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## Session 6. Molecular Mechanism of Cancer Development and Progression

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### Lectures

#### L6.1

### Uncovering gene networks in cancer by computational systems biology

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Cancer is a very complex disease that occurs from accumulation of multiple genetic and epigenetic changes in individuals who carry different genetic backgrounds and have suffered from distinct carcinogen exposures. These changes affect various pathways which are necessary for normal biological activities and gene networks are driving these pathways in disorder in the center. By intensively using the supercomputer system of Human Genome Center (225TFLOPS at peak) and K computer (10PFLOPS at benchmark) at RIKEN Advanced Institute of Computational Science, we are challenging for development of systematic methodology for unraveling gene networks and their diversity lying over genetic variations, mutations, environments and diseases. We first present a statistical/computational method that will exhibit how gene networks vary from patient to patient based on gene expression profiles according to a modulator, which is any score representing characteristics of cells, e.g. survival. We defined an EMT (epithelial-mesenchymal transition) modulator and analyzed gene expression profiles of 762 cancer cell lines. Network analysis unraveled global changes of networks with 13 508 genes of different EMT levels. By focusing on E-cadherin, 24 genes were predicted as its regulator, of which 12 have been reported in the literature. A novel EMT regulator KLF5 was also discovered in this study. We also analyzed Erlotinib resistant networks using 160 NSCLCs with GI50 as a modulator. Hubness analysis exhibited that NKX2-1/TTF-1 is the key gene for Erlotinib resistance in NSCLCs. Our microRNA/mRNA gene network analysis with Bayesian network method also revealed subnetworks with hub genes (including NKX2-1/TTF-1) that may switch cancer survival. We also developed a statistical/computational method for modeling dynamics in cancer cells from time-course gene expression profiles and revealed dynamic network changes against anti-cancer drugs and network differences between drug-sensitive and drug-resistant cancer cells. We devised a state space model (SSM) with dimension reduction method for reverse-engineering gene networks from time-course data, with which we can view their dynamic changes over time by simulation. We succeeded in computing a gene network with prediction ability focused on 1500 genes from data of about 20 time-points after EGF stimulation with/without Gefitinib dose. We applied this SSM model to human normal lung cell treated with (case)/without (control) Gefitinib, and we identified genes under differential regulations between case and control. This signature of genes was used to predict prognosis

for lung cancer patients and showed a good performance for survival prediction. On-going cancer research using K computer is also introduced.

## L6.2

### Targeting signaling pathways to prevent drug resistance and metastasis

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Over the years, clinicians and scientists have been interested in being able to specifically target molecules involved in growth which may be aberrantly regulated in cancer and result in drug resistance and metastasis. Classical chemo- and radiotherapy are in general not specific and often toxic to normal cells. Moreover, a common problem with traditional cancer therapies is the development of drug resistance and relapse. These phenomenon often are due to the emergence of cancer stem cells, more precisely referred to as cancer initiating cells (CIC). The CICs have different growth properties than the non-CICs which are often referred to as bulk cancer cells (BCs). CICs are often dormant, drug resistant and not rapidly proliferating while the BCs are rapidly proliferating and respond to chemo- and radio-therapy. So routinely after various therapeutic approaches, the BCs will die off or be removed (after surgery), however, the CICs will persist and eventually re-emerge and result in relapse. Development of methods to target the CICs are imperative for improved cancer therapies. One pathway which is critical for the regulation of cell growth is the PI3K/PTEN/Akt/mTORC1 pathway. Inhibitors to key components have been developed and clinically evaluated. Some of them exert promising effects on eradicating CICs. Rapamycin is a mTORC1 blocker which has been used to prevent transplantation rejection since 1999 and is now being used to treat certain cancer patients. It turns out that Rapamycin is effective in suppressing the growth of various CICs. The LKB1/AMPK activated protein kinase (LMPK) pathway has been determined to be a key pathway in metabolism (diabetes) as well as cancer. The LKB1/AMPK network remains functional in a wide range of cancers and can be stimulated by drugs, such as Metformin. Metformin has been used to treat diabetes patients since the 1940's. LKB1/AMPK signaling induces cell cycle arrest, caspase-dependent apoptosis or autophagy in various tumors. Metformin inhibits mTORC1-controlled oncogenic protein translation, which does not occur with allosteric mTORC1 inhibitors, such as Rapamycin and its derivatives. Metformin also targets CICs, the critical target for cancer eradication. Thus the LKB1/AMPK pathway is critically involved in regulating proliferation and survival of malignant cells. Glycogen synthase kinase-3 (GSK-3) is well documented to participate in a complex array of critical cellular processes. It was initially identified in rat skeletal muscle as a serine/threonine kinase that phosphorylated and inactivated glycogen synthase. This versatile protein is involved in numerous signaling pathways that influence metabolism, embryogenesis, differentiation, migration, cell cycle progression and survival. Recently, GSK-3 has been shown to be important in CICs and may be an appropriate target. Lithium is an inhibitor of GSK-3 and has been used to treat psychiatric patients for decades. Lithium may also have significant effects on various cancers including CICs. Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) which targets  $\beta$ -catenin and can be used to inhibit CICs. In this presentation, we will discuss the abilities of these and other drugs to target CICs, prevent drug resistance and metastasis which may eventually improve cancer therapy.

## L6.3

### Role of microRNAs in tumorigenesis and angiogenesis

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MicroRNAs are a group of small, non-coding RNAs, which by targeting 3' untranslated regions (3' UTRs) of many different mRNAs decrease their stability and suppress translation. MicroRNAs are the important modulators of gene expression, influencing both physiological and pathological processes, often in a cell-dependent manner. The role of microRNAs in tumors is related not only to their functions as tumor suppressors or oncogenes, but also to effect on cell differentiation and regulation of the blood vessels formation.

