Session 2. Heterogeneity, Plasticity and Microenvironment of Cancer

Lectures

L2.1

Heterogeneity of cancer and its diagnostic/therapeutic consequences

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Cancer in many ways is defined by its heterogeneity. The large number of cellular contexts multiplied by the extreme number of known and not explored carcinogenic pathways lead to the plethora of cancer phenotypes, not sufficiently understood to provide the adequate treatment tailoring. Not only are tumors heterogenous among individuals, the next level of variance are the differences between multifocal cancers in the same individual, primary focus and its metastases and even various regions of the same tumor. The highest level, the heterogeneity of individual cells is poorly understood and not yet analyzed sufficiently in the context of healthy tissue variability.

The first significant systematic insight into cancer genomic heterogeneity was obtained by analysis of tumor specimens by microarray transcriptome profiling. I will present our data obtained by microarray analysis of breast cancer core biopsies, obtained in triplicate from almost 80 patients. The impact of gene expression profile heterogeneity on the stability of prognostic/predictive multigene signatures will discussed in the context of their potential clinical application – concentrating on the relatively high heterogeneity of immune response/stroma signatures versus low variance of *in vitro* chemosensitivity testing-derived gene sets.

The new wave of results was obtained by cancer genome sequencing and related technologies, including The Cancer Genome Atlas Project. Only some of the results were aiming at individual tumors heterogeneity, while the majority were oriented to the variability among different individuals. The first of landmark studies by Gerlinger *et al.* (NEJM 2012) has led to the number of further insights, even at the level of single cells.

The heterogeneity of cancer is related to the number of tumor plasticity phenomena, like epithelial-mesenchymal transition, stem-cell like phenotype or dedifferentiation. The bi-directional interaction of microenviroment and tumor cells regulate their plasticity and influence its heterogeneity, potentially impacting clinical outcome.

Clinical consequences of tumor heterogeneity, both in the terms of disease resistance and the impact on clinical decision making will be also shortly discussed, as well as the issue of the development of imaging modalities to visualize cancer phenotype variability *in vivo*.

Acknowledgements

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L2.2

Plasticity of cancer

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Cancer is a complex and heterogeneous disease. Genomic instability and the plasticity of cancer cell phenotype are major sources of diversity in tumor cell populations. The plasticity of cancer cells may result from phenotypic switches, including transitions between differentiated and de-differentiated states in response to stochastic events or microenvironmental cues. Reversible process of epithelial-mesenchymal transition (EMT) contributes to cancer progression and enables cancer cells acquisition of mesenchymal traits and metastatic phenotype as well as generates cells with properties of stem-cells. The cancer stem-like cells are thought to be responsible for cancer development, progression and the disease recurrence. Transdifferentiation of cancer stem-like cells into other cell types of a different lineages and bidirectional conversion between more differentiated cancer cells and more malignant cancer stem-like cells has been demonstrated. Cancer treatment procedures, including chemotherapy, ionizing radiation or surgery can induce stem-like phenotype features in differentiated cancer cells. The plasticity of cancer cell phenotype contributes to chemotherapy resistance and different drug tolerant states have been linked to reversible bidirectional phenotypic changes, including transitions of sensitive non-stem-like cells and resistant cancer stem-like cells. It suggests that both cancer stem-like cells and the bulk tumor population of more differentiated cancer cells must be effectively targeted to attain the best therapeutic outcome.

L2.3

Heme oxygenase-1 and carcinogenesis: cell type matters

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Heme oxygenase-1 is an antioxidative and cytoprotective enzyme degrading heme to biliverdin, ferrous ions, and carbon monoxide (CO). Population analyses suggest that higher activity of HO-1 pathway, associated with HO-1 promoter polymorphism, correlates with lower incidence of some type of tumors, especially head and neck cancers. On the other hand, expression of HO-1 can be upregulated in growing tumors and further increased in response to chemo-, radio- or photodynamic therapies, what commonly results in augmented tumor cell resistance and reduced efficacy of therapeutic treatments. Thus, HO-1 is a protective agent in healthy tissue, but then it plays a similar role in neoplastic cells.

Apart from the general cytoprotection, HO-1 may also play the tissue specific roles influencing only some types of tumors. We have shown that HO-1 overexpression potently inhibits maturation of myoblasts, acting mainly through CO-dependent reduction of cEBPδ binding to the myoD promoter and blockage of myoD expression. Accordingly, HO-1 inhibits myogenic differentiation in rhabdomyosarcoma (RMS) cells. The underlying mechanism is, however, distinct and relies mainly on downregulation of miR-206. Expression of HO-1 is higher in the more aggressive, alveolar RMS (aRMS) than in the benign embryonal RMS (eRMS), and inhibition of HO-1 decreases the proliferation of RMS cells and slows down the tumor growth in murine xenograft model.

HO-1 in tumors influences also stromal cells and tumor infiltrating immune cells. We found that HO-1 affects the immune response, targeting mainly myeloid-derived suppressor cells (MDSC) and mesenchymal stem cells. Thus, in the squamous cell carcinoma tumors with increased expression of HO-1, proportion of MDSC in leukocytic fraction is lower, while proportion of tumor associated macrophages (TAM) is higher, what is accompanied by faster tumor progression.

In summary, HO-1 affects tumor progression acting both on neoplastic and stromal cells, and can be regarded as a potential therapeutic target.

L2.4

The Cancer Genome Project Data (TCGA): The public resource for analysis of oncogenic pathways and and development of novel cancer diagnostics

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The Cancer Genome Atlas (TCGA) project is a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genome analysis technologies. The National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) launched TCGA as a 3 year pilot project in 2006, and have since beginning of 2010 initiated the second phase of the project to study at least 20 additional cancers over 5 years. The Greater Poland Cancer Centre became a part of TCGA network in May 2010 and so far provided more than 250 high-quality tumour samples along with pathology and clinical annotations, as well as participated in data analyses through active membership in the Analysis Working Groups for breast (BRCA), stomach (STAD), head and neck cancers (HNSC) and melanoma (SKCM). The tumour samples from each cancer type were analysed across five profiling platform including genomics (CGH arrays, DNA sequencing), transcriptomics (RNA sequencing, miRNA profiling) and proteomics (RPPA). The integrated bioinformatics effort identified major molecular subtypes of each of these tumours types across all five molecular profiling platforms. Further analyses correlating integrated molecular profiles with patients clinical data provided novel insights into tumor biology and oncogenic signalling (*Nature* **490**: 61–70, 2012 (breast cancer), Nature 2014 accepted (stomach cancer), Nature 2014 accepted (head and neck cancer)). Building upon these datasets we aim to translate these genomics profiles into diagnostic tools that can be used in every day medical practice. In the first step, we have generated tissue microarrays (TMAs) from FFPE BRCA and STAD samples obtained from the patients enrolled into the TCGA project. Next, the TMA slides were used for immunohistochemistry (IHC) using about 100 antibodies specific for major cancer markers, including oncogenic kinases frequently activated or overexpressed in breast cancer. The obtained IHC results have been scored and verified independently for each marker by two board-certified pathologists. To identify markers that correlate with or are specific to the molecular subtypes of BRCA, the TMA/IHC read-outs from each tumor sample were correlated with all TCGA genomic data. In addition, we integrate the pathology and genomics results with clinical data obtained from the TCGA-enrolled patients, including up to 4 years follow-up. Finally, we apply system biology tools to integrate the genomic data from TCGA with the proteomic analysis to understand causalities between changes in DNA, transcriptome and signal transduction pathways. Our long-term goal is to identify novel diagnostic biomarkers that will precisely identify each molecular subtype of BRCA and which may be predictive of patient response to therapy thus paving the way for novel personalized therapies for cancer.

Oral presentations

O2.1

Phenformin-resistant variants of ovarian cancer cell line IGROV1 — new model of cancer cell plasticity

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"Cancer stem-like cells" are thought to be responsible for cancer development, progression and disease recurrence. Recent studies suggest that the reversibility of cancer stem cell-like phenotype and bi-directional interchange between more differentiated tumour cells and cancer stem-like cells. An anti-diabetic biguanide drug, phenformin has been recently found to exert anti-cancer activities. We have previously observed that phenformin strongly inhibits the anchorage-independent clonogenic growth in soft agar in the ovarian cancer cell lines, including IGROV1, suggesting potential for targeting cancer stem cell-like cells. We observed that prolonged exposition of IGROV1 cells to phenformin results in small fraction of drug-tolerant cells, which can be continuously propagated in the presence of phenformin (IGROV1/PHEN). Here we characterize the IGROV1/PHEN cell line variants as a model for the studies of the plasticity of cancer stem-like phenotype. We found that IGROV1/PHEN cells display decreased clonogenic anchorage-independent growth potential, lower expression of "stemness" genes, including *Lin28A* and *HMGA2*, and increased sensitivity to cisplatin and decreased cell migration compared to the parental IGROV1 cells. Remarkably, IGROV1/PHEN cells propagated in the drug-free medium (IGROV1/DF) display an increase of anchorage-independent growth and cell migration, increased expression level of *Lin28A* and *HMGA2*, as well as decreased sensitivity to cisplatin compared to the IGROV1/PHEN cells. Subsequent re-administration of phenformin to culture of IGROV1/DF cells (IGROV1/DF/PHEN) resulted in decreased anchorage-independent growth potential and cell migration, confirming the reversibility of the phenotypic switches in IGROV1 cells. Flow cytometry analysis of IGROV1 variants, described above, revealed that changes in expression of selected ovarian cancer stem cell-like markers, including CD24 and CD44, further indicate that the tumour cells can reversibly acquire a transient stem cell-like phenotype. Our data show that tumour cells may undergo reversible phenotypic changes that determine a gain or a loss of cancer stem-like cells phenotype features. A better understanding of the cancer cell plasticity may help to develop new efficient anti-cancer treatments.

O2.2

Fenofibrate enhances barrier function of endothelial continuum within the metastatic niche of cancer cells

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Penetration of endothelial layers by circulating cancer cells (cancer cell diapedesis) is a crucial step of metastatic cascade that determines the effectiveness of malignant dissemination. Therefore, cancer cell diapedesis has been pinpointed as a potential target of anti-cancer strategies based on vasoactive (vasoprotective) drugs. We aimed at estimating the effect of fenofibrate, a commonly used anti-atherosclerotic drug, on the efficiency of this process. For this purpose, we adopted co-cultures of human umbilical vein endothelial cells (HUVEC) with DU-145 and A549 cells, as an in vitro model of their "metastatic niche". The local impairment of endothelial barrier function in the proximity of DU-145 and A549 cells, paralleled by the activation of HUVEC motility, cytoskeleton rearrangements and recruitment of vinculin to focal adhesions, was efficiently counteracted by fenofibrate. Improvement of endothelial barrier function to cancer cells by fenofibrate was correlated with strengthened HUVEC adhesion to the substratum rather than with the direct fenofibrate effect on intercellular communication networks within the metastatic niche. Maturation of focal adhesions observed in fenofibrate-treated HUVEC was accompanied by the inhibition of their motility and concomitant activation of PPAR-dependent and PPAR-independent ROS signaling, Akt and FAK phosphorylation, in the absence of cytotoxic effects. These observations demonstrate a new augmenting effect of fenofibrate on endothelial barrier function against cancer cell invasion. Our data provide a mechanistic rationale for extending the clinical use of fenofibrate, and for the combination of this well tolerated drug with the existing multidrug regimens used in cancer therapy.

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O2.3

In pursuit of the X factor triggering, together with HGF/MET axis, EMT-like changes in cervical carcinoma

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Survival of women suffering from cervical cancer is directly negatively connected with metastatic potential of tumor cells. The activation of HGF/MET axis is responsible for epithelial to mesenchymal transition (EMT) in numerous tumors. Morphological changes occurring during EMT lead to acquiring malignant and invasive abilities of expanding tumor

The aim of our study was to investigate the influence of HGF alone or in combination with EMT-associated factors (SDF-1, TGFb1, TGFb3, EGF, FGFb1, S1P, WNT1, WNT3a, WNT5a) on cervical cell line scattering. We also investigated EMT-like changes induced by serum. We used C-4I cell line, which biological properties correspond with primary cervical tumor *in situ*. We examined intracellular pathways responsible for cells scattering by blocking signal transduction cascades. Gene expression patterns related to metastatic progression were evaluated by Real-Time PCR analysis and Western Blot. Differences in cellular localization of adhesion molecules and cells cytoskeleton organization were estimated by immunofluorescent staining, while morphological changes were examined with light microscope

We found that HGF was essential but not sufficient to enable robust scattering of C4I cells. Treatment with HGF alone had a little impact on cells morphology and scattering, but was significantly augmented by addition of WNT5a. Similarly incubation of C4I cells with HGF and human AB serum had profound effect on cells morphology and scattering. None of others examined EMT-associated factors had such effect neither alone, nor in combination with HGF. By blocking MET receptor kinase activity we clearly showed that activation of HGF/MET axis is fully responsible for gaining mesenchymal-like phenotype in cervical cells. Gene expression analysis of stimulated cells demonstrated increase in the mesenchymal markers such as SNAIL, SLUG, ZEB1 and Vimentin, while expression of GSK3B kinase, responsible for degradation of EMT transcription factors, was downregulated. Surprisingly, the level of E-cadherin adhesion molecules was increased, however its localization was rather cytoplasmic than membranous. Moreover, actin cytoskeleton of stimulated cells exhibited complete rearrangement in comparison to untreated cells

Recognition of molecular mechanisms responsible for tumor metastasis may be useful in improving treatment efficiency. Out results clearly show that blocking of HGF/MET axis might be beneficial for cervical carcinoma patients at both early and late stages of the disease.

O2.4

Heme oxygenase-1 affects p38 kinase axis and influences rhabdomyosarcoma pathology

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Abrogation of the myogenesis related factors (MRFs) or muscle specific miRNAs (myomirs) may lead to development of myoblast derived tumor – rhabdomyosarcoma (RMS), the most common soft tissue sarcoma of children or adolescents. We have shown that myoblast differentiation is abolished by activity of heme degrading enzyme, heme oxygenase-1 (HO-1). This effect is mediated by carbon monoxide, which blocks interaction between cEBP delta transcription factor and its target gene myoD, the master switch for myogenesis. HO-1 modulates also activity of p38 kinases, including p38 alpha (that promotes myoblast differentiation) and p38 gamma (that promotes myoblast proliferation). The aim of this study was to evaluate a crosstalk between HO-1 and p38 kinases in RMS.

Experiments were performed using *in vitro* model of six human RMS cell lines of different clinical aggressiveness. Histological staining and gene expression analyses were also done in 31 clinical primary tumor samples. Additionally, to evaluate role of HO-1 and p38 in tumor stroma cells the RMS cells were seeded on feeder layer of primary mesenchymal stem cells (MSCs) expressing (HO-1 WT) or not (HO-1 KO) HO-1. Finally, effect of HO-1 inhibition was evaluated *in vivo* using RMS xenograft spheroid assay in the mice.

We found that expression of HO-1 is elevated in the RMS cell lines and in clinical primary tumors of more aggressive phenotype, namely in the alveolar RMS (aRMS). Moreover, upregulation of HO-1 can be induced in the mild form of embryonal RMS (eRMS) cells by forced expression of fusion Pax3-FoxO1 gene, the oncogene typical for aRMS. Induction of HO-1 is associated with decreased activity of pro-myogenic p38 alpha and increased activity of pro-proliferative p38 gamma kinases. Accordingly, pharmacological stimulation of RMS cells with HO-1 inhibitor (tin protoporphyrin, SnPP) led to upregulation of markers of myogenic differentiation, like myosin or miRNA206 in RMS cells. No effect of HO-1 inhibition was found in case of p38 alpha, however the p38 gamma activity was downregulated by SnPP. Interestingly, co-culturing of RMS cells with MSCs isolated from HO-1 KO mice resulted in a more pronounced myogenic differentiation in comparison to HO-1 WT co-culture, indicating a plausible role of HO-1 activity not only in tumor cells but also in stroma cells. Finally, inhibition of HO-1 in *in vivo* setting by systemic treatment with SnPP led to reduction of tumor growth, decrease in tumor vascularization, downregulation of p38 gamma activity, and promotion of myogenic differentiation.

Summing up, HO-1 is upregulated in RMS tumors and its expression is higher in more aggressive RMS form. Inhibition of HO-1 activity both in stroma and tumor cells promotes myogenic differentiation and abrogates proliferation. This effect might be connected with concomitant downregulation of p38 gamma, the pro-proliferative and anti-differentiating p38 isoform.

O2.5

Activated MET signaling enhances rhabdomyosarcoma progression and vascularization

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Rhabdomyosarcoma (RMS) is a soft tissue sarcoma, most often diagnosed among children and adolescents. Two major RMS types can be distinguished: embryonal (ERMS) and alveolar (ARMS), which in general has worse prognosis. Most likely, RMS derives from defect in differentiation either of the satellite cells or of the mesenchymal stem cells. During tumorigenesis normal cells of satellite or mesenchymal origin may acquire an excessive expression of a receptor for hepatocyte growth factor (HGF) called MET. The aim of our studies was to investigate the role of the activated MET signaling in development of RMS.
In RMS samples from patients MET level positively correlated with MyoD expression, suggesting association of MET with a defect in myogenic differentiation in those tumors. We have previously demonstrated that downregulation of the MET protein, the receptor for hepatocyte growth factor (HGF), forces ARMS cells differentiation and inhibits its growth (Miekus *et al.,* 2013). Moreover, we have shown that in less malignant ERMS tumors expression of MET receptor is lower than in ARMS tumors (Püsküllüoglu *et al.,* 2010). Therefore, we constitutively activated MET signaling pathway in ERMS SMS-CTR by transduction with viral vectors encoding TPR-MET. Consequently, those cells displayed uncontrolled overstimulation of MET downstream pathways, such as AKT and MAPK, regardless of HGF treatment. Accordingly, the cells with constitutively activated MET signaling displayed increased migratory capabilities in a scratch assay and enhanced migration towards gradients of HGF and SDF-1, suggesting their more invasive phenotype. Accordingly, expression of proangiogenic mediators, such as VEGF, MMP9 and miR-378 was increased.
In vivo, tumors formed by cells with activated MET signaling after subcutaneous implantation to immunodeficient NOD-SCID mice grew significantly faster than control tumors. Moreover, they developed necrosis and were characterized by more undifferentiated morphology compared to control tumors. They were formed by pleomorphic cells that did not shape into structures resembling muscle fibers. Additionally, constitutive activation of MET signaling resulted in higher number of capillaries inside tumors and enhanced metastasis to lungs.
To conclude, constitutive MET activation blocks differentiation and enhances growth, vascularization and metastasis of RMS. This finding might lead to future innovative therapies modulating RMS differentiation.

