Session 4: Molecular aspects of cancer development and progress

Lectures

L4.1

Epithelial Mesenchymal Transition in the progression of carcinoma

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Epithelial-Mesenchymal Transition (EMT) is a fundamental mechanism governing morphogenesis that employs multiple signaling pathways to shape the embryo. The intriguing possibility that similar mechanisms operate in carcinoma has been documented repeatedly in experimental models, with the discovery of mesenchymal phenotypes in tumor subsets. The transition from an epithelial carcinoma to a mesenchymal-like state is potentially associated with increased stemness, therapeutic resistance, and immune escape. I will describe the EMT spectrum of a large collection of human tumors and cell lines and emphasize the broad distribution of scores measured in distinct tumor histotypes and their corresponding cell lines. We have validated several compounds as viable EMT inhibitors. I will discuss the need for inhibiting cooperating signaling pathways in mesenchymal-like bladder carcinoma lines to abrogate their invasive properties *in vivo*. I will also discuss the contribution of EMT to immune avoidance using an MCF7 breast cancer cell model system, and show that MCF7 cells rendered mesenchymal-like by the forced expression of Snail or by a chronic TNF-α exposure become refractory to a specific cytotoxic T lymphocyte (CTL) clone. We have recently developed a 3D screening method to interfere with EMT triggered by endothelial cells that are organized as a vessel in a microfluidic device. This system delivers a more accurate representation of the *in vivo* situation. We also have analyzed the role of polarized macrophages in promoting carcinoma cell dissemination. Finally, I will discuss the importance of EMT in the genesis of CTCs. We have explored the phenotype of CTCs *in vitro* following their expansion in short-term culture and found that numerous CTCs express EMT markers. I will describe how these findings are consistent with other reports, and discuss how the potential for CTCs to transiently expand *in vitro* may be used as a surrogate marker for ascertaining therapeutic responses.

L4.2

Does inflammation promote carcinogenesis?

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Inflammation is a carefully orchestrated process, designed to eliminate pathogens, injured cells and chemical irritants. Generally, inflammation is considered as a therapeutic process ending with resolution that rely on a rapid and programmed clearance of inflammatory cells: neutrophils, macrophages, dendritic cells and invasive agents from the inflamed place. Prolonged or chronic inflammation is a causative factor in a variety of cancers as inflammatory cells release factors, such as proinflammatory cytokines, chemokines, that have an impact on the cancer development by fostering proliferation, survival and migration.
MCPIP1 is an important regulator of inflammation. It regulates the level of transcripts coding for proinflammatory cytokines and also transcription factors, that are key regulators of gene expression in inflammatory processes. It acts as an RNase recognizing stem loop structures in 3' end of its target and degrading them. PilT N-terminus domain (PIN domain), present in MCPIP1 is responsible for nucleolytic activity.
The level of MCPIP1 is diminished in several types of cancers, including clear cell renal cell carcinoma (ccRCC). Overexpression of MCPIP1 in ccRCC cell line, Caki-1, decreases proliferation rate, slows down cells migration, induces caspase 3 and 7 activity. On molecular level, MCPIP1 diminishes amount of transcripts coding for IL-6, VEGF-A, GLUT4. Subcutanous injection of Caki-1 with diminished level of MCPIP1 into NOD-SCID mice results in a faster growth and increased weight of tumor.
MCPIP1 mediates inflammatory state influencing tumor growth, metabolism and angiogenesis.

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L4.3

Roles of Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Matrix Metalloproteinase 9 (MMP9) in therapeutic sensitivity

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Neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase-9 (MMP-9) have important roles in cancer development. NGAL is a lipocalin and has many important functions. NGAL forms complexes with MMP-9 and increases its stability. NGAL is also involved in the transport of iron and other small molecules. We and others have determined that NGAL and MMP-9 can be detected in the urine of various cancer patients, but it not readily detected in the urine from "healthy" individuals. NGAL has the capacity to bind small hydrophobic molecules and also plays critical roles in iron transport. We have observed that altering NGAL expression by infection of cells with a retrovirus encoding NGAL increased the invasiveness of prostate cells which normally lack NGAL expression. In contrast, suppressing NGAL expression in cells which normally express large amounts of NGAL by infection with a lentivirus encoding a shNGAL sequence inhibited the invasiveness of the cells. NGAL and MMP-9 expression are regulated by the transcription factor NF-kappaB. NF-kappaB is detected at higher levels in advanced prostate cancers and is also a key factor in disease progression. Targeting NF-kappaB might be an approach to inhibit invasiveness of certain cancers. In addition, altered NGAL expression also affected the sensitivity of cancer cells to targeted therapy. There is a complicated regulatory loop between NGAL and TP53. TP53 is an important tumor suppressor gene which is frequently mutated in human cancer. Wild-type TP53 expression can decrease NGAL levels and increase the sensitivity to chemotherapy and radiotherapy. Whereas mutant TP53 activity can increase NGAL levels and decrease sensitivity to both chemotherapy and radiotherapy. Combined, these results indicate complex regulatory loops between NF-kappa-B, NGAL, MMP9 and TP53 which are important in therapeutic sensitivity and invasiveness. Increased NGAL expression could also alter the sensitivity to certain small molecule inhibitors as well as plant natural products which are used in traditional medicine to treat various diseases. As certain cancer patients express large amounts of NGAL and MMP-9, they could have altered responses to certain inhibitor treatments. These results suggest that prior screening of cancer patients for MMP-9 and NGAL may be beneficial. Thus NGAL and MMP-9 play important roles in key processes involved in metastasis as well as response to therapy.

Oral presentations

O4.1

lncRNA profile in head and neck cancers

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Introduction: Head and neck squamous cell cancers (HNSCC) belong to the group of tumors with poor prognosis and high patients' mortality. Long non-coding RNAs (lncRNAs) are a new class of non-coding RNA larger than 200 bp. Deregulation of lncRNAs is shown in many diseases, including cancer, so changes in their expression can be used potentially as new biomarkers and can lead to treatment personalization. In this study expression of 90 lncRNAs in tumour and healthy samples obtained from HNSCC patients were analyzed.

Methods: Tested cancers from different localisations of HNSCC and matched healthy samples came from 11 HNSCC patients. Total RNA was isolated using TRI reagent and qualified using Nano-drop spectophotometer. lncRNA profiling was performed using lncRNA profiler™ qPCR array (System Bioscience) consisting of 90 lncRNAs and 6 controls. cDNA library were synthesized by reverse transcription after polyadenylation and annealing of oligo-dTs, and RT-qPCR reaction was performed using SYBR Green master mix (Roche) according to the manufacturer's protocol. All real-time PCR data was calculated using the $\Delta\Delta C_t$, normalized against expression mean of controls and t-test analyzed.

Results: Comparison of all examined HNSCC samples with matched healthy ones revealed significant diffrences in the expression levels of DLG2AS familly ($p=0.0367$) and snaR ($p=0.0429$). For H19 some diffrences was noticed ($p=0.0586$). In the group of oral and tongue cancers the significant changes in the expression of DLG2AS familly ($p=0.0415$), H19 ($p=0.0375$), PCGEM1 ($p=0.0347$) and TMEVPG1 ($p=0.0356$) were indicated. The pharyngeal cancers were characterized by changes in expression of HOXA3as ($p=0.0172$), NTT ($p=0.0398$), snaR ($p=0.0081$) lncRNAs. Other examined lncRNA did not changed statistically significant (*t*-test, $p<0.05$).

Conclusions: To date, this is the first preliminary lncRNA profile study in HNSCC indicating new lncRNAs involved in HNSCC. We observed that the tumor localization has pivotal influence on lncRNA profile of HNSCC and lncRNAs can serve as indicators for distinguish tumor and healthy tissues. Hovewer, the exact role of changed lncRNA is still not discoverd.

O4.2

Patient-derived tumour xenografts development for preclinical studies with new oncology drugs

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To successfully move drugs from bench to bedside, a high-quality and reproducible animal models used both in drug discovery and development phase are required to provide assurance that drug targets are valid. In years 2010-2012 the lack of efficacy accounted for 59% and 52% reasons of failure in Phase II and Phase III oncology drugs trials [1], respectively, highlighting the urgent need for better models than cancer cell lines grown in culture in drug discovery and development phase. Earlier this year the National Cancer Institute (NCI) announced the withdrawal of cancer cell lines from its drug-screening program and replacing them with cancer models that are derived from patient tumor (PDTX) and grown in immunodeficient mice [2]. Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology is a major Polish oncology facility with 78 000 admissions and 355 000 outpatient visits annually. Thanks to growing interdepartmental collaboration and facing the lack of PDTX models in Poland, in 2014 we started establishing PDTX models mainly focusing of colorectal cancer (CRC). We will present our up to date experience with developing PDTX CRC models combined with histopathological and next-generation sequencing-based genomic and transcriptomic evaluation, as well as the results of our preclinical studies with new anti-cancer drugs.

Reference:

1. Arrowsmith J, Miller P (2013) *Nat Rev Drug Discov* **12**: 569.

2. Ledford H (2016) *Nature* **530**: 391–391.

O4.3

The impact of *TWIST1* silencing on the sensitivity colon cancer cells to 5-fluorouracil

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Colorectal cancer is classified at the forefront of causes of cancer death around the world. 5-Fluorouracil (5FU) has been the first-choice chemotherapy drug for colorectal cancer for many years, but the main obstacle affecting its effectiveness is a drug resistance. TWIST1 protein is a basic helix-loop-helix transcription factor, which plays an essential role in cancer metastasis.

The aim of our study was to examine the relation of *TWIST1* expression level to 5FU chemosensitivity of human colon cancer HCT-116 and HT-29 cell lines. The suppression of *TWIST1* expression in cancer cells was achieved by transduction with a lentiviral vector carrying the *TWIST1* silencing sequence (shRNA).

The suppression of *TWIST1* reduced the proliferation rate of HT-29 and HCT-116 cells, and increased their sensitivity to serum withdraw. Moreover,suppression of *TWIST1* enhanced chemosensitivity of HT-29 and HCT-116 cells to 5FU by increasing apoptosis. However, the cytotoxic effect of 5FU was significantly higher in HT-29 cell cultures. Silencing of *TWIST1* resulted in altered expression of genes encoding enzymes metabolizing 5FU. This was also associated with changes in expression of genes encoding *epithelial–mesenchymal transition (EMT)* markers. Decreased level of TWIST1 protein was associated with reduced expression of vimentin and N-cadherin. In HT-29 cells suppression of *TWIST1* promoted increase expression of E-cadherin. Molecular analyses showed that the expression level of gene encoding dihydropyrimidine dehydrogenase (*DPYP)* and thymidylate synthase (*TYMS)* decreased significantly in HT-29sh*TWIST1* cells but not in HCT-116sh*TWIST1* cells. In both cell lines with suppressed *TWIST1* we observed increased expression of thymidine phosphorylase (*TYMP*) and uridine phosphorylase 1 (*UPP1*). The expression level of thymidine kinase 1(*TK1*) increased in HT-29sh*TWIST1* and decreased in HCT-116sh*TWIST1*.

In conclusion, our observations suggest that TWIST1 might be a target protein to increase the sensitivity of colon cancer cells to 5-FU. We also suggest that the elevated expression of TWIST1 could be a marker of lovered sensitivity of colon cancer to 5FU.