Recently we have elucidated the role of microRNAs in the origin and growth of two types of tumors: rhabdomyosarcoma and non-small cell lung carcinoma (NSCLC), addressing the microRNA interactions with the antioxidant enzyme, heme oxygenase-1 (HO-1). Interestingly, overexpression of HO-1 in myoblasts, the precursors of skeletal muscles impaired myoblasts differentiation, affecting strongly the expression of microRNAs (Kozakowska *et al.*, 2013, *Antioxid Redox Signal*). The HO-1-microRNAs interactions have been then confirmed in the clinical rhabdomyosarcoma. Accordingly, inhibition of HO-1 and restoration of the microRNAs expression may be considered as a therapy for rhabdomyosarcoma.

Interestingly, HO-1 modulated also the expression of microRNAs in human NSCLC, inhibiting the expression of microRNAs involved in tumor growth and angiogenesis, and accordingly, attenuating the NSCLC tumor growth. Among several angiomiRs described so far, the role of miR-378, a mirtron encoded in the first intron of PGC-1beta gene appears to be of particular interest (Skrzypek *et al.*, 2013, *Antioxid Redox Signal*, *in press*).

In this talk the significance of described interactions for tumorigenesis and blood vessel formation will be discussed.

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## L6.4

### Glutamate signaling in cancer — new therapeutic targets

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Because of the low efficacy of the traditional chemotherapy, new types of targeted therapy, acting selectively on specific metabolic pathways pathologically deregulated in cancer cells, are still needed. In the last decade evidence has emerged implicating a role for glutamate as a signal mediator in different tissues in autocrine and paracrine manner, as well as a growth factor in tumors, which renders glutamate signaling as a new potentially therapeutic target in cancer. Glutamate receptors (GluRs) are differentially expressed in human tumor cells corresponding with the formation of functional ionotropic channels. Blockade of GluRs by genetic modifications or synthetic antagonists decreases proliferation in several neuronal and non-neuronal cancer cell types. GluRs inhibition interferes with key metabolic pathways involved in cancer cell proliferation, such as ERK1/2 and Akt kinases cascades, resulting in aberrant gene expression. Encouraging by the recent results showing that AMPA-receptor inhibitor (Talampanel) improves survival rates in patients with glioblastoma, GluR anagonists open new perspectives to target therapy of these types of tumors.

## L6.5

### Chemoimmunotherapeutical approach in neuroblastoma treatment

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Neuroblastoma is the most common extracranial tumor in children. Despite application of multimodal treatment regimens, patients diagnosed with high risk disease are at risk of poor outcome. Therefore, we seek new strategies to enhance killing of the cancer. Here we show, that this can be achieved by dual targeting of two vital molecules of cancer cells, namely GD2 ganglioside (GD2), and aurora A kinase. Both targets are over-expressed in various human cancers. Our recent studies showed that the GD2-specific mouse monoclonal antibody 14G2a (mAb) exhibits cytotoxic effects on IMR-32, LA-N-1, CHP-134, and HTLA-230 neuroblastoma cell lines in time- and dose-dependent manners. We also measured activity of caspases-3/7 in the mAb treated cells. Moreover, we observed significant inhibition of expression of both phosphorylated and unphosphorylated aurora A, B and C kinases upon 24 and 48 hours of the GD2-specific mAb treatment in MYCN amplified IMR-32 and LA-N-1 cell lines. Additionally, we analyzed changes in expression and localization of three substrates of aurora A kinase such as MYCN, P53 and PHLDA1 in IMR-32 and CHP-134 cells (both with wild type P53) treated with the 14G2a mAb for 2, 6, 24, 48 h. It should be stressed that the cytotoxic effect is correlated with statistically significant increase in total PHLDA1 protein, nuclear P53 accumulation, and decrease in nuclear MYCN level in the two cell lines. We also tracked changes in a transcription factor HSF1, known to be involved in direct *PHLDA1* gene activation, and chaperone proteins HSP40, HSP70 that are participating in regulation of cell death mediated with PHLDA1 (Hayashida N *et al* (2006) *EMBO J* **25**: 4773-4783). We showed that HSF1 statistically significantly increased in nuclei of IMR-32 cells 48 h of the 14G2a mAb-treatment. On the contrary, as expected HSP40 and HSP70 proteins statistically significantly decreased in whole cell extracts of IMR-32 and CHP-134 cells.

In separate experiments, we introduced a novel aurora A inhibitor MK-5108 and showed that it decreased cell viability of IMR-32 and LA-N-1 cells in a dose dependent-manner. Combination of the GD2-specific 14G2a mAb with the inhibitor exerts enhanced cytotoxic effect on IMR-32 and LA-N-1 cells *in vitro*. We showed that treatment with the inhibitor did not change GD2 expression on the cells 24 and 48 h of the treatment. Additionally, we analyzed influence of the inhibitor, and the two agent regiment on: phosphorylation status of aurora kinases A, B, C, and expression levels and localization of the transcription factors P53, MYCN, HSF1, and total expression of PHLDA1, HSP70, and HSP40 proteins.

The results of these studies may contribute to broadening of current treatment strategies and may improve the outcome of neuroblastoma patients.

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## L6.6

### Telomerase inhibition provokes broad spectrum of morphological and physiological changes in cancer cells

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Telomerase has been recognized as a relevant factor distinguishing cancer from normal cells since it is either undetectable or has a low level of activity in normal somatic cells. Thus, it has become a very promising target for an anticancer therapy. It was revealed in many studies that regulation of telomerase in mammalian cells is a multifactorial process, involving expression of telomerase subunits coding genes, posttranslational protein-protein interactions, and protein phosphorylation. Thus the complexity of telomerase control is studied in the context of stem cell renewal, tumor development, as well as aging. The studies across many tumor types have shown that the vast majority of tumors (~85 %) express telomerase and hence, are able to maintain a stable and homogenous telomere length. It was suggested in numerous studies, that telomerase silencing is accompanied by abundant genes expression modulation which implicates that cell death following telomerase downregulation is not always related to telomere shortening.

