
Session IV: Cancer biology

Lectures

L4.1

Tumor microenvironment in cancer progression

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Keywords: tumor microenvironment; Heat-shock Factor 1 (HSF1)

Billions of years of evolution through changing environments led organisms to develop an arsenal of cytoprotective pathways to promote their survival under stressful conditions. We hypothesized that tumors exploit these mechanisms to support their own survival as they rapidly develop and evolve in a hostile and stressful environment. For tumors to form, progress and metastasize, they must recruit and reprogram normal cells in their microenvironment into a protumorigenic stroma. Recently we have shown that Heat-shock Factor 1 (HSF1), master regulator of the heat-shock response, plays a crucial role in this process. Across a broad range of human cancers, HSF1 is activated not only in the malignant cells themselves, but also in cancer-associated fibroblasts (CAFs). In early stage breast and lung cancer, high stromal HSF1 activation is strongly associated with poor patient outcome. HSF1 drives a transcriptional program in CAFs that complements, yet is completely different from, the program it drives in adjacent cancer cells. This CAF program is uniquely structured to support the malignant potential of cancer cells in a non-cell-autonomous way. Here we further explore the HSF1-dependent cross talk between cancer and stroma. We dissect the mechanism of stromal HSF1 activation, identify key components of the HSF1-dependent stromal transcriptional program and highlight the prognostic implications of cell-autonomous and non-cell-autonomous activation of HSF1 in cancer.

Posters

P4.1

Apoptosis mediated by ROS on melanoma cells after treatment by liposomal anacardic acid, vitamin C and mitoxantrone cocktail

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Keywords: glioblastoma; temozolomide; polymeric carrier; apoptosis; doxorubicin

Vitamin C influences the anticancer activity of several anticancer drugs by increasing their toxic activity toward cancer cells and simultaneously decreasing their toxic activity toward normal cells. Our data showed that mitoxantrone encapsulated in anacardic acid (AA) containing liposomes by a vitamin C ion gradient increased the number of surviving normal cells (NHDF-normal human dermal fibroblasts), in comparison to liposomes where mitoxantrone was encapsulated by an ammonium sulfate gradient. The opposite effect was observed in cancer cells (A375, Hs294T-melanoma cell lines). These results suggest that our liposomes have a dual mechanism of action, depending on the type of cells they interact with.

Apoptosis is characterized by cell shrinkage, chromatin condensation, membrane blebbing, protein fragmentation and DNA degradation. Many proteins are involved in this complex process. Caspases, a family of cysteine-dependent aspartate-directed proteases, play a critical role in the initiation and execution of apoptosis. Among this family of caspases, caspases 3 and 7 are believed to be some of the caspases most commonly involved in the execution of apoptosis in various cell types.

The aim of this project was to verify the ability of anacardic acid and vitamin C to protect NHDF cells against mitoxantrone. One of the proven mechanisms of mitoxantrone toxicity is ROS (reactive oxygen species) production. We determined the level of ROS and the level of antioxidant agent-gluthathione as well as the level of executive caspases.

Our results suggest that ROS production is a key mechanism in our anacardic- and ascorbic acid-containing liposomes.

P4.2

From irritable bowel syndrome to colorectal cancer - impact of estrogen receptors

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Keywords: GPER; ER α ; ER β ; IBS; CRC; microRNA

Estrogens appear to be involved in the regulation of intestinal epithelial cell metabolism and in the microarchitecture of the intestine. The aim of this study was to investigate the participation of estrogen signaling and to determine the potential relationship between functional gastrointestinal disease and predisposition to neoplastic transformation of the large intestine. Our analyses revealed that expression of GPER at the mRNA and protein levels in irritable bowel syndrome (IBS) with constipation and diarrhea predominance (IBS-C and IBS-D), as well as in colorectal cancer (CRC) patients, as compared to healthy controls, are elevated. Moreover, GPER expression appears to be associated with a different methylation pattern on one of the CpG islands of the promoter region of the GPER gene in all studied cases. We did not observe any changes in the expression of canonical estrogen receptors in IBS, while the mRNA level of ER α but not ER β in CRC patients was found to be altered. Furthermore, increased expression of miR-148a and miR-145, involved in GPER signaling and cancer cell proliferation, in patients with IBS-D and CRC, respectively compared to healthy controls has been stated. Results suggest that abnormalities in the estrogen signaling network contribute to the development of IBS and CRC. Furthermore, it seems that IBS may be one risk factor for the development of CRC.