Posters

P4.1

The modification of UGT and P450 isoenzymes activity by C-1305 and C-1311 antitumor agents in colon, liver and breast cancer cell lines

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Analysis of the compounds' metabolic pathways is highly important in drug discovery processes for knowing the structures of metabolites and the impact they have on metabolic enzymes. The possible drug-drug interactions can lead to the amplification of side effects as well as to the reduction of therapeutic effect. Therefore, it is important to know the impact of potential therapeutics on cellular metabolism of other drugs. Cytochromes P450 and UDP-glucuronyltransferases (UGT), represent the I and II phase metabolic enzymes responsible for the biotransformation of a lot of therapeutic agents.
The most active compounds within the group of acridinone derivatives developed in our laboratory are C-1305 and C-1311. Our group revealed previously that both of them are metabolized by several UGT isoforms, whereas they were not metabolized by P450 isoenzymes. In contrast, C-1311 inhibited the activity of P450 3A4 in noncellular systems. Thus the aim of the present studies is to test the ability of these compounds to modulate the activity of CYP3A4 and UGT isoenzymes in cancer cell lines of colon (HCT116, HT29), breast (MCF-7) and liver (HepG2), relying on diverse enzyme activities in these tumors.
The influence of acridinone derivatives on the enzyme activities was investigated by preincubation of cells with one of the studied compounds and the following incubation with specific substrates for UGT and CYP3A4: 7-hydroxy-4-(trifluoromethyl)-coumarin and testosterone, respectively. The metabolic rates of these substrates were measured with the aid of HPLC analysis. We also performed the control (without the drug) and the reference experiments (with irinotecan antitumor agent).The results showed that both C-1305 and C-1311 strongly increase the activity of UGT in HepG2 and HCT116 cells. Lower level of enzyme induction was observed in HT-29 and MCF-7 cells. However, irinotecan decreased the rate of standard substrate glucuronidation. By contrast with UGT, CYP3A4 activity was reduced by both acridinone derivatives. The modulation effect of irinotecan, which is known as inducer of CYP3A4 activity, was once more in opposition to acridinone drugs. In conclusion, the obtained results indicated that C-1305 and C-1311 potentially applied in multidrug therapy might modulate the effectiveness of other drugs sensitive to P450 and UGT mediated metabolism.

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P4.2

Studies on the mechanisms of selective pro-differentiating activities of double-point modified analogs of vitamin D_2

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1,25-dihydroxyvitamin D_3 (1,25D) has a high anti-cancer potential, but its ability to mobilize calcium to the blood serum limits it therapeutic potential [1]. This has led to the synthesis of 1,25D analogs with improved anti-proliferative and pro-differentiating activities and lower calcemic effects [2]. In our studies we analyzed activities of two, new double-point modified analogs. These analogs have been shown to be less calcemic than 1,25D. Using acute myeloid leukemia cells and cells which are responsible for calcium transport, we studied the mechanisms of their selective actions. In addition we tested affinities of these analogs to vitamin D receptor (VDR) protein, which is the only known receptor for these compounds. We found that there is no correlation between the affinity of given analog to VDR and its pro-differentiating effect. We also did not find correlation between affinity of given analog to VDR and its calcemic activity. Therefore, we suggest that there is another receptor which participates in semi-selective activities.

References:

1. Holick MF (2006) *Prog Biophys Mol Biol* **92**: 49–59.
2. Nadkarni S *et al.* (2015) *Curr Pharm Des* **21**: 1741–63.

P4.3

Improving 3D renal cancer cell culture biological parameters

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Three-dimensional cell cultures are becoming a more and more common tool in molecular oncology and t the same time are a more adequate and reliable testing tool than monolayer cell cultures, which do not reflect complex cell-cell interactions and which lack the capability of recreating the specific cancer microenvironment. Biological parameters of 3D cultures are therefore crucial and rely on both media used in the research as well as on special 3D precoated plates. Those may impact on changes in cell cycle, viability and proliferation of cells. In this research, 769-P, Caki-1, and ACHN cell lines were used. Plates were coated with either laminin, collagen I, poly-D-lysine or with both laminin and poly-D-lysine. 8 different 3D culture media were added to the cultures seeded on the plates in various combinations. Muse Merck Millipore cytometer-like device was used alongside with dedicated tests also by Merck Millipore and cell cycle analysis, count and viability assay as well as Ki67 proliferation test were performed. The results presenting the most visible differences among the cultures of the same cell lines are presented herein.

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P4.4

Neuromedin U is upregulated by SNAIL at early stages of EMT in colon cancer cells

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Background: The epithelial-mesenchymal transition (EMT) is considered a core process that facilitates the escape of cancer cells from the primary tumor site. The transcription factor Snail was identified as a key regulator of EMT; however, the cascade of regulatory events leading to metastasis remains unknown and new predictive markers of the process are awaited. Hence, in our study we described the global, early transcriptomic changes induced through stable overexpression of Snail in HT29 colon cancer cells.
Results: HT29 cells overexpressing Snail showed changed morphology, functions and transcriptomic profile associated with EMT induction. Our data indicate that Snail upregulation results in incomplete phenotype conversion, up to the intermediate epithelial state; cells express mesenchymal markers and become more motile but still well proliferate and have weak capability to form new colonies. Next, to identify transcriptomic changes of expressed genes upon Snail,Affymetrix Gene Chip Human Genome U133 Plus 2.0 analysis was performed. A total of 541 genes were altered in two analyzed clones. Among those, 340 genes were down-regulated, while 201 genes were upregulated.To associate biological functions and diseases with the transcriptomic data we performed functional enrichment analysis using Ingenuity Pathway Analysis software. The results showed, as expected, that gene expression altered through Snail in CRC cells contributed with the highest degree to changes in the *cellular movement* characteristics for *cancer* and *gastrointestinal diseases*. We observed changes in expression of 324 genes, previously correlated with cell motility but significant number of those genes were not reported as Snail activity-associated. Neuromedin U was the second highest upregulated gene in HT29-Snail cells. This increase was validated by real-time PCR. Additionally elevated level of NMU protein was detected by ELISA in cell media.
Conclusions: These results show that Snail in HT29 cells regulatesearly phenotype conversion towards an intermediate epithelial state. We provided the first evidence that neuromedin U is associated with Snail regulatory function of metastatic induction in colon cancer cells. Neuromedin U, small secreted neuropeptide with widespread distribution emerged as potentially interesting player in the cancer progression process.

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P4.5

Influence of 3D culture on gene expression in renal cancer cells

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Background: Tissue architecture strongly determines activity of constituent cells and final biological functions are an effect of subtle interactions of different cell types. Biomedical sciences face a challenge to recapitulate these phenomena in controlled laboratory conditions. In cancer research it is a crucial requirement to successfully develop novel treatment strategies. As primary drug screening is based on *in vitro* cell cultures, it is indispensable to work out efficient models that combine feasibility with adequacy. It was reported that culture of cells in 3D structures more precisely mimic tumour development *in vivo*. Aim of the study was to develop efficient 3D culture models of renal cell cancer (RCC) cell lines and how such conditions influence expression of genes crucial in cancer progression.
Methods: RCC cell lines 786-O, Caki-1 and ACHN were cultured in variable media and substrates to obtain morphologically different 3D structures. Standard 2D culture served as control, together with HEK293, non-cancerous cell line. Level of gene expression was measured with real-time PCR; TaqMan probes and primers for: *oct4, sox2, nestin, nanog, E-cadherin, N-cadherin, hif1, hif2, cd105, cd133, vhl, pax2* were used.
Results: All tested cell lines were able to form 3D structures (spheres, aggregates etc) but required conditions (medium, surface) were mostly different for particular cell lines. Culture conditions affected gene expression in all tested cell lines. Formation of compact spheres resulted in up-regulation of hypoxia and stem cell related genes. Also, gene pattern characteristic for EMT was observed as E-cadherin dropped with simultaneous N-Cadherin enhancement.
Conclusion: RCC established cell lines can be successfully cultured in 3D conditions. Formation of 3D structures induces changes in expression of genes related to tumour progression. Gene expression pattern suggests that RCC cells can display different behaviour ex. in response to drugs. Therefore such model system can be valuable tool in screening for novel therapeutics.

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P4.6

In vitro study of tumour biology – development of the three-dimensional heterogeneous model of breast cancer

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For decades most of the *in vitro* studies investigated tumour cells in a monolayer cell culture. However, the morphology, proliferation and gene expression profiles of cells cultured in flat, two-dimensional (2D) cultures, do not resemble those of an *in vivo* system. Moreover, the presence of various cell types including fibroblasts, endothelial cells, immune cells or adipocytes, modify the tumour microenvironment drastically. To provide more relevant *in vitro* model of cancer we integrated 3D scaffolds made of silk fibroin with co-culture of cancer cells and stromal fibroblasts.
Porous scaffolds were manufactured by salt-leaching method using silk fibroin solution extracted from *Bombyx mori* silk cocoons. Two cell lines were used during the experiments: murine breast cancer cell line – EMT6 – and murine fibroblasts – NIH 3T3, both were modified by lentiviral vectors to express fluorescent proteins. Cells were cultured in 2D and on the 3D scaffolds as a monoculture and co-culture in different ratios. Morphology of cells was visualized using confocal and scanning electron microscopy. Total DNA quantity measurement was used to determine cell proliferation. Toxicity of Doxorubicin was measured by Alamar Blue assay. Gene expression profiles of cells cultured in different conditions were examined using fluorescent cell sorting followed by real time RT-PCR analyses. In the heterogeneous co-cultures lower rate of proliferation of both cell types was observed, as compared to both mono-cultures in 3D and 2D conditions. Cells grown on 3D silk scaffolds were approx. 10 times more resistant to doxorubicin as compared to 2D culture. When cancer cells where co-cultured with fibroblasts, a significant decrease in the expression of *Tgfb*, *Il6* and *Hif1* was detected, with a simultaneous increase in *Mmp9*, *Ccl24* and *Vegfa* expression. The fibroblast expressed more extracellular matrix proteins such as fibronectin 1, collagen IV, tenascin-C, and desmin, as compared to 2D culture. Additionally, there was an increase in the expression of Cd44 and decrease in caveolin-1 expression in fibroblasts co-cultured with cancer cells on the scaffolds.
We optimized methods of scaffold preparation, cell seeding and long-term 3D culture. We characterized our model using microscopic visualisations, cell proliferation assays, cytotoxicity assays and gene expression analyses. This system may lead for better understanding of tumor biology, and further progress in oncological therapies.

P4.7

HIPPO signaling is involved in cancer stem cell formation and oxidative stress in melanoma

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Melanoma belongs to the most aggressive human cancers. In metastatic phase it is resistant to systemic treatment. Despite a recent progress in identification of a number of therapeutic molecular targets or inhibitors of immune check-points, metastatic melanoma is still uncurable. Such a high invasiveness and metastatic potential of melanoma result from several mutations and activation of different signal transduction pathways. Two of them- PI3K/Akt (AKT) and ERK/Raf/Ras (MAPK) are particularly involved in melanoma. Effective signal transduction however requires their coordination with other signaling pathways. Our study indicated a relationship between AKT, MAPK and HIPPO signaling. HIPPO pathway is responsible for a growth control and differentiation of tissues and organs. It is also largely involved in tumor formation and metastasis by affecting epitelial-to-mesenchymal transition (EMT) and cancer stem cells (CSC). Cancer stem cells have recently focused a significant attention. It is believed that this population is responsible for the formation of tumor metastases and resistance to treatment. Although the concept of CSC is generally accepted for various tumors, the existence of such cells in human melanoma has been the subject of debate. The discovery of CSC was associated with identification of specific markers. In melanoma the population of the cells with stem-like properties is termed melanoma initiating cells (MIC) and may be characterized by enhanced aldehyde dehydrogenase ALDH1 activity. Our study showed a significant correlation between the levels of HIPPO pathway proteins- LATS1 tumor suppressor and its oncotarget YAP, and the expression of MIC markers. We also demonstrated that LATS1 knock down sensitizes melanoma cells to the oxidative stress which in turn is linked to Akt activation. Further analysis of HIPPO cascade proteins, their interactions with other major signaling pathways involved in tumorigenesis, and with melanoma initiating cells, will provide a better understanding of the mechanisms of melanoma pathogenesis and identification of its specific clinical subtypes. This in turn will allow for identification of specific therapeutic molecular targets for a more effective treatment.