We analyzed the influence of TMPyP4, potent telomerase inhibitor, on morphology and metabolism of MCF7 and MDA-MB-231 breast cancer cells *in vitro*. The cells were analyzed by flow cytometry as well as with the use of migration assays, adhesion assays and qPCR.

It was shown that after treatment with TMPyP4 (0.5 μM) the cells were much more difficult to detach using trypsin during passaging. Since the compound revealed capability for cell adhesion modulation we performed a scratch assay. This experiment demonstrated a significant decrease of the migration potential of both cell lines comparing to control cells. Additionally, treatment of cancer cells with telomerase inhibitor provoked a significant shortening of telomeres. Surprisingly, telomeres were getting shorter up to 19 passages and after that, they were restored to the primary length. We assume that this might be provoked either by the mechanism of Alternative Lengthening of Telomeres (ALT) or a resistance to the compound might arise. Interestingly, telomere length changes did not provoke any changes in cell cycle.

It was concluded that inhibition of telomerase by TMPyP4 could affect the motility of cancer cells which consequently could provoke decreased ability of cancer cell to metastasize. This, in turn, might constitute an interesting observation in the context of cancer therapy.

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## Oral presentations

### O6.1

#### Grainyhead-like 1 (GRHL1) transcription factor in signaling pathways and in development of skin cancers

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The Grainyhead-like 1 (GRHL1) protein belongs to evolutionarily conserved family of transcription factors, which is involved, among others, in epidermal barrier formation and maintenance. Two closely related proteins, GRHL2 and GRHL3, have been implicated in cancerous transformation, but their roles in this process are contradictory, as depending on tissue-context they can be pro- or anti-tumorigenic.

Here we present evidence for skin tumor suppressive properties of GRHL1. In carcinogenesis experiments *Grhl1*<sup>(-/-)</sup> mice developed more cutaneous carcinomas, with an earlier onset, than their wild type littermates. To explain the observed phenotype we examined the properties of *Grhl1*<sup>(-/-)</sup> keratinocytes.

Previously we showed that GRHL1 directly regulates expression of epidermal desmosomal cadherin - desmoglein 1 (*Dsg1*). This led us to investigate epidermal barrier function in these animals. Histological analysis of their epidermis demonstrated its thickening and mild impairment of terminal differentiation of keratinocytes. Furthermore, in these mice we observed increased expression of antimicrobial peptides, mast cells infiltration of the dermis, and increased blood level of inflammation-associated cytokine TSLP. These results indicate an abnormal status of epidermal barrier accompanied by mild skin inflammation.

To elucidate mechanisms by which the deletion of *Grhl1* leads to described phenotype we measured the levels of expression of a number of genes potentially regulated by GRHL1, including PTEN. Recently it has been shown that a close homologue of GRHL1 — GRHL3 — directly regulates the expression of PTEN, and *Grhl3*-deficient mice develop aggressive squamous cell carcinoma (SCC) induced by PTEN-dependent activation of PI3K/AKT/mTOR signaling. In contrast, our results demonstrated lack of changes in the expression of PTEN upon *Grhl1* deletion. This suggests that the mechanism of increased tumor susceptibility in *Grhl1*<sup>(-/-)</sup> mice is different from *Grhl3*<sup>(-/-)</sup> animals. It is likely to involve EGFR/MAPK signaling.

In other reports it has been demonstrated that decreased level of DSG1 and improperly functioning desmosomes result in failure in inhibition of EGFR/MAPK pathways during keratinocytes' differentiation. Furthermore, activation of MAPK pathway in suprabasal keratinocytes is sufficient to induce inflammatory response in the skin. The analysis of these pathways in *Grhl1*<sup>(-/-)</sup> mice skin is in progress, and the latest results will be presented at the Meeting.

## 06.2

### The regulation of *STATs* and *NANOG* genes in the aspect of changes in metastasis of T24 bladder cancer cells

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**Introduction and the aim of the study:** Signal transducer and activator of transcription 3 (*STAT3*) can promote the process of metastasis, as it can activate the expression of human matrix metalloproteinases (MMPs), as MMP-9 [1, 2]. *STAT5* is a necessary survival factor for many types of cancer cells, including breast cancer, bladder cancer and prostate cancer [3]. *NANOG* is a transcription factor which is involved in the self-renewal of embryonic stem cells (ECs). It has been proved, that expression of *NANOG* gene is observed not only in embryonic-derived malignancies, but also in breast cancer, ovarian cancer, cervix cancer or bladder cancer. *NANOG* overexpression is correlated with high activity of MMP-2 and MMP-9 [4, 5]. The aim of the study was to evaluate the changes in the metastasis of T24 cells with modulated expression of *STAT3*, *STAT5* or *NANOG* genes.

**Material and methods:** Human urinary bladder cancer cells T24 (HTB-4) were cultivated under standard conditions. Transfection of the cells with silencing constructions was performed with the application of Lipofectamine 2000 (Invitrogen) reagent. The evaluation of changes in the expression level of individual genes was performed using Real Time<sup>TM</sup> PCR. Changes in the protein level of individual genes were in turn performed using Human ELISA Kit (Abcam). The migration capability of transfected cells was tested using Matrigel Invasion Chambers (BD Biosciences). **Results:** The Real Time<sup>TM</sup> PCR evaluation showed that silencing the *STAT3* gene led to the decreasing of mRNA for *MMP-9* gene to the level of 10.1% and silencing the *STAT5* caused the decreasing of mRNA for *MMP-9* gene to the level of 62% according to control. In T24 cells with silenced *NANOG* gene, the mRNA for *MMP-9* decreased to the level of 52% comparing to the control. The cells with modulated expression of examined genes migrated slower in the Matrigel Invasion assay. The number of cells with silencing *STAT3* gene, which migrated to the inner side of insert's membrane and to the bottom of the plate were reduced to the 45% of control cells number. The number of cells with silenced *STAT5* and *NANOG* genes were reduced respectively to the 29% and 38% of control cells number. The results above were confirmed during immunoenzymatic tests.