This work was supported by a grant (2015/17/N/NZ5/00336 to DJ) from the National Science Center, Poland.

P4.3

Expression and functionality of novel splice variants of SRSF2 3'UTR in renal cancer

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Keywords: SRSF2; splice variants; renal cancer; nonsense-mediated mRNA decay; 3'UTR; biomarker

SRSF2 is a multifunctional protein that regulates alternative splicing and contributes to carcinogenesis. Recently, we identified three novel splice variants of SRSF2 3'UTR expressed in renal cancer. These isoforms differ in the length of the 3'UTR and presence of microRNA binding sites. The role of the novel splice variants of SRSF2 3'UTR in renal cancer has not been studied yet. Therefore, in this study, we analyzed the expression and functionality of these splice variants in renal cancer. Twenty-three pairs of human renal cancer and control samples were used, along with the renal cancer-derived Caki-2 cell line. The expression of novel splice variants of SRSF2 3'UTR was analyzed using real-time PCR in renal cancer and control samples. Transcript stability and nonsense-mediated mRNA decay (NMD) was analyzed in Caki-2 cells by using actinomycin D and cycloheximide, followed by RT-PCR. We observed statistically significant upregulation of expression of the three novel splice variants of SRSF2 3'UTR in renal tumors compared with controls. Incubation of Caki-2 cells with cycloheximide increased the level of all analysed splice variants. Although the novel splice variants of SRSF2 3'UTR are probably non-functional and undergo NMD, we suggest their potential use as cancer biomarkers due to their upregulation in renal tumors.

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P4.4

Three-dimensional growth reconstitution of renal cell carcinoma

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Keywords: renal cell carcinoma; hypoxia; three-dimensional cell culture

Renal cell carcinoma (RCC) is the most common type of kidney cancer and is responsible for more than 78,000 deaths each year worldwide. New therapeutic strategies, which might prolong the progression-free survival of RCC patients, are critically necessary. However, the majority of innovative therapeutic agents are tested using monolayer cytotoxicity assays, and many potentially crucial molecules drop out during screening. Due to tissue architecture, three-dimensional (3D) microenvironments appear more similar to *in vivo* than standard and commonly used 2D microenvironments. In the literature, many studies have supported the effect of culture dimension on gene expression profile changes. It is strongly likely that many pivotal therapeutic agents can be eliminated due to different gene expression profiles. Furthermore, the oxygen partial pressure within the human body and tumors (hypoxia) is reduced compared with that of standard cell culture systems (hyperoxia). In this study, we analyzed various 3D growth methods of RCC cell lines under different oxygen partial pressures (hypoxic 1% and hyperoxic 20%). We cultured the RCC cell lines from primary and metastatic tumors in hanging drop and colony formation assays, and we compared them to an innovative 3D system with methylcellulose. We propose a high-throughput, 3D RCC cell culture system that strongly mimics *in vivo* conditions.

The research was funded by a grant obtained by DM from the National Science Center (no. UMO-2014/15/N/NZ5/0056).

P4.5

Activation of ROS-mediated death induced by pseudofactin II in ovarian and breast cancer cell lines

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Keywords: biosurfactant; cancer; ROS

Biosurfactants are extensively studied because they induce apoptosis in cancers. Pseudofactin II (PFII) is a novel cyclic lipopeptide biosurfactant produced by *Pseudomonas fluorescens* BD5. PFII has pro-apoptotic properties in melanoma A375 cells. It causes DNA fragmentation, actin condensation, lactate dehydrogenase (LDH) release, and caspase-3 activation [1]. Our investigations indicate that cell death is caused by generation of reactive oxygen species (ROS) [2]. We found that PFII affects cell viability of a panel of ovarian and breast cancer cell lines via ROS. Possible molecular mechanisms of ROS generation and cell death following treatment of ovarian and breast cancer cell lines with PFII will be discussed.

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P4.6

Establishing reference genes for the ovarian cancer SKOV-3 cell line

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Keywords: ovarian cancer; reference genes; hypoxia

Introduction: Ovarian cancer is the most aggressive gynecological neoplasia. Most patients suffer from disease recurrence within two years after initial treatment. One of the hypotheses to explain its poor prognosis focuses on the presence of cancer stem-like cells (CSLCs) in tumor masses. The features of these cells can be influenced by certain experimental conditions and assessed by analyzing gene expression. Precise analysis requires the application of proper reference genes.