P4.8

Posttranscriptional regulation by PUMILIO proteins of SPINDLIN family genes encoding cell cycle regulators, in human seminoma

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It was previously demonstrated that a translational regulator Pumilio1 controls the amount of the male germ cells in the mouse testes by modulating apoptosis, thus being crucial for the male fertility. Here, in TCam-2 seminoma cell-line representing a model of the human testis germ cell tumor (TGCT), we show that PUMILIO1 itself does not influence apoptosis directly but by repressing translation of its anti-apoptotic paralogue, PUMILIO2. We show that these effects are mediated at least by two novel PUMILIO mRNA targets encoding a previously identified as tumorigenic SPINDLIN1 protein and its paralogue SPINDLIN3. First, we show both mRNAs to be enriched in anti–PUMILIO1, as well as in anti–PUMILIO2 immunoprecipitations. Second, we demonstrate that PUMILIO1 knockdown yields higher expression of endogenous SPINDLIN3, while PUMILIO2 knockdown increases the expression of SPINDLIN1. Therefore, both proteins, SPINDLIN 1 and 3, seem to be under control of distinct PUMILIO paralogues. Moreover, PUMILIO1 as well as PUMILIO2 cause a repression of luciferase reporter carrying 3'UTR of *SPINDLIN1* or *SPINDLIN3* mRNA, both mRNAs carrying the known PUMILIO-binding motifs in their 3'UTR. Furthermore, we show that both SPINDLIN1 and SPINDLIN3 are anti-apoptotic. Studies aimed to clarify influence of SPINDLINs and PUMILIO proteins on TCam-2 cell cycle and cell proliferation are underway to understand the significance of SPINDLIN regulation by PUMILIO proteins in human seminoma.

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P4.9

Transient BAZ1B knock-down decreases *in vitro* proliferation and inhibits CL-11 colorectal cancer cell line xenografts growth

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Background: Disturbance of epigenetic regulations may lead to cancer initiation and progression. Bromodomains (BRD) containing proteins are group of chromatin associated proteins recognizing and binding acetylated lysine residues of histones to further elicit respective transcriptional responses. William Syndrome Transcription Factor (WSTF) is encoded by *BAZ1B* gene and acts as a BRD containing transcription regulator. *BAZ1B* overexpression was described in colorectal adenomas and adenocarcinomas.
Aim: To provide a proof whether therapeutic inhibition of WSTF may be a promising strategy in oncology
Materials and methods: CL-11 colorectal cancer cell line, overexpressing *BAZ1B*, was used. Transient knock-down of *BAZ1B* expression was performed with the use of siRNA and RNAiMAX lipofectamine. Efficiency of knock-down was assessed with qPCR and immunoblotting. In the clonogenic cell survival assay cells were seeded in 6 well plates ($1x10^4$ cells/well) and cultured for 2 weeks followed by crystal violet staining. Ability to form subcutaneous tumors was assessed by injection of siRNA transfected CL-11 cells to Nu/J mice. Xenografts growth was monitored for 3 weeks.
Results: Transient knock-down with siRNA reduced *BAZ1B* expression by ~80% and was sustained for ~14 days following transfection. The clonogenic assay revealed decreased ability of *BAZ1B* depleted cells to form colonies. The growth of *BAZ1B* siRNA transfected cells when compared to non-targeting siRNA was slower as subcutanous xenografts.
Conclusion: These data suggest that WSTF protein may be a promising molecular target for therapeutic inhibition. Further ongoing studies, including obtaining of stably transfected cell lines with inducible *BAZ1B* knock-down system, will validate the relevance of *BAZ1B* as a target for drug discovery and development.

P4.10

SWI/SNF remodelling complex promotes triple negative breast cancer progression

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Breast cancer is one of the most common medical problems of women nowadays. Among these, cancers with negative IHC staining for estrogen receptor (ER-), progesterone receptor (PgR-) and HER2 (HER2-), called triple negative breast cancer (TNBC), are very aggressive type, linked with poor prognosis. They represent 10% to 20% of all breast cancers and are characterized by high degree of metastasis and chemotherapy-resistance. So far, no targeted therapy has been developed against TNBC.
Such aggressive cancers are claimed to undergo the process called epithelial-mesenchymal transition (EMT), promoting the invasive phenotype. EMT leads to the loss of epithelial characteristics, such as adherence and polarity, and gain of migratory features. This phenotype is demonstrated by downregulation of such epithelial markers as E-cadherin and cytokeratines, as well as upregulation of vimentin, N-cadherin and cadherin-11.
Our results suggest, that SWI/SNF chromatin remodelling complex (SWI/SNF CRC) may play a crucial role in the EMT and hence in the development of TNBC breast cancer metastasis. We identified direct interactions between SWI/SNF CRC and EMT promoting/driving transcription factors – SNAIL and SLUG in living cells. We also found that the interactions between SNAIL family transcription factors and SWI/SNF CRC may be important for the proteasomal control of stability of this complex in estrogen dependent breast cancer cell line. Furthermore, we postulate that disruption of SWI/SNF complex stoichiometry may lead to development and progression of cancer.

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P4.11

The endoglin-based DNA vaccine with IL-12 polarizes tumor-associated macrophages phenotype from M2 (tumor growth-promoting) into M1 (tumor growth-inhibiting)

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Progression of tumor strongly depends on the tumor microenvironment. Cells that form tumor milieu, especially tumor-associated macrophages (TAMs), play a significant role in at least two key processes underlying neoplastic progression: angiogenesis and immune surveillance. M1 macrophages can inhibit tumor growth whereas M2 macrophages stimulate it. M1→M2 phenotype polarization is an important element of tumor progression: it contributes to proangiogenic and immunosuppressive tumor microenvironment.

We examined possible polarization of TAMs from M2- to M1-like phenotype in B16-F10 murine melanoma exerted by a combination of endoglin-based DNA vaccine (constructed by us) and interleukin 12 (IL-12). The DNA vaccine is directed against endoglin (ENG), a protein which occurs on the surface of endothelial cells co-forming tumor blood vessels, as well as on certain cancer cells. IL-12 is a pleiotropic cytokine that activates both specific and nonspecific immune responses.

The study consisted of post-therapeutic analyses of excised tumor material after combined therapy (orally-delivered ENG vaccine and intratumorally injected plasmid DNA encoding murine IL-12 gene). The analysis included identification of M1- and M2-like phenotypes of macrophages. It was also investigated whether TAMs depletion (using Clodronate liposomes) reduces the therapeutic effect of combining antiangiogenic ENG vaccine with IL-12.

Our results demonstrate that combination of ENG-based DNA vaccine with IL-12 significantly increases the percentage of the tumor-infiltrating M1-like macrophages and reduces percentage of the tumor-infiltrating M2-like macrophages. Furthermore the ratio of non-M2-like (antitumor) TAMs to M2-like (protumor) TAMs was more than three times increased. Depletion of TAMs decreased the growth of control tumors, indicating that TAMs were predominantly of the M2 tumor-promoting phenotype. But in mice treated with combined therapy we observed enhanced tumor growth after TAMs depletion, showing that TAMs was more M1 tumor-inhibitory phenotype. This indicated the importance of macrophages in the therapeutic effect of ENG vaccine and IL-12.

To summarize, the endoglin-based DNA vaccine with IL-12 polarizes tumor-associated macrophages phenotype from M2 (tumor growth-promoting) into M1 (tumor growth-inhibiting).

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P4.12

Progesterone mediates resistance to herceptin treatment in breast cancer cells *in vitro*

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Breast cancer (BCa) is the most common cancer affecting women worldwide. Overexpression of human epidermal growth factor receptor 2 (HER2) or amplification of *HER2* gene are reported in approximately 15–20% of primary breast cancers. HER2, a tyrosine kinase receptor that mediates signalling pathways of cell growth, division, motility and repair, plays a crucial role in BCa progression. HER2 overexpression has been associated with more aggressive phenotype involving high-grade tumors, increased metastatic potential, and lower overall survival rates. Herceptin, humanized antibody against HER2 is a standard therapy in HER2 overexpressing cases, however, approximately one-third of patients still relapse despite treatment. Numerous studies have investigated molecular mechanisms associated with herceptin resistance. Previous studies have shown cross-talk between progesterone receptor (PR)- and heregulin (HRG)/HER2-initiated signalling pathways, but the effect of this relationship on herceptin resistance has not been studied.

Herein, we analysed influence of steroid hormone – progesterone (Pg) on herceptin-mediated cell growth inhibition. We showed that herceptin-inhibited proliferation of breast cancer cell lines overexpressing HER2 (BT474 and MCF/HER2) in 3D culture is strongly impaired by Pg. Moreover, Pg treatment induced shift of herceptin-dependent G1 cell cycle arrest towards S and G2 phases. We also found that Pg treatment resulted in increase of HER2, HER3 and HRG expression and led to activation of HER2/HER3 signalling pathway.

These results demonstrate for the first time Pg involvement in the failure of herceptin treatment *in vitro*. Our observations suggest that cross-talk between Pg- and HRG/HER2-initiated signalling pathways may lead to acquisition of resistance to herceptin in BCa patients.

P4.13

The comparison of the effects of Wnt pathway modulators – lithium chloride and PKF118-310 and epigenetic modulators – decitabine and panobinostat on Wnt target gene expression in normal, dysplastic and oral carcinoma cell lines

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The alteration in Wnt canonical signaling has been indicated as one of the mechanisms of head and neck carcinogenesis. Enhanced transcriptional activity of β-catenin leads to increased expression of genes associated with cell proliferation and motility. The epigenetic silencing of genes which act antagonistically towards the Wnt pathway is hypothesized to be important in supporting β-catenin downstream signaling in oral tumors.
The aim of the present study was to compare the effects of epigenetic modulators – decitabine (DNA demethylating agent) and panobinostat (histone deacetylase inhibitor) and direct modulators of Wnt signaling – lithium chloride (stimulates Wnt signaling *via* inhibition of GSK-3β) and PKF118-310 (inhibits Wnt pathway by disrupting β-catenin-TCF/LEF interaction) on the expression of *β-catenin* and its target genes: *CCND1*, *MMP7*, *c-MYC*, *NEDD9* and *survivin* in oral cell lines.
Squamous cell carcinoma cell lines derived from tongue (CAL 27 and SCC-25 cell lines), dysplastic keratinocytes from tongue (DOK cell line) and normal keratinocytes from floor of the mouth (OKF4/TERT-1) were used. Cell viability was assessed using the MTT assay. Cells were incubated with selected doses of the compounds and subsequently fractionated with the Universal DNA/RNA/Protein Purification Kit (EURx). Total RNA was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo) and subsequently amplified in LightCycler 96 (Roche) using HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne) with the addition of specific primers.
The induction of *MMP7* gene expression by LiCl was observed in OKF4/TERT-1 cells while *NEDD9* expression was upregulated by LiCl in DOK cells. Overall, CAL 27 cells were most responsive to Wnt pathway modulation by all the tested compounds. The expression of *MMP7* and *NEDD9* increased after the exposure to LiCl and decreased (also *c-MYC*) after PKF118-310 treatment of CAL 27 cells. Panobinostat reduced *MMP7* and *NEDD9* level more potently than PKF118-310. However, treatment with decitabine gave a more differential response in this cell line.
The results indicate that CAL 27 cell line is a good model for research on Wnt signaling modulation. It can be concluded that the expression of *MMP7* and *NEDD9* and not *c-MYC* or *CCND1* is responsive to the modulation of Wnt pathway in oral cell lines.

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P4.14

miRNA gene expression and DNA methylation as epigenetic modifications in human cancer

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Posttranscriptional regulation of gene expression by noncoding miRNA, DNA global hypomethylation, post-translation histone modification (i.e., deacetylation and methylation), promoter hypomethylation of tumour suppressor gene are key mechanisms for epigenetic regulation. In our study we focus our attention on first two of these molecular processes.

References:

Klimczak-Bitner A *et al* (2016) *Oncology letters*.
Kaufman-Szymczyk A *et al* (2016) *International Journal of Molecular Sciences*.

