

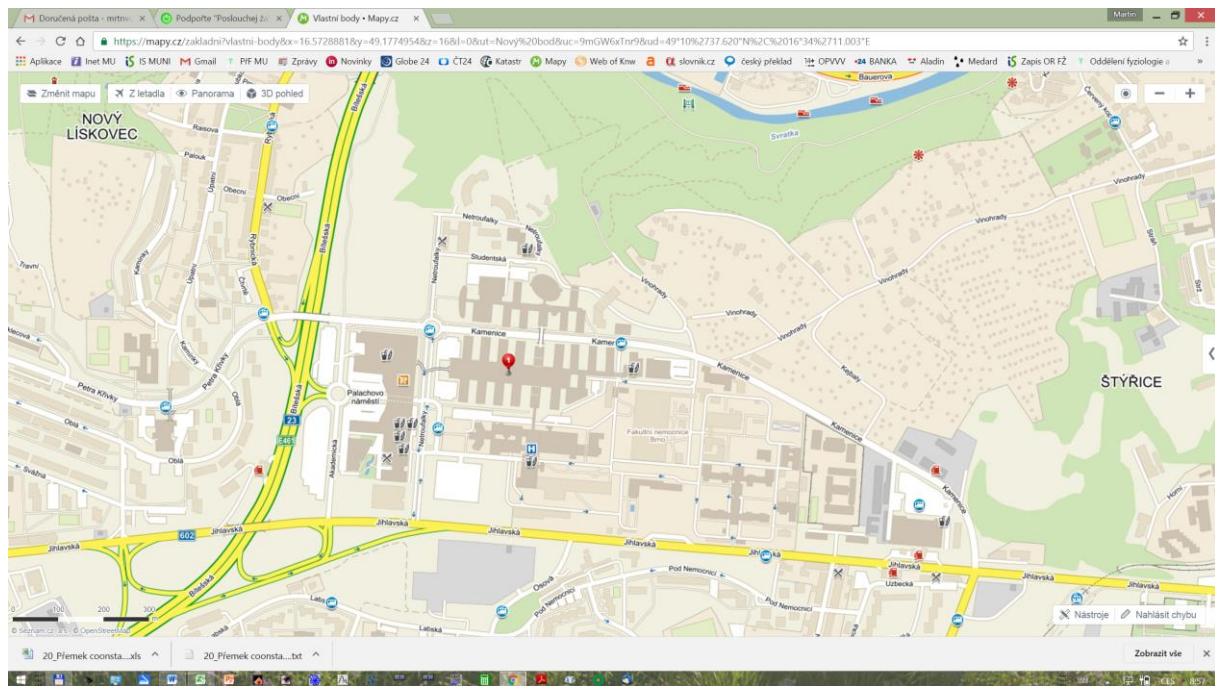
Workshop doktorandů 3. semestru Programu Fyziologie, imunologie a vývojová biologie živočichů na Přírodovědecké fakultě MU Brno

Sborník abstraktů

Čtvrtek 13.2. 2019 Univerzitní kampus Bohunice
Kamenice 5 – budova A11, učebna 205
Od 8.30

M U N I





Application of plasma polymers on nanofibrous mats for tissue replacement therapies

Mgr. Petra Černochová

Supervisor: doc. Mgr. Lenka Zajíčková, Ph.D.

Consultant: Mgr. Jiřina Medalová, Ph.D.

Most promising issue of tissue engineering is development of synthetic polymers modified by plasmachemical processes. Their production is rapid, ecological, and economical. Plasma polymerization improves surface properties of polymers such as hydrophilicity, cell attachment and proliferation. It was demonstrated that modification by plasma treatment can improve cell adhesion and growth of cells.

This project focuses on Petri dishes and nanofibres made from polycaprolactone both plasmachemically coated with non-toxic cyclopropylamine, which forms layers rich in positively charged amine groups. We compared effect of four types of amino-rich treated samples on various types of cells (fibroblasts, keratinocytes, vascular smooth muscle cells and three various endothelial cell lines) and we found that these layers have different influence on them. Studied cell types with the exception of endothelial cells had very high resistance to trypsin, increased rate of attachment and slightly decreased motility on all four types of surface. The endothelial cells are exceptional as they are trypsin resistant only on particular surfaces, and what is interesting, cells from different veins prefer different type of surface. To sum up, amine rich surfaces affect the ability of cells to be trypsinized and also their attachment and proliferation.

Jméno studentky: Petra Černochová

Hodnocení vystoupení studenta

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Excretory/secretory products of entomopathogenic nematodes in the host-parasite interactions

Mgr. Sara Eliáš

Supervisor: asoc. prof. RNDr. Pavel Hyršl, Ph.D.