**Conclusion:** The transcriptional activity of *STAT3*, *STAT5* and *NANOG* genes is connected with bladder cancer cells metastasis and has the influence on *MMP-9* expression level.

#### Acknowledgements:

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## 06.3

### MicroRNA expression profiling studies in brain tumors

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Altered miRNA expression has been associated with development of human cancers, including brain tumors. The most frequent brain tumors in adults are malignant gliomas, arising from glial cells or their precursors. Glioblastoma multiforme (GBM) represents the most common and aggressive type. It is still perceived as a daunting challenge to reveal miRNA-target networks, miRNA functions and expression profile of miRNAs specific for tumor/cancer type. While malignant gliomas represent highly heterogeneous group of tumors, miRNA profiling could be advantageous not only for further research but also for diagnostic and therapeutic purposes.

We have profiled global miRNA expression in adult malignant gliomas, margins of tumor tissues and non-tumor brain tissues using miRNA microarrays. We have identified 97 miRNAs that are significantly differentially expressed in GBM in contrast to normal, healthy brain. Comparison of tumor margin tissues (adjacent peritumoral tissues) with normal brain samples, showed 6 miRNAs with elevated expression and 16 miRNAs down-regulated in borders of tumors.

We also preformed meta-analysis of differentially expressed microRNAs in malignant gliomas. The main source of available data comes from miRNA microarray and PCR array studies. Meta-analysis revealed 314 deregulated miRNAs that were previously reported for malignant gliomas, from which 162 was found to be up-regulated and 152 to be down-regulated. Based on miRNA microarray research and meta-analysis, we found a set of 37 miRNAs which expression is the most frequently deregulated. Our results provide a comprehensive overview of miRNA signature in malignant gliomas, mainly GBM. It can be useful in development of miRNA-based therapies or diagnostic applications.

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## 06.4

### Tetraspanin CD151 regulates expression of fibroblast growth factor receptor-2 (FGFR2) in breast cancer

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CD151 is a member of evolutionary conserved transmembrane-4 superfamily. CD151 was the first tetraspanin associated with cancer development and its role in promotion of invasion and migration has been demonstrated in numerous *in vitro* and *in vivo* models. Clinically, high levels of CD151 are correlated with poor prognosis in a variety of tumours including breast cancer. Increased expression of CD151 was observed in both premalignant form of breast cancer - ductal carcinoma *in situ* (DCIS), and invasive ductal carcinoma (IDC). There is an increasing evidence that the FGF-FGFR2 signaling axis plays an important role in breast cancer. Elevated expression of FGFR2 transcripts was associated with a higher incidence of breast cancer. On the other hand *in vitro* and animal studies show that FGFR2 inhibits tumour progression. Here we demonstrate for the first time that expression and function of fibroblasts growth factor receptor-2 (FGFR2) in an immortalized mammary epithelial cell line (HB2) and breast cancer cell lines (SKBR3, MCF7) is regulated by tetraspanin CD151. FGFR2 expression was assessed by western blotting and qPCR in cell lines (all are CD151+) and their CD151-negative variants. CD151-dependent responses to various FGFs were evaluated in cells grown in 3D collagen. In order to reveal molecular pathways underlying CD151-mediated FGFR2 expression, cells were incubated with chemical inhibitors of a panel of kinases and analysed for the presence of FGFR2 protein.

We found that CD151 knock-down upregulated expression of FGFR2 at both mRNA and protein level, without affecting other FGF receptors. This was reflected in abolishment of CD151-negative cells response to FGF2 (ligand for FGFR2) grown in 3D collagen. Analysis of signaling pathways likely to be responsible for these effects revealed that CD151 impairs activation of p38 kinase which controls FGFR2 expression level. Inhibition of p38 activity or its forced overexpression, impaired or elevated FGFR2 level, respectively. We also identified Sox9 as a transcription factor which mediates p38 activity towards FGFR2 expression.

The results demonstrate for the first time that tetraspanin CD151 interacts with the FGF-FGFR2 signaling axis in mammary epithelial and cancer cells and regulates expression and function of FGFR2 gene and protein. Clinical and *in vivo* studies are in process to define the role of these interactions in breast cancer progression.

## 06.5

### Molecular mechanisms of pharmacological restoration of the TAp73 tumor suppressor function

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In the past 30 years, an impressive amount of clinical and basic research has focused on the p53 tumor suppressor protein, mainly because its inactivation occurs in almost all of the cancer types [1]. In mammals, p53 belongs to a small family that includes two additional paralogues, p73 and p63. They are structurally and functionally similar to p53, and have important functions in embryonic development and neuronal differentiation [2]. Of notice, p73 and p63 are also involved in tumor suppression so the entire p53 family can be regarded as a “network” controlling cell proliferation, differentiation and cell death [3].

It is now established that pharmacological manipulation aimed at restoring the functions of the p53 protein can induce tumor regression *in vivo*. Considering that p73 is rarely mutated in cancer, pharmacological activation of the tumor-suppressive activities of p73 represents an attractive alternative strategy to treat cancer cells, in particular those where p53 is lost or mutated [4].

The *TP73* gene encodes the tumor suppressive full-length TAp73 and N-terminal-truncated ΔNp73 isoforms that act as dominant negative inhibitors of TAp73 [5]. The overall effect of p73 in oncogenesis is thought to depend on the TAp73 and ΔNp73 isoforms’ ratio [6]. Here we show that TAp73 can be a promising target for anticancer therapies, especially in tumors with *TP53* loss or mutations. Pharmacological activation of TAp73 by small molecules induces p73-dependent apoptotic program through induction of *BAX*, *PUMA* and *NOXA* expression. We also reveal that activation of TAp73 may overcome so called ‘oncogene addiction’ of cancer cells through transcriptional trans-repression of key oncogenes. This function adds up to the proapoptotic propensity of TAp73 and further supports the druggable potential of this protein. The mechanism of TAp73 activation by small molecules works through its stabilization on protein level through disruption of inhibitory complexes.