P4.15

A new mechanism of transmesothelial invasion of ovarian cancer cells involving senescence of normal peritoneal mesothelium

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Peritoneal cavity is a prime site of ovarian cancer metastasis. There is agreement that the colonization of the peritoneal cavity by ovarian cancer cells is related to their interactions with the peritoneal mesothelium (HPMCs). At the same time, the exact role of HPMCs in this process is a matter of an ongoing debate.
According to a group of scientists, HPMCs play a passive role as "the first line of defense", the disruption and concomitant penetration of which allows cancer cells to start interacting with the tissue stroma and to freely disseminate. To the greatest extent, this assumption stems from their observation that the biopsies of ovarian tumors present in the peritoneum did not contain HPMCs in close proximity to the proliferating cancer cells.
These findings differ from our results showing the presence of intact HPMCs in proximity to cancerous tissue in both mouse xenografts and tumors from patients with ovarian malignancy. Importantly, we also found a fraction of senescent HPMCs laying directly above cancer cells. Accordingly, we propose a theory that senescent HPMCs display features that promote ovarian cancer cell progression, in particular their transmesothelial invasion. More specifically, we predict that monolayered HPMCs containing a fraction of senescent cells exhibit lower integrity, which allows cancer cells to invade more efficiently. This increased penetrability is plausibly associated with decreased expression of junctional proteins, e.g. connexin 43 and E-cadherin. In addition, our previous studies using colorectal cancer revealed that senescent HPMCs may induce their epithelial-mesenchymal transition (EMT), which helps cancer cells to invade with higher efficacy across enlarged intercellular spaces in the mesothelial cell layer. Last but not least, it is possible that cancer cells may elicit the EMT in the remaining, not-senescent HPMCs, which could also contribute to their improved dissemination.
Collectively, we propose a new mechanism by which ovarian cancer cells may colonize the peritoneal cavity using specific features of senescent HPMCs. Experimental background for this conception is presented nearby in the poster by Dr. Justyna Mikuła-Pietrasik and Krzysztof Książek entitled "Transmesothelial invasiveness of ovarian cancer cells is associated with their ability to induce premature senescence and EMT in the peritoneal mesothelium".

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P4.16

Role of Gli2 phosphorylation in the activation of Hedgehog/Gli signaling

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The hedgehog (Hh) signaling pathway is a conserved pathway essential for embryonic development, stem cell self-renewal, and tissue homeostasis. It is aberrantly activated in many tumors. Gli proteins are transcription factor effectors of Hh signaling. Their regulation depends on many phosphorylation events: Gli proteins are phosphorylated by protein kinase A (PKA), glycogen synthase kinase 3β (GSK3β) and casein kinase 1 (CK1), which triggers Gli2/3 polyubiquitination and partial processing of Gli2/3, yielding Gli2/3 repressor forms, which leads to the inhibition of Hh signaling.
We have identified a serine/threonine cluster that is located in a strongly conserved region of *Drosophila*, *Xenopus*, human and mouse Gli2/3. Phosphorylation of these amino acids, which we named Pc-g, plays a positive role in Gli2 ativity. However, the kinase or kinases that phosphorylate Pc-g are not yet known. Our goal is to use proteomic methods to find kinases that take part in Pc-g phosphorylation and Gli2 activation. The understanding of biochemical steps that lead to Gli2/3 activation in Hh signaling will help us better understand the abnormal activation of the Hh pathway in cancer cells and may pave the way for future therapies.

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P4.17

Modification of biological activity of trifluoperazine in cancer cells by fluvastatin

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Phenothiazine derivatives, such as trifluoperazine (TFP), apart from being anti-psychotic drugs, strongly influence cancer cells. In high concentrations phenothiazines are cytotoxic to cells, induce apoptosis and modulate the properties of cellular membranes. TFP was one of the first identified modulators of multidrug resistance of cancer cells. The modulators are able to restore the sensitivity of resistant cells to chemotherapeutics. Unfortunately the use of TPF in combined cancer therapy (anticancer drug plus modulator) was precluded by side-effects that resulted from the use of high concentrations of TFP. Statins are inhibitors of one of the key enzymes of cholesterol biosynthesis pathway, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), and are widely used in therapy of hyperlipidemia and in prevention of cardiovascular diseases. It was observed that the presence of fluvastatin in cultures of human colorectal adenocarcinoma cells resistant to doxorubicin (LoVo/Dx) allowed for ca. 10-fold reduction of TFP concentration required to induce various biological effects. Fluvastatin, in the concentration in which it was itself inactive towards cancer cells, significantly increases TFP cytotoxicity to LoVo/Dx cells. Also the concentration of TFP required for doxorubicin resistance reversal was reduced in the presence of fluvastatin. Fluvastatin decreased also the concentration of TFP needed for induction of apoptosis in cancer cells, as well as increases the susceptibility of cellular membranes to peroxidation caused by TFP. The results suggested that the reduction of membrane cholesterol content in cancer cells resistant to doxorubicin sensitized the somehow to TFP. The elucidation of the precise mechanism of the observed effect requires further study.

P4.18

The LSH/HELLS homolog Irc5 is involved in cohesin association with chromatin and contributes to genomic stability in yeast

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Accurate chromosome replication and segregation are essential for every living cell as failure to execute these functions results in genome instability that manifests in carcinogenesis and developmental disorders. Irc5 from *Saccharomyces cerevisiae* is a poorly understood homolog of human LSH/HELLS protein and a member of the conserved Snf2 family of the ATP-dependent DNA translocases. Here we identify Irc5 as a novel interactor of the cohesin complex. Irc5 associates with Scc1 (Rad21 in humans) and Scc3 (SA1 or SA2 in humans) cohesin subunits and contributes to the efficient cohesin binding to chromatin. Disruption of *IRC5* decreases cohesin levels at centromeres and chromosome arms causing premature sister chromatid separation. Moreover, reduced cohesin occupancy at the rDNA region in cells lacking *IRC5* leads to the loss of rDNA repeats as a result of increased unequal sister chromatid exchange. We also demonstrate that *IRC5* disruption impairs cohesin accumulation at stalled replication forks resulting in replication resumption defects. Finally, we show that Irc5 interacts physically with the Scc2/Scc4 (NIPBL/MAU2 in humans) cohesin loading complex and is important for cell survival when the Scc2/Scc4 complex is impaired. Taken together our results indicate that Irc5 promotes efficient cohesin deposition onto chromosomes to maintain genome stability under normal conditions and during replication stress.

P4.19

The role of HAX-1 protein in induction of pro-inflammatory response of cancer cells

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Recent epidemiological and clinical data clarified the close association between inflammation and carcinogenesis process. During carcinogenesis, microenvironment influence cancer and cancer influence microenvironment. Surrounding cells like activating lymphocytes, macrophages, stromal fibroblasts and other cells produce pro-inflammatory factors to support the cancer growth. Inflammation is tightly associated with the development of cancer and growing cancer promote the expression of pro-inflammatory factors. RNase MCPIP (Monocyte chemoattractant protein-induced protein) has a well-established role in inflammation and indirectly, in carcinogenesis. In contrary, knowledge about the role of HAX-1 (HCLS1-associated protein X-1) protein in this process still needs further examination.
To verify the hypothesis that HAX-1 can interact with MCPIP1 protein we performed the co-immunoprecipitation experiment and found out that both proteins co-precipitate. We also observed that HAX-1 protein level as well as MCPIP1 level decreased in clear cell renal carcinoma clinical samples.
The intended studies should clarify whether HAX-1 and MCPIP1 can regulate transcripts involved in inflammation process during carcinogenesis. The studies are expected to bring the new knowledge about mechanisms how and where HAX-1 and MCPIP1 cooperate in the cells to maintain the internal homeostasis between cells and inflammation process.

P4.20

Regulation of vitamin D receptor expression by two different signaling pathways in acute myeloid leukemia cells

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Acute myeloid leukemia (AML) is a disease characterized by the accumulation of abnormal myeloid progenitors which lose their ability to differentiate and apoptosis (Grove CS & Vassiliou GS (2014) *DMM* **7**: 941-951). A very attractive approach to treatment is 'differentiation therapy' which employs agents that modify cancer cell differentiation with concomitant loss of proliferative capacity (Leszczyniecka M *et al* (2001) *Pharmacology & Therapeutics* **90**: 105-156).
All-*trans* retinoic acid (ATRA) and 1α,25-dihydroxyvitamin D_3 (1,25D) are active compounds which regulate cellular proliferation and differentiation. Both compounds exert biological effect trough their respective nuclear receptors: retinoic acid receptors (RARs): α, β and γ and vitamin D receptor (VDR) (Bastien J & Rochette-Egly C (2004) *Gene* **328**: 1-16). 1,25D is capable of inducing monocytic differentiation of leukemic blasts, while ATRA induces differentiation towards granulocytes (Brackman D *et al* (1995) *Leukemia Research* **19**: 57-64). The most successful differentiation therapy agent is ATRA, which is used to treat a subtype of AML, named acute promyelocyte leukemia (APL) (Saeed S *et al* (2011) *British Journal of Cancer* **104**: 554-558). Although compounds such as 1,25D and ATRA can reverse the maturation block, the mechanisms of their combined actions haven't been elucidated. Previous reports on combination of 1,25D and ATRA in AML cells are sometimes conflicting and demonstrate either synergism or an additive effect, depending on the cell line used, while other findings revealed antagonism (Marti J *et al* (1997) *Leukemia Research* **21**: 173-176; James S Y *et al* (1997) *Biochemical Pharmacology* **54**: 625-634; Bastie J-N *et al* (2004) *Molecular Endocrinology* **18**: 2685-2699).
In our studies, we examined reasons for AML cell resistance to 1,25D-induced differentiation and interactions between nuclear receptors: RAR and VDR. We used AML cell lines which differ in their susceptibility to 1,25D and ATRA-induced differentiation and in the expression levels of respective nuclear receptors. We found for AML cells that *VDR* gene is transcriptionally regulated by ATRA and the main isotype responsible for this regulation is RARα. Additionally, we showed that the *cis*-regulatory element, used by RARα, is located in the VDR promoter region of exon 1a. We also found that the constitutive activation of STAT1 signal transduction resulted in high expression of IFN-stimulated genes and resistance to 1,25D-induced differentiation which was reversed by disruption of the gene encoding constitutively active fusion kinase *FOP2–FGFR1*.

P4.21

UDP-glucuronyltransferases, the role in drug detoxification or in drug resistance?

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The final therapeutic effect of the drug candidate, which is directed to specific molecular target strongly depends on its absorption, distribution, metabolism and excretion (ADME). Enzymatic system of II phase metabolism are necessary to transform nonpolar endogenous and exogenous compounds into their hydrophilic derivatives, what impairs the undesired accumulation of lipophilic agents, facilitating their excretion outside the organism or out of cells. Conjugation of uridine-5'-diphospho-α-D-glucuronic acid (UDPGA) with the functional group of aglycone (hydroxyl, amine, carboxyl, sulfhydryl) catalysed by UDP-glucuronyltranferases (UGTs) seems to be crucial among phase II metabolic pathways. The first biological function discovered for UGTs was deactivation of endogenous substrates (bilirubin, bile acids, lipid acids, steroid and thyroid hormones and lipid soluble vitamins) to maintain their balance in the organism. The main exogenous UGTs substrates are environmental pollutants, carcinogenic compounds and numerous groups of therapeutic agents, which undergo detoxification. UGT-mediated drug resistance can be associated with: (i) inherent overexpression of the enzyme, named intrinsic drug resistance or (ii) induced expression of the enzyme, named acquired drug resistance observed when enzyme expression is induced by the drug or other factors, as food-derived compounds. Very often this induction occurs via ligand binding receptors as AhR (aryl hydrocarbon receptor) PXR (pregnane X receptor), or other transcription factors. The effect of UGT dependent resistance is strengthen not only by coordinate action but also a coordinate regulation of the expression of UGTs and ABC transporters. This coupling of UGT and multidrug resistance proteins has been intensively studied, particularly in the case of antitumor treatment, when this resistance is "improved" by differences in UGT expression between tumor and healthy tissue. The detoxification role of glucuronidation is well known for irinotecan, etoposide and epirubicin antitumor drugs, whereas tamoxifen, breast cancer therapeutic agent is activated by N-glucuronidation. The studies on acridinone antitumor agents, compounds C-1305 and C-1311, developed in our group revealed that UGT isoenzymes could be involved in the observed resistance of cancer cells to these drugs.