Aim: The aim of the study was to establish the set of reference genes that enables a reliable comparison of ovarian cancer cell line SKOV-3 gene expression among various experimental conditions.

Materials & methods: We cultured the SKOV-3 cell line in normoxic or hypoxic conditions with the addition of chemotherapeutics or in supplemented media for 48 hours. We used isolated RNA to select the best references among 20 gene candidates using the geNorm algorithm.

Results & summary: Applied procedure make it possible to select the most suitable reference genes for SKOV-3 cell lines in various test conditions. Some of the genes that we analyzed were not suitable for this setting, although they had been used as references in previous experiments with SKOV-3 cells. Our results highlight the need for carefully choosing reference genes since they may significantly affect the interpretation of the data. Such procedure should be mandatory before each qPCR analysis.

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P4.7

Metastatic melanoma cells respond to EGF, HGF, and TGF β differently than primary melanoma cells

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Keywords: melanoma; invasion; EGF; HGF; TGF β ; signal transduction

Melanoma development is strongly connected with homeostatic disorders that disrupt the melanocyte environment. Epidermal growth factor (EGF), hepatocyte growth factor (HGF), and transforming growth factor beta (TGF β) are involved in melanocyte tumorigenesis; however, their impact does not correlate clearly with receptor overexpression or down-regulation. We found that melanoma cells from different tumorigenesis stages respond to EGF, HGF, and TGF β differently in terms of their invasiveness. For instance, in the case of TGF β treatment, we observed divergent cellular responses depending on the cell line origin. For primary cell lines, this cytokine stimulates invasion by enhancing extracellular matrix degradation, increasing invadopodia formation and modulating actin polymerization by decreasing the amount of cytoplasmic filamentous actin. In contrast, in metastatic cell lines, TGF β inhibits invasion and increases the F:G actin ratio.

In our study, we also evaluated EGFR, MET, and TGF β R1 expression in the melanoma cell lines. Simultaneously, we performed immunocytochemical analysis on permeabilized and unpermeabilized cells to determine if there were any differences in the cellular localization of these receptors. Some discrepancies in receptor expression and localization were observed among the melanoma cell lines; however, the differences were not sufficient to explain the different cellular responses. We next examined receptor internalization to determine if the differences in observed in the cellular responses result from clathrin- or caveolin-mediated endocytosis. The type of receptor internalization pathway distinguished cells based on their altered intracellular signaling transduction.

P4.8

miR-25-3p targeting ITGA5 inhibits migration of renal cancer cells

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Keywords: renal cell carcinoma; microRNAs; cancer metastasis

Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney cancer (80%) and well known for its therapy resistance and capacity to metastasize. Consequently, the search for new therapeutic strategies to treat ccRCC has been an ongoing subject of investigation in the literature. MicroRNAs are short, non-coding RNA molecules that downregulate expression of target genes. We recently found that decreased expression of microRNA miR-25-3p in ccRCC tumors contributes to the upregulation of ITGA5, which is a gene that encodes for integrin α_5 , an important initiator of prosurvival intracellular cell signaling and mediator of contacts with the extracellular matrix. This work further investigated the impact of miR-25-3p on the ITGA5 protein level and properties of the renal cancer cells, i.e., proliferation and migration.

To achieve our scope, we transfected ccRCC-derived cell line Caki-2 with an miRNA mimic or scrambled control and performed the following functional assays: BrdU proliferation incorporation assay and Wound Healing assay. The regulation of ITGA5 by miR-25-3p was determined on the protein level in the Caki-2 cell line as well as in the cancer tissue samples obtained from patients who had undergone a radical nephrectomy.

Transfections of miR-25-3p mimics in Caki-2 cells led to diminished ITGA5 protein levels (i.e., by about 60%; $p < 0.0001$) and caused an inhibition of the migration of the cancer cells (i.e., by 22%; $p < 0.05$). miR-25-3p did not influence the proliferation rate.

Based on this data, we conclude that miR-25-3p targets ITGA5 and inhibits the migration of renal cancer cells, which may suggest that it could play an important role in renal cancer metastasis.