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## 06.6

### Doxorubicin loaded silk bionanoparticles for targeted cancer therapy

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Spider silk proteins have been explored as biomaterials for cell culture and tissue engineering because of their excellent mechanical properties such as strength, toughness, versatility in processing, biodegradability and biocompatibility. Bioengineered silk is based on the repetitive consensus sequences found in corresponding native silk. The bioengineered silk proteins may be further modified to gain new functions. The strategy of hybrid protein constructed at the DNA level combines the sequence coding bioengineered spider silk, which is responsible for biomaterial structure, with sequences coding polypeptides for functionalization. The ability of silks to self-assemble into micro- and nanospheres renders this family of structural proteins important candidates for drug delivery applications.

The bioengineered silk protein (MS1) and its hybrid variants (MS1 fused to Her2 binding domains: H2.1MS1, H2.2MS1, MS1H2.1 and MS1H2.2) were obtained. Control and functionalized spider silk proteins were used to produce spheres by inducing a nucleation and particle growth upon addition of potassium phosphate. Silk spheres were characterized by confocal laser scanning microscopy. Obtained silk spheres were tested in Her2 binding assays. The binding efficiency was examined by flow cytometry and confocal microscopy. The anticancer therapeutic doxorubicin was used as a model drug for loading of silk spheres. Cytotoxicity of spheres with incorporated doxorubicin and control spheres without drug was investigated using MTT assay.

The spheres made of functionalized spider silk indicated considerably higher binding to the Her2 overexpressing cells comparing with Her2-free cells and the control spheres without the functional domain. Significant internalization of functionalized spheres was observed for Her2-positive SKOV3 and SKBR3 cells in contrast to Her2-negative MSU1.1 cells. The functionalized spider silk spheres, unlike control spheres, loaded with doxorubicin substantially higher reduced viability of Her2 overexpressing cells. The observed cytotoxicity was not due to the spider silk.

The obtained results encourage the delivery of a therapeutic agent confined in silk spheres to the specific tumor microenvironment. We propose that such Her2-targeted biodegradable silk spheres are likely to be a fine drug delivery carrier for cancer treatment.

## 06.7

### Caffeine as an interceptor of aromatic carcinogens: inhibition of Trp-P-1 and Trp-P-2 mutagenic activity

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Caffeine (CAF) is the most widely consumed alkaloid worldwide, mainly in many popular beverages. Numerous epidemiological studies demonstrated that CAF can reduce the risk of several cancer types. Despite extensive studies conducted to reveal mechanisms of CAF protective effects, its exact mode of action still remains unclear. It has been proposed, among many others suggested mechanisms, that CAF can directly interact with aromatic carcinogens, sequestering them thus diminishing their bioavailability. In this work we intended to establish the protective role of CAF against two well-known food-borne carcinogens – Trp-P-1 and Trp-P-2, belonging to heterocyclic aromatic amines (HCAs).

In order to investigate CAF impact on a biological activity of aromatic carcinogens, we employed bacterial mutagenicity assay, based on *Salmonella typhimurium* TA98 strain. CAF caused a significant, dose-dependent decrease in Trp-P-1 and Trp-P-2 mutagenic activity, both with and without exogenous activation. UV-Vis spectroscopy studies enabled us to examine direct interactions between CAF and chosen carcinogens. Observed spectral changes revealed mixed complexes formation between analyzed compounds. We calculated concentrations of all mixture components and determined neighbourhood association constant values ( $K_{AC}$ ) with the statistical-thermodynamical model of mixed aggregation. This analysis also revealed that for the highest CAF concentrations (CAF : carcinogen ratio about 300 : 1), as many as 90% of mutagen molecules is complexed with CAF. Moreover, we observed a strong linear correlation between Trp-P-1 and Trp-P-2 mutagenic activity and their free form molar fraction in each mixture with CAF. Isothermal Titration Calorimetry (ITC) studies revealed that Trp-P-1 – CAF and Trp-P-2 – CAF hetero-complexation is thermodynamically favourable, with enthalpy values about -7 kcal/mol, which serves as a further indication that observed interactions are based on stacking complexes formation.

The findings of this work indicate that CAF not only inhibits mutagenic activity of aromatic carcinogens – Trp-P-1 and Trp-P-2, but also directly interacts with them by formation of stacking hetero-complexes. These observations strongly suggest a key-role of stacking complexes formation in CAF protective effects against food-derived aromatic carcinogens.

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## 06.8

### Change of expression of CART and GAL in the gastric mucosa affected by carcinoma depending on the size of the tumor in the TNM classification (T3 and T4)

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**Introduction:** Both cocaine- and amphetamine-regulated transcript-peptide (CART) and galanin (GAL) are regulatory peptides expressed in the central nervous system, as well as in the gastro-interstitial tract (GIT), where they fulfill many functions. It is suggested that both neuropeptides play a very important role not only in the neurotransmission, but also in the modulation of GIT functions.

**Aims:** The present study was aimed at disclosing the distribution and colocalization pattern of CART and GAL within the tissues of the human stomach challenged by the cancerogenesis of different tumor stage.

**Material and methods:** Tissue samples comprising all layers of the gastric wall were obtained during surgery from both surgical margin and neoplastic regions. Specimens of stomach tissues were histologically assessed in order to classify the tumor stage in the TNM classification. They were fixed by immersion in buffered paraformaldehyde solution. Cryostat sections were processed for triple-labelling immunofluorescence to study the distribution of the intramural nerve structures (visualized with antibodies against protein gene-product 9.5) and their chemical coding using antibodies against CART and GAL.