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P4.22

Involvement of FGFR2 in mammary epithelial cell transformation

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Polarity of epithelial cell determines morphology and physiological function of mammary gland. Stimuli which originate from tissue microenvironment are responsible for maintaining polarity of epithelium. At the molecular level cell polarity is maintained by formation of tight junctions (TJs) and adherens junctions (AJs) between adjacent cells as well as hemidesmosomes (HDs) attaching cell to basement membrane components (i.e. collagen IV or laminins). Deregulation of signalling pathways may lead to disruption of basoapical polarity of epithelial cell followed by disorganization of the gland structure and subsequent oncogenic transformation. Fibroblast growth factor receptor 2 (FGFR2) is an essential mediator of communication between tissue microenvironment and mammary epithelial cell. Apart of significant physiological functions, FGFR2 is involved in development of several carcinomas, i.e. breast cancer, stomach cancer, lung cancer and pancreatic cancer. On the other hand, FGFR2 was proven to inhibit bladder cancer, prostate cancer and melanoma progression. FGFR2 role in maintaining epithelial cells polarity has not been described so far.

The aim of the study was to analyse FGFR2 function in early steps of breast cancer development i.e. regulation of mammary epithelial cell polarity. To this end we used two variants of HB2 epithelial cells derived from mammary gland: wild-type cells (with high expression of FGFR2) and cells with FGFR2 knock-down (established with specific shRNA cloned into pLKO.1 vector). Western blot analysis revealed that FGFR2 knock-down results in decrease of tight junctions proteins occludin and claudin-1, suggesting FGFR2 importance in TJs formation and maintenance. Immunostaining and confocal microscopy were used to test FGFR2 role in establishment of acinar-like structures and distribution of TJs proteins.

These preliminary results elucidate FGF/FGFR2 signalling pathway action in breast cancer development based on regulation of tight junctions establishment and maintenance.

P4.23

Transmesothelial invasiveness of ovarian cancer cells is associated with their ability to induce premature senescence and EMT in normal peritoneal mesothelium

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One of the most important elements of ovarian cancer cell progression in the peritoneal cavity is their transmesothelial invasion towards stroma. Although the role of certain features of the peritoneum-lining mesothelial cells in this process is indisputable, the effect of their senescence has never been studied before. Here we examined if ovarian cancer cells (A2780, OVCAR-3, SKOV-3) may induce a premature senescence in primary human peritoneal mesothelial cells (HPMCs), and if so, if senescent HPMCs may favour the transmesothelial invasion of the cancer cells.
Experiments showed that young HPMCs subjected to conditioned medium (CM) generated by the cancer cells display various signs of senescence-like phenotype, including decreased lifespan, increased expression of SA-β-Gal, increased content of γ-H2A.X and 53BP1 foci, and decreased expression of junctional protein, connexin 43. Analysis of the cancer cells' secretome, followed by intervention studies with recombinant proteins and neutralizing antibodies revealed that their pro-senescence activity was related to the production and activity of hepatocyte growth factor (HGF) and HGF-dependent activation of p38 MAPK, JNK, AKT and NF-ϰB. Apart from changes associated with HPMC senescence, cancer cell-derived CM elicited in HPMCs the epithelial-mesenchymal transition (EMT), which was evidenced according to decreased expression of E-cadherin and increased expression of vimentin. In order to verify if hypertrophic, senescent HPMCs, undergoing additionally the EMT may facilitate the invasiveness of cancer cells, we prepared co-cultures of young and senescent (0, 1, 5, 10, 25, 50, 75%) HPMCs and then performed an invasion assay. The study showed that the higher was the percentage of senescent cells, the greater was the invasive potential of the cancer cells. Lat but not least, analysis of tumors excised from the peritoneum of patients with ovarian malignancy showed that HPMCs lying in a close proximity to cancerous mass display features of senescence, such as the presence of SA-β-Gal and γ-H2A.X foci, as well as decreased expression of Cx43.
In conclusion, our results indicate that ovarian cancer cells can induce senescence in HPMCs and thus they may evoke in the latter morphological and functional features that facilitate their transmesothelial invasion and the formation of solid tumors within the tissue stroma.

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P4.24

The impact of CPR and CYP3A4 overexpressions on metabolism of C-1748 acridine antitumor agent in pancreatic cancer cell line AsPC-1

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Pancreatic cancer results in the highest mortality rate of all major cancer diseases because of the limited treatment options. The poor prognosis of pancreatic cancer results from its tendency to late presentation, early metastasis and poor response to chemotherapy. Common resistance toward gemcitabine, the leading chemotherapeutic against advanced pancreatic cancer, imposes the research for new active compounds. The compound C-1748, is one of the most potent 1-nitroacridine derivative developed in our department. Strong cytotoxic activity against colon cancer cell lines and high antitumour activity against prostate and colon carcinoma xenografts, along with low mutagenic potential are the promising features, which allowed C-1748 to be selected for preclinical studies. Preliminary results indicated therapeutic potential of C-1748 also against pancreatic cancer. Metabolic pathway recognition is a crucial step in the studies on molecular mechanisms responsible for the biological activity of anticancer agents. Biotransformation of C-1748 with human liver microsomes, human cytochrome P450 reductase (CPR) and cytochrome P450 recombinant isoenzymes, as well as in hepatic cancer cells has been studied so far. The aim of the current study is to know the role of phase I metabolizing enzymes: CPR and P450 isoenzyme CYP3A4 in C-1748 metabolism in pancreatic cancer cell line AsPC-1. Recently, we have shown that several pancreatic cancer cell lines significantly differ in the expression levels of phase I metabolizing enzymes what may underlie their diverse susceptibility to undergo apoptosis following C-1748 treatment. In the present studies we investigated whether the exogenous reintroduction of CPR or CYP3A4 to AsPC-1 cells would influence C-1748 metabolism. The cell culture media were analyzed by HPLC/UV-Vis after drug treatment and the metabolic profiles of C-1748 in CPR- and CYP3A4-transfected cells were compared with that of wild type cells. We observed that enzyme transfections resulted in higher metabolite levels and in slight changes of metabolite profiles. In conclusion, our results indicated that both CPR and CYP3A4 participate in C-1748 metabolism in pancreatic cancer cells and that different expressions of these enzymes could be responsible for diverse sensitivity toward C-1748 observed among pancreatic cancer cell lines. Moreover, C-1748 as a substrate for P450 isoenzymes, may cause drug-drug interactions in combined therapy of pancreatic cancer.

P4.25

Gli transcription factors – regulation by posttranslational modifications and intracellular transport

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Gli proteins are transcription factor effectors of Hedgehog (Hh) signaling. Their deregulation can lead to malignant transformation in the brain, skin, breast, pancreas, and other organs. Gli proteins can play the role of activators or repressors of transcription, with the mammalian Gli1 and Gli2 proteins primarily playing the roles of activators, and Gli3 primarily that of repressor. We use a combination of molecular cloning, cell imaging, and proteomics to explore various determinants of Gli transcriptional activity: conserved protein domains, posttranslational modifications, such as phosphorylation, acetylation, and ubiquitination, and protein trafficking into the nucleus and the primary cilium. Other than answering fundamental questions in transcription factor biology and signaling, our goal is to design targeted therapies to inhibit the growth of Hh-dependent cancers.

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P4.26

Requirements for the internalization of antibodies directed against FGFR1

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Fibroblast growth factors (FGFs) and their cognate receptors (FGFRs) form complex signaling circuits that govern developmental processes and metabolism. Dysregulation of FGFR signaling leads to the development of cancer and severe metabolic diseases. Numerous tumors overproduce FGFR and this is correlated with poor patient prognoses. One of the most promising therapeutic approaches for targeted treatment of FGFR-dependent cancers are Antibody Drug Conjugates (ADC). In this strategy, antibodies that are physically linked to the cytotoxic drug (ADCs) selectively recognize cancer cells that overproduce appropriate receptor. Next, ADC-receptor complexes reach cellular interior via receptor-mediated endocytosis. The cellular vesicular transport system directs ADC-receptor complexes to the lysosomes for degradation, which leads to the release of the cytotoxic drug and cell death. Although the cellular trafficking of FGFRs in complex with their natural ligands has been recently studied, the requirements for the efficient internalization of antibodies that recognize FGFR remain unknown.

Here we studied the endocytosis of antibody fragments raised against FGFR1. Antibodies in the ScFv and diaboody formats are not able to reach cellular interior. However, identical ScFv's fused to the Fc fragment undergo efficient internalization into the cells that contain elevated levels of FGFR1. Similarly to the FGF1, binding of ScFv-Fc antibodies to the FGFR1 induces receptor degradation, suggesting that endosomes containing ScFv-Fc-FGFR1 complexes are destined for lysosomal degradation. The endocytosis of the ScFv-Fc-FGFR1 complex, similarly to the FGF1-FGFR1 complex, is dependent on the activities of clathrin and dynamin. Interestingly, we observed that the internalizing antibodies, in contrast to the natural ligand FGF1, do not activate FGFR1-dependent signaling pathways, suggesting that the kinase activity of FGFR1 is not critical for receptor endocytosis.

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P4.27

The chemopreventive and anticancer potential against colorectal cancer cells of evening primrose flavanol preparation

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The aim of our study was to evaluate the impact of evening primrose flavanol preparation (EPFP) on insulin-like growth factor (IGF-1), its receptor (IGF-1R) and matrix metalloproteinase-9 (MMP-9) in the human colorectal cancer cells SW-480.

IGF promotes cell proliferation and inhibits apoptosis and is thereby implicated in carcinogenesis. Additionally, MMP-9 plays a pivotal role in the development of several malignancies, including colorectal cancer. High expression of MMP-9 is responsible for matrix remodeling and collagen IV degradation which is associated with invasion, metastasis, growth, migration, angiogenesis, and poor prognosis of colorectal cancer.

To gain insight into the regulation of IGF, we determined that EPFP inhibits the growth of SW-480 cells previously stimulated with 50 ng/ml IGF-1. We also established using Q-PCR analysis that EPFP effectively reduces in a concentration-dependent manner IGF-1 and IGF-1R gene expression. EPFP at the concentration of 100 μM catechin equivalents/CE caused almost 90% and 70% reduction of IGF-1 and IGF-1R mRNA expression, respectively. Compared with the control cells, Western Blot analysis also showed nearly a 35% decrease in IGF-1R protein level at the same concentration of EPFP. Finally, enzyme-linked immunosorbent assay (ELISA) demonstrated that tested extract also downregulates IGF-1R protein in SW-480 cells. We observed a 53% decrease of MMP-9 mRNA expression in SW-480 cells with 100 μM CE of EPFP using RT-PCR analysis. Comparably, zymography assay also indicated that activity of MMP-9 was significantly suppressed after treatment of SW-480 with EPFP.

These results demonstrate that EPFP may influence on both SW-480 cells proliferation and induction of apoptosis, which could play a chemopreventive role in colon anticancer strategy.

P4.28

Extracellular vesicles secreted by colorectal cancer cell line HT29 overexpressing Snail affect endothelial cells and can contribute to modification of pre-metastatic niches

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Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Invasiveness of CRC is enhanced by Snail, a key transcription factor involved in the epithelial-mesenchymal transition. Extracellular vesicles (EVs), that include microvesicles (MV) and exosomes, from tumor cells has been recently recognized as a carrier of proteins and nucleic acids and they are considered messengers in intercellular communication, mediate the formation of metastatic niches and affect cancer cells invasion and metastasis.