P4.9

In vivo model for tumor initiating cells/cancer stem cells isolated from clear cell renal cell carcinoma cell line

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Keywords: clear cell renal cell carcinoma (ccRCC); tumor initiating cells/cancer stem cells; CD105+/- cells; magnetic resonance imaging (MRI); positron emission tomography (PET)

Many research have proven that cancer stem cells/tumor initiating cells (CSCs/TICs) are responsible for development of tumor, disease progression, aggressiveness, metastasis spread and resistance to drugs. The existence of CSCs/TICs has been proven in many tumor tissues including RCC. *In vivo* tumorigenic potential of CSCs/TICs isolated from established RCC cell lines has never been investigated. CD105+ cells were isolated from RCC cell line caki-1. Sorted CD105+ cells were cultured in FreeStyle™ 293 Expression Medium before injecting into mice (NOD/SCID). Mice were scanned with Bruker 7T tomograph, Structural MR images to quantify tumor volume were acquired with T2-weighted TurboRARE. Tumor volume calculation were performed with a region growth algorithm by MeVisLab Software. PET and CT images were obtained with Bruker Albira small animal dedicated system after injection of 18F-deoxyglucose (FDG). CD105+ cells grow as adherent and floating cells in normal monolayer culture condition in comparison to CD105- cells. Approximately two week of culture, floating cells were able to create floating tumorspheres, which were further live stained using CD105 antibody. Immunocytochemistry for CK7 and CD10 was performed on total caki-1 cells confirming the RCC subtype. Our results confirmed that tumorsphere formed by sorted CD105+ cells were also positive for CD105 marker. Caki-1 cells were positive for CD10 marker. Tumor growth using MRI confirmed faster growth of CD105+ induced tumors and higher FDG uptake.

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P4.10

Calcium ions and electroporation influence on the breast adenocarcinoma cells cytotoxicity

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Keywords: electroporation; calcium; adenocarcinoma

Calcium is an important factor that can play different functions in human organism. Electroporation (EP) is based on electric field interaction with the cell surface.

The main objective of the presented work was investigation of anticancer activity of calcium introduced to malignant cells with electroporation into breast adenocarcinoma cell lines. Two human cell lines were used: MCF-7/WT and MCF-7/DX. To electroporation following parameters were selected: 800, 1000, 1200 and 1400 V/cm; by 8 pulses of 100 μ s, pulse duration 1 Hz. The following calcium concentrations were applied: 0.25, 0.5, 1 and 5 mM. Viability was analyzed by SRB assay. The determination of the cytotoxic activity was carried out by measuring the amount of cellular protein.

The best anticancer effect was observed after the application of Ca^{2+} delivered by electroporation. The cell survival rate of Ca^{2+} + EP significantly decreased in comparison to the single method. EP with calcium stimulated antitumor effect of Ca^{2+} action. EP or Ca^{2+} application alone did not cause such a strong antitumor effect. The most effective results were obtained at higher voltages: 1200 and 1400 V/cm (independently of the concentration of calcium). The decrease of survival rate was observed after 48 h, especially at high voltages. As we observed, MCF-7/DX cells were more sensitive to applied therapy. The EP application for regulation of Ca^{2+} transport to both cell death and proliferation offers the opportunity for a set of new drug targets in cancer.

P4.11

Lipid damage of primary lung metastasis cells after electroporation with cisplatin

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Keywords: lung metastasis; primary cells; electroporation

The lung cancer at a later stage of disease has a high tendency to metastasize. Approximately one-third of patients diagnosed with a malignant tumors have a lung metastasis. The goal of treating metastatic lesions is total removal of the tumor weaves, which are a source of further spread. This is often difficult to perform, and in such cases, chemotherapy is the most common choice; however, many tumors are resistant to treatment. Moreover, high concentrations of therapeutics cause many side effects, and thus, in our study we propose application of electroporation (EP). We used primary lung metastasis cell lines from different organs. The aim of our study is to investigate the influence of EP in combination with cisplatin on the cellular structure, proliferation and lipid peroxidation in cells. The effect of EP (with and without cisplatin) was determined using the MTT assay. The cell membrane state was visualized using a DHCC marker. Measurement of lipid peroxidation is based on determining the concentration of malondialdehyde using thiobarbituric acid. In EP experiments, different voltage values (from 0 to 1200 V/cm), an 8 pulse duration of 100 μ s, and 1-s intervals between pulses were used. Our results indicate that the combination of EP and chemotherapy can improve cancer cell destruction.

