and stained with cresyl violet. Images of the specimens were acquired using a microscope equipped with a digital camera. Volumes of the hippocampi were estimated using Cavalieri principle and point grid method. Besides absolute hippocampal volume, we have evaluated also the values adjusted to body weight which is much lower in PWD/Ph mice.

Results: There was no significant difference in absolute hippocampal volume between PWD/Ph and C57Bl/6 mice. However, if adjusted to body weight, PWD/Ph mouse hippocampi were larger that hippocampi of the C57Bl/6 mice. The hippocampi were symmetrical with no marked lateralization in both strains.

Conclusion: Although, the hippocampus is involved in both behavior as learning and memory, marked differences in these functions between wild-derived PWD/Ph mice and C57Bl/6 strains representing Mus musculus domesticus subspecies are not accompanied with gross changes in anatomy of this structure. The difference was apparent only if the volumes were adjusted to mouse body weight, what does not necessarily reflect functional capacity regarding learning ability.

This study was supported by Cooperatio (research area NEUR) and GAUK No. 49724.

STEREOLOGICAL ASSESSMENT OF CHANGES IN MICROGLIAL CELLS IN INDIVIDUAL LAYERS OF LURCHER MICE CEREBELLA

O. Yakushko (2nd year of DSP), D. Jelinkova (2), J. Cendelin (2), Y. Kolinko (1)
(1) Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University
(2) Department of Pathophysiology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Mgr. Y. Kolinko, Ph.D. (1)

Background: The cerebellum is indispensable for normal brain functions. Its structural and functional involvement in neurodegenerative disorders is linked to the decline of both motor and non-motor functions, including cognition, mood, and behavior. Microglial cells have a significant role in the neurodegeneration of the cerebellum, as they are responsible for eliminating dead cells, protein aggregates, and other particles that may endanger the central nervous system. Lurcher mice are well known model for investigating the extensive degeneration of cerebellar Purkinje cells and a majority of cerebellar granule neurons. Despite extensive research on this model, a comprehensive characterization of the microglial condition during such profound degeneration remains still incomplete. Thus, the objective of our study was to present stereological analysis of microglial distributions across different layers of the cerebellum in Lurcher and wild-type mice.

Methods: The frozen cerebella collected from 6 three-month-old mice (3 from B6CBA Lurcher mutants and 3 from healthy wild-type mice) were processed into 60-μm-thick serial sections and stained using anti-allograft inflammatory factor 1 (Iba1) antibodies (Jackson Immuno Research, UK). The volumes of individual cortical layers, white matter, cerebellar nuclei, as well as the total number of microglial cells and lengths of their processes were determined using the stereological approaches. The statistical analysis was conducted using the Statistic 13 software. The Mann–Whitney U test was used to examine between-group effects, whereas the Kruskal-Wallis test was employed to evaluate the distributions of cells across individual layers.

Results: Our early results revealed that the distribution of cerebellar microglia was uneven between specific histological layers of the cerebellum in wild-type mice: microglial density was noted to be lowest in the molecular layer, intermediate in the granular layer and white matter, and highest in the cerebellar nuclei. However, this pattern of microglial distribution in the Lurcher mice cerebellum was disrupted: the lowest density was observed in white matter, intermediate in the molecular layer and granular layer, and remained high in the cerebellar nuclei. It was found that the total number of microglial cells and the total lengths of their processes decreased in Lurcher mice, but the cell density across various cerebellar compartments increased compared with wild-type mice. Power analysis indicated that increasing the number of cases would result in a statistically significant difference between groups.

Conclusion: Our findings revealed the activation of microglial cells in response to Purkinje cell degeneration, as well as variances in microglial density between Lurcher and wild-type mice. Studying the behavior of microglia during degeneration is critical to inform treatment strategies.

This study was supported by EMBO, grant number SLG 5433 and Charles University Cooperatio Program (MED/DIAG).

OCCURRENCE OF RESISTANCE TO NEW BETA-LACTAM ANTIBIOTICS IN CARBAPENEM RESISTANT GRAM-NEGATIVE RODS IN THE CZECH REPUBLIC

K. Vlkova (4th year of DSP; 1,2), K. Chudejova (1,2), A. Sramkova (1,2), V. Heringova (1,2), V. Paskova (1), V. Jakubu (3), H. Zemlickova (3)

(1) Biomedical Center, Faculty of Medicine in Pilsen, Charles University

(2) Department of Microbiology, Faculty of Medicine in Pilsen, Charles University

(3) National Reference Laboratory for Antibiotics, National Institute of Public Health, Prague

Supervisor(s): prof. Ing. J. Hrabak, Ph.D. (1,2)

Background: Bacterial resistance to antibiotics is increasing worldwide. The biggest problem is the increasing number of strains from the family Enterobacterales and Gram-negative non-fermenting rods (Pseudomonas aeruginosa, Acinetobacter species) resistant to carbapenems, which belong to backup antibiotics. Due to this, the treatment options for infections caused by these bacteria are decreasing. For that reason, there is a need for the development of new antibiotics, which will be effective against strains resistant to carbapenems. There are antibiotics, which can be used for treatment of such infections. It is ceftolozan-tazobactam (CET), ceftazidime-avibactam (CAV) and cefiderocol (CID). However, despite the potential effectiveness of these antibiotics against multiresistant strains, resistance is already occurring. It is a multifactorial type of resistance that can be caused by efflux pumps, porins, beta-lactamase production and changes in iron uptake. Mapping the frequency of resistance to new beta-lactam antibiotics (ceftolozane-tazobactam, ceftazidime-avibactam and cefiderocol) in carbapenem resistant Enterobacterales and Gram-negative non-fermenting rods.

Methods: During February and March 2024, a collection of carbapenem resistant strains was carried out through the National reference laboratory for antibiotics. All strains were tested for susceptibility to CET, CAV and CID based on the disk diffusion assay. Disc diffusion tests were evaluated based on the EUCAST 2024 guidelines.

Results: As part of the collection, a total of 231 strains from the family Enterobacterales (147 Klebsiella species, 45 Escherichia coli, 15 Enterobacter species, 24 other) and 122 strains of non-fermenting rods (116 Pseudomonas aeruginosa, 6 Acinetobacter species) were collected. Resistance to CET was detected in 226 strains from the Enterobacterales family, most commonly Klebsiella pneumoniae (132) and E. coli (43), and in 87 strains of P.aeruginosa and 6 strains of A. species. Resistance to CAV occurred in 126 strains of enterobacteria (most often K. pneumoniae-83 and E. coli-16), in 66 strains of P. aeruginosa and 6 strains of A. species. Resistance to CID was in 117 strains of enterobacteria (80 K. pneumoniae, 9 E. cloacae complex), 19 strains of P. aeruginosa and 1 strain of A. species. Resistance to CET was observed in the majority of carbapenemase producing strains (97,7 %) and overall in 90,3 % of submitted strains. Resistance to CAV was detected in most of metallobetalactamase producing strains (97,2 %) and in total in 56,1 % of sent strains. Resistance to CID was detected in 38,8 % of the submitted strains.

Conclusion: In this work, a short-term collection of isolates was carried out to determine the occurrence of resistance to novel antibiotics. The work showed that the occurrence of resistance to new beta-lactam antibiotics in strains resistant to carbapenems in the Czech Republic is relatively high. Monitoring of the occurrence of resistance to these antibiotics will continue in the upcoming months. At the same time, whole-genome sequencing will take place in selected strains to determine the genetic basis of this resistance.

AN IMAGE OF DIFFERENT TYPES OF PHYSIOLOGICAL AND PATHOLOGICAL LEUKOCYTES IN RAMAN SPECTROSCOPY

L. Vaňková (3rd year of DSP; 1), D. Macečková (2), J. Bufka (3), P. Šigutová (4), A. Štambachová (4)

(1) Department of Histology and Embryology, Faculty of Medicine in Pilsen Charles University
(2) Laboratory of Tumour Biology and Immunotherapy, Biomedical Center, Faculty of Medicine in Pilsen, Charles University
(3) Department of Paediatrics, University Hospital Pilsen
(4) Department of Clinical Biochemistry and Haematology, University Hospital Pilsen

Supervisor(s): Mgr. Monika Holubová, Ph.D. (2), MUDr. Věra Křížková, Ph.D. (1)

Background: Raman spectroscopy is a nondestructive analytical method based on the interaction of electromagnetic radiation with the molecules of the sample. It provides information about the geometry and vibrational states and reflects the molecular symmetry and chemical groups present. In the past, multiple types of cellular metabolites have been characterised by this method. Raman spectroscopy and microscopy have also been explored for many biomedical applications, including imaging of the chemical composition of cells and tissues and analysing cellular metabolism. Because of its noninvasiveness, adaptability and speed of measurement, it appears to be a potentially useful diagnostic method that is also applicable for studies of molecular processes in haematology. Our work aimed to measure the spectra of physiological leukocytes and the spectra of different haematological tumour cell lines (THP1, MOLM13, etc.) under different conditions with their differentiation and component analysis.

Methods: Leukocytes from healthy donors were processed in a standard blood smear in panoptic staining. Different types of leukocytes were recognized based on their typical morphology and measured in the smear using an immersion objective as part of the Raman spectrometer. Tumour cell lines were cultured in a standard way. Some of them were treated with various drugs (midostaurin, antisense oligonucleotides, etc.). Then, a modified protocol for the blood smear was used for their preparation with subsequent measurement on a Raman spectroscope. The control sample was created by combining all the chemicals used, i.e. methanol, May-Grünwald and Giemsa-Romanowski dyes, phosphate buffer, and immersion oil. The background signal (especially the immersion oil spectrum) was subtracted from each spectrum, and a multipoint baseline was corrected in the entire spectral region. The positions of the strongest Raman peaks were determined, and the fluorescence was normalized. The peaks were assigned based on the literature.

Results: The Raman spectra of cell groups have good reproducibility. After processing the measured spectra, we were able to distinguish some groups using multivariate statistical analysis. For others, the differences remained below the detection capabilities of this method. Common bands by groups that we identified in their spectra, were bands of carotenoids, proteins, lipids, nucleic acids, and cytochrome C. The differences and specific positions of measured and interpreted chemical bands will be described during the conference.

Conclusion: We established a protocol for obtaining, processing, and measuring samples for Raman spectroscopy. The band assignment demonstrated the feasibility of utilizing Raman microscopy to describe the molecular composition of leukocytes and their pathological

equivalent and identify some molecular fingerprints. The results indicate good reproducibility and low interindividual variability of the spectral parameters.

The study was supported by the grant SVV no. 260 651 and the Cooperatio program area MED/DIAG supported the study.