Study Aim: In a broader study of contribution of Snail to modify the pre-metastatic niche, we hypothesized that EVs from CRC line HT29 that overexpress Snail will affect the release of the interleukins from endothelial cells and influence their migration.

Methods and Materials: MV and exosomes released from HT29 cells transfected with Snail (HT29/Snail) or control vector (HT29/pcDNA) were obtained via sequential centrifugations and ultracentrifugation. Incorporation of EVs by human umbilical vein endothelial cells (HUVECs) were evaluated by confocal microscopy. HUVECs were seeded in 96-well plates (3×10^4 cells per well) and incubated with MV or exosomes (10 μg/ml). After 24 hrs the supernatants were collected and evaluated for the presence of IL-6 and IL-8 using ELISA kits. HUVEC migration test was performed using the "wound healing" assay. The images of cell-free spaces of the confluent HUVEC monolayer were taken up to 26 hrs after wounding and percent of wound recovery quantitated using the equation: R(%) = [1-(wound area at Tt/wound area at T0)]×100, where T0 is the wounded area at 0h and Tt is the wounded area after indicated time.

Results and Conclusions: Both MV and exosomes from HT29/Snail and HT29/pcDNA were incorporated into HUVECs. The MV and exosomes delivered from HT29/Snail as compared to HT29/pcDNA cells augmented secretion of IL-6 and IL-8 in HUVECs. They also increased migration of HUVECs up to 24% after 9h of stimulation. Both, enhanced migration and increased release of proangiogenic chemokines, confirm the role of Snail transcription factor in modulating the cells constituting metastatic niche of CRC. Our study provides preliminary insides into the mechanism by which Snail and tumor cell extracellular vesicles affect angiogenesis and contribute to modification of pre-metastatic niches.

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P4.29

CYP3A4 and UGT1A10 overexpression modulates biological response of breast cancer cells, MCF-7 treated by antitumor compounds, C-1305 and C-1311

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The effectiveness of anticancer drugs can be influenced by several issues: patient individual levels of proteins responsible for the molecular mechanism of drug activity and transport, drug-drug interactions and their impact on proteins involved in drug metabolism and the control of cell survival and death. Triazoloacridinone C-1305 and imidazoacridinone C-1311 derivatives are anticancer compounds. They intercalate to DNA and inhibit topoisomerase II. They are not metabolized by cytochrome P450 enzymes, but inhibit enzymatic activity of CYP1A2 and CYP3A4. UDP-glucuronosyltransferases, especially 1A10 isoform, are involved in biotransformation of acridinone derivatives.

The aim of the presented studies was: (I) to show the impact of enhanced expression of CYP3A4 and UGT1A10 enzymes in MCF-7 cells on metabolism of C-1305 and C-1311 agents, (II) to demonstrate the drug influence on the level of selected P450 and UGT isoenzymes in cells and (III) to determine the cellular response of three MCF-7 cell lines triggered by compounds.

MCF-7 cells exhibited expression levels of CYP1A2, 2C9, 2D6 comparable to liver hepatoma cells, HepG2, and very slight of CYP3A4. Among UGT family, 1A1, 1A9 and 2B7 isoforms were present in MCF-7 cells and there was no expression of 1A4 and 1A10. MCF-7 cells with stable overexpression of CYP3A4 and UGT1A10 were developed. Higher level of CYP3A4 did not changed metabolism of both compounds in MCF-7 cells. However, the presence of UGT1A10 in these cells led to the glucuronidation of C-1305 and C-1311 and their glucuronides were excreted out of cells and observed in media. Both acridinones caused strong induction in CYP1A2 expression and protein level. Cytotoxicity of C-1305 and C-1311 in MCF-7-CYP3A4 were higher in comparison to MCF-7-EV cells (containing empty vector). The examination of cellular response showed that treatment with acridinones resulted in DNA degradation (increase in sub-G1 fraction), changes in nuclear morphology and induction of necrosis (PI staining) in slightly bigger population of MCF-7 cells when CYP3A4 was overexpressed than in EV cells. Furthermore, MCF-7 cells with UGT1A10 overexpression were significantly more sensitive to C-1305, what is an unique observation, whereas C-1311 partially lost its cytotoxic activity. Prolonged C-1305 treatment did not show any differences in the cellular response of MCF-7-EV and MCF-7-UGT1A10 cells, while C-1311 induced necrosis in a lower extend.

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P4.30

Inhibitors of human recombinant cytochrome P450 1A2, C-1305 and C-1311 acridinone antitumor agents, induce the overexpression of this isoenzyme in human breast cancer cell line MCF-7

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The multidrug therapies of tumour diseases are needed to cope with drug resistance. Cytochrome P450 is a family of isoenzymes responsible for I phase biotransformations of majority of therapeutics. Although there are at least 50 different P450 isoforms, the drug metabolism in humans involves most likely CYP1A2, CYP3A4, CYP2C9, CYP2C19, and CYP2D6. The identification of P450 isoenzyme responsible for drug metabolism is important with respect to drug-drug interactions, which can result in modulation of pharmacological effects and adverse drug reactions.

The acridinone derivatives 5-dimethylaminopropylamino-8-hydroxytriazoloacridinone (C-1305) and 5-diethylaminoethylamino-8-hydroxyimidazoacridinone (C-1311) were determined to be the selective inhibitor of liver microsomal and human recombinant cytochrome P450 (CYP) 1A2 and 3A4 isoenzymes. Moreover, preliminary results from our laboratories indicated that C-1305 is able to induce the activity and the expression of CYP3A4 and CYP2C9 in HepG2 tumour cells in a concentration- and time-dependent manner. Therefore, C-1305 and C-1311 might modulate the effectiveness of other drugs sensitive to metabolism and used in multidrug therapy.

The present work was designed to test whether and which one of P450 isoenzymes is able to modulate the metabolism of acridinone antitumor agents (C-1305 and C-1311) in other than hepatoma tumor cells.

The gene expression analysis by reverse-transcription quantitative PCR (qPCR, Real-Time PCR) and Western blot methods were used to determine the mRNA and protein expression levels of CYP1A2, CYP3A4, CYP2C9, CYP2D6 in human breast MCF-7 cancer cell line after treatment with different concentration (0,1; 1; 10 μM) of C-1305 and C-1311 for 24 hours.

Our results indicated that CYP1A2 was the primary P450 isoform, which was induced in MCF-7 cells after C-1305 and C-1311 treatment. Both acridinone derivatives induced CYP1A2 expression of mRNA and the increase of protein levels in a concentration-dependent manner, however these effects were higher in the case C-1311.

Summing up, the presented results indicated that C-1305 and C-1311 treatment resulted in overexpression of CYP1A2 enzyme in human breast cancer cells MCF-7. Further investigations are needed to provide relevant information whether C-1305 and C-1311 act as CYP1A2 modulators under *in vivo* conditions and whether CYP1A2 overexpression affects the final cellular response in tumor cells.

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P4.31

Combined transcriptomic and metabolomic analysis reveals link between beta-alanine metabolism pathway and progression of renal cancer

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Introduction: Renal cell cancer (RCC) is highly aggressive and fast growing subtype of kidney cancer. Previous studies suggested that in RCC cellular metabolism is disturbed. However, the impact of these alterations on patients survival is weakly understood.
Aim: To find changes in metabolic pathways that contribute to RCC progression and influence survival of RCC patients.
Material/Methods: 70 human tissue samples (35 RCC tumors and 35 matched-paired non-tumorous controls) were used for methanol:MTBE extraction of metabolites and RNA/miRNA isolation (miRCURY RNA Isolation Kit, Exiqon). The extracts were analyzed using GC-MS. RNA/miRNA was analyzed with qPCR Custom Panel arrays (Roche Diagnostics) targeting the expression of 93 metabolic genes. Survival analysis was performed using SurvExpress platform and TCGA data of 468 RCC patients.
Results: Top metabolites with concentrations changes in tumors included: beta-alanine (+4.2-fold), glucose (+3.4-fold), succinate (-11.0-fold), myo-inositol (-4.6-fold), adenine (-4.2-fold), uracil (-3.7-fold), hypoxanthine (-3-fold), creatinine (-2.4-fold), xanthine (-2.3-fold), inosine (-2.2-fold), and glycerol (-2.2-fold). Top genes with expression changes in tumors included: HK2 (+17.9-fold), TYMP (+9.8-fold), IL4I1 (+8.5-fold), HK3 (+7.8-fold), ADA (+6.1-fold), AGXT (-172.4-fold), UPP2 (-136.8-fold), PAH (-101.3-fold), G6PC (-37.9-fold), TREH (-16.0-fold), MIOX (-16.2-fold), ASS1 (-12.2-fold), DHDPSL (-10.9-fold), ALDH6A1 (-8.9-fold), GADL1 (-7.7-fold), ALDH4A1 (-7.2-fold), CKMT1B (-6.1-fold), DDC (-5.9-fold), CKMT1A (-5.8-fold), CKMT2 (-5.4-fold), ETNK2 (-5.3-fold). Poor survival of RCC patients was linked with altered expression of genes involved in metabolism of beta-alanine (HR=3.25, CI:2.27~4.65, p=1.035e−11), glucose (HR=3.25, CI:2.27~4.65, p=9.195e−12), and succinate (HR=2.96, CI:2.09~4.2, p=0.534e−10).
Conclusions: Combined metabolome/transcriptome analysis revealed that RCC tumors are characterized by alterations of glycolysis, TCA cycle, as well as metabolism of glycerol, amino acids, adenine and uracil. Disturbances in glycolysis, TCA cycle and metabolism of beta-alanine affect survival of RCC patients. This is the first study reporting alterations in beta-alanine metabolism in kidney tumors.

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P4.32

Proteins identified in ectosomes isolated from human melanoma WM266-4 cells

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Background: Cancer cells are known to release extracellular vesicles (EVs), which are small, membrane-enclosed particles that can mediate the transfer of different signalling factors as well as structural proteins, nucleic acids and lipids [1]. Ectosomes (also referred as microvesicles or microparticles) represents rather heterogeneous population of EVs, ranging between 100–1000 nm in diameter, which are secreted by shedding or budding from the plasma membrane [2]. The aim of this study was to identify proteins carried by ectosomes isolated from human melanoma WM266-4 cells.
Methods: WM266-4 cells, which is a is a metastatic cell line obtained from skin metastasis [3], were cultured in RPMI 1640 medium with GlutaMAX-I supplemented with 10% foetal calf serum and antibiotics at 37°C and 5% CO_2. After reaching 80% confluence cells were grow in serum-free medium. After 24 h, conditioned media was collected and ectosomes were isolated by sequential centrifugation (140×g for 10 min, 7000×g for 10 min, 18000×g for 20 min).The protein level was quantified using the micro-BCA assay. Protein bands obtained after 8% SDS-PAGE were excised from the gel and subjected in-gel digestion with trypsin. The resulting peptide mixture was analysed using UltiMate 3000RS LCnanoSystem coupled with Coupled with MicroOTOF-Q II mass spectrometer using Apollo Source ESI nano-sprey equipped with low-flow nebulizer. Mass spectrometry was operated in standard DDA MS/MS mode with fragmentation of the most intensive precursor ions. Analysis of mass spectrometry data was done with Data Analysis 4.0 software (Bruker).
Results: Ectosome proteins were separated into 16 bands ranging from 27 kDa to 225 kDa. In ectosome cargo cytoskeletal and ER elements, cell adhesion molecules, heat shock proteins, MHC class I/II, down-stream effectors and protein kinases, matrix metalloproteinases and enzymes involved in glycosylation and ubiquitination were identified.

References:

1. Minciacchi VR *et al* (2013) *Semin Cell Biol* **200**: 373-383.
2. Harhett LA *et al* (2013) *Pulm Circ* **3**: 329-340.
3. Westermark B *et al* (1986) *Proc Natl Acad Sci* USA **83**: 7197-7200.