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P4.12

The inhibitory effect of nitroglycerin on proliferation of WM239 melanoma cells line

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Keywords: aldehyde dehydrogenase; nitroglycerin; melanoma cells

A majority of literature data have evidenced antimitotic and, thus, carcinogenesis-inhibiting action of aldehydes. Hence, from the point of view of anticancer therapy, the use of aldehyde dehydrogenase (ALDH) inhibitors seems beneficial because it should increase aldehyde level and, consequently, lead to proliferation inhibition. In this study, we examined nitroglycerin (glyceryl trinitrate; GTN) in this context, since it was reported to undergo biotransformation in the ALDH-catalyzed reaction which inactivates this enzyme. The studies were conducted on cultures of WM239 melanoma cell line. Preliminary results indicated that proliferation of melanoma cells cultured in the presence of GTN at a concentration of 200 μ M for 48 h was inhibited by 60% in comparison with control values. Proliferation was tested with the use of crystalline violet. It was also shown that GTN at the tested concentrations was not toxic. However, the study results indicated only trace activity of ALDH in GTN-untreated melanoma cells. Thus, our results are interesting and surprising because they suggest that the inhibitory effect of GTN on cell proliferation was not connected with the inhibition of ALDH activity. This suggests a new mechanism of action of GTN on tumor cell proliferation.

P4.13

Down-regulation of adaptor protein Ruk/CIN85 in Lewis lung carcinoma cells results in suppression of tumor cells proliferation and migration *in vitro* as well as tumor development and metastasis *in vivo*

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Keywords: Ruk/CIN85; Lewis lung carcinoma; tumor progression

Ruk/CIN85 is an adaptor/scaffold protein that plays a crucial role in cell signaling, proliferation, adhesion, motility and invasion. Clinical data indicate that increased expression of Ruk/CIN85 is associated with higher malignancy of breast cancer. However, there is insufficient data about the role of Ruk/CIN85 in the processes of malignant transformation of cells, transmigration, and invasion.

The aim of this work was to investigate the effect of stable Ruk/CIN85 down-regulation in Lewis lung carcinoma (LLC) cells on their oncogenic properties *in vitro* as well as on tumor development and metastasis *in vivo*. B1 and B3 sublines of LLC cells with suppressed Ruk/CIN85 expression were obtained by shRNA interference technology using Ruk/CIN85 specific shRNA lentivirus. Cell proliferation rate *in vitro* was estimated by direct cell counting and MTT assay, and cell migration was studied with scratch assay. For *in vivo* studies, control and Ruk/CIN85 down-regulated LLC cells (1×10^5 cells) were subcutaneously injected into the right hind leg of female C57BL/6 mice. Dynamics of tumor growth and lung metastasis were assessed for the period of 29 days.

It was demonstrated that cells of B1 and B3 sublines are characterized by decreased proliferation rate and viability as well as migratory properties *in vitro* in comparison to control. Primary tumor growth rate, number and size of lung metastases were significantly reduced in animals inoculated with B1 and B3 cells in comparison to control. The data obtained demonstrate the role of Ruk/CIN85 in tumor progression and are prospective for development of targeted anti-cancer therapy.

P4.14

DNA repair pathways contributing to resistance to photodynamic therapy and as possible therapeutic targets

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Keywords: cervix cancer; vulvar cancer; photodynamic therapy; DNA repair

Photodynamic therapy (PDT) is a treatment method for epithelial cancers, such as skin, cervical, or vulvar cancers or neoplastic changes. It is less damaging to normal tissue than surgery, radiation, or chemotherapy. In PDT, photosensitizers (PS) are applied systematically or topically to the diseased tissue. PS generate highly cytotoxic free radicals in response to light. In addition, PDT damages the tumour vasculature and induces an inflammatory reaction that can lead to the development of systemic immunity. Limitations of PDT are tumor location, all cancer types do not respond well to this therapy, and acquisition of resistance to PDT during treatment.

Several factors contribute to PDT resistance among which are proteins that affect the photosensitizer and its derivatives from accumulating in cells or PS degradation pathways. Also the mitochondrial iron transporter, various heat shock proteins, antioxidant enzymes, and proteins that regulate PDT by inducing apoptosis or participating in the repair of lesions may cause PDT resistance as well as glutathione over-expression.

Our transcriptomic and proteomic analyses of PDT-resistant and -sensitive cells may identify novel gene products that contribute to PDT resistance. Because different types of cancers may have specific changes in transcriptomes and proteomes, comparing resistant and sensitive cell lines of different origins may identify gene products universally responsible for PDT resistance. We also investigated new modes of sensitization to PDT by using inhibitors of DNA repair proteins.