THE CULTURE OF 3D PRIMARY HUMAN HEPATOCYTES SPHEROIDS – PRELIMINARY DATA

P. Jančová (4th year of DSP), L. Vištejnová
(1) Biomedical center, Faculty of Medicine in Pilsen, Charles University
(2) Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Ing. Bc. Lucie Vištejnová Ph.D. (1, 2)

Background: In order to study metabolic dysfunction-associated steatotic liver disease (MASLD, recently renamed from NAFLD – non-alcoholic fatty liver disease), we need functional tools. In the case of in vitro models of MASLD, long-term cultivation of hepatocytes remains a huge challenge. Primary human hepatocytes (PHH) are the preferred cell type to study liver diseases, however, they are difficult and expensive to cultivate, they dedifferentiate easily and cells from different donors do not have the same properties. In our previous experiments, we cultured 3D spheroids of the HepG2 cell line as an easy-to-culture and cheap alternative to PHH. However, HepG2 cells proliferate even in fully formed spheroids, with the size becoming too large for oxygen diffusion leading to necrotic core formation during the first week of culture. Therefore now, we aim to characterize the PHH spheroids despite the obstacles involved in their cultivation. Our goal was to establish a 3D method of hepatocyte culture that is suitable for long-term (4 weeks) cultivation.

Methods: PHH were cultured in 96-well ultra-low attachment (ULA) plates designed for spheroid formation. In each well 1500 cells were seeded, and after one week the spheroids were fully formed. From this time point, we cultivated them for another 4 weeks and several analyses were performed at days 0, 3, 7, 14, 21 and 28: photos in the culture wells were taken and their diameter was measured, the viability was assessed by intracellular ATP content and albumin production, morphology was studied by histological analysis of hematoxylin and eosin-stained tissue sections.

Results: Albumin production and cell viability slightly decreased during the first week of culture but remained stable after that. The spheroid diameter remained between 250 and 300 μm, which seems to be sufficient for oxygen and nutrients to diffuse in the whole cell mass because a necrotic core was not observed. Moreover, PHH maintained their typical morphology including the presence of binucleated cells. More types of histological and IHC stains will be used to further characterize PHH spheroids by quantitative histology and to compare them with HepG2 spheroids.

Conclusion: 3D PHH spheroids seem to be suitable for long-term culture. The spheroids maintained their size, with stable viability and morphology of the cells during the whole 4-week period.

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OPTIMIZING PCL/PLGA SCAFFOLD BIOCOMPATIBILITY USING GELATIN FROM BOVINE, PORCINE, AND FISH ORIGIN

A. Nekleionova (3rd year of DSP), M. Ghafouri Azar, L. Wiesnerova, J. Dvorakova, P. Chocholata, V. Babuska

Department of Medical Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): doc. Ing. Václav Babuška, Ph.D

Background: Our hypothesis suggests that combining bioresorbable polymers with gelatin will not only improve biocompatibility, but also form porous structures that support cell penetration and the use in tissue engineering. To evaluate the morphology, mechanical, and biological properties of the biodegradable composite films fabricated by combining PCL/PLGA with gelatin from different sources, including bovine, porcine, and fish.

Methods: Five samples of scaffolds with different compositions were prepared: the pure polycaprolactone (PCL) as the basic sample, the combination of poly (lactic-co-glycolic acid) (PLGA) and pure PCL, and combinations of PCL/PLGA with bovine, porcine and fish gelatin. The resulting solutions were poured onto a glass surface and then dried to form 0.5 mm thick films. The films were evaluated for morphology, mechanical properties, thermal stability, biodegradability, hemocompatibility, cytotoxicity, cell adhesion and proliferation. Biological testing was performed using human dermal fibroblasts (HDFs).

Results: Our findings indicate that the incorporation of gelatins into the films changes their mechanical properties, resulting in a decrease in tensile strength but an increase in elongation at break. This indicates that the films become more flexible with the addition of gelatin. Gelatin incorporation has a limited effect on the thermal stability of the films. The composites with the fish gelatin show higher biodegradability with the highest weight loss. The films demonstrate high hemocompatibility with minimal observed hemolysis. The gelatin has a dynamic effect on cell behavior and supports long-term cell proliferation. All composite films reveal extremely low levels of cytotoxicity.

Conclusion: Gelatin-based samples have an acceptable level of mechanical properties, very good degree of biodegradability and good biocompatibility. The results of our research can be used for further studies, especially the incorporation of scaffolds with gelatin in tissue engineering applications.

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DECIPHERING GENOMIC BACKGROUND OF SYNCHRONOUS AND METACHRONOUS COLORECTAL CANCER LIVER METASTASES WITH WHOLE-EXOME SEQUENCING

M. Rajtmajerová (3rd year of DSP), P. Vodička, L. Vodičková, J. Brůha, L. Červenková, P. Hošek, V. Liška, P. Souček, A. Trailin, V. Hlaváč, F. Ambrozkiewicz, K. Hemminki
Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Prof. Kari Hemminki, MD, Ph.D

Background: Colorectal cancer (CRC) remains the third most frequently diagnosed cancer worldwide. Metastatic disease (often arising due to late diagnosis) results in more than 700, 000 deaths per year. CRC is known to be highly heterogeneous in terms of its genetic background, leading to exceptionally high number of driver mutations (APC, TP53, MLH1, PIK3CA, KRAS, BRAF etc). Metastases are usually classified as either synchronous (diagnose dat the same time as the primary tumor) or metachronous (diagnosed after primary tumor removal). We hypothesize that underlying genetic pathways are responsible for differences in tumor progression and spread. We aimed to find crucial differences in mutational profiles responsible for tumor early or delayed progression of CRC.

Methods: We performed whole-exome sequencing of paired samples of primary tumor and liver metastases in 89 patients and singleton samples (either metastatic or primary CRC) of another 37 patients. All samples were obtained by dissecting FFPE blocks coming from University Hospital in Pilsen and University Hospital in Hradec Králové.

Results: Whilst sequencing is still ongoing and the subsequent analysis is therefore not complete yet, we plan to depict specific mutational signatures corresponding to synchronicity and metachronicity. Further, data obtained from WES will be correlated with results of other ongoing projects including immune cell densities within tumor and telomere lenghts measurements.

Conclusion: We were able to identify all known CRC driver mutations (TP53, PIK3CA, APC, KRAS and others) in our subset of samples whose sequencing has already been completed. After completion of the remaining samples we will continue to decipher genetic signatures of CRC progressiveness.

GENETIC ANALYSIS OF ONCODRIVERS AND PHARMACOGENES RELATED TO 5-FLUOROURACIL RESISTANCE IN COLORECTAL CANCER PATIENTS

L. Heczko (3rd year of DSP) (1), V. Hlaváč (1), V. Liška (1,2), O. Vyčítal (1,2), O. Fiala (1,3), P. Souček (1)

(1) Biomedical Center, Faculty of Medicine in Pilsen, Charles University
(2) Department of Surgery, University Hospital in Pilsen and Faculty of Medicine, Charles University
(3) Department of Oncology and Radiotherapeutics, University Hospital in Pilsen and Faculty of Medicine in Pilsen
(4) Department of Biology, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): Mgr. V. Hlaváč, Ph.D. (1), doc. RNDr. P. Dvořák, Ph.D. (1,4)

Background: Colorectal cancer (CRC) is the third most common cancer overall and a second leading cause of cancer-related deaths. CRC poses a challenge in treatment due to its highly heterogenous nature. In hereditary CRC, CRC arises either from inherited mutations in high-penetrance susceptibility genes or from common genetic polymorphisms with low penetrance or their combinations while in sporadic cases, it arises from the accumulation of sporadic mutations as a result of exposure to environmental carcinogens. Standard treatment includes surgical tumor removal and administration of adjuvant chemotherapy for stage II patients in high risk of disease progression. Patients in advanced stages receive adjuvant or palliative chemotherapy. The main drug used is 5-fluorouracil (5-FU), also widely used for other cancer types; however, the response in patients with the advanced CRC to 5-FU alone is only 10-15%. This number can be improved by combined chemotherapy or by application of a targeted treatment but this is based on patients' mutational status and thus not available for all patients. This highlights the need for a deeper understanding of toxicity and resistance to 5-FU. The aim of this project was to analyze the genetic background of 558 genes related to 5-FU resistance according to PharmGKB, DGIdb and DrugBank, major oncodrivers according to the latest tier 1 and tier 2 Cancer Gene Census, and actionable genes (COSMIC) in 83 patients with CRC.

Methods: In 83 patients diagnosed with sporadic primary CRC tumors at various stages, the paired samples of tumor tissue and blood were collected between years 2015 – 2019. From all biological samples, DNA was isolated and libraries for targeted sequencing were prepared. We performed hybridization capture reaction for the above specified genes, which were then sequenced on the NovaSeq 6000 platform. The obtained data were bioinformatically analyzed.

Results: In our cohort, mutations in APC, TP53, and KRAS were most frequent. Patients in advanced stages of CRC exhibited significantly more mutations in these genes whereas certain mutations, such as frameshift in APC and 12D mutation in KRAS, were associated with a shorter RFS and worse response to adjuvant treatment. Additionally, mutations in genes ANK2, ABCA13, and COL7A1 showed prognostic value irrespective of adjuvant chemotherapy administration. Notably, mutations in FLG, GLI3, and UNC80 were predictive for patients who did not receive any chemotherapy, while COL6A3, LRP1B, NAV3, RYR1, RYR3, TCCH, and TENM4 for those patients who did.

Conclusion: Overall, our findings are shedding light on the genetic landscape of CRC, revealing associations between disease stage, specific gene mutations, and patient outcomes.

Knowledge of biomarkers of chemotherapy response and patient prognosis could potentially lead to personalized treatment strategies for CRC patients, improving therapeutic efficacy and clinical outcomes.

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GENE FUSIONS IDENTIFIED IN AGGRESSIVE TESTICULAR LEYDIG CELL TUMORS LIKELY REPRESENT EVENTS SECONDARY TO GENOMIC INSTABILITY WITHOUT A DRIVER ROLE

A. Straková Peteříková (3rd year of DSP), P. Martínek, S. Gupta, K. Cornejo, J. Cheville, H. Miyamoto, K. Collins, M. Idrees, M. Michal, T. M. Ulbright, A. M. Acosta, K. Michalová
(1) Department of Pathology, Faculty of Medicine in Pilsen, Charles University
(2) Bioptical Laboratory Ltd., Pilsen
Supervisor(s): doc. MUDr. Květoslava Michalová, Ph.D

Background: Approximately 10% of adult testicular Leydig cell tumors (LCT) demonstrate aggressive clinical behavior and metastasize. Given that malignant LCTs respond poorly to systemic therapy, there is a special interest in identifying biomarkers that may predict aggressive behavior in this tumor type. FH alterations and MDM2 amplification have been identified in subsets of aggressive LCTs, but recurrent genomic findings have not been found in significant proportion of malignant LCTs. Recently, a study demonstrated recurrent exon 2 TERT fusions in 3 of 7 malignant LCTs, suggesting that they might represent a useful biomarker (PMID: 33741265). To further test this hypothesis and explore the spectrum of gene fusions potentially driving oncogenesis in LCT, we evaluated a series of aggressive and non-aggressive LCTs using a fusion RNA sequencing panel.