O2.6

The influence of microenvironment on invasion of colon cancer cell lines representing different modes of movement

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Cell migration plays a pivotal role in many physiological and pathological (e.g. metastasis) processes. A better understanding of the molecular mechanisms of cell locomotion is therefore of high clinical relevance. Mesenchymal and ameboid types of movement are two main modes of cancer cells migration. Mesenchymal cell motility is Rac- and protease-dependent and associated with occurrence of F-actin rich protrusions such as lamellipodia and invadopodia (adhesion structures with proteolytic activity). Mesenchymally migrating cells have elongated morphology. Ameboid mode of motility is Rho/ROCK kinases-dependent and cells moving in this way are round and form numerous membrane blebs. The mode of cancer cell migration depends largely on the tumor microenvironment - composition of the extracellular matrix and character of growth signals coming from the environment. We decided to check if invasion abilities of tested colon cancer cells differ when cells are cultured in two-dimensional or three-dimensional extracellular matrix (ECM). Additionally we estimated the influence of selected ECM proteins (collagen, fibronectin, matrigel) on invasion potential of tested cells.
In this study two human colon adenocarcinoma cell lines differing in mode of movement were used: BE (mesenchymally moving) cells and LS174T (ameboidally moving) cells. Actin cytoskeleton organization was analyzed with the help of confocal microcopy. In 2D environment (the cells were seeded on coverslips coated with fibronectin or gelatin) the BE cells formed lammellipodia and prominent invadopodia. We did not observe any stress fibres. The LS174T cells on the contrary created bleb-like pseudopodia with microfilaments forming a cortical ring under the cell membrane. In 3D environment (collagen) or after passing a Transwell™ filter the cells changed their morphology and shape of protrusions. Additionally, we have noticed that both BE and LS174T cells migrated with different efficiency and speed. It depended on the dimension and composition of microenvironment. We focused in detail on invadopodia in the BE cells in relation to various types of substrate, on which the cells were grown. The amount and size of these structures were calculated and we examined the localization of MT1-MMP metalloprotease and cortactin – essential proteins for proper functioning of invadopodia.

O2.7

Heterogenity of transitional cell carcinoma

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Transitional cell carcinoma (TCC) is classified as low-grade non-muscle invasive cancer, and it is the most common tumor of the entire urinary system. Even though it is not immediately life-threatening, lifetime cost for patients with TCC is the highest among all cancer due to its high propensity for recurrence. As the etiology of bladder cancer is poorly understood we assume implementation of comprehensive study of bladder cancer heterogeneity an appealing task. To identify subtypes of TCC we used gene expression profiling (meta-data set of 170 cancer samples provided by our collaborators from MD Anderson Cancer Center). To alleviate non-linear spurious signals, data was processed with Robust PCA algorithm that divides matrix of gene expression into low-rank "signal" matrix and sparse "noise" matrix. Samples and genes were clustered using agglomerative hierarchical clustering using Ward's method with appropriate distance function.The process of gene selection and clustering was repeated iteratively until clustering stabilization. Based on marker genes, we were able to identify stable clusters corresponding to molecular subtypes of cancer. We discovered that low-grade bladder cancer can be divided into at least two intrinsic molecular subtypes, termed "luminal" and "basal-like", that resembled established molecular subtypes of breast cancer. Both subtypes have characteristics of different stages of urothelial differentiation, and have clinically meaningful differences in progression and outcome. With the goal of defining the biological basis for the molecular heterogeneity that is observed we applied functional analysis using Ingenuity Pathway System. We performed an integrated analysis of canonical pathways differing both subtypes. We also conducted a comparative analysis of upstream regulators We found that basal TCC were characterized by p63 activation, and inactivation of FGFR3, as well as aberrations in Hras and Hif1 signaling. We argue that our observations may explain more aggressive disease at presentation in basal subtype of conventional cell carcinoma in comparison to Luminal subtype. Our results have also important implications for prognostication, the future clinical development of targeted agents, and disease management with a novel subtype specific therapies.

O2.8

Global control of eukaryotic gene expression depends on chromatin remodeling by SWI/SNF complexes

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ATP-dependent chromatin remodeling complexes (CRCs) regulate the structure, activity and organization of chromatin and play critical roles in maintenance, transmission and expression of eukaryotic genomes. Different types of CRCs are defined by the type of central SNF2-type ATPase and unique composition of other subunits, including auxiliary proteins. The SWI/SNF class of ATP-dependent chromatin remodeling complexes, a prototype of which was first described in *Saccharomyces cerevisiae*, is conserved from fungi to mammals and plants. The SNF2 ATPase is associated with a small set of highly conserved „core" subunits including homologues of yeast SWI3 and SNF5-type proteins, which when reconstituted in vitro, have full SWI/SNF remodeling activity. In multicellular eukaryotes, the lack or aberrant stoichiometry of individual core subunits causes embryolethality or severe defects in development, and in animals also leads to carcinogenesis. The studies of human CRCs represent an important area of cancer research. Depending on subunit composition, distinct mammalian SWI/SNF complexes can act as either activators or repressors of transcription, and may thus exert opposite effects on cellular activities and serve as the interface for integration of various processes. Genetic and molecular analyses confirm that different classes of plant SWI/SNF complexes are involved in regulation of specific processes

Here we show by comparative functional analysis of plant and human SWI/SNF CRCs their conserved involvement in control of evolutionary most ancient signaling and regulatory processes, including those involved in regulation of metabolic status and energy homeostasis. Our findings suggest that impairment of SWI/SNF CRCs activity may be one of the main reasons of metabolic switch observed in some types of cancer. Our study justify using *Arabidopsis thaliana* as attractive model for investigation of molecular mechanisms controlling highly conserved processes in Eukaryotes.

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O2.9

CRNDE (a novel marker of poor prognosis in patients with cancer) encodes the CRNDEP protein, a component of stress granules

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Background: The *CRNDE* gene,currently known as non-protein coding, has been found to negatively affect prognosis in patients with colorectal and ovarian carcinomas when overexpressed in cancer at mRNA level. Identification of a protein product encoded by this gene may be important due to its potential role in cancer prognosis and prospective usage in ovarian cancer screening.
Material and methods: The study was performed on RNA samples isolated from normal endometrium, ovarian cancer and HeLa cells. 5' RACE/3' RACE techniques and reverse transcription PCR followed by nested PCR were employed to identify complete splice variants of the *CRNDE* gene. Next, based on bioinformatics analyses, the most promising open reading frame (ORF) was chosen and cloned into five expression vectors. These vectors were used to elicit overexpression of the hypothetical peptide in bacteria (pQE30, pET201) and HeLa cells (pCDNA3.1(+)). They were also utilised in cellular localisation studies under a fluorescence microscope (pEGFP-N1, pDsRed Monomer-C1). In addition, a polyclonal antibody against the peptide was developed in rabbits. It was used in western blot hybridisation and immunohistochemical experiments.
Results: The 5' RACE and 3' RACE experiments revealed two different splice variants of *CRNDE*. Additional PCR experiments, inspired by the results of other research teams, proved the existence of several other splice variants of *CRNDE*. Some of them seemed to be tissue-specific, but the variant recognised here as protein-coding was ubiquitous. This shortened variant encodes the CRNDEP peptide, consisting of 84 amino acids. This peptide localises to stress granules in HeLa cells, and its upregulation stimulates the formation of these granules. Given these results and the outcome of bioinformatics analyses, CRNDEP seems to exhibit oxidase activity, thus enhancing risk of oxidative stress when overexpressed. The presence of CRNDEP in the variety of human tissues was confirmed by our team with the use of immunohistochemical methods. The existence of a protein product encoded by the *CRNDE* gene has never been reported before in the literature.
Conclusions: *CRNDE* emerges as a protein-coding gene. The product of this gene, the CRNDEP peptide, was identified herein as a component of stress granules, and its overexpression seems to stimulate the formation of these granules.

O2.10

Development of PIM/FLT3 kinase inhibitors for cancer treatment

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Acute myeloid leukemia (AML) is a group of heterogeneous diseases with a common feature of abnormal levels of myeloblasts in bone marrow and in circulation, where current standard of care including chemotherapy and allogeneic hematopoietic stem cell transplantation results in less than 40 % cure rate with little progress over the last decades. One of the most extensively investigated approaches in targeted therapy of AML are FLT3 inhibitors that address a significant population of patients harboring FLT3 mutations. These mutations result in high relapse rate and decreased overall survival when compared to patients lacking such mutations. Therefore inhibition of the constitutively active mutants of FLT3 is a promising therapeutic approach. Unfortunately, most of the tested FLT3 inhibitors failed to achieve robust and sustainable responses in clinical trials, that could translate into significant overall survival benefit of AML patient. One of the main mechanisms of resistance are tyrosine kinase domain mutations and clonal selection upon treatment with selective FLT3 kinase inhibitors. In parallel, the role of PIM kinases, especially PIM1/2 in FLT3 mediated leukemogenesis attracted attention of pharmaceutical industry with currently two phase I programs targeting PIM kinases in hematological malignancies. PIM kinases are important downstream effectors of FLT3 signaling and play a crucial role in cell survival and inhibition of apoptosis upon expression.
Selvita has developed a potent and selective dual PIM/FLT3 mutant kinase inhibitor - SEL24-B489 which is highly active in *in vitro* and *in vivo* AML models. Developed compound shows high inhibitory activity against mutated FLT3 (FLT3-ITD and TKD mutations such as D835H, D835Y, N841I) and all three PIM kinase isoforms, comparable to activity of selective inhibitors of FLT3 (A220) and PIM (AZD1208). Head to head comparison of SEL24-B489 in cellular models reveal high activity across tested cellular AML models and biomarker inhibition on protein translation in line with expected kinase profile. Most importantly, apart from single agent activity, SEL24-B489 showed also strong synergistic effect in combination with current standard of care, such as cytarabine, but also other targeted inhibitors. Activities of B489 *in vivo* in xenograft models of AML and lymphomas after oral administration were higher than compared to selective PIM inhibitor and lead to remissions in certain models. SEL24-B489 is a successful example of rational drug design and is currently in preclinical development. It was designed as a compound with strictly controlled kinase activity promiscuity and represents a promising therapeutic approach which addresses alternative survival pathways downstream of FLT3 apart from the activity on resistant FLT3 mutant kinases, which hopefully will translate into improved survival of AML patients in clinical trials.

Posters

P2.1

Effect of gold nanoparticles on the human cancer cell lines and biological activity of curcumin

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Lung cancer and colorectal cancer are one of the most common cancers in the world. The development of the disease can be associated with genetic risk factors as well as with environmental risk factors like long term cigarette smoking for lung cancer and drinking alcohol, high intake of red meat and obesity for colorectal cancer. Therapeutic strategies for those types of cancers are surgical resection, chemotherapy and radiotherapy. Nevertheless, those cancers still characterize high mortality rate [1]. Therefore, it is important to looking for the new therapeutic strategies
Different metal nanoparticles are widely examined in biomedical applications nowadays. One of the most commonly studied are gold nanoparticles (GNPs) [2]. They are applied for therapeutic purpose for example as a vector for antitumor drug deliver thanks to their high affinity to tumor cells, surface modification ability and special optical properties [3]. Moreover, the therapeutic effect of GNPs can be size, dose and cells type dependent.
The aim of work was to compere the biological effects of spherical gold nanoparticles on viability of human colon adenocarcinoma cell line (LoVo) and non-small-cell lung cancer (A549). Moreover, in this study was examined impact of GNPs on biological activity of curcumin – polyphenol, known as popular Asian spice with anti-cancer properties [4].
In order to assess A549 and LoVo cells viability after 24h, 48h and 72h of incubation with investigated compound, MTT staining method was used. We report that GNPs reduce viability of both investigated cell lines in time and dose dependent manner. However, the strongest effect was observed in different time of incubation. GNPs in concentration 25μg/ml reduced viability of LoVo cells to 62% of control after 48h of incubation, whereas 65% viability of A549 was noted after 72h of incubation for the same concentration of GNPs. Moreover, A549 cells viability increased after treatment with 12 μM curcumin with addition of GNPs (1–25 μg/ml), while this effect was not observed in LoVo cells. The Single Cell Gel Electrophoresis (SCGE) was used to indicate whether GNPs induce DNA damage in examined cancer cells. Additionally, double staining method with Hoechst 33342/propidium iodide showed that GNPs not induce apoptosis in both cancer cell lines in concentration 1–25 μg/ml.
Our experimental data proved that GNPs reduced viability of LoVo and A549 cells in time and dose dependent manner. However, biological activity of curcumin was decreased by GNPs only in A549 cells.

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P2.2

In vivo and *in vitro* study of interaction between human thymidylate synthase and dihydrofolate reductase, the enzymes involved in thymidylate biosynthesis

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Thymidylate synthase (TS) and dihydrofolate reductase (DHFR) catalyze sequential reactions in cellular sole *de novo* thymidylate biosynthesis, forming indispensable precursor for DNA synthesis and repair - thymidine monophosphate (TMP). The lack of either enzyme activity leads to apoptosis of a cell, making them both highly important drug targets. DHFR inhibitors are used extensively in the therapy of haematological malignancies and TS inhibitors are primary drugs in chemotherapy of solid tumors of the gastrointestinal tract, head and neck. However, the development of drug resistance has become an escalating problem over the recent years.
TS and DHFR function in most of the organisms as a separate proteins, however in protozoa and plants they form a bifunctional enzyme consisting of distinct protein domains interacting with each other. Taking this into consideration and knowing that TS is one of the most conservative proteins, we postulated that human TS and DHFR enzymes need to form a protein complex during active TMP synthesis. Such interaction may accelerate the biosynthesis cycle to reach the needed efficiency, necessary for maintaining the accurate nucleotide balance for DNA synthesis. The aim of our study is to verify the existence of human TS-DHFR enzyme complex *in vivo* and to provide the kinetics parameters of the binding in *in vitro* experiments. Obtained information might be of great importance for potential anticancer drug development, as it could allow for creation of inhibitors preventing the enzyme-enzyme interaction in rapidly proliferating tumor cells.
Using purified recombinant human proteins we characterized, by means of microscale thermophoresis and quartz crystal microbalance, TS-DHFR interaction and calculated complex kinetic parameters. Moreover, we estimated the binding stoichiometry and mechanism of conformational protein adjustment during the interaction. Studying cancer cells *in vivo* by flow cytometry, we computed distribution of the enzymes in the cell cycle. We also investigated the colocalization of the proteins using confocal microscopy. In addition, we compared the enzyme amount and their colocalization during cycle phases in cells treated and not treated with TS or DHFR inhibitors. Obtained results gave us insight into the interactions between thymidylate synthase and dihydrofolate reductase occurring in human cells.

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P2.3

The *CRNDE*, *VAV2* and *CEBPA* genes as new negative prognostic factors in ovarian cancer patients

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Background: Ovarian cancer is the leading cause of death from gynaecological malignancies. Mortality in this disease is exceptionally high due to the absence of specific symptoms at early phases and the lack of good screening methods. The majority of patients are diagnosed at late stages, characterised by poor prognosis. Identification of new molecular prognostic markers, potential targets of molecular therapy, may facilitate the fight against this neoplasm.
Material and methods: The genes examined in this study were selected based on the results of preceding gene expression microarrays. The prognostic value of their expression at mRNA level was evaluated in ovarian cancer patients treated with either cisplatin and cyclophosphamide (the PC regimen, N=32) or taxanes and cisplatin (the TP regimen, N=74). *HGPRT* was chosen experimentally as a reference gene. In qPCR reactions, inventoried TaqMan assays (Life Technologies) were used, except for the *CRNDE* gene, expression of which was evaluated with two personally designed TaqMan assays, specific to two different splice variants. The amount of genomic DNA contamination was assessed and taken into account if necessary (i.e., for *CEBPA*, an intronless gene). The results were analysed statistically using univariate and multivariate Cox proportional hazards models.
Results: Elevated mRNA expression of *CRNDE* (two different splice variants), *VAV2* and *CEBPA* genes negatively influenced prognosis by significantly increasing risk of death and/or recurrence. For *CEBPA*, this association was mainly observed in a group of patients treated with PC. The clinical significance of *VAV2* overexpression seemed to be related to the TP treatment, though a negative impact of upregulation was also visible in the group of all patients analysed. Overexpression of *CRNDE* negatively affected prognosis without clear discrimination between the chemotherapies administered. In addition, some clinical associations of *CRNDE* seemed to depend on TP53 accumulation status.
Conclusions: Considering the Real-Time qPCR results, the *CRNDE*, *VAV2* and *CEBPA* genes emerged as novel cancer-promoting factors and potential molecular markers in ovarian cancer patients. The clinical meaning of their protein products should be further evaluated in a bigger, well characterised group of tumours through immunohistochemical staining.