Entomopathogenic nematodes produce excreted/secreted products (ESPs), which are a mixture of small molecules, proteins and nucleic acids with various functions. Some of them are able to interact and even diminish host immune system.

The aims of my PhD project are: 1. characterization of ESPs molecules produced by nematode *Heterorhabditis bacteriophora*, 2. description of potential differences among selected isolates of this species, 3. comparison of ESPs produced by *H. bacteriophora* to other species of entomopathogenic nematodes such as *Steinernema carpocapsae*, 4. comparison of the effect of ESPs on different insect species.

We optimized the protocol for isolation of ESPs and already tested the effect of ESPs obtained from *Heterorhabditis bacteriophora* on immune system of *Galleria mellonella* larvae. The results indicate suppression of phenoloxidase activity after administration of isolated ESPs. Ongoing analysis of active ESPs is focused on identification of specific molecules responsible for the observed effect by mass spectroscopy. This part of the project is summarised in manuscript which we prepare for submission at the beginning of this year. Our next goal is to characterise the effect of ESPs on the antimicrobial activity of *G. mellonella*, because in preliminary experiments we have observed an inhibitory activity of ESPs in this type of immune response.

Until now, the entomopathogenic nematodes were mostly used as a biological control of insect pests as the whole organism, however the specific molecules characterised within my PhD project could offer new possibilities of application as well as the improvement of efficacy of nematode-based biocontrol. It is of note that products of some entomopathogens involve also compounds affecting the human immunity or acting as the antibiotics which makes the ESPs of nematodes an interesting source of bioactive molecules with potential use in pharmacology.

Jméno studentky: Sara Eliáš

Hodnocení vystoupení studenta

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Using advanced proteomics for the analysis of cell signaling

Mgr. Kristína Gömöryová

Supervisor: prof. Vítězslav Bryja, Ph.D.

Co-supervisor: Mgr. David Potěšil, Ph.D.

Wnt pathway is one of the key evolutionary conserved signaling cascades. Its deregulation leads to a variety of diseases including cancer, neurodegenerative and metabolic diseases. In my PhD project we decided to focus on the Wnt/Planar Cell Polarity (PCP) pathway which plays crucial role in the maintenance and establishment of cell polarity and migration. Although it has been extensively studied in the past years, only little is known about the signal transduction in the Wnt/PCP pathway. To address this issue, we will study dynamic changes in the protein interactions (interactome) of key Wnt/PCP proteins. I am using so called BioID approach where the key molecules of Wnt/PCP pathway were fused to BirA* ligase, which upon addition of biotin biotinylates proteins in the radius of 10 nm. Upon mass-spectrometry based proteomic analysis this allows us to identify even transient interactions that take place in the cellular environment.

For the analysis of BioID data, I developed specific pipelines using the software container environment (KNIME platform). Several new tools have been developed along the way for the statistical analysis (imp4p, proDA) and visualization (e.g. UpSet plots, interactive volcano and violin plots). I combined our results with the existing tools such as CRAPOME/SAINT analysis followed by ProHits and with the Human Cell Map project. All these approaches finally allowed us to select a list of potentially interesting Wnt/PCP interactors, which will be further investigated both in the cellular overexpression systems and in the zebrafish.

In my PhD project I plan to follow with the integration of other BioID datasets with the Wnt/PCP one. In the next step I will also analyze the global proteome and phospho-proteome of CRISPR cell lines lacking the key proteins involved in the Wnt/PCP pathway.

Jméno studentky: Kristína Gömöryová

Hodnocení vystoupení studenta

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The role of endogenously produced hyaluronan in pro-fibrotic transition of mesothelial cells in the course of peritoneal adhesions formation

Mgr. Anna Kocurková

Supervisor: Mgr. Gabriela Ambrožová, Ph.D.

Peritoneal adhesions are severe problem following intra-abdominal surgery causing infertility, bowel obstruction and chronic pelvic pain. Mechanical injury is main cause but adhesions can occur also in undisturbed sites of peritoneum. Thus, others factors are involved, including inflammation, hypoxia, fibrinolysis and desiccation of peritoneal cavity during surgery. Prevention of adhesions is based on effort to avoid mentioned conditions and on application of antiadhesive barriers.

Molecular mechanism of peritoneal adhesion formation is not fully understood. However, pro-fibrotic transition of mesothelial cells (MCs) plays an important role contributing to extracellular matrix (ECM) over-production. Changed levels and metabolism of hyaluronan (HA), component of ECM, can influence plenty of fibrosis-related pathologies but exact mechanism remains unknown. So, we hypothesize that HA produced by MCs effects their pro-fibrotic transition in peritoneal adhesion formation.