Methods: We analyzed 14 aggressive LCTs (defined as metastasizing tumors or primary neoplasms with 2 or more of the following features: size over 5 cm, necrosis, >3 mitoses per 10 HPF, atypical mitoses, lymphovascular invasion or infiltrative growth) and 12 non-aggressive primary LCTs using the Illumina TruSight RNA Pan-Cancer fusion assay, which targets 1354 genes and can detect fusions with non-targeted genes as long as 1 of the partners is included in the structural variant.

Results: Thirteen malignant and 9 benign LCTs were sequenced successfully. Two aggressive LCTs revealed gene fusions: NAV3::ASB8 and RAB3IP::TAFA2, respectively. Both were present in-frame (i.e., predicted to be productive), but they have not been previously reported in other cancer types; therefore, their biological significance remains uncertain. Of note, no TERT fusions were identified in aggressive LCTs and no gene fusions were detected in non-aggressive cases.

Conclusion: TERT fusions were not identified in this series of LCTs, with 2 aggressive LCTs harboring non-recurrent NAV3::ASB8 and RAB3IP::TAFA2. These structural variants have not been reported previously and likely represent events secondary to genomic instability without a clear pathogenic role (PMID: 34103665). Methylation analysis is underway to reveal potentional differences in epigenetic signatures between malignant and benign Leydig cell tumors.

OOCYTE TRANSCRIPTION FACTOR A, MITOCHONDRIAL (TFAM) DEPLETION AFFECTS REPRODUCTIVE OUTCOME, WHILE TFAM IS DISPENSABLE FOR EARLY EMBRYONIC DEVELOPMENT

M. Shavit (4th year of DSP), K. Grygarová, M. Mahrouk, J. Nevoral
Laboratory of Reproductive Medicine, Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. Ing. Jan Nevoral, Ph.D.

Background: The overall quality of the oocyte mitochondrial pool has increasingly emerged as a key determinator of oocyte developmental competence, influencing fertilization, cleavage, and implantation, and consequently the overall chances of a successful reproductive outcome. Transcription factor A, mitochondrial (TFAM) is a key factor for mitochondrial biogenesis. We hypothesized oocyte TFAM insufficiency would affect the quality of the oocyte mitochondrial pool, and subsequently impair oocyte developmental competence and reproductive outcomes. The aim of the study was to describe TFAM dynamics during early embryonic development and characterize the effects of germline TFAM knockout in a mouse model, including an assessment of the quality of the oocyte mitochondrial pool, oocyte developmental competence, and reproductive outcomes.

Methods: The mitochondrial pool of the in vivo ovulated (IVO) oocytes of female wild type (WT) and TfamloxP/loxP;Zp3-Cre (cKO) mice was characterized via a combination of Immunocytochemistry and quantitative PCR (qPCR) to assess the oocyte mtDNA copy number. Embryo and parthenotes were generated from WT and cKO IVO oocytes via IVF and parthenogenic activation. The cleavage and blastocyst rates were examined, and TFAM expression was characterized via Immunocytochemistry. To establish the reproductive phenotype, cKO females were mated with WT males. All data was analyzed using GraphPad Prism 8.1.1 (GraphPad Software Inc., USA).

Results: TFAM was effectively depleted in cKO oocytes. The quality of the mitochondrial pool of cKO IVOs was significantly affected as evidenced by a significant reduction in mtDNA copy number ($P \leq 0.0001$) and impairments in mitochondrial membrane potential as evidenced by JC1 staining. Surprisingly, the cleavage and blastocyst rates of embryo and parthenotes were unaffected, in spite of evidence of TFAM expression linked to the embryonic genome activation. While pregnancy rates were unaltered, the littersize of cKO females was reduced.

Conclusion: Taken together, our results show that while TFAM is dispensable for early embryonic development, oocyte TFAM deficiency may limit the subsequent progression through the stages of implantation and postimplantation development. In light of the current state of the art in the field, our data points to a clear role of mitochondria in embryo implantation. We thus believe our findings will help in bridging the knowledge gap explaining the seemingly contradicting Warbug effect, and the crucial dependency of mitochondria for oocyte developmental competence.

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THE IMPACT OF DIABETES MELLITUS ON THE NPB/W SIGNALLING SYSTEM IN DIFFERENT PARTS OF THE STOMACH IN ZDF RATS

T. Chmelíř (1st year of DSP) (1), S. Pandey (2,3), D. Jarkovská (1,3)
(1) Department of Physiology, Faculty of Medicine in Pilsen, Charles University
(2) Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University
(3) Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. M. Chottová Dvořáková, Ph.D. (1,3)

Background: Diabetes mellitus (DM) is a serious chronic metabolic disease, the incidence and prevalence of which has been increasing in recent years. One of the frequent complications of this disease is the impairment of the autonomic nervous system, which negatively affects on the functioning of a number of organs and organ systems. Affected systems undoubtedly include the organs of the gastrointestinal tract (GIT), where the exact pathophysiological mechanism of the action of DM has not yet been fully clarified. Diabetes-induced damage to the autonomic innervation of the GIT, collectively referred to as diabetic gastroenteropathy, has a number of unpleasant symptoms in patients such as nausea, vomiting, diarrhea or constipation, abdominal pain or flatulence. In addition to classic mediators such as acetylcholine and noradrenaline, a number of other substances are involved in the autonomic innervation of the GIT. Neuropeptide B (NPB) and neuropeptide W (NPW) constitute NPB/W signalling system, along with their receptor NPBWR1. The location and function of NPB/W signalling system have been predominantly detected and mapped within the CNS, including its role in modulation of inflammatory pain or neuroendocrine functions. However, their presence was also confirmed by few studies in the organs of the GIT. It seems that NPW could slow gastric emptying by acting on the pyloric sphincter, but in general the function of these neuropeptides in the organs of the GIT has not yet been systematically investigated. Confirm the expression of the genes for NPB, NPW and NPBWR1 in 3 different parts of the rat stomach – cardia, corpus and pylorus. Determine the impact of type 2 DM on gene expression for NPB, NPW and NPBWR1 in 3 different parts of the stomach in 12 and 34 week old rats.

Methods: Zucker diabetic fatty (ZDF) and lean Zucker (control) rats were sacrificed by decapitation at week 12 or 34 of age. The stomach was rapidly excised, rinsed with cold physiological solution, and then divided into individual parts – cardia, corpus and pylorus. Total RNA was isolated by the phenol chloroform method. Obtained RNA was reverse-transcripted and subsequently quantitative PCR analysis was done. Relative expression of mRNA of NPB, NPW and NPBWR1 was expressed as a ratio of target gene concentration to control gene.

Results: The expression of the gene for NPB, NPW and NPBWR1 was confirmed in all parts of the rat stomach. Nevertheless, the expression of NPBWR1 in the pylorus was too low that we could not work with this value further. We have observed the significant downregulation of mRNA for NPB in the corpus (0,54-fold, p=0,015) and in the pylorus (0,72-fold, p=0,035) in 12 week old diabetic rats. On the contrary, expression of mRNA for NPB in pylorus was significantly upregulated (1,58-fold, p=0,035) in 34 week old diabetic rats. No significant differences were observed in other cases.

Conclusion: Our study confirmed the impact of type 2 DM on the expression of the NPB gene in corpus and pylorus of the rat stomach. Altered expression in the pylorus can affect its contractions and thus the passage of digestate throught the GIT.

HMGA2 GENE ALTERATIONS DEFINE A DISTINCTIVE CANALICULAR SUBTYPE OF SALIVARY PLEOMORPHIC ADENOMA

N. Klubíčková (4th year of DSP) (1,2), F. Loghides (3), P. Martínek (2), P. Šteiner (2), T. Vaněček (2), A. Skálová (1,2)

(1) Department of Pathology, Faculty of Medicine in Pilsen, Charles University
(2) Bioptical Laboratory Ltd., Pilsen
(3) Department of Anatomical Pathology, Canterbury District Health Board, Christchurch Hospital, Christchurch, New Zealand

Supervisor(s): prof. MUDr. Alena Skálová, CSc.

Background: Pleomorphic adenoma (PA) is the most common neoplasm of salivary glands, usually occurring in the parotid gland of adults. It is driven by PLAG1 and HMGA2 gene rearrangements, and expression of the respective proteins is detectable by immunohistochemistry. Such genetic background was not reported in canalicular adenoma of minor salivary glands. We present a subset of PA defined by HMGA2 alterations and a distinct canalicular and trabecular growth pattern.

Methods: Institutional archives were searched for unusual cases of PA with increased cellularity and canalicular and trabecular architecture. Clinical, morphological and immunohistochemical data of the 39 cases were cataloged. Targeted RNA sequencing using customized NGS kits was performed in 38 cases. FISH analysis of HMGA2 and PLAG1 rearrangement was performed in 24 and 18 cases, respectively.

Results: All cases occurred in adult patients (mean 56 years, median 66 years) and slightly more commonly in women. While the parotid gland was the most common location, minor salivary glands and the submandibular gland were affected in rare cases. The monophasic (n=24) or biphasic (n=15) tumors exhibited canalicular and trabecular growth pattern of anastomosing strands of cells. A component of classic biphasic PA with a sharp or gradual transition into the canalicular areas was identified in 13 cases. One biphasic case showed invasive growth into surrounding adipose tissue and was diagnosed as myoepithelial carcinoma ex-PA. The tumor cells uniformly expressed HMGA2. Cytokeratin 7, S100 protein, and SOX10 displayed either diffuse positivity or highlighted the luminal and abluminal cell populations, respectively, in all cases tested. Markers p63 and p40 were positive in 46% and 39% of cases, respectively, most of these cases had biphasic morphology. Areas with oncocytoid transformation of luminal cells were seen in 6 biphasic cases. Whereas the luminal cells in these cases had voluminous lightly eosinophilic cytoplasm, slightly enlarged round or oval nuclei, and an occasional prominent nucleolus, the abluminal cells were small, flat, and in some instances almost indiscernible. Immunohistochemical analysis revealed diffuse AR positivity in the luminal cells. HMGA2 rearrangements were detected by RNA-sequencing in 29 cases. The most common alteration was an HMGA1::WIF1 fusion, but several novel fusion partners were also identified. In addition, FISH revealed HGMA2 break-apart in 9 cases where targeted sequencing failed to detect any alteration and in 1 case where RNA sequencing was not performed.

Conclusion: These cases provide evidence for a subset of PA driven by HMGA2 rearrangements which are characterized by distinctive canalicular, trabecular and often monophasic morphology. This entity is distinct from canalicular adenoma of minor salivary glands which

does not harbor HMGA2 or PLAG1 alterations. Notably, all of the detected HMGA2 rearrangements lead to the loss of the 3' untranslated region that binds regulatory mi-RNA let-7. This event might be the basis for aberrant activation of the corresponding HMGA2 protein. Awareness of this rare variant ensures appropriate diagnosis and clinical management.

PHYSICAL FITNESS & LIFESTYLE OF MEDICAL STUDENTS

A. Zdráhal (4th year of MSP), A. Zdráhalová
Department of Sports Medicine and Active Health Sciences, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. P. Brož, Ph.D., doc. MUDr. A. Kroužecký, Ph.D.