P2.4

New approach in chemo- and radiotherapy of head and neck cancer

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Malignant tumors of the head and neck region differs natural clinical outcome and prognosis depending on the histological diagnosis and location. Despite that the diagnostic and therapeutic problems are similar. The gold standard of therapy these tumors, with a view to maximal radicalization of treatment, is combined therapy involving the local (surgery, radiotherapy) and systemic treatment (chemotherapy). Great interest among clinicians has a individual selection of cytostatics to patients with various malignancies.
The aim of the studies is to develop and implement in clinical practice a rapid test for chemotherapeutic effect assessment of tumor cell cytotoxicity as well as to evaluate correlation of double-strand breaks (DBS) marker γH2AX with radiation effect in the mucous membranes of the mouth, throat and skin.
Ex vivo colony forming assay (FLAVINO assay) on cancer cell lines derivated from HNSCC tumors (head and neck cancers are squamous cell carcinoma) will be carried out. The preliminary results indicate that this assay allows to assess the effectiveness of chemotherapeutic (cisplatin, docetaxel and cetuximab). In the part of the project were also estimated the impact of these drugs on the induction of apoptosis, cell cycle and cell proliferation rate *via* immunofluorescence staining and cytometer analysis. DNA damage marker γH2AX during radiotherapy and chemoradiotherapy of head and neck cancers and its correlation with DNA damage level was measured via flow cytometry
Obtained results could have a direct translational character due to the rapid assessment of cytotoxicity of drugs on tumor cells derived from an individual patient. Application of such personalized therapy definitely increases its efficiency, reduce the toxicity of chemotherapeutics in patients and thereby increase quality of life.

P2.5

Methodological discrepancies in the research on clear-cell renal cell carcinoma drug resistance

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Background: Concerning the *in vitro* research on drug resistance in cancer, methodology has always been a matter of discussion. Authors of most of the published data do not show their preliminary results, which at the same time does not explain their specific decisions on used methods. Discrepancies concern: chosen viability test, the number of cells seeded onto specific surfaces, drug concentrations and chosen control. Those discrepancies occur since ccRCC belongs to the group of highly heterogeneous tumors.
Materials and methods: Differences in the research on clear-cell renal cell carcinoma (ccRCC) drug resistance to tyrosine kinase inhibitors were taken into account. The study comprised two ccRCC cell lines: 786-O (primary tumor) and ACHN (secondary tumor), as well as one human kidney cancer stem cell line (Celprogen®) which is described as CD133+. Cells were seeded on 96-well plates in different and the same number per well for each cell line. Different drug concentrations of a tyrosine kinase inhibitor sunitinib (Sutent®) were used. While performing those studies, Alamar Blue viability test was performed.
Results: While CD133+ and 786-O cells had a similar growth curve, ACHN grew more slowly and therefore reached a log phase of growth later than 786-O or CD133+. Improper cell seeding density resulted in not reaching the logarithmic phase of growth by slowly growing cell lines. At the same time cell lines with a shorter doubling time, seeded in too high concentrations, became overgrown before reaching the planned end of the experiment. ccRCC cells cultured in monolayer were shown to be more "robust" to higher drug concentrations than patients.
Discussion: Here, data from our preliminary research from various studies in this field were presented, showing that seeding the same amount of cells on a 96-well plate brings different results than seeding different cell numbers, depending on the cancer cell line specific features. What is more, serum/plasma peak drug concentrations in patients' organism should be taken into consideration while planning the experiments. Proper control during cell viability tests should be also used. Moreover, it was demonstrated that drug concentrations have to be adjusted to the molecular weight of a drug as well as to the specific drug features, for instance to its accumulation properties.

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P2.6

MicroRNA-mediated regulation of expression of heterogenous nuclear ribonucleoprotein A1 (hnRNPA1) in renal cancer

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Introduction: hnRNPA1 is involved in the regulation of alternative splicing process by antagonizing the function of SR proteins. Our previous studies showed disturbances in expression of hnRNPA1 in clear cell renal cell carcinoma (ccRCC), which is the most common type of renal cancer. ccRCC is characterized by high mortality and frequent metastasis at the early stage of the disease. Disturbed expression of hnRNPA1 in ccRCC may be caused by different factors, one of them can be microRNAs (miRNAs). These short, non-coding RNA molecules regulate gene expression via binding to 3'UTRs of mRNAs, leading to inhibition of translation or degradation of transcript. In this work we hypothesize that hnRNPA1 expression may be regulated by miRNAs in ccRCC.
Material and methods: The expression of hnRNPA1 was measured using Real-Time PCR in 34 pairs of ccRCC and control samples. Using Pick & Mix microRNA PCR Panels (Exiqon) we analysed the expression of miR-1, miR-149-5p and miR-206 miRNA molecules potentially binding to 3'UTR of hnRNPA1. ccRCC-derived cell line, Caki-2, was transfected with miRNAs precursors or scrambled controls using Lipofectamine 2000. Reference genes were analysed using NormFinder, whereas statistical analysis was performed using GraphPad Prism.
Results and discussion: We observed that hnRNPA1 expression was decreased in 29.4%, increased in 17.6% and unchanged in 53% of tumor samples when compared to control samples. Correlation matrix of tumor-specific changes of mRNA expression demonstrated statistically significant negative correlation between miR-1 and hnRNPA1 (Spearman $r=-0.55247$, $p=0.001$). Transfection of Caki-2 cells with synthetic precursors: pre-miR-1, pre-miR-149-5p and pre-miR-206 led to statistically significant reduction of hnRNPA1 mRNA level, by about 28% ($p=<0.0001$), 25% ($p=0.009$) and 26% ($p=0.0045$), respectively, when compared with cells transfected with scrambled control miRNA precursors.
Conclusions: miR-1, miR-206 and miR-149-5p are potential regulators of hnRNPA1 expression in ccRCC. We are currently starting functional studies aiming to confirm direct interactions between these miRs and hnRNPA1 transcript. To our knowledge, this is the first study describing the role of miRNAs in the regulation of splicing factor in ccRCC.

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P2.7

Curcumin as a cytostatic agent in renal cancer *in vitro*

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The renal cancer is relatively rare (3% of all cancers in adults), however it's mortality rate is high (about 45%), due to diagnosis in advanced and refractory stage of the disease. The five – year survival after standard therapy (chirurgical resection of the tumor) is rary (2–5%) and life expectancy after diagnosis is poor (4 months to 1 year). Therefore it is crucial to improve already existing therapies and to find alternative methods of treatment. Supplied with daily diet polyphenol curcumin (spice, food colouring E100) is characterized by anti-cancer properties and low toxicity. Thus it was decided to evaluate the effectiveness of potential therapy of renal cancer *in vitro* with curcumin. In present work we used A498 and Caki-1 cell lines as a model of **C**lear **C**ell **R**enal **C**ell **C**arcinoma (ccRCC). This type of cancer is the most common histological subtype (85% of all kidney cancers). We tried to evaluate the curcumin influence on proliferation, viability, genotoxicity, mobility and induction of **R**eactive **O**xidant **S**pecies (ROS) in renal cancer cell lines.
MTT assay showed that curcumin significantly suppress proliferation of investigated cell lines in dose – dependent manner. The results showed a higher cytostatic activity of curcumin in the case of A498 cells (lower IC_{50} coefficient than in case of Caki-1 cells). Cytotoxic properties of curcumin (assesed by double staining method with Hoechst 33342/propidium iodide) was weak. However, Caki-1 cells have been more sensitive than A498 (viabilty decreased by 20% and 10% respectively). Genotoxic properties of curcumin (obtained by Comet Assay) was directly proportional to the concentration used. But Caki-1 cells were more susceptible than A498 (32% and 13% of DNA damage in relative to control, respectively). Wound healing assay showed that polyphenol inhibits A498 cells migration in dose – dependent manner. Dichlorofluorescein assay showed that curcumin stimulated cellular production of ROS only by 20%. But in presence of hydrogen peroxide (strong oxidant) curcumin was a scavanger. Elimination of ROS by polyphenol was dose-dependent. The flow cytometer analysis showed that curcumin influenced the cell cycle of investigated cell lines.
Our results suggest that curcumin is a weak cytostatic agent in ccRCC treatment, which act like a cell death inductor, probably by production of reactive oxidant species within cells. In other hand it has a strong antioxidant properties. Furthermore curcumin effect is cell line dependent

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P2.8

Expression of cell-cell adhesion-related genes in colorectal tumors

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Epithelial tissues achieve their highly organized structure due to cell-cell junction complexes: tight junctions, adherence junctions, gap junctions and desmosomes. They connect the cells, provide the passage of signals and metabolites between adjacent cells and are also responsible for maintaining tissue integrity.
Cancerogenesis is accompanied by the change of cells intereactions and tissue morphology, often described as epithelial to mesenchymal transition.
We analyzed expression of genes encoding cell-cell junction proteins, that have been identified as differentially expressed in three types of colorectal tissue samples (cancer, adenoma and normal mucosa; fold change>2) in previous genome-wide expression profiling. The expression of 20 selected genes have been assessed with qRT-PCR in 26 colorectal cancer, 42 adenoma and 24 normal mucosa samples.
We observed dramatic changes of mRNA levels of genes encoding adherence junction proteins: upregulation of *CDH3, CDH11* and downregulation of *CDH19*; tight junction proteins: upregulation of *CLDN1*, *CLDN2*, *CLDN12* and downregulation of *CLDN5*, *CLDN8*, *CLDN23*, *CLDN15*, *JAM2*, *CGN*; desmosomes: upregulation of *DSC3* and downregulation of *DSP*, *DSC2*, *PKP2* as well as decrease of expression of some other genes involved in intercellular connections: *PCDHB14*, *PCDH7*, *MUPCDH* and *PTPRF*. The differences between tissue samples types reached statististical significance and resulted in separate sample clustering in hierachical clustering analysis.
Morphological changes of neoplastic cells are often described as a result of the disturbed expression of single key surface molecues, like cadherin 1 and cadherin 2. Our results show that this process is complex and expression switch of number of genes encoding elements of almost all junction complexes can be observed.

P2.9

microRNA detection in cancer — comparison of two methods for accurate microRNA quantification by RT-qPCR

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Background: microRNAs (miRNAs) are small, 20-22 nucleotide, non-coding RNAs, which post-transcriptionally regulate gene expression influencing e.g. cell growth, proliferation and differentiation. miRNAs expression was found to be deregulated in many diseases. Importantly, miRNA can act not only within cell in which is expressed but can be released and influence even distant organs. Accordingly miRNA expression profiles from tissue or tumor biopsy samples and/or patient's serum can be used as potential biomarkers. However, due to short length and high sequence similarities between certain miRNAs their detection is challenging. Here we compare two methods based on RT-qPCR for miRNA analysis using unmodified or LNA-modified primers focusing on their specificity.
Methods: miRNAs were chosen on basis of their similarities close to 3' end. Pair of miR-99a-5p and miR-100-5p has perfect complementarity at the last 5 nucleotides, whereas pair miR-146a-5p and 146b-5p has two mismatches within last 5 nucleotides but not at the very last position. Construction of four plasmid vectors for overexpression of pre-miR-99a, -100, -146a and -146b, transfection of HEK293T and measurement of given miRNA level with RT-qPCR using unmodified or LNA-modified primers were performed.
Results: After overexpression of miR-99a and miR-100 standard primers designed to detect miR-99a-5p or miR-100-5p recognized both microRNAs at similar level, which indicates unspecific recognition of both miRNAs. LNA primers showed much higher specificity as unspecific signal was below 5% when compared to specific. In case of miR-146a-5p and miR-146b-5p both standard and LNA primers were specific in detection of given microRNA.
Conclusions: Using unmodified primers for microRNA detection by RT-qPCR can be misleading in case of some microRNAs which share high sequence similarity, especially close to 3' end. Use of LNA-modified primers helps to significantly increase specificity of RT-qPCR in microRNA measurements.

P2.10

Copy number analysis of microRNA and microRNA processing machinery genes in lung cancer

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Background: Lung cancer is the leading cause of cancer related deaths worldwide, with the mortality higher than in colon, breast, and prostate cancers combined. It is mostly diagnosed at an advanced stage, while the survival of patients depends significantly on early detection. Thus, early diagnosis and prognostic biomarkers have become the target of interest. Among them are microRNAs, a class of small, noncoding RNAs that regulate mRNA and protein expression. Recently, two independent meta-analyses revealed a group of microRNAs with the most frequently altered expression in lung cancer cases.
Purpose: We examined one of the potential causes of microRNAs expression changes – copy number variations of the microRNAs genes. In addition to the most frequently up- and down-regulated microRNAs, we examined two other microRNA genes, potentially associated with lung cancer, and two genes encoding proteins, which belong to the microRNA processing machinery, DICER and DROSHA.
Materials and methods: We analyzed DNA samples from 216 NSCLC cases, using multiplex ligation-dependent probe amplification (MLPA). We designed two specific MLPA probes for each microRNA gene-containing region, and three probes for both *DICER1* and *DROSHA*.
Results: Performed analysis allow to identify genes undergoing significantly increased (frequently amplified) and decreased (deleted) copy number. Among the genes undergoing gain/amplification the most frequently are *MIR30D* (42%/16%), *MIR30A* (41%/15%), and *DROSHA* (40%/11%). On the other hand, genes undergoing deletion the most frequently are *MIR31* (13%), *MIR126* (13%) and *MIR155* (11%).
Conclusions: Our study showed, that some of the investigated genes undergo high copy number variation, higher even than the key lung cancer oncogenes, such as *EGFR* or *MET*. Such variation may be the mechanism underlying the changes in their expression, and may confirm oncogenic role of microRNAs.

P2.11

Semi-synthetic organo-selenium compound Selol affects apoptosis and CAMs expression in prostate cancer PC3 cells

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Prostate cancer is the second leading cause of male cancer mortality and its metastasis to bones has been observed in 90% of died patients. Thus, one of the key problems of prostate cancer therapy is the prevention of metastases. Selenium is one of the trace elements which proved to be a key component of the diet important in the chemoprevention of cancer. Prostate cancer PC3 cell line derives from metastases to the bone and characterizes by high invasiveness (stage IV). Cell adhesion molecules (CAMs) play an important role at each stage of the process of prostate cancer cells metastasis. PC3 cells exhibit inhibition of apoptosis and increased ability to migrate through increased activity of MAPK (mitogen activated protein kinase) and FAK (focal adhesion kinase). PC3 cells manifest also increased expression of IKK (IkappaB) kinase which stimulates activity of the nuclear transcription factor NF-kB, the main regulator of many cell survival proteins functions.
Selol is a mixture of selenitriglicerides synthesized from sunflower oil containing selenium (Se IV). The synthesis of seleninotriglycerides was carried out at the Department of Drug Analysis at Medical University of Warsaw (Pol. PL 176530 (Cl. A61K31/095). In our studies we exam the effect of Selol in PC3 cells on the apoptosis and expression of proteins involved in adhesion process.
To elucidate the effect of organic Se on PC3 cells we carried out an *in vitro* study aimed at assessing generation of reactive oxygen species (ROS), apoptosis, cellular distribution of NF-κB, and expression of cell adhesion molecules ICAM-1 and ALCAM-1. The growth inhibition effect on cells by Selol was determined by measuring the MTT day absorbance by living cells. ROS generation was evaluated by spectrofluorometric method using the DHR 123, DCFH-DA or HE. Analysis of NF-kB distribution was performed with laser scanning confocal microscope. Cell surface expression of CAMs was analyzed by flow cytometry.
We have observed that Selol generates ROS, decreases level of apoptosis, and selectively affected an expression of adhesion molecules on the surface of PC3 cell.

P2.12

Tumor-derived integrin ligands induce pro-invasive polarization of microglia

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Transformation of microglia or brain infiltrating macrophages to reactive states in response to pathology is a multifaceted process. Infection or brain injury leads to induction of inflammatory responses in microglia. However, clinical and experimental data show that microglia/macrophages recruited by malignant gliomas instead of initiating the anti-tumor response, contribute to tumor growth by supporting invasion, promoting angiogenesis and suppression of anti-tumor immunity. The molecules and signaling pathways that direct tumor-associated macrophages toward the pro-invasive phenotype are largely unknown. Using HPLC and a tandem mass-spectrometry we analyzed glioma secretome, and identified osteopontin and lactadherin as candidate factors involved in "glioma-induced microglia activation". Both proteins, known to interact with αVβ3 and αVβ5 integrins *via* RGD motif, were abundantly expressed in glioma cells, but not in non-transformed astrocytes. The response of primary rat microglia cultures to conditioned media (GCM) from rat C6 glioma cells was attenuated by a blocking peptide containing RGD motif. Recombinant osteopontin or lactadherin overexpressed in 3T3/NIH fibroblasts produced changes in microglial morphology, increased phagocytic activity and induced changes in gene expression associated with a pro-invasive phenotype of microglia. Mutation in the RGD motif in osteopontin or lactadherin abolished the effects of recombinant proteins towards microglia. Our findings demonstrate an involvement of integrin-linked signaling in polarization of microglia into tumor-supportive cells.