**Results:** Microscopic observations revealed that in the human cancerous stomach (T3 and T4) the number of myenteric plexuses and the number of nerve cells within them were lower than that found in the fragments from surgical margin of the stomach wall. On the other hand, it has been revealed that within the pathological tissue of T4 tumor stage the number of cell bodies inside the myenteric plexuses showing CART were higher than that found in surgical margin. However, the number of neurons containing GAL within plexuses of carcinoma-changed stomach wall, with T3 tumor stage was lower compared to the unchanged regions. However, we were not able to find statistically significant differences in the number of cell bodies containing studied substances, irrespective the TNM classification stage.

**Conclusions:** This is the first scientific report on the expression of CART and GAL in the *ganglion* cells in the intramural plexuses within human stomach wall, especially in regions affected by carcinoma. A increase in the expression of CART within nerve bodies on the area of tumor destroyed myenteric plexuses (T4) and a decrease of the number of GAL-positive neurons (T3) may suggest an important role of these peptides in the neuromodulation in studied areas. Observed changes weren't depend on the stage of the TNM classification. Hence, further studies are necessary to elucidate the detailed role of CART in studied process.

## Posters

### P6.1

#### Multidimensional mixture modelling as a possible way of improvement in analysis of nuclear magnetic resonance spectroscopy data — preliminary results

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Nuclear Magnetic Resonance (abr. NMR) is a promising technique that is widely used in research of tissue chemical structures. One of its most complex application is spectroscopy that is applied for determination of metabolism. There exist two types of NMR spectroscopy. In vitro that requires high induction field and returns a very precise information and in vivo that requires way smaller field but with worse result at the end. The second technique, because of the low field required, is very frequently used for diagnosis and during treatment of human tumours. Signal obtained as a result must be processed in order to acquire information relevant from the medical point of view. Authors presents techniques of signal so called pre-processing that were established during previous research and are used to filter out unwanted signal components such as: phase error, baseline and noise.

Pre-processed signal should be then modelled to obtain area under spectrum peaks that represents certain chemical compounds-metabolites in analysed case. This task is not trivial since the peaks very frequently overlaps. Authors proposed decomposition of spectra into Gaussian Mixture Model (abr.GMM) in which each single metabolite are represented by a single component or group of components in mixture model. Such a solution is novel and differs from standard, based mainly on signal reconstruction by Singular Value Decomposition. In previous research authors proposed application of the model for single unit-voxel only. In case of in vivo test as a compromise between quality and time of the NMR scan voxel size is quite large and final information is obtained for a volume that may consists of different kind of tissue. In this work authors propose to switch from local voxel based modelling into global one based on all possible voxels. As a solution authors propose a modified GMM that was successfully used for analysis of each voxel separately. Previously obtained model was analysed and appropriate extension based on introduction of additional dimension was proposed. As a result authors obtained method that allows to observe global trends and changes into tissue metabolism. However since obtained results are preliminary authors will continue their research in the nearest future.

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## P6.2

### UPLC-MS and NMR metabolomic analysis of mesothelioma cells after L-arginine degradation by arginine deiminase (ADI-PEG20)

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**Background:** L-arginine is involved in a growth and development of certain cancer types, including hepatocellular carcinoma, melanoma and malignant pleural mesothelioma (MPM). These cancers are characterized by deficiency of argininosuccinate synthetase 1 (ASS1) that is a rate-limiting enzyme in arginine biosynthesis. The arginine-auxotrophy of ASS1-negative tumor cells is well-known strategy in evaluation of the effectiveness of anticancer arginine-degrading drug – pegylated arginine deiminase (ADI-PEG20). This study was aimed to determine metabolomic profiles of ADI-PEG20-treated mesothelioma cell lines by the use of ultrahigh performance liquid chromatography with mass spectrometry (UPLC-MS) and nuclear magnetic resonance spectrometry (NMR) techniques.

**Methods:** Three ASS1-negative (MSTO, JU77, H2591) human malignant pleural mesothelioma cell lines were treated with ADI-PEG20 (750 ng/ml) for 24 hours. After preparing methanol cell extracts, the UPLC-ESI-MS and <sup>1</sup>H-NMR (700 MHz) analyses were performed to quantify metabolic changes induced by ADI-PEG20.

**Results:** 1626 metabolites were identified in all tested cell lines during UPLC-MS analysis. Of them, 141 metabolites with more than 2 fold up- and down-regulation (*p* 0.05) were selected for further analysis. Besides L-arginine deprivation in all ADI-PEG20-treated cell lines, qualitative and quantitative changes in other metabolites were observed among these cell lines. We also revealed down-regulation of compounds involved in uracil and thymine synthesis, such as orotic acid, ureidosuccinic acid, thymidine and methylmalonate. Additionally, <sup>1</sup>H-NMR spectrometry confirmed UPLC-MS data regarding changes in concentrations of L-arginine, L-citrulline, L-glutamine and glutamate metabolites in ADI-PEG20-treated MPM cells.

**Conclusions:** The study represents an innovative approach in evaluating potential markers for MPM and provides a deeper insight into the ADI-PEG20-induced metabolic pathways alterations in mesothelioma cells in the context of arginine deprivation.

## P6.3

### RSK2 kinase is involved in FGFR2 signaling pathway in human breast epithelial cells

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FGFR2 (Fibroblast Growth Factor Receptor 2) plays a critical role in proliferation, differentiation and survival of cells. Mutations of *FGFR2* gene, regulating its expression, were found to be responsible for higher breast cancer risk. We previously found that tetraspanin CD151 that is also associated with progression of a breast cancer inhibits expression of FGFR2 in human breast epithelial cell line HB2 – *in vivo* model of DCIS (*Ductal Carcinoma in Situ*).

Here we show that CD151-negative cells have higher activity of RSK2 (p90 ribosomal S6 kinase 2) which controls multiple cellular processes including growth, proliferation, survival and motility. Treatment with unspecific inhibitors of FGF receptors: PD173074, PD166866, AZD4547 and AZD2127 (Cediranib) demonstrated that only the last one strongly abolished FGFR2 activation. Interestingly, inhibition of FGFR2 with Cediranib was associated with massive decrease in RSK2 activation what suggests functional link between FGFR2 and RSK2. Searching for molecular mechanism we investigated the effects of inhibition of ERK, p38 and Src (potential upstream regulators) on RSK2 activation. It appeared that only inhibition of p38 significantly decreased the amount of phosphorylated RSK2.