This thesis is focused on studying peritoneal adhesions on newly developed mouse model of hypoxia-enhanced diffuse peritoneal adhesions and in vitro model of primary mouse MCs. Fibrotic tissue from in vivo model and content of peritoneal fluid have been analysed. Effect of HA on pro-fibrotic transition of MCs in peritoneal adhesions formation will be determined in both models by modulation of the synthesis, degradation and interactions of HA. In vivo model is already used for testing newly developed antiadhesive barriers.

Jméno studentky: Anna Kocurková

Hodnocení vystoupení studenta

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Role of tumor microenvironment in the triple-negative breast cancer

Mgr. Barbora Kvokačková

Supervisor: Mgr. Karel Souček, Ph.D.

Triple-negative breast cancer (TNBC) is an extremely heterogeneous subtype of breast cancer characterized by the lack of molecular targets for therapy (ER-, PR-, HER2-), therefore conventional chemotherapy remains only validated treatment option. TNBC solid tumors are dynamic complexes, comprised of a variety of different cancer cell populations with specific signatures, which might be responsible for aggressive behavior and bad prognosis as often seen in the clinic. Moreover, besides cancer cells, tumor tissue comprises stromal cells, known to shape tumor behavior and contribute to therapy resistance. To address the issue of intratumoral heterogeneity, microenvironment and cancer plasticity we plan to introduce, optimize and validate the innovative method of mass cytometry for analysis of “TNBC cytome” in patient tissues. Next, identified populations of interest will be sorted and specific transcriptome signature will be analyzed and match with clinical observations. We will focus in more detail on stromal populations exhibiting tumor modulation properties.

We are currently optimizing single cell, antibody-based protocol for detection of > 40 surface and intracellular markers and collecting fresh patient samples. Also, we previously described 10-molecule surface signature which reflects the epithelial-mesenchymal plasticity in breast cancer. To further analyze cancer subpopulations from identified 10-signature we plan to introduce a single tube multicolor protocol using conventional/spectral cytometry, subsequently sort the populations of interest and perform RNA-seq using obtained clinical samples or generated patient-derived xenograft (PDX) models. Furthermore, we hypothesized that defined 10-signature is most likely under control of a certain set of transcription factors and performed qPCR screen, which identified genes deregulated in epithelial/mesenchymal breast cancer cell lines. In addition, due to the lack of relevant TNBC models suitable for preclinical testing and often limiting amount of patient material, we also started with the generation of PDX models in immunodeficient mice. We believe that our setup might bright insight into the complexity of TNBC, identify the specific signature of selected cancer cells subpopulations associated with plasticity (and unravel new markers associated with prognosis and metastatic dissemination) and help elucidate the role of tumor stroma in cancer progression.

Jméno studentky: Barbora Kvokačková

Hodnocení vystoupení studenta

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Preclinical models of cancer progression and therapy

Student: Mgr. Markéta Pícková

Supervisor: Mgr. Karel Souček, PhD

The tumor cells dissemination into distant organs is the main cause of cancer-related deaths in the world. It is presumed that the main mediators of cancer dissemination are the circulating tumor cells (CTCs) released from primary tumors into blood as a consequence of epithelial to mesenchymal transition (EMT). The EMT supports the plasticity of CTCs which enhance their survival in the bloodstream as well as their adaptation to the different microenvironment and successful colonization of the target organ. In breast and prostate cancer patients were found CTCs in different EMT stages which predicts their metastatic potential and CTCs are used as prognostic markers in these types of malignancies in clinics. Additionally, the EMT status of CTCs together with tumor heterogeneity is associated with the development of drug-resistance which represents a serious issue in prevention of metastasis as well as their elimination in patients.

The experimental mouse models are an essential tool to understand each step of metastatic cascade including plasticity and heterogeneity in detail. The main aim of this Ph.D. project is to establish an experimental models of cancer with a focus on isolation and characterization of EMT markers on circulating tumor cells. For that purpose we injected orthotopically breast 4T1 12B luc mCherry and prostate RM1 luc mCherry cancer cell lines into both immunocompetent and immunodeficient mouse strains and monitored the tumor progression in time using the IVIS Lumina imaging system. For the validation of presence of CTCs we introduced flow cytometric detection on Attune Classic flow cytometer combined with the clonogenic assay for CTCs-derived clones isolation and further analysis. An other approach for establishment of breast cancer mouse model was injection of single CTC-derived 4T1 12B luc mCherry clones which were isolated from blood of experimental Balb/c mice and expanded in vitro. Based on our data the CTCs-derived clones had decreased metastatic capacity and were overall less tumorigenic than the parental cell line.