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P2.14

Effect of epsilon-viniferin on hippocampal (mHippoE-18) and neuroblastoma (Neuro-2a) cells

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A lot of extensive investigation proved that polyphenols might slow progression or even prevent chronic diseases – cardiovascular, ischemic, neurodegenerative, diabetes. As the latest data show it also might improve chemotherapy. Many efforts have been made to evaluate the mechanisms responsible for the effect of polyphenols in cell. However there are still many which pharmacological activity has not been extensively investigated, for example epsilon -viniferin. Epsilon-viniferin is a natural polyphenolic compound, most abundant present in grapes and wines. There are a few evidences that it has multiply biological properties.
Taking into account the above considerations, we decided to investigate the effects of epsilon-viniferin on hippocampal (mHippoE-18) and neuroblastoma (Neuro-2a) cells. The mHippoE-18 and Neuro-2a cells were treated with epsilon-viniferin concentrations ranging from 2.5 μM to 50 μM. After 24 and 48 hours of incubation, we observed reduced viability of cells *in a dose- and time-dependent manner*. Comparing cells viability, it is decreasing more rapidly in cancer cells (Neuro-2a) than in normal (mHippoE-18). We also observe that percent of apoptotic cells substantially increased in cancer cells comparing with normal. In both case we used concentration of polyphenol which caused 50% reduction in cell viability (IC_{50} value).
It's needless to say that new therapies and safer drugs are essential. Our data indicate that epsilon-viniferin might be applied in combination with chemotherapy or radiotherapy but still more evidences and further investigations of its action and its molecular targets are needed to use it wisely as a drug.

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P2.13

Prognostic significance of circulating tumor cells detection in rectal cancer patients treated with preoperative radiotherapy

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Objective: Circulating tumor cells (CTC) can be detected in peripheral blood (PB) of cancer patients who have no evidence of overt metastases. The presence of CTC in PB has proven to be of prognostic significance in patients with metastatic colorectal cancer. For non-metastatic colorectal cancer clinical significance of CTC is being investigated. The aim of this study was to evaluate the prognostic value CTC in non-metastatic rectal cancer patients treated with short-term preoperative radiotherapy.
Methods: In this single-center trial, 162 patients with rectal cancer after preoperative short-term radiotherapy (5x5 Gy) were recruited in 2008–2011. Clearance of CTC was determined in 91 patients enrolled to the molecular analysis. CTC presence was evaluated with real-time reverse transcription polymerase chain reaction assay (qPCR) based on the expression of three tumor genetic markers: carcinoembryonic antigen (CEA), cytokeratin 20 (CK20) and/or cancer stem cells marker CD133 (CEA/CK20/CD133)
Results: We found that: (1) CTC detection 7 days after surgery was of prognostic significance for the local recurrence (P-value=0.006), (2) CTC detected preoperatively and 24 hours after resection had no prognostic value in cancer recurrence, however there was a significant relationship between CTC prevalence 24 hours after surgery and lymph node metastasis (pN1-2). We also confirmed a significant clearance of CTC in peripheral blood (PB) 24 hours after surgery.
Conclusion: Preoperative sampling is not significant for prognosis in rectal cancer patients treated with short-term radiotherapy. Detection of CTC in PB 7 days after surgery is an independent factor predicting local recurrence in this group of patients.

P2.15

Intelligent game of glioma with immune responses of the host

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Introduction: Histopathology and flow cytometry studies have shown infiltration of human gliomas with activated microglia and peripheral macrophages. Despite their accumulation and activation in tumor microenvironment, anti-tumor immune responses are defective in glioblastoma. Tumor-educated immune cells induce an immunosuppressive environment, support glioma invasion and progression. In this study we analyzed by flow cytometry accumulation of microglia ($CD11b+CD45^{low}$), macrophages ($CD11b+CD45^{high}$) and T lymphocyte subpopulations ($CD4^+$, $CD4^+FOXP3^+$, $CD8^+$) in rat glioma-bearing hemispheres, and their impact on immune microenvironment of gliomas.
Methodology: Adult Wistar rats were sham-operated or intrastriatally transplanted with rat C6 glioma cells. Animals were sacrificed 21 days after implantation and after mechanical fragmentation with a neural dissociation kit, immune cells were isolated and analyzed by flow cytometry. Brain samples were cut and double staining with anti-Iba1 (a microglia/macrophage marker) and anti-Arginase 1 (a protumorigenic phenotype marker) antibodies was performed. RNA was isolated from sham-operated and glioma-bearing brains and transcriptome profiling with Affymetrix Rat Gene 2.1 ST microarray was accomplished.
Results: We found significant infiltration of gliomas with resident microglia, blood-derived macrophages and leukocytes at different times after implantation of C6 glioma cells. Glioma infiltrating microglia/macrophages presented the alternative phenotype as visualized by the presence of Arginase-1 positive staining ($Iba+/Arg1^+$). Detailed analysis of cell subpopulations indicated a significant increase in the percentage of blood-derived macrophages, T helper and T regulatory cells in rat gliomas, while the percentage of T cytotoxic cells was low. The analysis of global gene expression showed overexpression of immunosuppression-related genes in glioma-bearing brains.
Conclusions: Accumulation of protumorigenic microglia/macrophages, T helper and T regulatory cells in glioma and the reduced presence of T cytotoxic cells could be responsible for creating a highly immunosuppressive environment and lack of effective anti–tumor responses. Gene expression patterns in rat gliomas showed similarity to gene expression in subtypes of human glioblastoma. Our results indicate that rat gliomas adapt similar tumor strategy as human mesenchymal glioblastomas.

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P2.16

The proteolytic activity and oligomerization status of human HtrA3 protease functioning as a tumor suppressor

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HtrA3 protease belongs to the high-temperature requirement A (HtrA) family of serine proteases which take part in cellular stress response including heat shock, inflammation and cancer. They are oligomeric proteins characterized by the presence of a trypsin-like protease domain with the catalytic triad His-Asp-Ser and a PDZ domain at the C-terminal end. The latter serves as a substrate or regulator binding domain and may participate in oligomerization. Down-regulation of *HtrA3* in tumors, shown by other groups and us, suggests HtrA3 involvement in oncogenesis [1]. HtrA3 acts as a proapoptotic protein and is suggested to function as a tumor suppressor. It promotes cytotoxicity of etoposide and cisplatin in lung cancer cell lines [2, 3]. To date, HtrA3 has been poorly characterized from the biochemical point of view, mainly due to the fact that it is difficult to purify recombinant HtrA3. We were able to express in bacterial system and purify HtrA3 in quantities sufficient to perform structural studies. The aim of this study was to characterize the proteolytic properties and quaternary structure of HtrA3 protease. Using β-casein and fluorogenic peptide (Ala(Mca)IRRVSYSF-ANB-NH2) we observed that the protease activity rose markedly with temperature and the enzyme was active in a wide range of pH. The kinetics of HtrA3 activity without and with inhibitor indicated that it is an allosteric enzyme. Size exclusion chromatography revealed that HtrA3 occurred as a homotrimer and the substrate (β-casein) had no influence on its oligomerization status. Deletion of the PDZ domain caused a significant decrease in activity and dissociation of the protease to a monomeric form. Our results indicate that HtrA3 is a trimeric allosteric protease whose activity and oligomerization depend on PDZ domain.

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P2.17

Detection of leukemia-specific *NPM1* mutations with the use of three different approaches

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Nucleophosmin, encoded by *NPM1*, is a ubiquitous and multifunctional phosphoprotein which shuttles between nucleus and cytoplasm, but is mainly localized in the nucleolus. It is implicated in the regulation of ribosome biogenesis, centrosome duplication, activation and stabilization of proteins, including tumor suppressors p53 and ARF. There are several lines of evidence that nucleophosmin can act both as an oncogene as well as oncosuppressor. The increased level of NPM1 was observed e.g. in gastric cancer, hepatocellular carcinoma and multiple myeloma, but leukemia is the only cancer where *NPM1* is frequently mutated. About 30% of acute myeloid leukemia (AML) patients and 40–50% of patients with normal karyotype AML (NK-AML) harbor the mutation in the last exon of *NPM1*, resulting in aberrant, cytoplasmic accumulation of the protein. The tetranucleotide duplication of TCTG (mutation A) is the most common somatic mutation of *NPM1*. Mutation A accounts for 75–80% of all NPM1 mutations found in leukemic patients. As *NPM1* mutations have prognostic importance in AML we developed several approaches to test and estimate a relative fraction of the mutated allele on the genomic (gDNA) and transcriptomic (mRNA) level. To detect *NPM1* mutation in AML samples (gDNA-level) we developed a new Multiplex Ligation-dependent Probe Amplification (MLPA) assay and compared its results with results of standard Sanger sequencing. Additionally, MLPA assay permitted us to determine copy number variation of *NPM1* in the analyzed samples. The results from both gDNA-based approaches were then confronted with the data from next generation sequencing (RNA-seq) of AML transcriptomes that enabled the quantification of both total *NPM1* expression and the relative expression of the mutated allele. The analysis of the NPM1 mRNA levels was additionally performed with the use of droplet digital PCR (ddPCR). In terms of the mutation detection all three approaches showed 100% concordance (perfect specificity and sensitivity). Up to date, we have detected *NPM1* mutation (mutation A) in 21% of the analyzed leukemic samples from Polish patients. We did not observe copy number variation of *NMP1* neither in the *NPM1*-wild type nor in *NPM1*-mutated samples. In some AML samples we observed substantial increase of *NPM1* expression but the level of *NPM1* mRNA was not correlated with the presence of *NPM1* mutation.

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P2.18

Combination of tumor cell-based vaccine and IL-12 gene therapy polarizes tumor microenvironment

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Necrotic cells release factors that may be involved in the induction of immune system response against tumor. In this study, we report the antitumor effect achieved by a vaccine from CAMEL peptide-treated tumor cell lysate. It has been reported that CAMEL causes mitochondrial swelling and disrupts mitochondrial membrane. Disruption of the latter leads to decreased intracellular ATP level triggering cell death. CAMEL-treated tumor cell lysates were used to immunize mice that had been challenged with B16-F10 melanoma cells. To further improve the therapeutic effect of such tumor cell-based vaccine we combined it with subcutaneous administration of murine IL-12 gene-containing plasmid construct. Interleukin 12 is a cytokine with antiangiogenic properties and plays a prominent role in activating the immune system, *inter alia* by enhancing cytotoxicity of $CD8^+$ T cells. We observed that the combination of tumor cell-based vaccine and IL-12 gene therapy resulted in much better inhibition of B16-F10 murine melanoma tumor growth than application of either agent alone. Furthermore, in the case of combined therapy, post-therapeutic analyses demonstrated a decreased number of tumor vessels, larger necrotic areas as well as enhanced immune cells' infiltration in tumor sections, in comparison with single-agent approaches. The combined therapy activated both non-specific and specific immune responses, with higher levels of tumor-infiltrating $CD4^+$ and $CD8^+$ lymphocytes as well as NK cells and decreased level of regulatory T lymphocytes. Cervical lymph nodes of the treated mice showed enhanced levels of $CD4^+$ and $CD8^+$ lymphocytes. Our results suggest that the tested tumor cell-based vaccine, upon combination with interleukin 12 gene therapy, induces an anti-tumor immune response and affects polarization of the phenotype of cells forming tumor microenvironment, from proangiogenic and immunosuppressive towards antiangiogenic and immunostimulatory. Polarizing the tumor milieu inhibits tumor growth and seems to be a promising therapeutic strategy.

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P2.19

Valproic acid: epigenetic modifier of rhabdomyosarcoma pathology

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Rhabdomyosarcoma (RMS) is one of the most common forms of soft tissue sarcomas in children. Two main groups of RMS are distinguished – embryonal (eRMS) and alveolar (aRMS) RMS. aRMS is characterized by a higher metastatic potential and malignancy, due to deregulation of Pax3/7-dependent pathways followed by improper action of downstream elements governing myogenesis. We have shown recently, that heme oxygenase-1 (HO-1), an antioxidant enzyme, inhibits muscle differentiation and is upregulated in RMS. Thus, one can speculate that HO-1 might be here a putative therapeutic target. The aim of our study was to screen for compounds with known therapeutic application that may decrease HO-1 levels in RMS.
We demonstrated that HO-1 expression is significantly higher in the more aggressive aRMS subtype than in eRMS. Importantly, transfection of eRMS with Pax3-FoxO1 fusion gene, characteristic for aRMS, led to upregulation of HO-1. We also found that valproic acid (VA), the anti-epileptic drug, potently decreases HO-1 levels both *in vitro* and *in vivo*. Moreover, it increases expression of miRNA-133a/b and miRNA-206, the microRNAs which are known to govern myogenesis. On the other hand, VA did not influence miRNA-29 family. Interestingly, local delivery of VA decreased progression rate of RMS tumors *in vivo* in mice, indicating that this compound may be considered as a molecule affecting RMS growth, possibly at least in part by influencing HO-1. In search for putative mechanism underlying the HO-1 downregulation by VA, we evaluated HO-1 promoter activity as wells as expression and activity of microRNAs targeting 3'UTR of HO-1. Surprisingly, microRNA activities were decreased, whereas HO-1 promoter activity was increased after VA stimulation. That may indicate that HO-1 downregulation occurs at protein stability and not at gene expression level. Indeed, incubation of RMS cells with the inhibitor of proteasomal degradation reversed the inhibitory effect of VA on HO-1.
In summary, HO-1 can be a protein of interest as a potential target in therapy of RMS and valproic acid seems to downregulate HO-1 in RMS by tackling protein stability.

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P2.20

Soluble receptor of Interleukin 6 as a mediator in renal cancer metastasis

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Molecular basis of communication ccRCC and the target cells of organs of metastasis are fragmentary and remain unknown. Literature data based on studies conducted on other types of cancers implies that in the metastasis of ccRCC the complex of interleukin-6 (IL-6) and its soluble receptor (IL-6sR; complex IL-6/IL-6sR) and the signal transduction pathway (gp130/STAT3) play a key role in this process.
Interleukin-6 (IL-6) is a multifunctional cytokine that regulates pleiotropic roles in immune regulation, inflammation, hematopoiesis, and oncogenesis. Soluble IL-6 receptor is derived from the extracellular part of the membrane-bound receptor by either proteolytic cleavage of their membrane moiety or by alternative splicing.
Our result revealed that under hypoxic (1% O_2) condition, which is a hallmark of tumor, renal cancer cell lines (ACHN, Caki-1, Caki-2) secrete statistically significant more IL-6sR as under normoxic condition (20% O_2). Secretion of IL-6sR by cells derived from non-malignant kidney was below detection limit. Moreover inhibition of complex IL-6/IL-6sR decreases proliferation of RCC cell line. Preliminary data from Western Blot analysis confirm contribution of IL-6sR pathway in RCC metastasis.
In RCC metastasis complex IL-6/IL-6sR seems to be responsible for enhanced proliferation rate of RCC cells. High secretion level of IL-6sR is characteristic for malignant changes in comparison to normal kidney confirms influence of IL-6/IL-6sR in kidney cancer development.

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P2.21

SRSF2 splicing factor contributes to regulation of apoptosis in clear cell renal cell carcinoma (ccRCC)

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Introduction: ccRCC is the most common subtype of renal cancer, characterized by high aggressiveness, fast growth and chemotherapy resistance. There are no successful therapies for treatment of advanced ccRCC. This may be associated with disturbances of apoptosis, leading to resistance of tumor cells to anti-cancer therapy. One of the powerful regulators of apoptosis is splicing factor SRSF2. In our previous study, we found that SRSF2 expression is disturbed in ccRCC. Here, we hypothesized, that disturbed SRSF2 expression may be involved in regulation of apoptosis in ccRCC.
Material and methods: SRSF2 expression in ccRCC-derived cell lines was silenced using siRNA transfections. RT-PCR and real-time PCR were used to analyze splicing patterns and expression of genes involved in apoptosis regulation in ccRCC cells with silenced SRSF2 expression as well as in ccRCC tissue samples. Luminescence assays were used to analyze caspase 8 and 3 activity in ccRCC-derived cells with silenced SRSF2 expression. Changed expression of SRSF2 in ccRCC tissue samples and in cells transfected with siRNA was confirmed using real-time PCR and Western blot. Scrambled siRNA was used in control transfections.
Results: Silencing of SRSF2 expression in ccRCC-derived cell lines resulted in disturbed splicing patterns of initiator caspases 8 and 9, and regulator of initiator phase of apoptosis, cFLIP. Furthermore, the expression of executive caspase 3 was also disturbed. In addition, we observed statistically significant 16% decrease of activity of caspase 8 and 11% increase of activity of caspase 3 in Caki-2 cells, transfected with siRNA against SRSF2 in comparison to Caki-2 cell line, transfected with siRNA scrambled control. Moreover, we found that splicing patterns of caspase 8, caspase 9 and cFLIP were also altered in 33 matched pairs of tumor and control ccRCC samples, in which SRSF2 expression was also disturbed.
Conclusion: SRSF2 affects alternative splicing and expression of genes involved in regulation of apoptosis in ccRCC and also influences activity of caspase 8 and 3, involved in initiator and executive phase of apoptosis, respectively. Altered expression of SRSF2 may possibly contribute to disturbances of apoptosis in ccRCC.

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P2.22

GRHL transcription factors in human non-melanoma skin cancers

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Background: Grainyhead-like genes (*GRHL*) encode a highly conserved family of tissue specific transcription factors. Research on model organisms has shown that GRHL TFs are responsible for skin barrier maintenance (by keeping balance between proliferation and differentiation of keratinocytes) and are implicated in wound healing. It was also shown that in knock-out mouse models GRHL1 and GRHL3 function as skin tumor suppressors. Furthermore, genes regulated by GRHL TFs (like CDH1, DSG1, PTEN, hTERT, PCNA) were previously linked to tumorigenesis. Based on literature and preliminary results we hypothesize that human non-melanoma skin cancers are accompanied by disrupted function of *GRHL* genes or impaired GRHL proteins function.
Materials and methods: Surgical specimens, including tumor and adjacent unaffected skin, were resected from 32 patients with histologically confirmed non-melanoma skin cancers. Nucleic acids were purified using Qiagen Kits. Target enrichment was performed with HaloPlex Kit (Agilent) and *GRHL* genes were sequenced with MiSeq Illumina System, 100-fold coverage depth. TaqMan Real-Time PCR of *GRHLs* was carried out with Applied Biosystems chemistry.
Results: In human non-melanoma skin cancers the expression of *GRHL1* and *GRHL3* genes is downregulated. Moreover, significant correlation between the levels of expression of both genes may indicate a common regulation of their expression.
Targeted deep sequencing was performed for healthy skin and tumor samples to find potential mutations caused by UV light. No *de novo* mutations were found in *GRHL* genes. To determine the relationship between skin cancer occurrence and single nucleotide polymorphisms distribution in the *GRHL* genes, SNP frequencies in the patient group were compared to frequencies in 1000 Genomes Database and outlier SNPs (assigned to coding regions, promoter regions and 3'UTRs) were pointed.
Conclusions: Disruption of *GRHL1* and *GRHL3* gene expression is linked to human non-melanoma skin cancers. Continued efforts are aimed at finding molecular regulators of *GRHL* genes expression and determining the influence of nonsynonymous SNPs in coding regions on phosphorylation of GRHL proteins.