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P4.33

The impact of cannabidiol on the MDA-MB-231 cell proliferation and invasion

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Human breast cancer is one of the most common, invasive and hardly curable cancer. Many scientists search for the new, effective, non-toxic and targeted drug which could stop the tumor progression. In the present study, we analyzed synthetic cannabidiol's (CBD) influence on breast cancer cells. CBD is a non-psychotropic constituent of fiber-type *Cannabis sativa* plant with a high therapeutic potential. PI3 kinase/Akt signaling is crucial, among others (MAPK/Erk or mTOR signaling), in proliferation and apoptosis regulation. The Akt/mTOR signaling pathways are mostly up-regulated in human cancers. The Akt dephosphorylation promotes apoptosis as well as autophagy. It's a serine-threonine protein kinase, which activates survival, inhibits pro-apoptotic proteins like Bad and transcription factors like FoxO. We demonstrate that CBD inhibits growth and proliferation of breast cancer cells in vitro by simultaneous decrease of Akt kinase expression (all isoforms) at the protein level. The western blot analysis of experiments is reported. These are preliminary results from first step of developing technology for obtaining plant-derivatived cannabinoids with high CBD and low THC content. The antitumor role and especially mechanism in which CBD-rich extracts act undoubtly has to be more understood.

Keywords: Cannabidiol, breast cancer, cancer growth, PI3 kinase/Akt signaling

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P4.34

Structural studies of the HVEM (14-39) peptide and its analogues interacting with BTLA protein

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Metastatic melanoma is responsible for 90% of deaths by skin cancers, because of poor prognosis with less than 10% of patients surviving after 10 years. Recently, immunotherapies against melanoma have demonstrated promising results, but further progress is still necessary. It was shown that BTLA (*B- and T-lymphocyte attenuator*) is involved in the negative regulation of T cell responses in cancer patients and can be targeted by immunotherapy. BTLA binds to a member of the TNF receptor superfamily, herpes virus entry mediator (HVEM) [1].

The main goal of the project is to design efficient and selective BTLA blockers that prevent its interactions with the HVEM protein. In our research we determined whether a fragments (14-39) of the HVEM protein and its variants can inhibit the interaction between BTLA and HVEM. We will describe chemical synthesis and NMR structural studies of peptides and biological evaluation.

Reference:

1. Compaan DM, Gonzalez LC, Tom I, Loyet KM, Eaton D, Hymowitz SG (2005) *J Biol Chem* **280**: 39553-39561.

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P4.35

Anticancer drugs could imply on CLL cell signaling as well as on apoptosis induction

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Chronic lymphocytic leukemia (CLL) belongs to the group of hematological diseases with unknown etiology. The heterogeneity of disease could be caused by the diversities in cell signal transduction, epigenetic modifications and/or apoptosis inhibition. This type of leukemia mainly occurs older individuals, but in younger's an aggressive form of the disease could lead to fast spectacular increase of leukemic cell numbers. In polish health system treatment options use for patient's administration corresponding to international standards. Beside of that some patients still do not respond to anticancer therapy. It could happened that patient was administered for almost 6 months with drug(s) he is resistant to. Because of differences in clinical development of the disease and patient's sensitivity to anti-cancer therapy, selecting an appropriate treatment approach for patients seems to be essential in optimizing the patient's response to the used drug(s). Therefore, for many circumstances it is more easy to incubate leukemic cells with anticancer agents to find out the optimal *in vitro* patient's cell response to the used drug(s) before its/their administration to patient.

The aim of our studies was to compare the apoptotic induction potential based on individual patient's sensitivity to drugs used in chronic lymphocytic leukemia treatment with expression of *Bcr* and *Stat3* in CLL cells incubated with the combinations of cladribine (C) or fludarabine (F) with mafosfamide (CM, FM), or CM combined with rituximab (RCM) or roscovitine (Rosc), kinetin riboside (RK), rituximab alone (Rit) or rituximab with complement (Ritc).

The obtained results by RT-PCR method revealed the differences in gene expression after CLL cell exposure to drugs/experimental agents, as well as in apoptosis induction potential. Personalized therapy analyses were performed using Vybrant Apoptosis Assay#4, differential scanning calorimetry and Western blot technique. The significant reduction of cell viability, increase of apoptosis rate and decrease or loss of thermal transition at 95±5°C in DSC profiles of nuclei isolated from CLL cells incubated with drugs/experimental compounds as well as PARP proteolytic cleavage were characteristic when treatment was effective.

The obtained results show that drugs/experimental agents could imply on signal transduction and on apoptosis process realization.

P4.36

Hierarchical clusterization of dose-dependent structural modifications in genome of radiosensitive and regular response cell lines

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Abstract: Ionizing radiation plays a key role in the damage of genetic material. Activated mechanism of genetic material damage repair may lead to the structural modifications in the genome. Changes related to sequences amplifications or deletions are called Copy Number Variation.

Aim: of the study was to identify the patterns of structural changes in genome of radiosensitive and normal sensitive cell lines.

Material and methods: Two cell lines of human fibroblasts derived from female donors: CCF71 classified as normal responders to radiation and CCT39 classified as radiosensitive were studied. Affymetrix CytoScan HD microarrays were used for detection of genome modifications. Each cell line was irradiated with 0.5Gy, 1Gy, 2Gy, 3Gy, and 4Gy acute doses. The signal $\log_2$ ratios (SLR) referenced to array internal standard and cell line control level (not irradiated cells) were calculated. Their distributions were approximated by Gaussian mixture model and the component representing "no significant response to irradiation" was used in one-*versus*-sample *t*-test. The obtained *p*-values allowed for selection of significant CNVs. Depending on the SLR value every mapped sequence was classified as deletion, insertion or "no response". Each CNV was represented by series of *p*-values corresponding to its stepwise dose-response. The obtained dose-responses were grouped and cluster specific mean responses were calculated. Hierarchical clustering of mean trends allowed for estimation of the similarity between dose response of analysed cell lines.

Results: Response to irradiation significantly differs between cell linee independently of dose. In case of radiosensitive fibroblasts, the dissimilarity to control line response increases with dose, and even low dose irradiation of 0.5 Gy causes its significant CNV modifications. In general, insertions over dominate deletions, independently of cell line radiosensitivity. Higher similarity (autocorrelation) in the cell lines response was observed if two dose shift for normal sensitive cell line was applied.

Conclusions: Our results suggest that radiation sensitive phenotype can be related to the efficiency of genome damage repair processes.

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P4.37

hTERT downregulation reveals non-canonical functions of telomerase in breast cancer cells

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Telomerase is being identified as a relatively specific marker for cancer cells. However, as suggested, telomerase activity or telomere length maintenance do not seem to be a critical issue for cancer cells drug resistance. It may be that hTERT (human telomerase reverse transcriptase) on its own, or in combination with ABC proteins or adhesion and migration molecules play a crucial role in this process. Thus, cancer cells deprived of telomerase/hTERT would be more susceptible to cancer drugs however this correlation remains unclear.

As suggested so far, sensitization of cancer cells to drugs may be associated not with telomere shortening but rather with alterations in telomerase subunit hTERT expression level or its translocation to mitochondria. Resistance of cancer cells to cancer drugs has been studied for a long time but this issue seems to be difficult to overcome.

We revealed that telomerase hTERT downregulation with lentiviral particles provoked a significant decrease in MCF7 and MDA-MB-231 breast cancer cells survival measured with MTT and clonogenic assays. We also demonstrated that this effect was followed by a significant decrease in migration and adhesion of cancer cells (PAX, B1-integrin and FAK decrease in WB analysis). Due to telomerase downregulation some proapoptocic genes were upregulated and an increased senescence was observed.

Thus, the mechanism associating telomerase and cancer cell resistance to cancer drugs seems to be related to the migration and adhesion potential of cancer cells.

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P4.38

Influence of 3-*O*-acetylaleuritolic acid from *Drosera spathulata* Labill on proliferation, migration and autophagy in cancer cells

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Introduction: Plants provide a broad spectrum of potential drug substances for cancer therapy with multifaceted effects and targets. Pentacyclic triterpenes are one group of promising secondary plant metabolites. Triterpene acids are known mainly for their antiangiogenic effects, anti-inflammatory, antioxidative, antiallergic and antitumor activities.

Autophagy is a type of cellular catabolic degradation response to nutrient starvation or metabolic stress. Recent studies suggest that autophagy plays an important role in the regulation of cancer development and progression. This process determines also the response of tumor cells to anticancer therapy.

Aim: In the present study we investigated the influence of 3-*O*-acetylaleuritolic acid on cell viability, proliferation and migration. Moreover we studied the mechanism of autophagy induction after treatment with 3-*O*-acetylaleuritolic acid.

Material and method: The tested compound was isolated from the leaf rosettes and roots of *in vitro* culture of *Drosera spathulata* Labill. Three cancer cell lines (HeLaWT, HT29, MCF7) and one non-cancer cell line (MCF12A) were tested. MTT assay was used for quantification of *in vitro* antiproliferative and cytotoxic effects. Cell cycle analyse was performed by flow cytometry.

Cell migration was assessed using a scratch assay. The technique of electrophoresis, Western Blot and immunoidentification were used to show the level of autophagy proteins (Beclin-1, Atg5, LC3, p62/SQSTM1 and mTOR).

Results: After 24 hours' treatment with 3-*O*-acetylaleuritolic acidwe observed higher IC_{50} *value*for non-cancer cell line (MCF12A) compared to IC_{50}*values* for cancer cell lines (HeLaWT, HT29, MCF7). All cancer cells treated with tested compound were less invasive compared to untreated control cells.Cell cycle analysis of HeLaWT, MCF7 and HT29 cells showed no proapoptotic activity of 3-O-acetyloaleuritolic acid.Furthermore Western blot analysis revealed higher level of Beclin-1, Atg5 and LC3 proteins and the lower level of p62/SQSTM1 and mTOR compared to control cells, which implies activation of autophagy process.

Conclusions: The 3-*O*-acetylaleuritolic acid shown an antitumor effect by inhibition of migration and activation of *autophagic programmed cell death.*

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P4.39

Loss of SWI/SNF core subunits correlates with metabolic switch in clear cell renal cellular carcinoma (ccRCC)

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ccRCC is a very aggressive type of kidney cancer with high mortality degree. This type of cancer develops from epithelial cells of proximal renal tubules and is featured by acumulation of glycogen and fatty acid in the cells. The changes in metabolic pathways observed in ccRCC are associated with stabilization of HIF1-transcription factor and mutations in VHL genes. Additionally, ccRCC is featured by the hyperactivation of mTOR kinase signaling pathway. The recent study on ccRCC showed alterations in expression levels of genes coding for metabolic enzymes and indicated that ccRCC uniquely relies on glycolysis for energy production, and almost all genes encoding glycolytic enzymes were found to be upregulated, while the *FBP1* expression was decreased. In this study we showed, using immunohistochemistry, dramatic decrease of BRM-central ATPase subunit and BAF155 another core SWI/SNF subunit, in almost all tested ccRCC clinical samples. Subsequently, we found in nearly all tested samples the loss of BRG1, another central ATPase of SWI/SNF complex. Observed loss of SWI/SNF subunits did not correlate with Fuhrman grade and stage of the disease. Additionally, we showed that BRM, directly binds to *FBP1* locus. Furthermore, the high-throughput analysis of GEO database indicated downregulation of genes coding for SWI/SNF subunits in ccRCC samples. Our findings are in line with results of recent study on mice and Arabidopsis indicating that the loss of SWI/SNF causes changes on metabolome-related genes.