Background: Physical and mental endurance is an essential parameter for a future doctor who should set an example for his/her patients. The purpose of the study is to map the level of lifestyle among current medical students of the Faculty of Medicine of the Charles University in Pilsen. Studying general medicine and dentistry are some of the most difficult study programs. Students are exposed to a greater degree of lack of time, excessive stress and increased sitting due to the demands and length of their studies. To test the physical fitness of the students of the Faculty of Medicine in Pilsen, Charles University in Prague and compare the obtained results with published data and at the same time compare individual years with each other. Another sub-goal is to evaluate the data obtained in the form of a questionnaire survey.

Methods: The tested sample of students for the study on „Physical fitness & lifestyle of LFP students" was selected based on voluntary interest in participating in the study. Despite the voluntary interest, the sample was very varied, consisting of students of all grades, athletes and non-athletes. Physical fitness was tested in the stress laboratory through stress tests such as the generally accepted Bruce protocol, the handgrip strength test, and the back-leg-chest dynamometer strength test. Part of the data collection was also a questionnaire survey, focusing on sports activities of students before and during medical studies, sleep, abuse and other lifestyle issues. The results of the investigated population were compared with the data published so far, at the same time we compared the individual years with each other.

Results: 73 volunteers (27 men, 46 women) have been tested so far, data are presented as median, minimum - maximum. Bruce protocol results (time in minutes:seconds) - men 14:32 (9:54-19:01), women 11:28 (8:38-15:04). The survey was completed by 80 respondents. Regarding physical activity - 63.3% of participants engaged in regular sports activities for 10 or more years before entering university, 55% stated that entering university definitely influenced their physical activity. 43.8% of students still engage in physical activity for more than 200 minutes per week.

Conclusion: The study on „Physical fitness & lifestyle of LFP students" follows on from last year's study and has been ongoing since the beginning of the summer semester this year. 13 students were evaluated in the 1st year, 2nd year – 17 students, 3rd year – 14 students, 4th year – 27 students, 5th year – 4 students, 6th year – 3 students. The results of the majority of students in both sexes were above average.

This output was created within the framework of the Cooperatio program, a scientific area of IMMU.

VALIDATION OF A NEW METHOD FOR MEASURING AUTONOMIC NEUROPATHY IN PATIENTS WITH DIABETES

A. Hluchý (5th year of MSP), A. Kičko, V. Weissová
1st Internal Clinic, University Hospital in Pilsen and the Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. M. Krčma Ph.D.

Background: Diabetic autonomic neuropathy is a non-inflammatory disease of the autonomic nerves based on metabolic-vascular pathogenesis. It is a microvascular complication of diabetes mellitus. These patients have a higher risk of cardiac arrhythmias, gastroparesis, erectile dysfunction, or orthostatic hypotension. Questionnaires and clinical examinations (gastric emptying measurement, quantification of erectile dysfunction) are used for diagnosis, capturing advanced forms of autonomic neuropathy (AN). In clinical practice, cardiovascular autonomic neuropathy is measured using heart rate variability assessment (spectral analysis and Ewing tests). Currently, there is no validated device available for measuring AN. Our clinic is using an older device, Varia Cardio TF 5 (MIE Medical Research, Leeds, UK), which can be considered the standard for examination; it has been used in several older studies. Its production was discontinued years ago. Validation of the accuracy and correctness of a new method – the Nutripro BodyFit device (Fitsport-komplex, Brno, Czech Republic) for measuring autonomic neuropathy based on heart rate variability (measured by limb leads as in standard ECG) compared to the available standard (measurement by chest strap) in a sample of patients with type 1 and type 2 diabetes.

Methods: Patients with type 1 and type 2 diabetes were selected from the pool of patients in the diabetic outpatient clinic. Under standard resting conditions, simultaneous measurements were performed using both methods, utilizing spectral analysis of heart rate variability and Ewing tests (deep breathing, orthostatic test). Ewing tests were used for device comparison due to international standardization of assessment.

Results: Data from 37 consecutive patients, 19 males and 18 females, with a mean age of 40 ± 15 years, were evaluated. Excellent correlation between methods was found in individual dimensions of the tests (RR interval variability in inspiration, expiration, in the orthostatic test) with correlation coefficients $R = 0.87$ ($p < 0.0001$); $R = 0.86$ ($p < 0.0001$), and $R = 0.73$ ($p < 0.0001$). After artifact correction and patient grouping according to neuropathy severity, results matched in 86% of patients, with differences observed in 14% of patients (3 times the finding was rated as borderline by the original method and normal by the new method, 2 times the opposite difference occurred). Agreement was observed in all cases of manifest AN.

Conclusion: Preliminary results indicate good accuracy of the new measurement method, which is simpler and less demanding to operate with a semi-automatic device. Limb lead data acquisition is less affected by artifacts compared to chest strap.

NEUTROPHIL TO LYMPHOCYTE RATIO IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA TREATED WITH IMMMUNOTHERAPY

L. Soukup (3rd year of MSP)
Department of Oncology and Radiotherapy, University Hospital in Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Ondřej Fiala Ph.D.

Background: Immunotherapy based on immune checkpoint inhibitors (ICIs) targeting the programmed cell death protein-1 (PD-1)/ligand-1 (PD-L1) pathway has revolutionized systemic treatment of metastatic renal cell carcinoma (mRCC) in the recent years. Relevant predictive biomarkers are necessary for personalized systemic therapy for mRCC. To assess potential prognostic and predictive role of neutrophil to lymphocyte ratio (NLR) in patients with mRCC treated with immunotherapy.

Methods: The data on outcomes of mRCC patients treated with nivolumab in the second or higher line were retrospectively collected in a multicentric study. The impact of baseline NLR on progression-free survival (PFS), overall survival (OS) and disease control rate (DCR) was assessed. Gehan-Wilcoxon test was used for asssessment of PFS and OS. Mann-Whitney test was used used for asssessment of DCR (patients achieving complete remission, partial remission or stable disease).

Results: The study included 310 patients with mRCC. Patients with baseline NLR<3.2 had significantly longer PFS ($p \leq 0.001$) and 0S ($p \leq 0.001$) compared to those with NLR≥3.2. Additionally, lower baseline NLR was significantly associated with higher DCR ($p=0.0134$).

Conclusion: Baseline NLR is a reliable and easy to use parameter for estimation of response and prognosis of mRCC patients receiving immunotherapy.

VAGINAL PACKING AND THE OUTCOME OF LAPAROSCOPIC SACROCOLPOPEXY IN ONE YEAR FOLLOW-UP

M. Chromý (5th year of MSP), V. Svoboda
Department of Gynaecology and Obstetrics, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Z. Rušavý, Ph.D.

Background: Laparoscopic sacrocolpopexy (LSC) is a reconstructive surgical procedure used to correct pelvic organ prolapse. Packing the vagina with antiseptic solution-soaked gauze after the surgery is deemed to improve surgical outcome. Vaginal packing could contribute to better mesh adherence to surrounding tissues, thus reducing the risk of recurrence. Unlike vaginal reconstructions, the use of vaginal packing after LSC has not received enough attention, leading to variability in practice. Our study from last year did not confirm a significant impact of vaginal packing on infections, pain, bleeding, and overall patient satisfaction in the early postoperative period.The primary objective was to compare the surgical outcome in one-year follow-up after the insertion or non-insertion of vaginal packing for 24 hours. The secondary objective was to compare the patient global impression of improvement in overall health after 1 year.

Methods: This randomized clinical trial was conducted in the Department of Gynaecology and Obstetrics, Faculty of Medicine in Pilsen, Charles University between 2016 and 2022. All women with POP ≥ stage 2 who underwent pelvic floor reconstruction were included. Exclusion criteria were malignancy, coagulopathy, concomitant hysterectomy, or intraoperative vaginal perforation. Before the surgery, patients signed consent for study enrollment and underwent urogynecological examination with quantification of prolapse stage according to POPQ. Patients were randomized using envelope method into groups with and without vaginal packing. In 1 year, follow-up, the patients were examined to evaluate the outcome of the surgery. Anatomical recurrence was defined POPQ stage>1. Composite surgical failure was defined as prolapse beyond the hymen, subjective feeling of prolapse or retreatment. Using the PGI-I score (Patient Global Impression of Improvement), patients evaluated subjective changes in overall health status. Basic characteristics and perioperative data were retrieved from the clinical database. Data were evaluated using the following statistical methods: Wilcoxon paired test, $\chi 2$ test, Fisher exact test, Median test with a significance level of $p<0.05$.

Results: A total of 455 women were included in the study. Vaginal packing was inserted to 214 (47%) patients. 249 patients underwent concomitant supracervical hysterectomy, 69 reconstructions with uterus preservation, and 137 had underwent hysterectomy previously. No significant differences in basic characteristics were observed between the groups. The mean age of patients was 61±11.36 years, with a mean BMI of 26.6±3.5. The frequency of anatomical recurrence (16.8% vs. 14.1%; p=0.42) and composite surgical failure (2.8% vs. 1.7%; p=0.53), was comparable. PGI-I was also comparable in both groups (1.52 vs.1.46; p=0.28).

Conclusion: The insertion of vaginal packing has neither a positive nor negative impact on the quality of pelvic organ prolapse reconstruction. PGI-I after 1 year was also not affected by the vaginal packing. Therefore, routine use of vaginal packing after LSC is not meaningful in practice.

PREVALENCE OF ANTIRETROVIRAL THERAPY RESISTANCE IN HIV-1 PATIENTS AT HIV CENTRE IN PILSEN

Z. Prášek (4th year of MSP), K. Vančurová
Department of Infectious Diseases and Travel Medicine, University Hospital Pilsen
Supervisor(s): doc. MUDr. Dalibor Sedláček, CSc.

Background: HIV-1 drug resistance has been studied since 1989. The increasing use of antiretroviral therapy (ART) for HIV-1 treatment may lead to the rise of antiretroviral drug resistance. Our initial hypothesis posits that there is a notable prevalence of resistance mutations among HIV-1 patients undergoing ART. Our study aims to evaluate the prevalence of antiretroviral therapy resistance mutations in HIV-1 patients under treatment at Teaching Hospital Plzeň, and to identify any associated factors contributing to the emergence of resistance.

Methods: We conducted a retrospective analysis of HIV-1 patients undergoing antiretroviral therapy at HIV centre, Department of Infectious Diseases and Travel Medicine, Teaching Hospital Plzeň between January 1999 to March 2024. Patient data including treatment history, HIV RNA, using PCR (viral load, VL) and CD4 lymphocyte T, using flow cytometry measurements, and genotypic resistance testing results were collected and analyzed.

Results: Among 203 HIV-1 patients tested for ART resistance, included in the study, 22,2% displayed some type of antiretroviral resistance mutations. The most prevalent resistance were found in NRTI and NNRTI drugs.