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P2.23

Evaluation of surface marker presence in ovarian cancer cells cultured in stem-prone conditions

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Ovarian cancer being most aggressive gynecological malignancy accounts for 4% of female cancers. More than 3/4 of patients suffer from disease recurrence within 24 months after initial treatment, 80% of whom develop chemoresistance. One of hypotheses aiming at explaining this phenomenon focuses on cancer stem-like cells. Such cells clinically were associated with certain surface proteins (CD133, CD44, CD117 and recently CD105). As such cells are diffucult to be isolated from tumor samples, there are methods developed to obtain such population *in vitro*. The aim of this experiment was to evaluate presence of these markers is ovarian cancer cells cultured in stem-prone conditions *in vitro*. Three cell lines were treated with specially componed media (without FBS, with supplements) for certain time points and chosen conditions were then evaluated for CD133, CD44, CD117 and CD105 with FACS (fluoresence-activated cell sorting). Analysis showed no significant difference in surface markers expression apart from elevation of CD105 in OvBH-1 cell line upon longer treatment period. Investigated conditions don't seem to affect the presence of surface markers mentioned above.

P2.24

Imatinib and vitamin D analogues modify expression of stemness-related genes in human colon cancer cell line HCT-116 during renewal following the preselection with 5-fluorouracil

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It is assumed that cancer stem cells (CSC) are responsible for tumor relapse after conventional cytoreductive chemotherapy. The human colon cell line HCT-116 is derived from a poorly differentiated, highly metastatic colon cancer. We have found previously that HCT-116 cells highly express stemness-related genes and express genes related to epithelial-mesenchymal transition (EMT). We have also found that the expression of genes associated with stemness and invasiveness increases in HCT-116 colon cancer cells preselected with 5-fluorouracil (5-FU). Therefore, HCT-116 cell line can be a used as an *in vitro* model in research aimed on CSC directed therapies combined with the conventional cytoreductive treatment.

Aim of the study was to determine whether imatinib and/or hypocalcemic analogues of vitamin D modulate the expression of genes related to stemness, EMT, drug resistance or angiogenesis in HCT-116 cells that survive after treatment with 5-FU. HCT-116 cells were cultured for 1 day with 5-FU (6 μg/ml) and for 3 days in the absence of 5-FU. After the passage, HCT-116 cells preselected with 5-FU (HCT-116/5-FU cells) were cultured for 4 days without or with imatinib (10 μM) or/and with active metabolite of vitamin D and synthetic analog of vitamin D (1μM). Gene expression was analysed by qRT-PCR.

The expression of stemness-related genes *ALDH1, CD44, CXCR4, LGR5, SOX2* increased in HCT-116 cells during their renewal after the exposure to 5-FU. The expression of these and other stemness-related genes decreased in HCT-116/5-FU cells exposed to imatinib or to the active metabolite of vitamin D. Synthetic analog of vitamin D did not down-regulate the expression of stemness-related genes in HCT-116/5-FU cells. Synthetic analog of vitamin D abolished the regulatory effect of imatinib on the expression of stemness-related genes if HCT-116/5-FU cells were simultaneously exposed to this analog and to imatinib. The relative expression of EMT-related genes *ZEB1*, *SNAI1*, and *MMP9* increased in HCT-116/5-FU cells exposed to imatinib. Vitamin D analogues did not substantially affect EMT-related gene expression in HCT-116/5-FU cells.

Imatinib and the active metabolite of vitamin D could be used to modify the expression of stemness-related genes in colon cancer cells refractory to the conventional cytoreductive chemotherapy.

Acknowledgements

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P2.25

Acetylenic synthetic betulin derivatives induce apoptosis in neuroblastoma and medulloblastoma cells

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Betulin (BE), a lupane-type triterpene has been demonstrated to elicit interesting anti-cancer activity *in vitro*. Acetylenic synthetic betulin derivatives (ASBDs) have been synthesized to enhance pharmacological potential of BE. Only few studies have reported activity of ASBDs towards human cancer cells *in vitro*.
Previously, we have reported that ASBDs decrease the viability and proliferation of human tumour cells in a dose-dependent manner, but no changes in cell cycle progression have been observed.
In this study, two children tumour cells: neuroblastoma (SK-N-AS) and medulloblastoma (TE671) cells were treated for 24 and 48 hours with ASBDs and evaluated for induction of apoptotic cell death by estimation of membrane phosphatidylserine translocation and caspase-3 activity.
ASBDs induce apoptosis in dose- and time-dependent manner in TE671 and SK-N-AS cells.
Further investigations are required to resolve the molecular pathway by which ASBDs trigger apoptosis.

P2.26

Bystander effect induced by UVA radiation in human malignant melanoma cells

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Bystander effect is a phenomenon in which non-irradiated cells change their behavior in response to molecular signals released by irradiated cells. However, non-exposed bystander cells can also release some molecular signals. This intercellular signaling can lead to cell damage, or inversely, to some protection of irradiated and/or neighboring cells.
Bystander effect, well described for ionizing radiation is less explored for UV radiation (UVR). Solar UVR consisting ~95% of UVA (320–400nm) is potentially hazardous for human health. It penetrates the skin tissue, reaching the dermis and acts predominantly through reactive oxygen species that we have shown in previous study for human dermal fibroblasts (NHDF) (Widel *et al.* 2014). Current study was carried out on human malignant melanoma (Me45) and NHDF cells using a transwell co-incubation system. Three experimental setups were used; in the first, irradiated Me45 cells were co-incubated with bystander Me45 cells, in second-irradiated Me45 with NHDF fibroblasts, and in third, irradiated Me45 cells were incubated alone. The Me45 cells were exposed to 20KJ/m^2 UVA radiation. ROS, superoxide radicals and nitric oxide were measured in irradiated and bystander cells by flow cytometry using DCFH-DA, MitoSOX and DAF-FM diacetate respectively. Proliferative activity was evaluated in MTS assay. Results were compared with that of NHDF *vs.* NHDF setup.
Me45 cells exposed to UVA radiation incubated alone and co-incubated with NHDF indicated a slight decrease in viability (~80% of control). However, co-incubation with non-irradiated Me45 cells diminished dramatically viability of irradiated melanoma cells, indicating reciprocal communication between UVA exposed and bystander Me45 cells. The viability of bystander melanomas and fibroblasts declined slightly to ~85%. In contrast, viability of bystander fibroblasts decreased significantly when co-incubated with irradiated fibroblasts. ROS, and particularly superoxide levels, significantly increased at 12h in irradiated Me45 cells incubated alone. The superoxide in irradiated Me45 cells co-incubated with bystander Me45 or NHDF cells, as well as in bystander Me45 cells slightly increased at 24h. Any changes of ROS and superoxide was observed in bystander NHDF co-incubated with irradiated Me45 cells. In contrast, significant increase of ROS and small increase of superoxide was seen in fibroblasts co-incubated with UVA irradiated fibroblasts.
Taking together previous and current results, we conclude that human dermal fibroblasts are more sensible to bystander effect induction by UVA than neoplastic dermal cells such as malignant melanoma. Undoubtedly, reactive oxygen species increasing in irradiated cells are involved in the induction of molecular signals of bystander effect. One of these signals may be interlukin-6 as was noticed in our previous experiments on NHDF.

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P2.27

Elucidation of a new aspect in the radiosensitizing potential of arginine withdrawal

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Arginine deprivation is undergoing clinical trials now. Targets for such a therapy are tumor types unable to synthesize arginine from its precursors, ornithine or citrulline. The main limitation of arginine deficiency as an anticancer approach is the ability of some individual tumors to induce expression of argininosuccinate synthetase, an enzyme involved in citrulline conversion to arginine, thus, avoiding starvation and becoming resistant to the therapy. The possible solution to this problem can be combination of arginine deficiency with other therapeutic approaches. Our recently published data have shown that arginine deprivation alone as well as in combination with a natural arginine analogue, canavanine, enhances radiosensitivity of cancer cells. The aim of the ongoing study is to elucidate the effect of arginine precursor citrulline on the radiosensitizing potential of arginine withdrawal with or without canavanine treatment.

Two human colorectal cancer cell lines (HCT-116, HT29), which are able to rely on citrulline for arginine synthesis, were used as models. Cells were grown as 3-dimensional (3-D) spheroid culture, which had previously been proven to be more resistant to single amino acid limitation in comparison to monolayer. Arginine deprivation was achieved by specially formulated arginine-free medium. To evaluate the radiosensitizing potential, spheroids were subjected to single dose irradiation up to 20 Gy after 5 days of treatment.

Artificially high (400 μM), but not physiological (50 μM) citrulline concentration partly restored spheroid growth upon arginine withdrawal. 100 μM canavanine only slightly inhibited proliferation under arginine-rich conditions. At the same time, 3 days of canavanine treatment in arginine-free medium was enough to induce apoptosis and spheroids' disintegration. 50 μM citrulline neither protected spheroids from falling apart, nor from cell death, which was induced by canavanine treatment upon arginine deficiency. 400 μM citrulline partly preserved the 3-D structure and reduced the fraction of dead cells. Arginine deprivation exhibited the 2-fold increase in spheroid radiosensitivity compared to control arginine-rich medium for both tested cell lines. The influence of citrulline in arginine-free medium on radiosensitization was concentration-dependent: 50 μM citrulline failed to rescue cells from irradiation while 400 μM citrulline maintained survival as efficiently as control medium. Addition of canavanine (alone or in combination with either concentration of citrulline) further enhanced the radiosensitizing potential of arginine withdrawal.

We conclude that combination of arginine deficiency with arginine analogue(s) could be a promising strategy for increasing the curative potential of radiotherapy regardless of the tumors' ability to utilize citrulline.

P2.28

The cytostatic effect of phenylbutyrate treatment on glioblastoma cell line LN-229

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Aim: This study was designed to evaluate the effect of histone deacetylase inhibitor – phenylbutyric acid (PBA), on survival and proliferation of glioblastoma cell line LN-229. Histone deacetylase inhibitors (HDACIs) are known to induce various effects, such as differentiation, growth arrest, and apoptosis in cancer cell lines. Phenylbutyrate is a HDAC inhibitor used clinically for treatment of urea cycle disorders. Because of its low cytotoxicity, cerebrospinal fluid penetration, and relatively high oral bioavailability, we investigated PBA as a potential cytostatic agent in treatment of glioblastomas.

Materials and methods: Two glioblastoma cell lines LN-18 and LN-229 were selected for the study. Phenylbutyrate was used at a concentration of 5 mM and 15 mM for 24 and 48 hours. MTT test was done to assess the cells viability. Flow cytometry was used to analyze the cell cycle distribution. Real time PCR was used to check the expression level of p21 and p53 genes. Light microscopy was applied to visualize morphological changes in PBA-treated cells.

Results: PBA treatment resulted in cytostatic effect in LN-229 cell line, but PBA-induced cytotoxicity was not observed in LN-18 cells. The viability of LN-229 cells decreased significantly at both, 5 mM and 15 mM concentration, independently of the incubation time (about 30% and 70%, respectively). There was no evidence of G1 arrest, however cells treated with 5 mM concentration of PBA showed increase in S phase population, and 15 mM PBA treatment resulted in G2/M cell cycle arrest. mRNA expression level of p53 was increased significantly in cells supplemented with 15 mM PBA concentration regardless of incubation time, and p21 level was significantly higher only in 15 mM PBA after 48 hours. Microscopic analysis of cells morphology showed lower cell density and changed more flattened phenotype of PBA-treated glioblastoma cells.

Conclusions: Phenylbutyrate causes the cytostatic effect in LN-229 glioblastoma cell line mostly by inhibition of cell proliferation in dose-dependent, but not time-dependent manner. PBA in high dosages are able to produce p53-dependent G2/M cell cycle arrest, while lower PBA concentrations can result in an arrest in G1/S checkpoint.

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P2.29

PARP-1 expression is increased in colon cancer and correlates with OGG1

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The ethiology of colon cancer is largely dependent on inflammation driven oxidative stress and 8-oxo-7,8-dihydroguanine (8-oxoGua), one of the oxidatively modified DNA bases, is a typical biomarker of the damage. Moreover, many observations indicate a direct correlation between 8-oxoGua formation and carcinogenesis *in vivo*. Our analysis of 8-oxodGuo level in leukocyte DNA of healthy controls (138 individuals), patients with benign adenomas (AD, 137 individuals) and with malignant carcinomas (CRC, 169 individuals) revealed a significant increase of the level of 8-oxodGuo in leukocyte DNA of AD and CRC patients in comparison to controls. The counteracting mechanism is base excision repair, in which OGG1 and PARP-1 play a key role. We investigated the level of PARP-1 and OGG1 mRNA and protein in diseased and normal tissues taken from AD and CRC patients and in leukocytes taken from the patients as well as from healthy subjects. In colon tumors PARP-1 mRNA level was higher than in unaffected colon tissue and in polyp tissues. A high positive correlation was found between mRNA level of PARP-1 and OGG1 in leukocytes of healthy volunteers (r=0.7829), AD patients (r=0.8139), and CRC patients (r= 0.6772). This suggests reciprocal influence of PARP-1 and OGG1 on their expression and/or stability, and may contribute to progression of colon cancer. PARP-1 and OGG1 proteins level was several fold higher in polyps and CRC in comparison to normal colon tissues. This could suggest that higher expression of PARP-1could predispose for further development of cancer. Individuals bearing *Cys326Cy*s genotype were characterized by higher PARP-1 protein level in diseased tissues than *Ser326Cys* and *Ser326Se*r genotypes. OGG1 326 Cys variant is a protein with decreased enzymatic activity, however revealed only upon oxidative stress. Aforementioned result may suggest that the diseased cells with polymorphic OGG1 recruit more PARP protein, which is necessary to remove 8-oxodGuo. Thus, our findings may have some clinical implications since patients with decreased activity of OGG1/polymorphism of OGG1 gene and higher 8-oxodGuo level may be more susceptible for PARP-1 inhibitors treatment.

P2.30

The effects of selected growth factors on actin cytoskeleton architecture of different melanoma cells lines

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Increasing number of data indicates crucial role of tumor microenvironment in tumor progression and metastasis (Hanahan & Weinberg, 2011). In the organism cancer cells receive signals from extracellular matrix components such as elements of the basement membrane and paracrine signals from the cells surrounding tumor tissue. Among molecules involved in signal transduction between the cells there are growth factors, interleukins and cytokines. On paracrine way cancer cells communicate with each other and with the cells surrounding them such as endothelial cells, immune cells and fibroblasts. Growth factors are responsible for control of cellular growth, proliferation rate, cellular differentiation and migration/invasion abilities. We decided to choose for our research as cellular model melanoma cell lines. This neoplasms development and metastasis are strongly dependent on interactions with keratinocytes and fibroblasts. We focused on the influence of transforming growth factor beta (TGFβ), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) on cells invasion abilities. Additionally, we checked impact of selected growth factors on actin cytoskeleton architecture, since actin cytoskeleton, being downstream of several signaling pathways triggered by growth factors involved in tumor progression and metastasis, plays an important role in invasiveness of cancer cells.

In our study we used human melanoma cell lines differing in origin. Two cell lines were derived from primary tumor (A375 and WM35) and another two were derived from metastases to lymph nodes (WM9 and Hs294T). Invasion ability of these cells in response to selected growth factors stimulations were checked using Transwell™ filter coated with Matrigel™. We have obtained diverse cells reactions to different growth factors stimulations. The amount of invading cells into Matrigel™ depended on the stage of tumor progression and used growth factor. We analyzed by immunocytochemical stainings actin cytoskeleton architecture and localization of some actin bindings proteins such as gelsolin and profilin in the cells, which were incubated with selected growth factors. We estimated as well actin polymerization state in these cells by measuring filamentous actin to monomer actin ratio (F:G ratio).

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P2.31

Interdependence of SOX9/SLUG/HER2/CD151 in breast cancer — implication for tumor prognostication

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Breast cancer (BCa) exhibits a relatively high degree of morphologically and molecularly diverse lesions. Human mammary ductal carcinoma develops through gradual transition from hyperplasia, through pre-invasive carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC) finally reaching a highly metastatic state. Despite rapid development of specific assays, heterogeneity of DCIS is poorly understood. A number of clinically suitable biomarkers have been proposed but so far, there are no prognostic indicators which would reliably identify the initial steps of DCIS progression into invasive disease.

Accumulating evidence supports the thesis that SLUG, a transcription factor also known as SNAIL2, is involved in promoting invasiveness of human cancer. Overexpression of SLUG in basal and mesenchymal subtypes correlates with poor prognosis and shorter overall survival of patients with aggressive BCa. Moreover, SLUG with another transcription factors - SOX9 plays a central role in activation of mammary stem cell. Importantly, HER2 (Human Epidermal Growth Factor Receptor 2) is found in more cases of *DCIS* (50–60%) as compared to HER2-positive invasive tumours (20–25%). Recent studies have shown that tetraspanin CD151 correlates with expression of HER2 and regulates its function in BCa cells.