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P4.40

The role of primary cilia in Gli protein activation in the Hedgehog signaling pathway

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Primary cilium is a tiny microtubule-based protrusion present on a surface of the majority of mammalian cells. Signaling pathway components concentrate in the cilia, suggesting a key role of this organelle for proper signal transduction. Defects in structure and function of the cilium lead to disorders called ciliopathies whose symptoms include skeletal abnormalities, kidney cysts and retinal degeneration. Some of these symptoms are also caused by perturbations in the Hedgehog pathway (Hh), which regulates embryonic development and adult tissue homeostasis. Protein localization studies show that primary cilia serve as signaling centers for Hh. When the Hh pathway is activated, some of its components translocate to the cilium. In particular, Gli proteins, which are Hh-dependent transcription factors accumulate at the ciliary tip. We hypothesize that there is a separate ciliary localization sequence in Gli proteins, which is also present in other ciliary proteins. Our goal is to identify domains of Gli proteins necessary and sufficient for their ciliary localization. Using molecular cloning and fluorescence imaging, we study the localization of short fragments of Gli proteins. We also hypothesize that cilia contain proteins that regulate Gli protein activity. We use immunoprecipitation and mass spectrometry to identify these proteins.
Our long term aim is to discover principles of intracellular sorting of proteins into different compartments, including the nucleus and the primary cilium and to shed light on the role of cilia in cellular signaling. This work will have implications for the treatment of human diseases, such as ciliopathies and Hh-dependent cancers.

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P4.41

Differences of lipids profile of HeLa-C, MCF7 and MDA-MB-231 cells after treatment with oxythiamine

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Thiamine is crucial for intensively proliferating cancer cells to cover an increased synthesis of essential nutrients and energy requirements. The reduction of the efficiency of those processes by oxythiamine (OT) can lead to inhibition of tumor cell division and increase their mortality and sensitivity to other drugs. Decrease of cell growth rate by OT may be caused by changes in lipid metabolism, through the inhibition of pyruvate dehydrogenase complex involved in production of acetyl-CoA indispensable for fatty acids synthesis. Therefore we considered OT as a potential anticancer agent which can alter the lipid metabolism. In order to compare cytotoxic effect of OT on three human tumor cell lines (cervical cancer: HeLa and breast cancer: MDA-MB-231 and MCF7), GI_{50} values were determined. Moreover we examined the effect of OT on the content of fatty acids and lipids profile in tested cancer cell lines. All cell lines (control and treated with OT 1.5-400 μg/ml) were maintained up to 90% confluency of control cultures in MEM with Earle's Salts supplemented with 10% FBS, 50 U/ml penicillin and 50 mg/ml streptomycin at 37 °C in the atmosphere of 5% CO_2. The changes in the level of specific fatty acids and modifications of the lipid profiles were examined using gas chromatography and thin layer chromatography respectively.

MDA-MB-231 cells were the most resistant to OT (GI50=210 μg/ml) in comparison to the HeLa-C (GI50=35 μg/ml) and MCF-7 (GI50=41 μg/ml) cells. MDA-MB-231 total fatty acids content was increased which was associated with elevated concentration of cholesterol esters and triglycerides. In contrast, the most sensitive HeLa-C cells were unable to respond to OT by changing of their lipid profile.

Our results indicated that the possibility of reorganization of lipid metabolism is very important for OT response of different kind of tumor cells. Consideration of OT as a potential antitumor agent should be precede by studies on adaptive capacity of individual cancer types even of the same organ origin. In conclusion, our results indicate that OT could be considered as a potential antitumor agent in the therapy of cervical cancer.

P4.42

Effect of overexpression of β- and γ-actin isoforms on actin cytoskeleton organization and migration of blebbing colon cancer cells

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Actins are eukaryotic proteins, which are involved in diverse cellular functions including muscle contraction, cell motility, adhesion and maintenance of cell shape. Cytoplasmic actin isoforms β- and γ- are essential for cell functioning. They differ only by four amino acids located at positions 1-3 and 10 in the polypeptide chain and are ubiquitously expressed. However, their unique contributions are not very well understood. The aim of this study was to determine the effect of β- and γ-actin overexpression on the migration capacity and actin cytoskeleton organization of human colon adenocarcinoma LS174T cells with rounded morphology. Migration of these cells is stimulated by GTPase Rho and kinase ROCK. They do not form mature focal adhesions or stress fibres composed of polymerized actin. LS174T cells form very dynamic, non-apoptotic migratory protrusions called blebbs. They are formed on the leading edge of the cell, which moves forward due to contractions occurring at opposite edge.

The examined cells were transfected with plasmids pAcGFP-C1-β-actin or pAcGFP-C1-γ-actin, containing cDNA for β- or γ-actin isoforms. In cells overexpressing β- or γ-actin, distinct cytoskeletal actin rearrangements were observed under the laser scanning confocal microscope. Overexpressed actins localized at the submembranous region of the cell body. Cells with increased β- or γ-actin level showed greater blebbs formation activity. The cells transfected with plasmids containing cDNA for β- or γ-actin were characterized by increased migration and invasion capacities. Actin isoforms overexpressing cells presented also the elevated filamentous to monomeric actin ratio.

In conclusion, the increased level of β- or γ-actin leads to actin cytoskeletal remodeling followed by an increase in migration and invasion capacities of human colon LS174T cells. These data suggest that expression of both actin isoforms has an impact on cancer cell motility and may influence invasiveness of blebbing colon cancer cells.

P4.43

Role of MAELSTROM as a potential PUMILIO cofactor in mRNA regulation, in human seminoma

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Pumilio represents RNA-binding family of proteins which are involved in posttranscriptional regulation of gene expression. This regulation involves PUF-domain mediated specific motif recognition within 3'UTR, followed by recruitment of protein cofactors. Specific ribonucleoprotein complexes which are formed, dependent on composition, direct a selected mRNA(s) towards translational activation, repression, degradation or cellular localization. Identification of a human PUMILIO interaction with MAELSTROM (MAEL), a marker of the human testis germ cell tumor, may imply that MAEL protein functions as a PUMILIO cofactor in human male germ cells. According to recently raised posttranscriptional RNA-operon model, by interaction with PUMILIO1 or PUMILIO2 paralogues, MAEL might contribute to selection of functionally related, but distinct for each PUMILIO, mRNA subsets for posttranscriptional regulation. To test that hypothesis we performed an anti-PUMILIO1, anti-PUMILIO2 and anti-MAEL RIP-Seq (RNA co-immunoprecipitation and sequencing) experiments based on a human TCam-2 male germ cell line. RNA was isolated from anti-PUM1, PUM2 and MAEL Co-IP using specific antibodies or IgG, as the negative control. RNA quality was analyzed using RNA NANO Bioanalyzer test, cDNA libraries were prepared using Illumina Truseq RNA Sample Prep V2 and the subsequent next generation sequencing was performed on Illumina HiSeq4000 platform. The obtained reads were mapped to hg19 database and BOWTIE analysis revealed mRNA pools common for PUMILIO1/MAEL (515) or PUMILIO2/MAEL (624) partners. Gene Ontology analysis using DAVID GOTerm tool revealed different molecular pathways for PUMILIO1/MAEL compared to PUMILIO2/MAEL mRNA targets. Bioinformatic analysis revealed potential cooperation of PUMILIO1 and MAEL in regulation of small GTPase mediated signal transduction, while a potential cooperation of PUMILIO2 and MAEL would result in regulation of processes related to protein localization and intracellular transport. These findings strongly support our hypothesis and encourage further studies using knock-down experiments of *PUMILIO1*, *PUMILIO2* or *MAEL* genes to confirm that MAEL cooperates with PUMILIO to regulate functionally related but distinct for each PUMILIO mRNA targets in TCam-2 cells. If yes, do the functions corresponding to the selected mRNA subsets correlate with the testis germ cell tumors?

P4.44

Mesenchymal Stromal Cells as carriers of IL-12 cDNA in treatment of mice bearing B16-F10 melanoma

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Mesenchymal stromal cells (MSC) *in vivo* are an important functional element of the hematopoietic stem cells niche and the integral part of blood vessels. MSC secrete antiapoptotic and immunostimulatory proangiogenic factors, stimulating cell proliferation. MSC isolated from murine bone marrow exert taxis towards tumor cells. MSC cells given into the bloodstream of animal bearing tumor localize primarily in a proximity of cancer cells. MSC specific tropism for tumor was confirmed in melanoma, glioblastoma, ovarian, breast and liver cancer.

IL-12 is a cytokine with a broad spectrum of activity. IL-12 exerts both immunostimulatory and antiangiogenic properties.

The aim of the project is an innovative attempt to use genetically modified MSC cells in the treatment of mice bearing B16F10 melanoma. MSC used as carriers of therapeutic IL-12 cDNA, specifically provide an immunostimulatory and antiangiogenic cytokine in hard to reach areas of the tumor. Cytokine IL-12 secreted by MSC should activate cells of the immune system in a close proximity of tumor cells.

Mesenchymal stromal cells (MSC) with Sca-1+ CD105+ CD90+ CD29+ CD44+ CD106- CD45- phenotype were isolated from murine bone marrow. The potential of MSC to differentiate into adipocytes and osteocytes and MSC migration capacity towards tumor cells were confirmed.

cDNA encoding the two subunits of IL-12 was cloned into MSC using adenovirus vector and the protein production was confirmed by ELISA. We examined the therapeutic properties of MSC containing the IL-12 transgene. The modified cells were given intradermally in the vicinity of murine melanoma B16-F10 tumors, what resulted in a significant decrease in the volume of tumors, comparing to control groups (PBS, unmodified MSC). The cells injected to the tail vein of mice bearing B16-F10 melanoma resulted in lower number of metastases in lungs comparing to control mice (PBS, unmodified MSC).

P4.45

Snail-induced epithelial-mesenchymal-like transition of melanoma B16F1 cells is attenuated by lumican *via* regulation of membrane metalloproteinase activity

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Background: Lumican, a small leucine rich proteoglycan, contribute to extracellular matrix assembly and organization. Our previous data demonstrated anti-tumorigenic properties of lumican in melanoma both in vitro and in vivo. The transcription factor Snail triggers epithelial-mesenchymal transition (EMT) endowing epithelial cells with migratory and invasive properties during tumor progression. The aim of this work was to investigate lumican effects on MMP-14 activity and migration of Snail overexpressing B16F1 (Snail-B16F1) and HT-29 (Snail-HT29).
Results: Murine melanoma B16F1 cells and human colorectal adenocarcinoma HT29 cells were transfected with human *SNAIL* cDNA to establish stable clones. Both, Snail -B16F1 and -HT29 clones showed slight EMT-like changes with cellular characteristics such as spindle and dendritic shapes. Since MMPs play an important role in cancer cell invasion, the activity of MMP-14 in control and Snail overexpressing -B16F1 and -HT29 cells was analyzed. In comparison with the mock cells, Snail-HT29 cells did not exhibit significant increase in MMP-14 activity. In contrast, MMP-14 activity was increased in Snail-B16F1 cells by 1.6 fold. The Snail induced MMP-14 activity in B16F1 was inhibited by lumican. At the same time lumican did not impair MMP-2 and MMP-9 activities in mock- or Snail-B16F1 cells. Lumican also inhibited Snail-B16F1 cells migration and growth. To study the potential inhibitory effect of lumican in vivo, mock- and Snail-B16F1 cells were implanted subcutaneously into wild type ($Lum^{+/+}$) and lumican-deleted ($Lum^{-/-}$) C57BL/6 mice. Melanoma primary tumors were detected 8 days after cell injection and their growth was measured for additional 7 days. The volume of tumors induced by Snail-B16F1 cells was drastically decreased in wild type $Lum^{+/+}$ mice as compared to $Lum^{-/-}$ mice. This result suggests that the endogenous lumican of wild type mice is an important inhibitor of Snail-B16F1 cell tumor development.
Conclusions: Our results suggest a link between the level of Snail protein expression in B16F1 cells and MMP-14 activity and indicate that Snail overexpression may affect MMP-14 activity differentially in each cell type. Further studies are necessary for better understanding the mechanism of interaction between lumican and MMP-14 and the effect of Snail in this process. However, our results show that lumican-based strategy targeting Snail-induced MMP-14 activity might be useful for melanoma treatment.

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