Conclusion: Our study underscores the importance of monitoring antiretroviral therapy resistance in HIV-1 patients. The prevalence of resistance mutations highlights the need for continual surveillance and optimization of treatment strategies as a prevention of the development of HIV-1resistance. Further research is warranted to unravel the impact of resistance on treatment outcomes and to explore novel approaches to managing drug-resistant HIV-1 strains.

IDENTIFICATION OF DRUG INTERACTIONS IN A GROUP OF POLYPRAGMATIC LONG-TERM PATIENTS

J. Ťažiarová (3rd year of MSP)
Department of Farmakology and Toxicology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. M. Jirásko

Background: Drug interactions as an adverse effect represent a significant factor that can potentially threaten the quality of life of thousands of patients in the Czech Republic. Aging results in a decrease in functional reserves and deterioration of the organism's adaptive capacity, an increase in the number of chronic and degenerative diseases, changes in the sensitivity of target tissues, reduced absorption of some drugs and reduced function of elimination organs. Polymorbidity and polypragmasia increase the risk of drug interactions and non-adherence to treatment. Increasing age and the amount of drugs used are significant independent risk factors for an increase in drug-associated complications. To identify potential drug interactions in selected polypragmatic long-term patients, evaluate their clinical relevance and propose solutions.

Methods: Pharmacotherapeutic medical history, age and diagnoses of patients were obtained anonymously from providers of health care facilities and homes for the elderly. The electronic compendium DrugAgency Drug Interaction Database was used to evaluate the severity of drug interactions.

Results: 7 facilities were investigated. History of pharmacotherapy was obtained from 373 patients. The mean age was 81 years. Most of the patients were taking medications chronically, of which 81.8 % were taking psychiatric medication. Opioids for chronic pain were used by 26.5 %, while chronic pain management by daily application of NSAIDs was observed in 41.3%. Potential interactions were divided according to clinical relevance into 6 groups. Significant interactions (groups 3 - 6) were identified in 96% of patients. Drug duplication was also frequent, occurring in 17.2 % of patients.

Conclusion: The study confirmed the seriousness of the situation in polypragmatic patients and pointed to the need for closer links between the social and health providers, ensuring quality coordination of care and monitoring of current medication. The introduction of the use of drug interaction compendia and software could significantly simplify the process.

COMPARISON OF THE EFFECT OF ANDROGEN RECEPTOR INHIBITORS BETWEEN ANDROGEN DEPENDENT AND ANDROGEN INDEPENDENT PROSTATE CANCER CELL LINES.

E. M. Debilio (4th year of MSP), V. Attar
Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Mgr. et Bc. Roman Viták

Background: According to data of the World Health Organization, prostate cancer was the third most frequently diagnosed type of cancer in men in 2020 (after lung and colorectal cancer). Prostate cancer is a malignant tumor arising from the epithelium of the prostate gland. The development and progression of prostate cancer depends on androgens such as testosterone and dihydrotestosterone. The actions of androgens are mediated via androgen receptor. By blocking the androgen receptor through androgen receptor inhibitors, we achieve a slowdown in the growth of cancer cells. The aim was: 1) To acquire fundamental knowledge in cell culture cultivation, focusing particularly on prostate cell lines. 2) To identify and compare androgen receptor expression in androgen dependent and androgen independent prostate cell lines by immunoanalytical technique western blot. 3) Compare the effect of androgen receptor inhibitors on growth of androgen dependent and androgen independent prostate cell lines by using WST-1 cell viability assay.

Methods: Two prostate cancer cell lines were chosen for this work: LAPC-4 (androgen-dependent) and PC-3 (androgen-independent). Both cell lines were cultured in the appropriate medium supplemented with 10 % of fetal bovine serum, 100 IU/ml of penicillin, and 100 µg/ml of streptomycin at 37 °C in a 5% CO2 incubator. Once the cells have reached the appropriate confluence, we perform protein extraction by using RIPA buffer. Subsequently, the amount of extracted proteins was determined using the Bradford protein assay.

Separation of proteins was performed by electrophoresis in a polyacrylamide gel in the presence of sodium dodecyl sulfate. The separated proteins were then transferred to a PVDF membrane using the semi-dry western blot technique. To identify the androgen receptor, the membrane was incubated with the primary antibody against the androgen receptor, followed by incubation with the secondary antibody and chemiluminescence detection.

Two non-steroidal antiandrogens Flutamide and Bicalutamide were used as androgen receptor inhibitors. LAPC-4 and PC-3 cells were seeded into a 96-well plate and treated with different concentration of Flutamide and Bicalutamide. After 72 hours incubation cell viability was determined using the WST-1 assay. The analysis was based on the reduction of tetrazolium salts into formazan; the rate of WST-1 cleavage by mitochondrial dehydrogenases correlates with the number of viable cells. The absorbance of each well was measured by using a microplate reader at 450 nm.

Results: Results from western blot analysis will be interpreted and discussed in the form of PVDF membrane images. Inhibition results will be presented in the form of IC50 for both drugs.

Conclusion: We have reached the necessary knowledge for the cultivation of cell lines and to become familiar with the immunoanalytical western blot method and the method of determining cell viability using the WST-1 assay.

OVARIAN CANCER – MAINTENANCE THERAPY WITH PARP INHIBITORS AND THE RISK OF DRUG INTERACTIONS IN REAL CLINICAL PRACTICE

M. Zronková (4th year of MSP)
Department of Oncology and Radiotherapy, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): prof. MUDr. Samuel Vokurka, PhD.

Background: Ovarian cancers are serious malignancy, and two thirds of the patients are diagnosed in advanced stages. The poly-(ADP-ribose)-polymerase inhibitors known as PARP inhibitors (PARPi) can prolong the time to progression/recurrence of the disease and the overall survival. The potential drug interactions can affect their safety and efficacy. Evaluation of drug interaction risks in the treatment with PARPi (niraparib or olaparib)

Methods: Retrospective analysis of comedications in patients with high-grade serous ovarian cancer on the treatment with PARPi, since 2018 to 2023, in the local Oncology and Radiotherap. Dpt. in Pilsen. Drug interactions in individual patients were primarily assessed by means of mobile phone application Mediately (Modra Jagoda, Slovenia; drug interactions based on MedBase, Finland) and were graded 0 to 4: 0= no risk; 1= no clinical relevance; 2= should be monitored; 3= medium risk where dosage should be rearranged; 4= high risk. The interactions were then verified by using mobile phone application OncoASSIST (Portable Medical Technology Ltd, Ireland), by the Medicalc4 software (Teaching hospital in Pilsen; drug interactions based on Databáze lékových interakcí, DrugAgency a.s., CZ) and by the Clinical Pharmacist (PharmDr. J.Červeňova, Clinical Pharmacy Dpt., Pilsen). The daily practice and attitude of oncologists (Dpt. Oncology and Radiotherap. in Pilsen.) to drug interactions issue was assessed by using printed questionnaires.

Results: 47 patients, median age 63 (42-78), on maintenance treatment with PARPi were evaluated: olaparib tbl 21/47 (44,7 %) and niraparib cps 26/47 (55,3 %). In total 31/47 (66 %) patients took more than 3 other drugs together with PARPi. The phone application OncoASSIST evaluated the drug interactions to be more common, however less clinically significant, compared to Mediately and Medicalc4. Final consultancy with Clinical Pharmacist concluded that the potential drug interactions (grade 2-4) occurred in 9/47 (19 %) patients, in olaparib 1/21 (4,7 %) and in niraparib 8/26 (30 %). The questionnaires were filled by 12 oncologists (recurrence rate 100%). 6/12 (50 %) check drug interactions regularly in every patient, in 4/12 (33 %) drug interactions are checked when unexpected reaction on treatment occurs, in 2/12 (16,6 %) the oncologist remembers and is aware of interactions the used medications have. To assess interactions, 8/12 (66,6 %) oncologists follow Summary of Product Characteristic (SPC), 7/12 (58 %) discuss interactions with clinical pharmacist, 3/12 (25 %) use Mediately, 3/12 (25 %) the Medicalc4 a 1/12 (8,3 %) OncoASSIST.

Conclusion: Drug interactions are very important issue in the treatment with PARPi and can cause unwanted reaction. In the total amount of 47 patients, there were 9 (19 %) with potentially significant interaction (grade 2-4). Majority of oncologists are aware of and consider drug interactions, however, assessing it by using SPC or mobile phone application may be somewhat confusing and Clinical Pharmacist consultancy could be the best option.

EXTRACTION OF MUSHROOM BIOACTIVE PEPTIDES (BAPS) FROM TRAMETES OCHRACEA, LAETIPORUS SULPHUREUS, AND AGARICUS CAMPESTRIS.

M. Lazarev (4th year of MSP), M. Marouf, A. Sepas, S. Pandey
Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): Dr. Shashank Pandey, Ph.D., Zdeněk Tůma, Ph.D.

Background: Mushrooms are a promising source of high-quality proteins and bioactive peptides (BAPs). BAPs can have physiological effects on the body, such as regulating blood pressure, improving immune function, and acting as antioxidants, antihypertensive and antimicrobial activities. In the past few decades some anti-cancers have been discovered such as Polysaccharide-K (Krestin, PSK) from Trametes versicolor and preliminary studies has confirmed the role PSK in gastric cancer, breast cancer, colorectal cancer, and lung cancer. Aim: Extraction of peptides from different species of fungus such as Orche bracket, Laetiporus sulphureus and Agaricus campestris. MALDI TOF analysis of extracted peptides.

Methods: We have picked our mushroom of interest – Trametes ochracea. After collection of the mushroom, we have included other species from different mushroom genera to test our methods of peptide extraction. The process of preparation and peptide extraction remained the same for all the species selected. For extraction of peptides, we first lyophilized the mushroom, after that we ground the mushroom bodies with mortar and pestle. The powder was solubilized in urea buffer, the solute was put through sonication and then centrifugation. After centrifugation, supernatant was collected and protein fraction were analysed by 10% SDS-PAGE. Peptides were extracted from each sample by using special type of filters with cut off 3kDa. 1% TFA was added into peptide filtrate and precipitate was removed. Solubilized peptides were extracted via ZipTip (C18) and mixed with matrix HCCA matrix for MALDI analysis.

Results: We have extracted the peptides from 3 different species Trametes ochracea, Laetiporus sulphureus, and Agaricus campestris. Peptides were administered to two distinct fibroblast cell lines cultured in dimethyl sulfoxide (DMSO), where they exhibited pronounced cytoprotective effects against DMSO-induced cytotoxicity.

Conclusion: We have standardized the method for extraction of peptide from mushrooms and MALDI analysis has confirmed that extracted peptides were below < 3kDa.

ENDOMETRIAL CARCINOMAS WITH NO SPECIFIC MOLECULAR PROFILE: DETAILED MOLECULAR GENETIC AND IMMUNOHISTOCHEMICAL (IHC) STUDY

N. Hejhalová (5th year of MSP), P. Holub
Bioptic laboratory, Ltd., Sikl´s Department of Pathology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. K. Michalová, Ph.D.