The aim of the study was to investigate interdependence of CD151/SOX9/SLUG/HER2 in DCIS progression into IDC in mammary epithelial cell line MCF10A. Cell growth and induction of invasive phenotype of generated mutant cell lines vs wild type were assessed in 3D cultures. We demonstrate that overexpression of HER2 and SLUG induces morphological alteration in MCF10A. Moreover, a high level of SLUG modulates 3D collagen invasiveness. In addition, we found that SLUG upregulates HER2, while HER2 and depletion of CD151 downregulates SOX9. Therefore we conclude that interdependence of CD151/SOX9/SLUG/HER2 is involved in a mechanism underlying the mammary epithelium progression towards invasive phenotype.

P2.32

The expression of neutral endopeptidase (CD10) and its involvement in proliferation of colon cancer cells

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Background: It is proved that neutral endopeptidase (CD10) can be differentially expressed by tumor cells and is involved among others in proliferation of some types of cancers. It can act through proteolysis of bioactive peptides, growth factors and cytokines to create tumor promoting micrenvironment [1, 2]. This function of CD10 was not explored in colorectal carcinoma.

The aims of the study were to ascertain whether cells of colon cancer cells lines originated from different grades of tumor development express CD10 and if it is involved in their proliferation.

Material and methods: HT-29, LS180, SW948 and SW620 colon cancer cell lines were chosen and tested for CD10 expression by immunofluorescence staining and flow cytometry. The engagment of this peptidase in tumor cell proliferation was explored by BrdU incorporation into DNA of actively proliferating cells and detection of nucleotide by ELISA method (*in vitro* proliferation assay). Thiorphan, inhibitor of neutral endopeptidase was used to examine its role as endopeptidase in cell proliferation. Non-toxic concentrations of thiorphan were used in *in vitro* proliferation assay. Thiorphan cytotoxic activity was determined by LDH acitivity assay and Neutral Red uptake assay (*in vitro* cytotoxicity assay).

Results and conclusions: Immunofluorescence staining revealed the expression of neutral endopeptidase in all tested colon cancer cell lines. Moreover, flow cytometry indicated differential profile of expression. HT-29 and SW948 cell lines presented the lowest level of CD10 expression. In these cases 0.7% and 1.1% of cells expressed CD10, respectively. Whereas, 95.36% of LS180 cells and 97.8% of SW620 cells expressed the neutral endopeptidase. The *in vitro* proliferation assay indicated that neutral endopeptidase is differentially involved in proliferation of tested cell lines. In case of cell lines originated from I (HT-29) and II (LS180) stages of cancer development, inhibition of neutral endopeptidase activity with thiorphan resulted in increased cells proliferation. Whereas, proliferation of colon cancer cells derivated from III (SW948) and metastatic (SW620) stages of development was inhibited. These results may indicate that the role of neutral endopeptidase in colon cancer cells proliferation depend on stage of tumor development, rather than the expression level.

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P2.33

Circumvention of drug resistance in tumor cells by landomycin antibiotics: evaluation of reactive oxygen species' role

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Rapid development of drug resistance in tumor cells caused by over-expression of the ABC-transporter proteins is one of the main shortcomings in cancer chemotherapy. Angucycline antibiotics, the landomycin E (LE) were shown to be capable of circumventing such resistance (Korynevska *et al.*, 2007, Panchuk *et al.*, 2012), however, the molecular mechanisms of such action of that and other known Ls remain poorly understood. Here we addressed the structure-functional relationships in the anticancer action of 7 Ls: LA, 11-deoxy LA, LB, 11-deoxy LB, LE, LD, 11-deoxy LD). A panel of 10 carcinoma and leukemia cell lines differing in their drug resistance were tested. It was found that anticancer activity of Ls tightly depended on the presence or absence of specific chemical groups in their molecule which affected the production of hydrogen peroxide in mitochondria of treated cells. At the same time, we did not reveal Ls' impact on the production of superoxide radicals, on the contrary to such action of well-known anticancer drug doxorubicin. Elimination of every next sugar residue in Ls' oligosaccharide chain decreased ROS production under the action of these antibiotics by 30%, and proportionally decreased their anticancer activity. Similar dependence was observed when OH-group at C11 atom of the aglycone structure of the Ls was removed. The results of MTT assay demonstrated that sensitivity to landomycins in the parental tumor cell lines and their While drug resistant sub-lines of tumor cells with over-expressing P-glycoprotein or bcrp did not differ significantly in their sensitivity to Ls, and cell lines over-expressing the MRP-1 demonstrated only 2-fold elevation in their resistance to Ls, resistance of such cells to doxorubicin (positive control) increased 80-100 fold, thus, indicating high efficiency of Ls in killing drug-resistant tumor cells. ROS production under Ls' action was also dependent on Ls' ability to overcome drug resistance in tumor cells. It was derected that in HL-60/adr (MRP-1+) cells which were found to be 2-fold resistant to Ls, ROS level increased 13-fold under the action of these antibiotics, and slowly decreased up to 24 h after starting drug treatment. In contrary, the parental wild-type cells of HL-60 line have demonstrated 5-fold increase in ROS level which was sharply decreased as soon as in 3 h after drug addition to cultured tumor cells. HL-60/vinc cells (P-gp+) demonstrated only weak (30%) increase in their resistance to Ls compared to parental wild-type HL-60 cell line, and it was not accompanied by rapid changes in ROS production taking place for 6 h. For studying the molecular mechanisms of anticancer action of Ls towards drug-resistant tumor cells, Western-blot analysis on a panel of 30 proteins participating in cell cycle regulation and apoptosis was carried out. It was found that early activation of the effector caspase-7 functioning upstream of the initiator caspases-8 and -9 was the most prominent event, which significantly differs Ls from the other known anticancer drugs. Such activation also depended on number of sugar residues in saccharide chain of the Ls, and LA (6 sugars) caused much earlier induction of caspase-7 (1 h) and DNA fragmentation (6 h), compared to such effects of LE (3 sugars) – 3 h and 12 h, correspondingly. The results of *in vivo* studies of LA action demonstrated its therapeutic effectiveness towards murine NK/Ly lymphoma, leading to a complete tumor remission.

In conclusion, early generation of hydrogen peroxide and subsequent activation of caspase-7 were shown to be crucial events in apoptosis induction by the Ls, and high potential of antibiotics in circumventing drug resistance in tumor cells is evident.

P2.34

The influence of the microenvironment of a cell culture derived from ovarian cancer on HEK 293 cells

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Introduction: Ovarian cancer is one of the most frequently occurring gynecological cancer. The study evaluated the effect of the microenvironment of a cell culture derived from ovarian cancer on HEK 293 cells, which were non-gynecological control. During the experiment, the analysis of *NANOG* gene expression was conducted. *NANOG* gene is one of the pluripotency markers.
Aim of study: The aim of the study was to evaluate the influence of the microenvironment *in vitro* cell culture (acellular media after *in vitro* cell culture derived from patient suspected of ovarian cancer) on HEK 293 cells. *NANOG* gene expression was analyzed at the mRNA and protein level.
Material and methods: Material used for the study was ascites derived from patient diagnosed with ovarian cancer (W67), on which the cell line was based. The culture medium of these cells was used for further experiments. The non-gynecological control were HEK 293 cells. For subsequent analysis RT-PCR was used along with Western blot.
Results: The microenvironment of the filtered culture media after *in vitro* cell culture originating from patient suspected of ovarian cancer have an effect on HEK 293 cells. It has been demonstrated increased *NANOG* gene expression at the mRNA and protein level relative to the control.
Conclusion: The cells indicated a more undifferentiated characteristics in comparison to cells untreated with filtrates. This is evidenced by increased expression of *NANOG* gene at the mRNA and protein level.

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P2.35

Effects of structurally different DSs on breast cancer cell behavior

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Breast cancer is connected with high mortality due to ineffective treatment. On the other hand, many studies have recently revealed that glycosaminoglycans (GAGs), linear heteropolysaccharides, can affect cell functions. Dermatan sulfate (DS) is a member of the GAG family which has high binding affinity to such functional proteins as some growth factors and/or their receptors, cytokines and adhesion molecules. Thus, DSs can be potentially used as modulators of various events in cancer cell biology, such as proliferation, metastasis, differentiation and angiogenesis. However, the DSs usage is not available up to date, due to high structural heterogeneity of DS chains as well as a poorly-known relation between DS structure and its biological effect. The identification of DS motifs displaying anti-cancer effect(s) may be important for cancer therapy
The aim of our study was to compare the influence of structurally distinct DSs on the viability and the proliferation of T47D and MCF-7 breast cancer cells.
DS chains isolated from human and pig proteoglycans were characterized with respect to their sulfation and glucuronosyl epimerization patterns by reverse phase HPLC and gradient PAGE, respectively. The T47D and MCF-7 cells were exposed to examined DS chains (1 and 10 μg/ml) for 48 and 24 h, respectively. The viability and the proliferation of cancer cells were estimated by WST-1 test and BrdU incorporation, respectively.
All examined DSs displayed distinct structural features which were especially manifested in: (1) contents of 2,4-O- and 4,6-O-disulfated disaccharides as well as 6-O-sulfated disaccharides in iduronate (IdoA) and glucuronate blocks, respectively; (2) size of IdoA blocks. DSs showed dose- and type-dependent effects on both the cancer cell viability and proliferation. When DSs were used at a high concentration almost all of them exerted inhibitory impact on the cells, though significant differences between some GAGs were apparent in respect to their effectiveness. In turn, a low concentration of DSs led to different cell responses from stimulatory to inhibitory one depending on the GAG structure.
Our results clearly indicate that breast cancer cell may be sensitive to DS structure and the GAG content in the cancer microenvironment.

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P2.36

Cell monolayer positioning with respect to beam field of photon radiation affects cell viability and causes apoptosis

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Purpose: RT treatment sessions are planned based on the assumption that the effects of radiation on cells are target-oriented and occur only in cells lying in the beam field. Recently it has been shown that there are other non-targeted mechanisms which may affect cells that are not directly exposed to radiation. These non-targeted effects, such as genomic instability, bystander effect, hyperradiosensitivity and adaptive response are more complex than those assumed by the LNT model and can be important in case of cells lying outside the radiation field and which were exposed to low dose of radiation.
In the present study we tested cytotoxic and genotoxic effects of photon radiation upon cells placed inside a water phantom in the beam axis or outside the radiation field during exposure. We also carried out cell cycle analysis of such cells.
Materials/Methods: Measurements were performed on 5.4 cm depth in a water phantom. As the irradiation source, a linear accelerator was used (Clinac 2300 CD) and 6 MV photon radiation was applied at 100 MU/min accelerator mode, field size 20x20 cm. On this depth, the dose of 5 Gy is in the beam axis, and ca. 0.2 Gy is outside of irradiated field.
Human lung cancer cells A549 were exposed to radiation, both in the beam field or outside of it.
The cytotoxic effect of irradiation was evaluated as cell viability (MTS assay); genotoxic effects was determined as induction of apoptosis measured by microscopic observation and annexin V-FITC flow-cytometry analysis; cell cycle was also analyzed by flow-cytometry.
Results: Our measurements revealed that cells placed within the beam field show decreased viability and increased frequency of apoptosis and micronuclei as compared to non-irradiated control cells.
Surprisingly, compared to non-irradiated cells, the cells placed during exposure outside the beam field show decreased viability and higher level of apoptosis. Cells irradiated in the beam field showed inhibition of cell cycle in G2 phase, whereas in outlying cells no cell cycle-related changes were observed in comparison with control.
Conclusions: This finding suggests that healthy cells lying outside the beam field may be killed as a result of irradiation during RT. This relationship observed for therapeutic radiation ought be taken into consideration in both treatment planning and in clinical practice.

P2.37

Influence of zapotin on mechanism of autophagy in cancer cells over-expressed constitutively active PKCε

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Introduction: Zapotin (5,6,2',6'-tetramethoxyflavone), a member of the flavonoids, was first identified in the tropical fruit zapote blanco *(Casimiroa edulis),* later isolated from *Sargentia greggii* and extracted from leaves of *Primula veris*. Zapotin is neuroprotective, cardioprotective and chemopreventive agent acting as modulator of protein kinases and lipid-dependent signaling pathways. Anticancer activity of the zapotin was demonstrated in many cancer cell lines. Protein kinase C epsilon (PKCε), a novel PKC isotype is characterized as a calcium-independent and phorbol ester/diacyloglycerol sensitive serine-threonine kinase. PKCε is involved in many signaling systems including adhesion, migration, proliferation, gene expression, differentiation and apoptosis. Only PKCε, among all PKC isozymes, has been reported to exhibit full oncogenic potential. This protein seems to participate in tumor development, tumor invasion and metastasis. Autophagy is a type of cellular catabolic degradation response to nutrient starvation or metabolic stress. Recent studies suggest that autophagy may be important in the regulation of cancer development and progression and in determining the response of tumor cells to anticancer therapy.
Aim: The aim of this study was to investigate the mechanism of autophagy induction in cancer cells over-expressed constitutively active PKCe after treatment with zapotin.
Material and method: HeLa PKCεA/E cells were treated by zapotin in different concentrations, corresponding to 0.5-2.0 IC_{50} values. The technique of electrophoresis, Western Blot, immunoidentification with appropriate antibodies were used to shown PI3K, Beclin-1, Atg5, LC3 and mTOR protein level. Moreover, we conducted the monitoring of early autophagic structures using fluorescence microscopy after treatment with zapotin. Localization of autophagosomes has been studied immunohistochemically using anti- LC3 antibody. Moreover we estimated the influence of autophagy modulator (rapamycin) on the level of pro-autophagic proteins.
Results: Western blot analysis showed the lower level of Beclin-1, ATG-5, PI3K and LC3 proteins in HeLa PKCεA/E cells compared to cells without doxycycline-induced PKCεA/E expression (control). In control cells and over-expressed PKCεA/E cells treated with rapamycin the level of those proteins increased. However, rapamycin in control and over-expressed PKCεA/E cells decreased the level of mTOR, respectively. Moreover, Western blot analysis indicated zapotin autophagy inhibition in a concentration-dependent manner. These results were confirmed by the lack of accumulation of autophagosomes.
Conclusions: Our results conclusively demonstrate the anti-autophagic potential of the zapotin.

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P2.38

Plasticity of replication origin activation induced by topoisomerase I inhibitor topotecan

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Key words: DNA replication, replication stress, topoisomerase inhibitors.

Background: Under conditions of replication stress a rate of DNA synthesis and the rates of fork movement are significantly reduced [1]. Stalling of replication forks also activates checkpoints which lead to inhibition of activation of new origins. However, drug-induced inhibition of DNA replication in eukaryotic cancer cells also induces activation of the dormant origins of replication. Three mechanisms of these processes were proposed: (i) different levels of replication stress lead to different signaling outputs - a short term stress is ignored by the cell, while a long term stress induces origin plasticity, (ii) two different independent checkpoints are responsible for the opposite effects – when the active replication regions are arrested, the first checkpoint blocks activation of new origins, while the second one compensates – it activates dormant origins, and (iii) activation of origins is local (in individual factories) but inhibition is global [2].

Goal. The aim of this work was to verify the three hypotheses pertaining to plasticity of replication origins under conditions of replication stress.

Methods: A549 pulmonary adenocarcinoma or HeLa cells were exposed to a topoisomerase I inhibitor, topotecan (Tpt), in order to induce replication stress. Replication sites were visualized by detecting the incorporated DNA precursor analogues (BrdU/EdU) or GFP-PCNA. Epigenetic histone marks (H4K20me1, H4K20me2, H3K4me2, H4K12ac, H3K14ac, H3K9ac) were detected using the unique FabLEM technology [3]. The levels of γH2A.X, ORC, ORCBP1 were detected using FLAG-tag system or immunocytochemistry staining. Confocal and superresolution Single-Molecule Localization Microscopy with extensive quantitative image analysis (based on algorithms written specifically for this purpose) were used.

Results: After short-term incubation (less than 2h) to Tpt a subpopulation of cells continued to replicate. After a long-term incubation (4h) the number and volume of replication foci decreased and the volume of nuclei increased. The replication was delayed and new replication foci were activated (i) in the same region, and (ii) in new regions, located afar of the stalled replication foci. Super-resolution microscopy revealed the numbers of replication forks active in individual factories. Live cell imaging revealed that replication is inhibited globally in the first minutes after incubation with Tpt. After few hours new replication foci become detectable. Tracking of changes of histone modifications enable correlation of replication with variety of processes, for example with timing of origin firing, formation of newly replicated chromatin, defining ORI and ORC formation.

Conclusions: All three mechanisms of activation of new ORI appear to operate, depending on the duration of replication stress induced by topotecan. A broad range of methods enables characterization of the phenomenon of replication origin plasticity.

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P2.39

Five-membered nitroxyl derivatives Pirolin and Pirolin can modify paclitaxel activity in human breast cancer cells

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Pirolin (2,2,5,5-tetramethyl-3-carbamoyl pyrroline-1-oxyl, PL) and Pirolid (2,2,5,5-tetramethyl-3-carbamoylpyrrolidine-1-oxyl, PD) are cell-permeable, five-membered stable nitroxide radicals. Though not toxic, they can affect the toxicity of chemotherapeutic agents. In particular, they can reduce the side effects of treatment while maintaining its effectiveness. Chronic cardiotoxicity, likely related to iron oxidation and oxygen free radicals is a long-term side effect of anthracycline chemotherapy, which often leads to cardiomyophathy associated with congestive heart failure. Paclitaxel (PTX) is often used in combination with doxorubicin in treatment of numerous solid tumors. The exact mechanism of PTX cytotoxicity against tumor cells is still under study. It is commonly accepted that the drug interacts with microtubules and induces apoptosis in various tumor cells. Used in combination with DOX enhances, however, DOX prooxidative action.