In summary, we established mouse models of breast and prostate cancer and we introduced methods for the CTCs detection and isolation. Our next work will be focused on the characterization of EMT markers on the CTCs-derived clones and its association with the predictive response to therapy.

Jméno studentky: Markéta Pícková

Hodnocení vystoupení studenta

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The effect of Pseurotin alkaloids on immune response

Mgr. Svitlana Skoroplyas

Školitel: doc. Mgr. Lukáš Kubala, Ph.D.

Konzultanti: RNDr. Milan Číž, PhD, Mgr. Ondřej Vašíček, PhD

Mycotoxins are important toxins contaminating food and agricultural feeds. Among them are pseurotins, secondary metabolites produced by many species of fungi such as *Aspergillus* sp. and *Penicillium* sp., with suggested significant biological activities. However, their effects on immune system response is unknown.

In my thesis, I focus on effects of natural pseurotins A and D on both innate and specific immune response. Primarily, I determined modulation of functions of polymorphonuclear neutrophils (PMNL). Data revealed that pseurotins did not affect unstimulated PMNL and had only limited inhibitory effects on oxidative burst and degranulation of PMNL induced by different activators. Next, I study effects of pseurotins on activation of mouse lymphocytes. I observed pseurotin mediated inhibition of proliferation of sorted mouse B-lymphocytes and their differentiation into plasma cells characterized by surface expression of CD19+, CD138+ and B220+. This is connected with inhibition of JAK/STAT signaling pathway. Further, the major part of my work is analysis of effects of pseurotins in vivo using different mouse models of acute or chronic inflammation and hypersensitivity responses. Particularly, pseurotin D significantly inhibits ovalbumin (OVA) induced hypersensitivity reaction and carrageenan induced inflammatory reaction in mouse.

These data together with other data obtained by my colleagues show that pseurotins can downregulate specific immune response. Next, pseurotin synthetic analogs could be seen as potential drugs for treatment of overwhelming immune response such as hypersensitivity reaction. During the rest of my studies I will continue on characterization of mechanisms how pseurotin affects lymphocytes. I would also like to complete thorough characterization of pseurotin mediated modulation of hypersensitivity response in mice.

Jméno studentky: Svitlana Skoroplyas

Hodnocení vystoupení studenta

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Understanding of the role of Trop-2 in tumor cell plasticity and dissemination

Mgr. Ondřej Vacek

Supervisor: Mgr. Karel Souček, Ph.D.

The cell surface glycoprotein and stem cell marker Tumor-Associated Calcium Signal TransDucer 2 (Trop-2, TACSTD2) is known to be overexpressed in carcinomas and its deregulation is associated with cancer progression and poor clinical prognosis. Trop-2 may represent promising yet not clearly characterized target for therapy. Biological function of Trop2 in tissue maintenance and tumorigenesis remains to be elucidated, although several cellular processes and signaling pathways have already been linked to Trop-2 role, most notably cellular adhesion and canonical Wnt signaling pathway. We suppose that revealing more details about Trop-2 role in tumor development and metastasis is essential to evaluate an advantage of its targeting. Aims of this study include 1) to unravel the functional role of Trop-2 in the tumor dissemination and 2) in the tumor tissue organization and 3) to characterize Trop-2 phenotypic plasticity in response to specific components of tumor/metastatic microenvironment.

Thus far, in vivo metastasis assays and in vitro functional assays were performed to analyze differences between Trop-2 expressing and Trop-2 deleted cancer cells. To connect observed in vivo and in vitro phenotype of Trop-2 deleted cells with processes on molecular level, we analyzed whether β -catenin as component of canonical Wnt signaling pathway is altered based on Trop-2 level. In vivo studies showed that Trop-2 presence positively affects growth of primary tumor although deletion of Trop-2 leads to increased ability to disseminate from primary tumor to lungs. Interestingly, in case of dissemination directly from vasculature, Trop-2 knocked out cells displayed decreased metastases occurrence in lungs. In vitro functional studies underlined role of Trop-2 in adhesion but not in migration or invasion. Results did not reveal any changes in β -catenin due to Trop-2 deletion however β -catenin and Trop-2 levels are presumably related as a consequence of overall epithelial or mesenchymal phenotype of cell.

In conclusion, our results indicate impact of Trop-2 level on the ability of breast cancer cells to disseminate from primary tumor in mouse in vivo model. Nevertheless, the role of Trop-2 seems to be ambiguous considering positive correlation with growth of primary tumor but negative correlation with dissemination from primary tumor. Our in vitro data supports involvement of Trop-2 in cellular adhesion but not direct effect of Trop-2 on β -catenin level. Further research is planned to focus on molecular events affected by the presence of Trop-2 to understand more deeply its role during carcinogenesis.

Jméno studenta: Ondřej Vacek

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