Background: Endometrial carcinomas (EC) are currently classified into 3 molecularly distinct groups: POLE mutated, hypermutated, and EC with p53 alteration. ECs lacking the diagnostic molecular features of these groups fall into the fourth and largest group named EC of no specific molecular profile (NSMP). The molecular features with pathogenic driver roles known in the 3 groups of EC influence the management of these patients, including therapy decisions. Given the heterogeneity of NSMP, such an approach cannot be uniformly applied for these patients, and therapy is guided by the grade and stage. Therefore, further stratification is warranted. In order to better characterize this diverse group of EC and potentially discover IHC/molecular features helpful for their further categorization, we conducted comprehensive molecular and IHC analysis of large cohort of ECs of NSMP.

Methods: Based on the results of molecular classification of EC routinely performed for all EC diagnosed at Faculty Hospital, 110 cases were selected. In addition to routine diagnostic procedures, including IHC (MMR proteins, p53) and molecular analysis (NGS- customized panel analyzing 18 relevant genes), we analyzed tumoral microenvironment using CD3, CD4, CD25, CD163 antibodies. Additionally, HER2 analysis and more extensive molecular analysis were performed using the NSG panel TruSight500.

Results: 110 cases were studied, with patient's ages ranging from 28 to 89. The size of the tumors ranged from 0,3 to 11 cm, stage ranged from pT1 to pT3. Follow-up was available for all patients, ranging from 1 to 45 months. Most patients were alive and well, 7 were alive with disease. All patients underwent surgery, with 60 receiving adjuvant therapy. 104 cases were endometrioid carcinomas, the remaining were mesonephric-like, undifferentiated, dedifferentiated, clear cell carcinoma, and carcinosarcoma. IHC, all cases were MMR proficient, p53 was normal in 96 cases, 5 cases showed over-/null expression. CD3 was positive in 24 cases, CD4 in 22, CD8 in 25, CD25 in 23, CD163 in 25, HER2 in 22 cases. The most common molecular alterations were: PTEN (44%), PIK3CA (30%), ARID1A (21%), KRAS (9%).

Conclusion: Study corroborates the findings of previous research, indicating that PTEN mutations are the most prevalent molecular abnormalities observed in EC of NSMP, followed by PIK3CA mutations. IHC analysis of the tumor microenvironment was not helpful to stratify these tumors, as the ECs exhibited overlapping immunophenotypes regardless of other characteristics (such as grade, stage, or molecular features). From a molecular standpoint, ECs of NSMP can be classified into at least two groups: one associated with PTEN mutation (most cases) and the other lacking PTEN mutation. Nevertheless, we did not observe any significant differences in clinical outcomes between these groups, which may be attributed to the short follow-up period. It is worth noting that the additional extensive molecular analysis did not aid in categorizing ECs of NSMP, suggesting that such thorough NGS analysis is not necessary in diagnostic workup, thus leaving the customized, more restricted NGS panel as the method of choice.

SUBPOPULATION OF T-LYMPHOCYTES IN PATIENTS WITH ENDOMETRIOSIS AND HEALTHY WOMEN

D. Uschkereit (3rd year of MSP), M. Kytýrová, K. Kabíčková, B. Sedláčková, A. Šimůnek, M. Grundmanová

(1) Gynecology and Obstetrics Clinic, Faculty of Medicine in Pilsen, Charles University, and University Hospital Pilsen

(2) Department of Physiology, Facuty of Medicine in Pilsen, Charles University

(3) Laboratory of Tumor Biology and Immunotherapy, Biomedical Center, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): prof. MUDr. J. Bouda, Ph.D. (1), doc. MUDr. M. Čedíková, Ph.D. (2, 3), Mgr. M. Holubová, Ph.D. (3), MUDr. L. Prajka (1)

Background: Endometriosis is an estrogen-dependent, inflammatory, systemic, and chronic disease characterized by the presence of ectopically located endometrial glands and stroma outside the uterine cavity. The aim of our study was to compare basic anamnestic data and analyze T-lymphocyte subpopulations in women with present endometriosis compared to healthy women.

Methods: The study group consisted of 53 women, including 24 patients with endometriosis and 29 healthy women. Basic anamnestic information was collected from each woman, including age, weight, height, body mass index (BMI), age at menarche, length of menstrual cycle, and duration of bleeding. T-lymphocyte subpopulations (TH-lymphocytes, TC-lymphocytes, double-negative T-lymphocytes, memory T-lymphocytes, regulatory T-lymphocytes, naive and transient T-lymphocytes) were evaluated using flow cytometry.

Results: There was no statistically significant difference in anamnestic parameters between patients and healthy women. Significant differences were observed in the number of activated T-lymphocytes among the studied T-lymphocyte subpopulations, but the total number and individual subtypes did not differ between groups.

Conclusion: Immune cells likely play a significant role in the pathophysiology of endometriosis. However, their specific role remains largely unknown, necessitating further detailed analysis and extensive research for clarification.

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CHROMOSOME 12P AMPLIFICATION AND P53 ABNORMALITIES APPEAR TO BE RARE EVENTS IN SPERMATOCYTIC TUMORS WITH CONVENTIONAL MORPHOLOGY

D. Trpáková (4th year of MSP)
Sikl's Department of Pathology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Květoslava Michalová, Ph.D.

Background: Spermatocytic tumors (ST) were historically perceived as akin to seminomas due to their morphological similarities. Nevertheless, their distinct pathogenesis has been reliably demonstrated in multiple studies, leading to their recognition as separate entities with different oncogenic drivers and biological behavior. The majority of STs are benign, characterized by conventional morphology. A subset of STs may display anaplastic morphology and some undergo sarcomatoid transformation, resulting in a highly aggressive clinical course. Recent studies have identified STs with anaplastic morphology exhibiting aggressive biological behavior, along with one case featuring conventional histology and a similarly aggressive course. These investigations have unveiled a molecular background potentially contributing to such aggressive behavior, namely the presence of either TP53 gene mutation or chromosome 12p amplification. Given the reported case of ST with conventional morphology displaying aggressive behavior, we conducted a study on a cohort of mostly conventional ST with the aim to investigate the potential presence of hallmarks of aggressiveness in such cases. Additionally, we sought to assess the reliability of SSX, a relatively novel immunohistochemical marker for ST.

Methods: Eighteen cases of ST with conventional morphology and one case of anaplastic ST were identified in the authors' consultation files. Paraffin blocks were available for all cases. Clinical information was retrieved from electronic medical records for cases diagnosed in Faculty Hospital; however, it was mostly unavailable for consult cases. Immunohistochemistry for SSX, p53, and OCT3/4 was conducted. The FISH method was used as a molecular technique for analyzing chromosome 12p abnormalities.

Results: The age of the patients ranged from 33 to 87 years (median 59 yrs). Tumor sizes ranged from 0,7 to 18 cm (median 4,5 cm). Follow-up was available for 5 patients, ranging from 9 to 118 months (median 82 months); one patient was lost to follow-up. All patients are alive and well. There is no available follow-up both for the case of anaplastic ST and the 3 cases of aberrant p53 expression. Immunohistochemically, 17/19 cases were diffusely positive with SSX, the remaining cases were focally positive and negative, respectively. Sixteen cases showed normal p53 expression while 3 cases showed aberrant p53 expression (focal overexpression in 2 cases, complete negativity in 1). All cases (19/19) were negative for OCT3/4. Similarly, FISH analysis did not reveal gain 12p in any of the cases (18 analyzable cases, including anaplastic ST)

Conclusion: The occurrence of aggressive features, such as 12p amplification and p53 abnormalities, appears to be infrequent in STs with conventional histology. Our findings suggest that routine evaluation of ST for the presence of 12p and p53 alterations is not warranted in spermatocytic tumors with conventional morphology; such assessments should be limited to tumors displaying histopathologic features associated with aggressive behavior. Furthermore, the SSX marker appears to be a useful immunohistochemical adjunct for the diagnosis of ST.

KRAS G12C MUTATED LUNG ADENOCARCINOMAS: A RETROSPECTIVE REVIEW OF 222 CASES

P. Slavík (5th year of MSP), M. Michal, P. Martínek
Bioptic laboratory, Ltd., Sikl´s Department of Pathology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. M. Bradová, Ph.D.

Background: KRAS G12C mutant lung adenocarcinoma is a malignant neoplasm of the lung in which the cancer harbors an oncogenic KRAS mutation at codon 12, resulting in the misplacement of glycine to cysteine. Advances in understanding the molecular genetics of this tumor have led to the development of targeted therapies that specifically inhibit the KRAS G12C mutation (e.g. sotorasib, adagrasib, etc.). According to some studies patients with KRAS-mutant adenocarcinomas carry poorer survival and have a higher frequency of distant metastases compared to those with wild-type KRAS. The aim of the study was to correlate the morphology and known molecular genetic background of these KRAS G12C mutant adenocarcinomas.

Methods: From the Registry of Lung Tumors of the Šikl´s Department of Pathology and Bioptic laboratory, Ltd., 222 cases of KRAS G12C mutant adenocarcinomas were selected from the years 2017-2022. These specimens were subjected to histological, immunohistochemical and basic statistical analysis.

Results: From the 222 cases studied, 98 were female and 124 male patients. The average age was 66,6 years (age range 29 to 91 years). Most of the cases were primary lung tumors (n=181, 82%). A diagnosis from the metastasis was performed in 40 cases (18%) including locoregional metastatic spread to pleura/pleural cavity/chest wall (n=17) and mediastinal and hilar lymph nodes (n=8). Distant metastases were encountered in 15 cases (15/40), including neck and axillary lymph nodes (n=5), skin (n=3), bones (n=2), brain (n=3), parotid gland (n=1) and abdominal cavity (n=1). Morphological features were assessed in 109 cases (49%). The tumors exhibited rhabdoid/plasmacytoid/epithelioid features in 61 cases, squamous features in 74 cases and sarcomatoid (spindle-cell) features in 24 cases. In 77 cases (71%) the tumors were solid. In these cases, with aggressive morphology, nuclei were pleomorphic, irregular and contained multiple nucleoli while the tumor cells showed multiple mitoses, even atypical. Classical markers of pulmonary adenocarcinoma TTF1, Napsin A and CK7 were positive in 87%, 86%, and 97% respectively. Non-classical markers p40/p63 and CK5/6 were expressed in 5% and 6% respectively. PD-L1 was positive in 107 cases (63%).

Conclusion: KRAS G12C mutant lung adenocarcinomas are morphologically aggressive neoplasms diagnosed at metastatic site in 18% of cases. These tumors often grow in a solid fashion with a rhabdoid/plasmacytoid/epithelioid tumor cell phenotype. Squamous features (polygonal cells and keratin pearls) are also often present and can be a misleading feature, resulting in an erroneous diagnosis of squamous cell carcinoma, for which genetic testing is not a standard procedure. Immunohistochemistry is therefore an important step towards the correct diagnosis as these tumors have a characteristic immunoexpression of TTF1, Napsin A and CK7 markers. The correct molecular-genetic stratification of these tumors provides access to an alternative therapeutic approach using KRAS inhibitors in advanced and inoperable cases.