Analysis of signaling and functional assays strongly suggests involvement of RSK2 in HB2 cell adhesion and motility. Stimulation of cells with FGF2 (FGFR2 ligand) significantly increased the number of focal adhesions. This increase was better visible in CD151-negative cells where RSK2 also localizes in focal adhesions. Soft agarose assay for anchorage-independent growth showed that CD151-negative cells form bigger colonies than wild-type line. Stimulation with FGF2 enhanced the increase in colony size and number and this effect was abolished by RSK2 kinase inhibitors (FMK and BI-D1870).

Our results for the first time show an association between CD151, FGFR2 and RSK2 in the mammary epithelium. We propose that tetraspanin CD151 regulates expression of FGFR2 which then activates RSK2 kinase through p38. RSK2 activation promotes anchorage-independent growth and formation of focal adhesions. Cross-talk between CD151, FGFR2 and RSK2 is likely to play a significant role in breast cancer development or/and progression.

## P6.4

### On the verge of the cell death: a cross-talk between PI3K, Akt, mTOR and P53 signaling pathways in GD2 ganglioside — targeted neuroblastoma

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Better understanding of tumor-relevant signaling pathways led to cancer treatment being shifted from conventional cytotoxic drugs to target-based agents. We aimed to study PI3K/Akt/mTOR and P53 signaling routes, being aberrantly regulated in neuroblastoma (NB) and altered upon GD2 ganglioside-targeted treatment with the 14G2a monoclonal antibody (mAb). PI3K/Akt/mTOR cascade is involved in oncogenesis and NB cancer progression [1]. The Akt kinase lies at the center of the pathway that promotes cancer cell survival and protects cell from apoptosis. Pathological activation of Akt occurs frequently in NB and correlates with poor prognosis [2]. Furthermore, Akt regulates cell growth through its effects on mTOR pathway including its p70S6K and 4EBP1 downstream effectors [3]. While mTOR pathway is one of the pro-survival signaling cascades, P53 pathway plays a crucial role in NB cell death [4]. Therefore, finding therapeutics that drive NB apoptotic fate after all is a new challenge in the coming decade.

In this study we performed profiling of phosphorylated proteins in the mAb-treated IMR-32 cells (as compared to untreated cells) by proteomic arrays. These results were confirmed by immunoblotting analysis of the expression and phosphorylation status of PI3K and Akt kinases in IMR-32, LA-N-1 and CHP-134 neuroblastoma cell lines for which cytotoxic effects of the mAb were observed. We found that phosphorylation of Akt was the most significantly decreased in IMR-32 cell line, especially 24 and 48 h after the mAb treatment, possibly as a consequence of simultaneous upregulation of phosphorylated form of PTEN phosphatase (Akt negative regulator). Inhibitory effects of the mAb were also noted for phosphorylated and unphosphorylated forms of mTOR and its downstream effectors: p70S6K and 4EBP1 in IMR-32 cells. We showed that in the mAb-mediated cytotoxicity toward IMR-32 and CHP-134 cells (both expressing wt P53) the statistically significant inhibitory effect on Akt/mTOR cascade was correlated with upregulation of P53 protein and no such correlation was found for the mAb-unresponsive BE(2)-C cells with mutated P53.

Combining of the mAb with PI3K kinase inhibitor (LY294002) significantly decreased the viability of IMR-32 and LA-N-1 cells. Dramatic reduction in cell survival as assessed by measuring cellular ATP content appears to arise from PI3K inhibition and already high cytotoxic effect induced by the mAb. Among three compared NB cell lines, we found the weakest effect of LY294002 inhibitor on BE(2)-C, shown to be mAb-insensitive.

Taken together, our findings suggest that the dominance of signals such as upregulation of proapoptotic P53 protein and inhibition of pro-survival PI3K/Akt/mTOR pathway in GD2-targeted IMR-32 cells may promote the commitment to apoptotic cell death. The results of these studies may contribute to designing of molecular therapies based on drugs targeting GD2 along with PI3K/Akt/mTOR pathway and improving the poor prognosis of NB patients.

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## P6.5

### Evaluation of the impact of nitroxides on proapoptotic activity of anticancer drugs in human breast cancer cells

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Doxorubicin (DOX) and taxanes (paclitaxel — PTX and docetaxel — DTX) have been recognized as efficient anticancer drugs, particularly in the treatment of breast cancer. Chemotherapy with their use, however, causes serious side effects such as cardiotoxicity, nephrotoxicity, neurotoxicity. Thus, there is a need for compounds which inclusion to chemotherapy could reduce systemic toxicity of cytostatic drugs without compromising their anticancer activity. Synthetic and nontoxic five-membered pyrroline and pyrrolidine derivatives could exhibit such activity.

The aim of the study was to evaluate the impact of nitroxides Pirolin (PL) and Pirolid (PD) on proapoptotic activity of doxorubicin and taxanes in estrogen-responsive breast cancer cells. The cells (MCF-7) were incubated for 1 h with PL or PD (50  $\mu$ M), and then for 2 h with IC<sub>50</sub> concentration of DOX (3.0 mM), PTX (0.4 mM) or DTX (0.5 mM). The drugs were used alone or in combination doxorubicin-taxane. For a drug effect only 2 h incubation was applied. Analogically for the effect of a nitroxide as a single agent 3 h incubation with was used. The percentage of live, apoptotic and necrotic cells was determined by flow cytometry at 0 h time point (in the end of incubation) and after 24 and 48 h post-treatment cell growth in a fresh medium, using FITC Annexin Apoptosis Detection Kit.