This study was aimed at investigating the effect of Pirolin and Pirolid on the anticancer activity of PTX against human breast adenocarcinoma cells with an emphasis on DNA damage and microtubule disruption.

MCF-7 breast adenocarcinoma cells were incubated for 2 h with IC50 concentration of PTX (0.4 μmol/l) and for 3 h with 50 μmol/l concentration of nitroxides. When combination of both compounds (a drug and a nitroxide) has been used the cells were pretreated with Pirolin or Pirolid for 1 h before the addition of PTX. DNA damage was analyzed using single cell electrophoresis (comet assay) immediately after the treatment and at the different time points of post-treatment incubation in drug-free medium (24-96 h). Inhibition of microtubule depolymerization was investigated by immunohistochemistry with monoclonal antibody against β-tubulin.

At the end of the incubation of cells with PTX a small amount of damaged DNA in the comet tail has been observed. Percentage of the damaged DNA considerably increased (11-12-fold) during the first 24 h of the post-treatment. After the following 24 h (48 h time period) DNA content in the comet tail drastically decreased, suggesting repair of most of the DNA damage. Then an increase of the tail DNA was observed again. Pretreatment with Pirolin attenuated genotoxic effect of PTX and caused a significant decrease in the percentage of damaged DNA in the comet tail at any of the investigated time period. Pirolid in contrast to Pirolin displayed greater effect on PTX genotoxicity and enhanced DNA damage caused by PTX. An increase in percentage of tail DNA was also observed in cells treated with a nitroxide alone. No significant effect of nitroxide on microtubule depolymerization was observed either when they were used alone or in combination with PTX. In conclusion, Pirolin and Pirolin seem to be promising for chemotherapy, exhibiting cytotoxic properties by themselves but enhancing the antitumor effect of PTX.

P2.40

Development and optimization of a novel assay for studying protein kinase CK2 enzyme interactions using quartz crystal microbalance with dissipation monitoring (QCM-D)

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Protein kinase CK2 is a ubiquitous, highly pleiotropic and constitutively active kinase that phosphorylates mainly serine/threonine residues. It has been observed, that an elevated CK2 activity underlies global human diseases, with special reference to cancer and tumor pathologies. Six major hallmarks for cancer have been reported and protein kinases, especially CK2, can contribute to each of these steps. Therefore, screening for selective CK2 inhibitors is of great importance. The standard method for study of CK2 and most protein kinases activity is based on a radiometric filtration binding system, involving the use of radioactive ATP as a phosphate donor. Radioactivity methods require special handling and involve high cost of waste disposal. Therefore, availability of non-radiometric technique is highly desirable.

The aim of this study is to develop and optimize a novel assay for investigating enzyme inhibition and protein-protein interactions using CK2 as a model substrate. This technique might serve as a powerful tool for fast and sensitive evaluation of potential anticancer agents. Our research includes a full analysis of the mechanism and kinetics of kinase-catalyzed phosphorylation and enzyme inhibition by known and novel CK2 inhibitors.

In our studies we use a quartz crystal microbalance with dissipation monitoring (QCM-D), a unique tool which can be applied for studying biomolecular interactions. Its main advantage is ultra-sensitivity and ability to simultanously measure frequency and dissipation changes in real time. In other techniques, applied for studying enzyme assays, only the result of the reaction is investigated. Here, it is possible to monitor changes in mass and viscoelastic properties of the adsorbed layer throughout the whole process. This gives novel insights into the structural and kinetic properties of the reactions investigated.

Our studies of CK2 activity have shown that QCM-D is a suitable device for this assay, as it allowed us to determine all enzyme-reaction kinetic parameters. Moreover, we have investigated the influence of inhibitors on CK2 activity and measured the inhibitor-CK2 interactions kinetic parameters. For this assay we have used two types of quartz crystal sensors: i) Au surface and ii) modified with Cu^{2+} chelated ions for HisTag label capturing. Finally, the influence of CK2 inhibitors on colonal cancer cells HCT 116 was analyzed using a QCM-D device equipped in a window module enabling simultaneous optical access to the sensor surface.

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P2.41

Antiangiogenic and immunostimulatory agents (digoxin and DMXAA) together inhibit better the growth of B16-F10 murine melanoma and GL261 murine glioma tumors

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During tumorigenesis cells acquire specific phenotype properties which include the ability of unrestricted formation of tumor blood vasculature. Parts of tumor with insufficient blood supply develop underoxygenated domains (hypoxic regions). HIF-1 transcription factor is the basic hypoxia regulator which controls expression of numerous proteins participating in adaptive cancer mechanisms. Inhibition of blood vasculature formation *via* digoxin-mediated HIF-1 inhibition appears as an effective anticancer strategy. Moreover, combination of antiangiogenic concept and immunotherapy seems to offer a very promising anticancer approach. Such a combination results in a switch from angiogenic/immunosuppressive phenotype (tumor-promoting) to antiangiogenic and immunostimulatory (which is tumor-inhibiting).
The aim of this study was to examine digoxin and DMXAA, two agents affecting tumor blood vasculature and showing immunostimulatory properties.
It was demonstratedthat digoxin does not affect proliferation of cancer cells with the exception of A-172 human glioma cells. On the other hand, its administration inhibits cell migration. An inhibitory effect of digoxin upon HIF-1α expression in cancer cells was observed. Therapy of mice burdened with experimental B16-F10 murine melanoma tumors or GL261 mouse gliomas resulted in considerable inhibition of tumor growth following DMXAA administration; additional application of digoxin enhanced the therapeutic effect. Following administration of both DMXAA and digoxin we were able to trigger both tumor vasculature destruction, as well as bring about extensive tumor infiltration by immune system cells.
Combination of these two drugs polarizes tumor microenvironment phenotype from angiogenic/immunosuppressive (tumor-promoting) to antiangiogenic and immunostimulatory (which is tumor-inhibiting).

P2.42

The impact of insulin and insulin-like growth factors on renal cell carcinoma

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Introduction: Renal cell carcinoma (RCC) is the 10th malignancy worldwide and the most frequent type of kidney cancer in adults. Insulin and insulin-like growth factors (IGFs) were shown to play a significant role in cancer development and progression, including RCC. IGFs are mainly identified as a proliferation regulators and insulin as a metabolism controlling hormone. The interplay between this molecules and their receptors might be crucial for RCC cell biology and progression.
Aim of the study: The present study was to evaluate the role of IGFs and insulin as well as their receptors in renal cell carcinoma cell lines.
Materials and methods: Cell lines, 786-0, 769-P, RCC6, CAKI-I, CAKI-II, SW156, ACHN, Hs891.T, PCS-400-010 and HEK293 were seeded in 96 wells plates/T75 flasks and cultured under standard conditions (37°C, 5% CO2). After 24 h 1% serum medium was added with different concentrations of insulin, IGF-1, IGF-2 (0, 1, 10, 100 ng/ml). Cells were tested for viability (AlamarBlue), insulin receptor (IR) and IGF-1 receptor (IGF-1R) expression (FACS, ELISA).
Results: The results showed that IGF-1 and IGF-2 have higher potential to promote cell proliferation than insulin. All RCC cell lines were shown to have IGF-1R expression and no IR expression on cell surface. Only control cell line - Human Embryonic Kidney 293 had detectable IR expression.
Conclusion: Both IGFs and insulin can affect RCC cells growth. These cell lines express IGF-1R and are responsive to IGFs stimulation. RCC cells do not express IR thus insulin in high concentrations may influence their growth through IGF-1R receptor stimulation. Both IGF-1 and IGF-2 together with IGF-1R can constitute an autocrine-paracrine loop of tumor cell stimulation, what may be one of the mechanism promoting renal cancer growth and progression.

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P2.43

Resveratrol derivative, 3,3',4,4'-tetrahydroxy-*trans*-stilbene, efficiently inhibits ovarian cancer cell progression via exacerbation of cellular oxidative stress

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Resveratrol (Res) is known to restrict the progression of various malignancies both in vitro and in vivo. Its low level of bioabsorption coupled with fast breakdown to less active metabolites jeopardize its routine usage in clinical practice. In order to avoid the flaws of this compound, keeping at the same time all its advantageous activities, a chemical structure of Res has been used as a template for synthesis of its derivatives, whose effects, at least in theory, should be more pronounced compared with Res itself.

In this paper we examined the effect of a new Res analogue, 3,3',4,4'-tetrahydroxy-*trans*-stilbene (3,3',4,4'-THS), on progression of ovarian cancer cells (A2780, OVCAR-3, SKOV-3) *in vitro*. The results showed that 3,3',4,4'-THS exerts stronger cytotoxicity than Res in all cancer cell lines studied. Moreover it induced more effectively apoptotic cell death, as evidenced according to enlarged fraction of cells with condensed nuclei and oligonucleosomally fragmented DNA. This effect was coupled with increased activity of caspase-3 and -9. Besides, 3,3',4,4'-THS significantly reduced cancer cell proliferation (decreased the percentage of cells in S phase of cells cycle) and accelerated their senescence (increased the activity of senescence-associated β-galactosidase). These effects were accompanied by markedly exacerbated generation of reactive oxygen species, decreased activity of antioxidants (superoxide dismutase and catalase), augmented accumulation of DNA damage (8-hydroxy-2'-deoxyguanosine and apurinic/apyrimidinic sites), and ineffective DNA repair, including down-regulated expression of DNA glycosylase I.The redox imbalance and the magnitude of DNA injury in cells subjected to Res were less pronounced.

Altogether, these observations indicate that 3,3',4,4'-THS may constitute a promising tool a fight against ovarian malignancy, and that the activity of this compound may be underlined by an augmentation of oxidative stress.

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P2.44

Significance of *ESR1* PvuII, *CYP2C19*2* and *UGT2B15*2* polymorphisms in the context of tamoxifen treatment in breast cancer

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Introduction: Breast cancer is estrogen receptor positive disease in around 60-70% of cases. Tamoxifen classified as a selective estrogen receptor modulator (SREM), is a prodrug which targets the estrogen receptor. Responsiveness to tamoxifen may be influenced by polymorphisms of certain genes which products are engaged in its metabolism, such as *CYP2C19, UGT2B15* or polymorphism is estrogen receptor itself (*ESR1*). *ESR1* encodes form α of ER, the target for tamoxifen. CYP2C19 is a monooxygenase which participates in bioactivation of tamoxifen. UGT2B15 is engaged in elimination of active tamoxifen metabolites [1,2]. The aim of the study was to compare the frequency and prognostic significance of selected polymorphisms: *ESR1* PvuII, *CYP2C19*2, UGT2B15*2* in the tamoxifen-treated and -untreated subgroups of breast cancer patients.

Materials and methods: The studied group consisted of 244 women diagnosed with stage I-III breast cancer, 113 of which were tamoxifen-treated, while 131 untreated. Polymorphisms of *ESR1* PvuII, *CYP2C19*2* genes were evaluated by PCR- restriction fragment length polymorphism, whereas *UGT2B15* by high resolution melting (HRM) in leukocytes genomic DNA. Correlations between polymorphisms and clinico-pathological factors and survival parameters were investigated.

Results and conclusions: No influence of genotypes on clinico-pathological factors was observed in the group of tamoxifen-untreated patients. However the correlation between *ESR1* copy number and *ESR1* PvuII polimorphism was observed in both group of patients. Previous research showed that the presence of at least one *UGT2B15* wt allele combined with the wt/wt *ESR1* PvuII genotype in tamoxifen treated patients was associated with decreased DFS and OS (P=0,03 and P=0,08, respectively) [3]. Altered metabolism of administered tamoxifen, which diminished therapeutic effect seems to be involved as no such correlation was observed in tamoxifen untreated group.

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P2.45

Microvesicles secreted from the colorectal cancer cell line HT29 can transfer into cells constituting metastatic niche

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Malignant tumors are complex structures that consist of cancer cells and the surrounding tumor stroma. A continuous crosstalk between cancer cells and local/systemic environment is required for effective tumor growth and dissemination. Studies show that microvesicles derived from activated tumor cells may participate in cell-to-cell communication and mediate the formation of metastatic niches. Moreover, epithelial to mesenchymal transition (EMT) is crucial in carcinoma metastasis. A key transcription factor involved in EMT is Snail that enhances proliferation, cell adhesion and migration of colorectal cancer cells (CRC).
Important question in above process is the role of CRC microvesicles that may transfer into metastasis niches. Further we would like to clarify the role of Snail in EMT of CRC. In this study we performed the analysis of microvesicles derived from CRC line HT29 and their incorporation into endothelial cells (HUVEC) and monocytes/macrophages cell line (THP-1). We expressed Snail in HT29 line by stable transfection using pcDNA 3.1 vector (HT29/Snail). In this report we show that HT29/Snail produce microvesicles, which can be transferred to the cells constituting metastatic niche (HUVEC and THP-1). We developed protocol and performed isolation of microvesicles from the supernatants of HT29/Snail. The incubation of HT29/Snail in experimental medium did not induce apoptosis and necrosis of the cells. The characterization of the microvesicles was performed by SDS-PAGE and Western blotting. In the purified microvesicles we detected CD63 and CD81, markers of microvesicles, whereas cytochrome c was not detected. Further the microvesicles were labeled using PKH67 Fluorescent Cell Linker kit to examine the uptake into HUVEC and THP-1. The cells with incorporated microvesicles were visualized under a confocal microscopy (Nikon D-Eclipse C1).
Our study show that microvesicles released by the HT29/Snail are internalized by HUVEC and THP-1. This observation is a starting point for our next investigation of the effect of the microvesicles and Snail on the response of cells constituting metastatic niche. It could be important for understanding the induction of immunosuppressive mechanism during cancer progression and inflammatory diseases and also may help to develop the future strategies to block metastasis.

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P2.46

Analysis of FGFR2-IIIb and FGFR2-IIIc isoforms role in breast cancer cells growth

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Fibroblast Growth Factor Receptor-2 (FGFR2) is one of the key mediator recognizing signals from fibroblast-rich tumour microenvironment. Cancer-associated fibroblasts were proved to promote breast cancer (BCa) progression by secreting number of growth factors including TGF-β, FGFs, PDGF or EGF. Single nucleotide polymorphisms (SNPs) in *FGFR2* gene, which are responsible for increased activity of receptor, correlate with higher incidence of breast cancer. However, some *in vitro* studies present contradictory role of FGFR2 in human BCa cell lines. FGFR2 exists in two splice variants: FGFR2-IIIb typical for epithelial and FGFR2-IIIc in mesenchymal cells with different specificity towards various FGFs. Expression of FGFR2 variants changes during disease progression in some carcinomas (e.g. prostate, bladder). It was observed that switch from FGFR2-IIIb to FGFR2-IIIc in prostate cancer correlates with epithelial-to-mesenchymal transition. Still little is known about their role in BCa initiation and progression In our studies we established stable overexpression of FGFR2-IIIb and FGFR2-IIIc in mammary epithelial (MCF10A) and breast cancer (MCF-7) cell lines. Presence of a proper isoform was detected by qPCR and enzymatic digestion (AvaI and HincII) of amplified *FGFR2* exon III. Their activity was confirmed by analysis of FGFR signalling pathway after stimulation either by FGF7 (specific for FGFR2-IIIb) or FGF9 (FGFR2-IIIc). We found that FGFR2-IIIb, as well as FGFR2-IIIc overexpression affect growth of MCF10A and MCF-7 cells in 3D cultures in Matrigel, but in opposite manner. Stimulation by employed FGF promotes growth of epithelial cells and inhibits growth of cancer cells.
These results indicate importance and complexity of FGFR2 isoforms in breast cancer. We are proceeding further investigation of specific roles of FGFR2-IIIb and FGFR2-IIIc in mammary carcinoma both using *in vitro* and clinical investigations.

P2.47

Influence of umbilical cord blood-derived MSCs on multidrug resistance phenotype of glioblastoma cells *in vitro*

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Investigation of interactions between tumour cells and mesenchymal stem cells may contribute to understanding the phenomenon of tumour plasticity and even create a base for searching for new therapeutic approaches [1–3].
The aim of the present study was to investigate the effects of human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs) on glioblastoma multidrug resistance phenotype *in vitro*. Co-cultures of hUCB-MSCs and glioblastoma-derived cells were performed in a transwell system. Analyses focused on assessment of expression of multidrug resistance-associated proteins (MRPs), anti-apoptotic genes and putative tumour stem cell markers (e.g. SOX2, Bmi-1, Msi-1, nestin). Gene expression at mRNA level was evaluated by quantitative real-time PCR and protein analysis was performed by immunofluorescence method.
The obtained results showed that the presence of hUCB-MSCs can alter the characteristics of glioblastoma cells in vitro with regard to examined multidrug resistance-associated protein genes and tumour stem cell markers. Comparative analysis revealed that glioblastoma cells co-cultured with hUCB-MSCs demonstrated decreased expression of the selected tumour stem cell markers but increased expression of MRP3 gene, which is regarded as new molecular therapeutic target [4, 5]. However, our investigation did not result in consistent findings for all tested glioblastoma samples. The observed contradictions may be caused by heterogeneous nature of glioblastoma.