MORFOLOGICAL, IMMUNOHISTOCHEMICAL AND MOLECULAR-BIOLOGICAL STUDY OF BAP1-INACTIVATED MELANOCYTOMAS-LIKE LESIONS

A. Bernklauová (4th year of MSP)
(1) Biopticka laborator s.r.o.
(2) Sikl's Department of Pathology, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): (1,2) prof. MUDr. D. Kacerovská, Ph.D., (1,2) prof. MUDr. M. Michal

Background: BAP1 (BRCA1-associated protein-1) -inactivated melanocytomas are characterized histologically by epithelioid cell morphology and genetically by inactivation of the BAP1 gene. They occur sporadically or in association with the BAP1 tumour predisposition syndrome (BAP1-TPDS). We report six pigmented lesions from different patients exhibited a BAP1-inactivated melanocytoma morphology but with preserved immunohistochemical BAP1 expression. In all cases molecular biologic study was performed to exclude BAP1 gene mutation.

Methods: This is a clinicopathological, immunohistochemical and molecular biological study complemented by a literature review.

Results: All six melanocytic lesions histopathologically revealed predominantly intradermal proliferation of large epithelioid melanocytes with abundant eosinophilic glassy cytoplasm consistent with BAP1-inactivated melanocytoma morphology. Immunohistochemically, in all cases a BAP1 nuclear expression was preserved. In 5 cases nests of conventional melanocytes/nevus cells were present. In all cases bi- or/and multinucleation, nuclear blebbing, micronuclei, shadow nuclei, nuclear pseudoinclusions were detected. In one case pseudorosettes structures were seen. Molecular biologic study revealed mutation in BRAF gene in all cases except one. No mutation of BAP1gene was not detected in any case.

Conclusion: A small subtype of melanocytic lesions with BAP1- inactivated melanocytoma morphology but with retained BAP1 nuclear expression may exist. In these cases the additional molecular investigation is necessary to exclude mutations of BAP1 gene.

ATYPICAL FEATURES OF PLEOMORPHIC ADENOMA: POTENTIALLY SIGNIFICANT DIAGNOSTIC PITFALL

K. Bělohlávková (4th year of MSP)
(1) Department of Pathology, Faculty of Medicine in Pilsen, Charles University
(2) Biopticka Laboratory Ltd., Pilsen
Supervisor(s): prof. MUDr. Alena Skálová CSc.

Background: Pleomorphic adenoma (PA) is the most prevalent benign salivary gland tumor. Its structure consists of epithelial and mesenchymal patterns. PA is a biphasic tumor composed of epithelial and myoepithelial components, including metaplastic changes and various forms of differentiation. The major differential diagnostic challenge lies typically in PAs presenting with high cellularity and/or atypical histological features, diagnosed as "cellular" or "atypical" PAs, respectively. In such cases, low-grade salivary carcinomas with biphasic growth pattern, such as epithelial-myoepithelial carcinoma, must be excluded.

Methods: A retrospective analysis of 66 PA cases, diagnosed between 1960-2023 was conducted on the Salivary Gland Tumor Registry of the Biopticka Laboratory Ltd., Pilsen and the Department of Pathology, Faculty of Medicine in Pilsen consultation files. Key words used in search included "pleomorphic adenoma," "atypical," and "cellular". The focus was given on data such as age, sex, site, size and uncommon histological features such as hypercellularity, necrosis, hyalinization, proliferative activity (MIB1 index), and presence of nuclear polymorphism.

Results: Most of the tumors were found in the parotid gland 51/66 (77,3%), followed by submandibular (9/66;13,6%), and minor salivary glands 2/66 (3%). The average age of the patients was 57 years (range 17-85). Hyalinization was present in 41/66 (62,2%) of the cases, hypercellularity in 58/66 (87,9%). Necrosis was present only in 8/66 (12,1%) of the cases. Atypical pleomorphic nuclei were found in 42/66 (63,6 %) of the cases. Proliferative activity measured by MIB1 index, had shown the median value 10% (0-40%).

Conclusion: We have confirmed that histological features such as hyalinization, hypercellularity and nuclear polymorphism seen in so called „atypical PAs" do not determine aggressive clinical and biological behavior of salivary PA, and should not result in over-diagnosing as malignancy.

SPONTANEOUS IN-VIVO RECELLULARIZATION OF DECELLULARIZED PIG LIVER GRAFT

M. Brousil (3 rd year of MSP), L. Červenková (1), V. Moulisová (1), P. Hošek (1), M. Jiřík (1), J. Rosendorf (1, 2), R. Pálek (1, 2), M. S. Massaro (1), S. Šarčevič (1, 2), J. Ševčík (1,2), T. Polenda (1), I. Matsviayonak (1), D. Volák (1)

(1) Laboratory of Cancer Treatment and Tissue Regeneration, Biomedical Center, Faculty of Medicine in Pilsen, Charles University

(2) Department of Surgery, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): MVDr. L. Červenková (1), RNDr. V. Moulisová, Ph.D. (1), prof. MUDr. V. Liška, Ph.D. (1, 2)

Background: The increasing demand for suitable liver transplants could be solved by the decellularization of a porcine liver followed by its subsequent recellularization with human cells. Our team designed a fast decellularization protocol and developed a new scoring system for the assessment of recellularization. This study explores the progress of spontaneous recellularization, via full-scale in-vivo study. This study aims to understand the process of recellularization and revascularization of the decellularized pig liver scaffold (liver extracellular matrix) implanted into the porcine omentum.

Methods: Small pieces of standard size cut out from the liver scaffold obtained by decellularization were implanted into the closed pockets created on the porcine omentum. Two groups of 8 animals were used – the first was sacrificed after 1 week of observation, the second after 4 weeks. The samples were taken out and processed by standard paraffine technique. Selected sections were immunohistochemically stained to measure the presence of cell nuclei and proliferation index (KI67), granulocytes, macrophages and monocytes (MAC-387), smooth muscle actin (SMA) and vascular endothelium (vWF). After microscopic scanning, we performed a quantitative analysis in QuPath. The implanted scaffold was detected and divided into polygonal zones of interest, 150 um thick (from the edge to the centre) to visualize recellularization in space. The two groups were then compared to understand the recellularization progress in time.

Results: Partial recellularization of the implanted scaffold was confirmed. Cells of the recipient were present in the scaffold and their concentration declined toward the central zone (some of them expressed proliferation activity which relatively increased toward the scaffold centre). A similar pattern could be observed in vessel density as well. Vessels, formed by endothelium and lined with SMA-positive cells, merged with the circulatory system of the recipient.

Interestingly, nuclei profile density was lower after the 4th week of recellularization compared to the 1st week. Similarly, proliferation activity gradually declined over time. After the acceptance of the material, nuclei profile concentration decreased. Presumably, the scaffold was colonised by myofibroblasts and slowly incorporated into the omental fibrous tissue.

Conclusion: It was confirmed that the decellularized liver scaffold is a favourable environment for cell growth and differentiation, especially thanks to its revascularisation.

This study verified scaffold biocompatibility presumed in the pilot.

In the next step, we are going to implant a human scaffold into the porcine omentum to exclude the cross-species reactivity against the scaffold.

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NON-PERFUSION DECELLULARIZATION OF PORCINE AND HUMAN LIVER – SEARCHING FOR IDEAL SCAFFOLD FOR ARTIFICIAL LIVER

I. Matsviayonak (5th year of MSP; 1), M. S. Massaro (1), M. Brousil (1), L. Červenková (1), V. Moulisová (1)

(1) Biomedical Center, Faculty of Medicine in Pilsen Charles University

(2) Department of Surgery, Faculty of Medicine in Pilsen, Charles University

Supervisor(s): MUDr. R. Pálek, Ph.D. (1, 2), prof. MUDr. V. Liška, Ph.D. (1, 2)

Background: Due to a dramatic gap between the number of available liver grafts and the number of patients on the transplantation waiting list a lot of effort was put into preparation of artificial liver by repopulation of decellularized liver scaffold. However, the ideal origin of the scaffold remains unknown. Scaffold from the porcine liver would be accessible in a sufficient number but is xenogeneic. On the contrary, scaffold from the human liver could be prepared only from livers unsuitable for transplantation but would be repopulated with allogeneic cells. Properties of human and porcine liver scaffolds and their recellularization capacity have not been compared. We are convinced that this is a critical step to be solved to improve the quality of organ substitutes based on decellularized scaffolds. To perform the functional comparison of the porcine and human liver scaffold, we first need to develop a methodology for successful decellularization of both human and porcine liver. Due to the unavailability of whole human livers, perfusion decellularization cannot be performed. This study aimed to develop a methodology for non-perfusion decellularization of human and porcine liver samples.

Methods: Samples of human livers were cut from the specimen of the resected liver during elective operations at the Department of Surgery. Samples of porcine liver were obtained during experiments in the Biomedical Center. All the samples were primarily frozen at -80°C. Right before decellularization, the tissue was cut using 8mm skin biopsy punch to obtain samples of comparable size. The decellularization process was performed by washing the tissue in the following solutions: 1% or 4% Triton-X 100, 1% SDS, and 0.1% ammonium hydroxide. Specific combinations of these solutions were used with repeated cycles of washing. A different number of cycles (4 to 10) and their different duration (60 to 240 min) were tested for both porcine and human liver tissue. After the washing process, the tissue samples were analyzed to evaluate the completeness of the decellularization and preservation of the extracellular matrix. The samples were stained with Hematoxylin and Eosin for overview evaluation, anti-cytokeratin-18 antibody was used to reveal potential residues of cytoplasm and immunofluorescence proved preservation of collagen, elastin, fibronectin, and laminin.

Results: The decellularization process was faster in the majority of human liver samples compared to porcine liver samples. However, we observed exceptions in the case of the human liver affected by steatofibrosis. To reach successful decellularization in the heterogenous group of samples a protocol with long-lasting cycles of washing in 1% Triton-X 100 and 0.1% ammonium hydroxide was evaluated as optimal.

Conclusion: The non-perfusion decellularization is affected by the amount of fibrotic tissue in the liver parenchyma. Despite the possible heterogeneity of the samples, the protocol for successful non-perfusion decellularization of porcine and human liver was established and will be used in the following studies.

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SELECTED BIOMARKERS AND ECHOCARDIOGRAPHIC PARAMETERS FOR PREDICTING SURVIVAL IN PATIENTS WITH LEFT-SIDED HEART FAILURE

L. Sanetrníková (3rd year of MSP)
Department of Clinical Biochemistry and Haematology, University Hospital Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. Daniel Rajdl, Ph.D.

Background: The essence of heart failure lies in the inability of the heart to provide sufficient blood output to meet the metabolic needs of the tissues. This condition often affects only the right or left side of the heart, but it can also involve both sides simultaneously. Heart failure kills more than 25 million people worldwide each year. The incidence increases with age, and after the age of 65, it becomes one of the most common causes of hospitalization. Unfortunately, repeated hospitalizations are not uncommon, and their increasing frequency places a significant burden on the healthcare system.Our goal was to select biomarkers that could appropriately identify patients with heart failure who are at increased risk of rehospitalization and death.