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P2.48

Radiobiological effect of indirect and scattered irradiation *in vitro* in MDA-MB-231 cell line

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Introduction: The radiation dose has a significant impact on the effectiveness of radiation therapy. Understanding the biological effects depend on the dose rate is crucial for the effective treatment of ionizing radiation and personalized treatment. Basically the reduction of the dose leads to a reduction in lethal effect, but in some circumstances the opposite effect of the dose rate may be observed, This phenomenon characterized by an increasing DNA damages and decreasing of cell survival as compared to equivalent doses of high power.
Aim of study: These studies were designed to examine survival fraction and radiation-induced in-field and out-of-field DSB-DNA damages in MDA-MB-231 cell line as a function of dose.
Material and methods: Triple negative breast cancer MDA-MB-231 cells were cultured under standard conditions and irradiated in the two dose range: a) dose range 1.5–3.0 Gy and b) 3.0–6.0Gy both in the beam axis (indirect radiation) and 10cm out-of-field (the scattered radiation) in the water phantom using Clinac 2300C. Sixty minutes after irradiation, cells were labeled by immuno-fluorescence using Apoptosis, DNA Damage and Cell Proliferation Kit BD Pharmingen™. Analysis was performed using a flow cytometer. In addition, clonogenic assays were performed with the aim of determining the value of SF (survival fraction).
Results: Ionizing radiation in beam axis cause different number of DSB in MDA-MB-231 cell line compared to cells treated 10cm out-of-field using the same dose-rate. Moreover, these cells have different proliferative potential.
Conclusions: Our study shows an influence of scattered irradiation on cell survival and DSB number. Knowledge about the effects of ionizing radiation out-of-field is essential for the proper planning of radiation therapy, resulting in improved quality of treatment and patient safety.
Key words: ionizing radiation, dose-rate, out-of-field irradiation, DNA damage, proliferation.

P2.49

Cumulative effects of vitamin D and 2-metoxyestradiol on inhibition of growth of osteosarcoma and melanoma cell lines

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Osteosarcoma is one of the most malignant bone tumors of childhood and adolescence while melanoma the deadliest skin cancer. Hundreds of drugs were tested to eradicate those malignancies, but the effective therapy has not been established, yet. High chemoresistance and metastatic potential limits usefulness of classic anticancer drugs, thus modulation of cell phenotype priory definitive treatment might be essential in order to eliminated tumor cells. Vitamin D is known to inhibit proliferation and stimulate differentiation of multiple cancer cells, while 2-methoxyestradiol (2-ME) as a natural derivative of 17β-estradiol, was found to reduce carcinogenicity and cancer angiogenesis.
Here we are presenting data indicating that the effects of treatment of highly metastatic 143B and MG-63.2 osteosarcoma cell lines with 2-ME were enhanced by vitamin D analogs. The combination of 2-ME and $1,25(OH)_2$ D_3 resulted in efficient inhibition of growth of osteosarcoma cells, while the effect of single treatment was less pronounced. Similar effect was shown for two other analogs 25-OH D3 and calcipotriol. Furthermore, 2-ME inhibited growth of SKMEL188 and A375 melanoma lines and synergistic effect of $1,25(OH)_2D_3$ and 2-ME was observed in WM98 melanoma.
It seems that vitamin D enhances expression of nNOS (recently discovered target for 2-ME), while 2-ME modulated expression of vitamin D-related genes in osteosarcoma cell lines. The effect of 2-ME on nNOS expression seems to be more complex, including induction of alternative splicing of nNOS mRNA. Interestingly, in melanoma line A375 treated with $1,25\text{-}(OH)_2D_3$, 2-ME decreased significantly expression of CYP24A1 (main enzyme responsible for deactivation of vitamin D). These observation might suggest that the effects of vitamin D analogs could be prolongated, what at least in part explain synergistic effect of vitamin D analogs and 2-ME.
In summary, our preliminary studies on osteosarcoma and melanoma cell models showed potential benefits of simultaneous treatment with 2-ME and vitamin D analogs.

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P2.50

Antitumor 1-nitroacridine derivative C-1748 induces significant apoptosis in pancreatic cancer cells.

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Pancreatic cancer is the fifth leading cause of cancer death and has the lowest survival rate of any solid cancer in the industrial countries. The poor prognosis of pancreatic cancer results from its tendency for late presentation, aggressive invasion, early metastasis, and resistance to chemotherapy. Gemcitabine still remains the best chemotherapeutic agent available for the treatment of advanced pancreatic cancer. However, gemcitabine treatment results in only a marginal survival advantage. Thus there is a strong need for the continual development of novel therapeutic agents to improve pancreatic cancer therapy.The compound C-1748 is the most active derivative of 1-nitroacridine antitumor agents developed in our laboratory. Strong cytotoxic activity against colon cancer cell lines (HCT8 and HT29) and high antitumour activity against xenografts in nude mice of prostate (LnCaP) and colon carcinoma (HCT8), along with low mutagenic potential and slight myelosuppressive properties allowed the selection of C-1748 for phase I clinical trials.The aim of the current study was to investigate and characterize the cellular response of human pancreatic cancer cell line MiaPaca-2 to C-1748 treatment. This cell line was selected due to its high sensitivity to C-1748.Cell cycle analysis revealed that between 24 h and 48 h of C-1748 treatment, MiaPaca-2 cells underwent transient accumulation in the G2/M phase which was followed by prolonged arrest in the G1 phase. Starting from 96 h of drug exposure, decrease in G1 phase population was accompanied by progressive increase in sub-G1 fraction, suggesting that initial G1 arrest led to cell death through apoptosis. Morphological changes of MiaPaca-2 cells in response to C-1748 treatment were observed using fluorescent microscopy. DAPI staining revealed that cells exposed to C-1748 exhibited features characteristic for apoptosis: condensed chromatin and apoptosis-body like structures. The drug induced apoptosis in time- and dose-dependent manner was also confirmed by flow cytometry analysis. Caspase-3 activation, phosphatydylserine externalization and mitochondrial dysfunction typical for apoptosis were detectable already after 24 h of treatment.To sum up, major cellular response triggered by C-1748 in MiaPaca-2 cells was the effective induction of apoptosis. These results highlight the therapeutic potential of C-1748 in pancreatic cancer and support rationale for its further investigation towards this type of malignancy.

P2.51

Cytotoxicity of doxorubicin on breast cancer cells co-cultured with fibroblast compared to mono-cultures in 3D cancer model.

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Introduction

Two dimensional (2D) cell culture is the most common tool for investigating cancer biology. However, cells grown in 2D conditions produce less or no extracellular matrix, they lack cell-cell and cell-ECM interactions, secrete less signaling molecules, display different morphology. On the contrary, three-dimensional (3D) cultures provide that environment and allow cells to form much more complex interactions, similar to those observed *in vivo*. We use three-dimensional scaffolds, made of natural silk protein to investigate cancer biology. Silk protein from *B. mori* is a perfect biomaterial, thanks to its self-assembling properties, biocompatibility and slow biodegradability.

Aim of this study

Aim of the study is to develop a 3D breast cancer model consisting of breast cancer cells and fibroblasts.

Methods

Water based silk solution extracted from silkworm cocoons was used to produce scaffold. Two cell lines were used – murine breast cancer cells and murine fibroblasts. Cells were cultured in mono and co-culture. Cells were seeded on the scaffolds and visualized at different time points using confocal and scanning electron microscopy. Cell proliferation was analyzed with AlamarBlue reagent and by total DNA quantification. Doxorubicin toxicity was examined on the cells cultured in 2D vs 3D and mono- vs co-culture experiments using AlamarBlue.

Results

Cell attachment, morphology and proliferation on 3D scaffolds were studied. Cells cultured on 2D plates differed in morphology from those cultured in 3D. In co-culture studies fibroblasts formed spherical structures, that were not observed in mono-culture. Cancer cells and fibroblasts cultured in 3D proliferated slower than in 2D conditions. Both cell lines proliferated well on the scaffolds in mono-culture, while in co-culture experiments cancer cells were overgrowing fibroblasts. Results show that cells cultured on the 3D scaffolds are about 13,2 - 19,2 times more resistant to cytotoxic reagent than those cultured in 2D conditions. Additionally, cells cultured in co-culture were 1,5- times more resistant than breast cancer cells and fibroblast grown alone.

Conclusion: *In vitro* three-dimensional cancer model consisting of cancer cells, fibroblast and in the future endothelial or immune cells can be better system to study cancer in an environment similar to that observed *in vivo*.

P2.52

Synergic and additive types of interaction between 5-fluorouracil and 2-oxohexyl isothiocyanate in concurrent treatment in breast cancer cells and not in colon cancer cells

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A new idea to enhance effectiveness of anticancer drugs through combined treatments with naturally occurring chemopreventive agents has been recently observed in scientific publications. The effects of using such combinations are increased efficacy of anticancer treatment and decreased toxicity in normal cells [1]. A chemopreventive compound that may be used in such combination is sulforaphane, one of isothiocyanates. The in-vitro studies show that it potentiates the activity of 5-fluorouracil, oxaliplatin and doxorubicin [2,3,4]. Anticancer abilities of sulforaphane were inspiration for the synthesis of 2-oxohexyl isothiocyanate, whose cytotoxic effect in cancer cells is stronger than the one of sulforaphane. Our previous studies indicated that 2-oxohexyl isothiocyanate acts antagonistically when combined with 5-fluorouracil in normal fibroblasts cell lines [5].

The aim of this study was to investigate and compare the types of interaction between 5-fluouracil, an anticancer drug, and 2-oxohexyl isothiocyanate in two colon cancer and two breast cancer cell lines after concurrent treatment.

The study was performed on cancer cell lines: breast ones (MDA-231 and MCF-7) and colon ones (Caco-2and Ht-29). The cytotoxic effects of single 5-fluorouracil or isothiocyanate treatments and their combination were evaluated by the MTT assay. The types of interaction were determined through the median effect analysis as described by Chou and Talalay and only when the level of cytotoxicity of the combination was more than 0.5 [6].

In HT-29 colon cell line, there was observed antagonism. In Caco-2 colon cell line, at the levels of cytotoxicity from 0.5 to 0.7, there were noticed additive effects, and at other levels of cytotoxicity there was observed antagonism.

In breast cancer cell lines, at all tested levels of cytotoxicity, the effects of the combination of compounds were stronger than the effects of single treatments. The effect was more prominent in MCF-7 cell lines than in MDA-MB-231 ones. Synergic types of interaction were shown for MCF-7 cell line and an additive effect for MDA-MB-231 cell line.

In conclusion, 2-oxohexyl isothiocyanate potentiates the activity of 5-fluorouracil better in breast cancer cell lines than in colon cancer cell lines

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P2.53

The role of SWI/SNF ATP-dependent chromatin remodeling complex in epithelial-mesenchymal transition (EMT) in breast cancer cells

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Epithelial to mesenchymal transition (EMT) is a process leading to the loss of epithelial cell characteristics and gain migratory and invasive properties manifested by the mesenchymal phenotype featured by reduced level of typical epithelial markers like E-cadherin and cytokeratins, up-regulation of vimentin, N-cadherin and cadherin-11. In cancer cells, EMT is associated with increased aggressiveness, as well as invasive and metastatic potential. Preferentially EMT occurs in breast tumors with the basal-like phenotype and EMT markers expression is associated with poor prognosis.

Triple negative breast cancer is a subtype of breast cancers that are associated with early recurrence and an aggressive metastatic progression. Tumors referred mostly as triple-negative cancers are usually classified as basal-like breast cancer (BLBC) which represents 10%–25% of all breast cancer tumors. BLBC is aggressive, metastatic and chemotherapy-resistant. In this type of cancer metabolic switch to glycolysis is observed. Furthermore, the epigenetic silencing of gene encoding fructose-1,6- biphosphatase (FPB1) enzyme by the transcriptional repressor Snail protein is obligatory and characteristic for the development of BLBC.

Our analysis of data deposited in ENCODE database indicated that SWI/SNF chromatin remodeling complex directly controls genes coding for glycolysis enzymes like e.g. *FBP1* by targeting their promoter regions. We have also shown that Snail protein co-precipitates from HeLa cells with BAF155, a core subunit of SWI/SNF complex. Therefore, our study provided new evidences for an important regulatory function of SWI/SNF complexes in control of *FBP1* expression and likely EMT process.

P2.54

Crosstalk between hypoxia and autophagy in response of leukemia cells to inhibition of FLT3 kinase by imidazoacridinone C-1311

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Internal tandem duplication mutations (ITD) of receptor tyrosine kinase FLT3 occur in 30% cases of acute myeloid leukemia (AML), and are associated with poor disease outcome. Despite promising *in vitro* results, clinical efficacy of FLT3 inhibitors is still limited: even if treatment rapidly eliminates circulating leukemic blasts, those from bone marrow (BM) hypoxic niche remain unaffected and may be responsible for AML relapse. Thus, new strategies that target both (i) intrinsic regulatory mechanism in AML cells, and (ii) supporting factors like BM environment that enhance cell survival, are necessary for complete eradication of residual AML cells.

Imidazoacridinone C-1311 is a small-molecule topoisomerase II inhibitor that has been show to inhibit FLT3 kinase activity in a cell-free system. We previously fund that in living cells, C-1311 significantly blocked constitutively active FLT3-ITD kinase, which led to down-regulation of PI3K/AKT and RAS/MAPK survival pathways and selective induction of apoptosis in FLT3-ITD mutants. Cells without FLT3 receptor were not affected by C-1311. Here we show that only in FLT3-ITD mutants, C-1311-induced apoptosis was supported by autophagy. Importantly, whereas blocking early steps of autophagy with 3-methyladenine (3-MA) moderately increased FLT3-ITD$^+$ cells proliferation, inhibition of late stages of autophagy with chloroquine (CQ) completely abolished C-1311 cytotoxicity. Under hypoxic conditions mimicking BM microenvironment, efficacy of C-1311 against FLT3-ITD mutants was reduced: despite induction of autophagy, cell proliferation increased with concomitant decrease in apoptosis. In contrast to normoxia, inhibition of early hypoxic autophagy significantly attenuated C-1311 cytotoxicity and further enhanced cell survival. Surprisingly, suppression of late hypoxic autophagy did not profoundly decrease C-1311 activity.

Our results suggest that autophagy, depending on FLT3-ITD AML microenvironmental conditions (normoxia/hypoxia), may serve as a functional regulator of cellular response to FLT3 inhibition. Moreover, given the growing interest in new therapeutic strategies involving combination of tyrosine kinase inhibitors with compounds that modify autophagic process, it seems that especially under hypoxia, only targeted modulation of autophagy, considering the nature of autophagy inhibitor and stage of the process may help to restore activity of FLT3 inhibitors against AML cells residing in hypoxic bone marrow niches.

P2.55

Intensification of the cytotoxic effect of Selol by sulforaphane and alyssin observed on Caco-2 and HT-29 colorectal cancer cell lines

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Selol is the Polish patent. It is an organic selenium compound, wherein the Se is present in the oxidation state +4. Selol currently is being intensively studied and it can possibly be used in therapy of cancer. [1,2,3]. Numerous studies also showed that isothiocyanates (ITC) potentiate the cytotoxic compounds used in antitumor therapy [4,5]. ITC are low-molecular compounds present in significant quantity in the vegetables of Brassicaceae family. Their chemopreventive activity is widely recognized in the literature [6]. Sulforaphane is a compound that is well known in the literature, alyssin is its new, patented analogue of stronger cytotoxicity against cancer cells.

The aim of the study was to investigate whether the administration of sulforaphane increases toxicity against cell lines of colorectal cancer.

Co-administration of Selol and ITC was performed on Caco-2 and HT-29 colon adenocarcinoma cell lines. The effect of Selol, sulforaphane (SFN) and alyssin toxicity was assessed after their administration separately and in combination. Interactions were tested using the Chou-Talalay's method [7].

Co-administration of Selol and sulforaphane and Selol and alyssin causes stronger (additive interaction) cytotoxic effect than when provided separate on cell lines tested. Thus making it possible to lower doses of the compounds used.

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P2.56

Biological Evaluation of new Vitamin D_2 analogues

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The active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 (1,25D, 1,25-$(OH)_2D_3$, also known as calcitriol) is a steroid hormone which plays a vital role in numerous biological processes. Besides being a potent anti-rachitic agent, 1,25D has also been shown to induce cell differentiation, cell proliferation as well as immunomodulation. Nevertheless, its clinical application is severely hampered by the dose-side effects: potent hypercalcemia and increased bone resorption, making it necessary to develop analogues with selective properties. Many vitamin D analogs with improved anti-proliferative and pro-differentiating activities, as well as lower calcemic effects have been designed. The studies on the mechanism underlying biological effects of 1,25D analogs provide important information that allows us to determine what structural modifications of 1,25D molecule are responsible for their changed biological properties. 1,25D induces most of its biological responses via the vitamin D receptor (VDR), a transcription factor that forms a heterodimer with the retinoid x receptor (RXR) and binds to the promoter region of a target gene. *CYP24A1*, a gene encoding an enzyme responsible for degradation of 1,25D and *CD14*, a gene encoding the macrophage receptor responsible for binding bacterial LPS, are among some of the many VDR-target genes. In this study we characterized the structure-function relationships of five new analogs of vitamin D_2 in comparison to both 1,25D and PRI-1907 (a lead vitamin D_2 analogue previously shown to be more active than 1,25D). The biological activities of these analogs were assayed against mice and the human acute myeloid leukemia (AML) cell line HL60. Our studies indicate that the ability to induce differentiation of the AML cell line HL60 of two of the tested analogues was much higher in comparison to 1,25D and to PRI-1907, while that of the other three were comparable to 1,25D. Calcium serum levels were evaluated in mice injected intraperitoneally with new compounds, as well as PRI-1907 and the natural ligand 1,25D. Our data indicated that calcemic activities of all new analogues and of PRI-1907 were lower in comparison to 1,25D. Finally, the ability of these analogs to bind VDR and to activate expression of CYP24A1 and CD14 genes is currently being carried out.

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