Methods: From a total sample of 39 patients with left-sided heart failure, there were 5 women (13%) and 34 men (87%). The average age of women was 61.3 years and men 59.5 years. This group was followed for 3.73 years, with a median follow-up of 3.55 years. The cause of heart failure was ischemic heart disease in 19 individuals (49%) and dilated cardiomyopathy in 20 individuals (51%). The following values were measured in the study population: B-type natriuretic peptide (BNP), cardiac troponin I and T (hscTnI, hscTnT; both measured by a high-sensitivity method), and the following echocardiographic parameters: the ratio of peak velocity of blood flow during left ventricular relaxation in early diastole to peak velocity flow in late diastole caused by atrial contraction (E/A ratio), left ventricular ejection fraction (LVEF), left ventricular mass, and left ventricular diameter. In descriptive statistics, the median was used as a measure of location parameter, and the interquartile range (IQR) as a measure of statistical dispersion. Survival analysis was used to predict complications and survival. The influence of each biomarker was graphically evaluated using Kaplan-Meier estimator, and the Cox's proportional hazards model was used for selected parameters.

Results: According to Kaplan-Meier curve analysis, promising biomarkers for predicting rehospitalization and death were selected, namely: hsTnI, hsTnT, B-type natriuretic peptide, furosemide dose, New York Heart Association (NYHA) classification of heart failure, and echocardiographic parameters (E/A, ejection fraction, left ventricular mass, and diameter). After adjustment for the above parameters, the final Cox model for rehospitalization due to heart failure identified B-type natriuretic peptide as the only statistically significant predictor of survival ($p = 0.0073$, Exp (B) = 1.0011, 95% CI 1.0003 to 1.0019), and for death, the echocardiographic parameter E/A ($p = 0.0167$, Exp (B) = 1.121, 95% CI 1.0209 to 1.2310).

Conclusion: From the measured results, we conclude that B-type natriuretic peptide may be helpful in predicting cardiovascular complications in patients with heart failure. The echocardiographic parameter E/A was found to be the most suitable for predicting death in this group.

NEZAŘAZENÉ ABSTRAKTY
ABSTRACTS NOT INCLUDED

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

V. R. Mallela (2nd year of DSP), P. B. Kasi (1), A Trailin (1), D. Shetti (1), L. Červenková (2), R. Palek (2), V Liška (2), K. Hemminki (1,3), F. Ambrożkiewicz (1)

(1) Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Plzeň, Czech Republic

(2) Laboratory of Cancer Treatment and Tissue Regeneration, Plzeň, Czech Republic

(3) Department of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany

Supervisor(s): Filip Ambrozkiewicz, Ph.D.

Background: 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 researches exploring the relationship between small nucleolar RNAs (snoRNAs) and HCC. We want to study the expression level of snoRA47 and snoRD126 in non-viral 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, decrease 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. Our aim of study is to provide a better understanding of how snoRNA expression affects patient's outcome in non-viral HCC.

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: SNORA47 expression was higher in tumor tissue than in non-tumor adjacent tissue. In contrary 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 ($p = 0.04$). 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, there was no association seen between expression and OS. No Correlations with clinical data was 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.

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TO ESTABLISH THE INVIVO CALCIUM IMAGING TECHNIQUE IN GECI POSITIVE MICE

S. Baindur (3th year of DSP)
Laboratory of Experimental Neurophysiology, Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Karel Jezek, Ph.D.

Background: Our study endeavors to establish the in vivo calcium imaging methodology in freely behaving transgenic GECI (Genetically Encoded Calcium Indicator) mice. This technique, akin to other imaging techniques like X-ray, MRI, and microscopy, facilitates deep-brain imaging in conscious animals through the utilization of fluorescent genetic indicators. This process enables the visualization of neuronal activity with a cellular resolution during free behavior, by surgically implanting a GRIN lens into the brain, thereby establishing a direct cell-to-function relationship in live animals.

Methods: Transgenic mice generated by the Genetically Encoded Neuronal Indicator and Effector (GENIE) Project express GCaMP6f in specific subsets of excitatory neurons in the brain. The transgene includes a mouse Thy1 promoter, GCaMP6f, woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), and bovine growth hormone (bGH) polyadenylation sequence. GCaMP6f-positive mice were identified via Polymerase Chain Reaction and gel electrophoresis using genomic DNA obtained from tail samples. Craniotomy, cortex vacuuming, and lens implantation were performed onto the CA1 layer of the brain, followed by baseplate positioning and cementing. Position data of the animal in the experimental apparatus was captured by a video camera placed above the track. Signal extraction was conducted using Inscopix's IDPS software, utilizing PCA-ICA or CNMFe algorithms to identify unique cells and extract calcium traces. Motion correction features minimized artifacts caused by animal movement. Positional data were synchronized with calcium events using custom MATLAB scripts.

Results: Stable fluorescent signal was detected in neocortex and hippocampus (CA1, CA3, DG) using a miniaturized fluorescent microscope attached to the implant while the animal was freely running on the track.

We observed >1500RLU luminiscence within the field of view, utilizing LED intensity between 80-100% with 2X gains. This illumination level facilitated the visualization of significant fluorescence events within cells. The cell spiking was corresponded directly to the calcium influx represented by the GCaMP6f fluorescence, viewed in the microscope attached to the freely moving animal. We achieved a stable fluorescence signal, exhibiting a high Signal-to-Noise ratio exceeding 10 standard deviations, against the background noise. As a large part of the pyramidal neurons in the CA1 hippocampus region comprise of Place cells, we mapped the spiking to the position of the animal on the maze. Rate maps for each individual unit were generated using MATLAB scripts from the acquired data.

Conclusion: We successfully established the in vivo calcium imaging technique in transgenic mice in our laboratory. Simultaneous recording from positional video cameras and the Inscopix Nvista 2.0 miniscope facilitated cell imaging and positional tracking. This methodology holds promise for further investigations into neural activity and behavior whenever a large population activity recording is required over a longitudinal section of the brain over months of study.

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IMPACT OF PREMATURE EJACULATION TREATMENT ON WOMEN´S QUALITY OF LIFE

R. Sajdlová (4th year of DSP)
Psychiatry Clinic, University Hospital in Pilsen and Faculty of Medicine in Pilsen, Charles University
Supervisor(s): doc. MUDr. Luděk Fiala, MBA, Ph.D.

Background: Men experiencing premature ejaculation (PE), defined as shortened sexual activity lasting up to two minutes, often endure stress due to this dysfunction. This condition is associated with dissatisfaction of their partners, frequently leading men to limit sexual activity. Such limitations exacerbate premature ejaculation, impacting not only men but the entire couple.

Women's sexual dysfunctions and disorders encompass various sexual issues affecting women of all ages. These include persistent or recurrent lack of sexual interest/desire, insufficient sexual arousal, difficulties in achieving orgasm, or pain associated with intercourse. The problem is often multifactorial, requiring a multidisciplinary approach focusing on biological, psychological, sociocultural, and relational factors. Women whose partners suffer from premature ejaculation (PE) experience decreased quality of life and increased stress related to their sexual lives. Treating men with premature ejaculation may improve the quality of life of their partners and alleviate their stress. The study aims to evaluate the impact of treating male premature ejaculation on the overall quality of life of their partners and their stress related to sexual life.

Methods: This study aims to recruit 50 couples, primarily focusing on women in these pairs. Couples will be recruited based on the examination of men visiting the Sexology Outpatient Clinic at the University Hospital in Plzeň. Men diagnosed with premature ejaculation will receive SSRI antidepressant therapy. The selection of participants will involve comprehensive assessments, including structured interviews, sexological examinations, and diagnostic questionnaires such as the Premature Ejaculation Diagnostic Tool (PEDT). Additionally, psychiatric histories will be obtained to exclude the use of other antidepressants, antipsychotics, or hypnotics, as well as regular consumption of addictive substances. Both partners will provide informed consent for participation.

Women in the study will complete questionnaires assessing their quality of life and sexual distress at baseline, after 4 weeks, and after 12 weeks of therapy. The assessments will include anamnesis regarding inflammatory or oncological diseases of the genital tract, endometriosis, and psychiatric history. The evaluation will focus on the WHOQOL-BREF questionnaire for overall quality of life and the Female Sexual Distress Scale-Revised (FSDS-R) for sexual distress.

Results: The results of this new research are pending. The hypothesis posits that women whose partners have PE experience reduced quality of life and increased stress related to their sexual lives. Treating men with premature ejaculation is expected to improve the quality of life of their partners and reduce their stress.

Conclusion: The study seeks to shed light on the impact of male sexual dysfunction on the sexuality of women and emphasizes the importance of considering the couple as a unit in diagnosis and therapy. Further data analysis is required to draw conclusive results.

HIPPOCAMPAL SPATIAL REPRESENTATION TRANSIENT INSTABILITY MODEL

P. Karkusova (3rd year of DSP), S. Leemburg, S. Kapl, K. Jezek
Laboratory of Experimental Neurophysiology, Biomedical Center, Faculty of Medicine in Pilsen, Charles University
Supervisor(s): MUDr. Karel Ježek, Ph.D.

Background: Schizophrenia is a serious mental disorder with considerable impact on the patient's life and the whole society. The etiology of positive symptoms of schizophrenia, such as hallucinations and delusions, is unknown. We hypothesized that these positive symptoms are the product of neuronal network instability. To model such conditions, we developed a behavioral protocol for transient destabilization of rat hippocampal representation by sudden rotation of orientational visual cues.

Methods: Long-Evans male rats were trained in a square-shaped environment with a single light cue on one of the walls while neuronal activity in CA1 was registered. During the test session when the rats behaved in the environment, the light cue suddenly switched off while at the same time an identical one appeared on an adjacent wall, effectively rotating the environment by 90°. This procedure was repeated several times in 2-minute intervals. Only CA1 place cell units were isolated and used for population vector analysis.

Results: Our population spatial correlation analysis showed the hippocampal map rotated respective to the visual cue shift. We observed that the network responded to the cue change within the first two seconds and remained unstable for another short interval before it stabilized in the respective map configuration.

Conclusions: In the next steps we plan to apply this procedure to pharmacological model of schizophrenia induced by injecting the rats with MK801, an NMDA receptor blocker. We will assess both the quantitative and qualitative changes of dynamical neural coding resulting from this intervention. We anticipate that MK801 will prolong the duration of the physiological instability period associated with the enforced adjustment of the expressed memory state.

REALIZAČNÍ TÝM
PROJECT TEAM

prof. PharmDr. Radek Kučera, Ph.D.
doc. MUDr. Miroslava Čedíková, Ph.D.
doc. Ing. Jan Nevoral, Ph.D.
Ing. Daniela Vyzrálová
Lenka Jetlebová
Mgr. Barbora Stachová