A progressive and time-dependent increase of the fraction of necrotic and apoptotic cells in analyzed samples was observed. Taxanes exhibited lesser proapoptotic activity than doxorubicin and did not hamper the proapoptotic properties of anthracycline. Neither PL nor PD affected apoptosis of MCF-7 cells induced by anticancer drugs. These results show that the investigated nitroxyl derivatives do not impede anticancer activity of doxorubicin and taxanes in human estrogen-responsive breast cancer cells and could be considered as good candidates for antioxidant protection against oxidative stress induced by chemotherapy in normal cells with impaired antioxidant defense, such as cardiomyocytes.

#### References:

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## P6.6

### Diallyltrisulfide and curcumin have a strong inhibitory effect on proliferation and survival of neuroblastoma and glioblastoma cell lines

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Cancer cell growth is of an utmost concern especially in the brain, where options for invasive treatments like surgical procedures are very limited. Many natural compounds including diallyltrisulfide (DATS) and curcumin are tested for their anti-cancer properties. Two cell lines, SH-SY5Y and U87MG derived from human neuroblastoma and glioblastoma cancer cells respectively were treated with either DATS or curcumin. Both compounds have strong impact on proliferation and survival of both cancer cell lines. DATS is a precursor of sulfane sulfur while curcumin is an inhibitor of thioredoxin reductase. To determine if the anticancer attributes of the compounds are connected with the metabolism of sulfane sulfur in cells, the level of sulfane sulfur, activity of  $\gamma$ -cystathionase (CST), rhodanase, 3-mercaptopyruvate sulfurtransferase (MPST) and expression of their genes were determined. Levels of the cysteine, cystine and reduced and oxidized glutathione were also measured to establish the impact of both compounds on the redox potential of the cells. Results will help to understand the mechanism of a protective role of DATS and curcumin in cancerogenesis and may contribute to the development of novel strategies in brain cancer treatment.

## P6.7

### Interactions of aromatic ligands with DNA in the presence of pentoxifylline

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Aromatic ligands are a prominent group of xenobiotics. Their mechanisms of action can be very diverse, including direct interactions with DNA (i.a. intercalation). This mode of action is observed for aromatic mutagens as well as anticancer drugs used in chemotherapy, so it can be said that the same mechanism is involved in both cancer emergence and its treatment. On the other hand, there is a large group of biologically active aromatic compounds, i.a. methylxanthines (MTX), which are shown to modulate direct interactions of aromatic ligands with DNA. Mechanism of this modulation is still disputed. One of postulated hypotheses suggests that this mechanism involves formation of mixed stacking aggregates between aromatic ligand and modulating agent. This effect may reduce the concentration of ligand in biologically active, free form. Additionally, one of MTX, pentoxifylline (PTX) is reported to exhibit anticancer activity itself. It allows one to speculate that administration of anticancer drugs together with PTX may reduce adverse effects of the therapy by reducing local concentration of drug free form.

This work presents analysis of interactions between aromatic ligands (ICR-191 — model aromatic mutagen/carcinogen, and mitoxantrone MIT — anticancer drug) and DNA in the presence of modulating agent — PTX. UV/Vis spectroscopy studies and calculations based on diverse statistical-thermodynamical models were accomplished to show interactions in the mixtures of either MIT or ICR191, DNA and PTX. Additionally, in order to investigate biological effects of interactions observed for calf thymus DNA, eukaryotic cells chromatin assay was conducted. Finally, mutagenic activity of ICR-191 in the presence of MTX was analyzed *in vivo* with bacterial mutagenicity assay (Ames test).

## P6.8

### Antitumor activity of VPA against larynx cancer cells *in vitro*

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Valproic acid (VPA) is widely used in neurological diseases, including epilepsy and seizures, bipolar disorders, migraine, clinical depression and schizophrenia. Recently, this drug was classified as histone deacetylase inhibitor (HDI), which possesses activity against hematological and solid tumors. It was showed that VPA induces differentiation and inhibits proliferation and angiogenesis of tumor cells.

The aim of our study was to investigate if VPA can also influence on proliferation of larynx cancer cells. Furthermore, we determined the molecular mechanisms that may be related to VPA action.

The viability and proliferation of larynx cancer cells (RK33 and RK45) were analyzed by MIT and BrdU test, respectively. Apoptosis was evaluated by the measurement of mono- and oligonucleosomes released to cytosol by apoptotic cells. Flow cytometry (FACS) and real-time PCR methods were applied to analyse cell cycle progression and expression of cell cycle-related genes, respectively.

Our studies showed that VPA inhibited viability/proliferation of RK33 and RK45 in a dose-dependent manner. FACS analysis revealed that VPA inhibited cell cycle progression at G1/S phase, accompanied by changes of genes expression, including up-regulation of p21<sup>waf1/cip1</sup> and down-regulation of cyclin D1 encoding genes. We also showed that VPA induced apoptosis in both larynx cancer cell lines.

Since valproic acid is well-tolerated drug, it could be considered as potential agent, alone or in combination with other chemotherapeutics, in larynx cancer treatment.

## P6.9

### Valproic acid as a potential therapeutic agent for lung cancer cells

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Valproic acid (VPA) belongs to a new generation of agents known as histone deacetylase inhibitors (HDIs) that are promising group of anti-cancer compounds. Mechanism of biological activity of HDIs is not fully examined. Clinical trials revealed that HDIs, either alone or in combination with other chemotherapeutics, have low toxicity against normal cells.

The purpose of this study was to investigate effects of valproic acid on proliferation, cell cycle progression and apoptosis in lung cancer cell lines.

The experiments were carried out on different types of lung cancer cell lines (A549 - non-small lung cancer), CRL5875 (adenocarcinoma lung cancer) and CRL5928 (squamous cell lung cancer). The viability/proliferation of cancer cells were analyzed by MTT test and BrdU test, respectively. Cell cycle analysis and apoptosis was performed by flow cytometric techniques.

Our studies showed that VPA significantly decreased viability/proliferation of lung cancer cells in a dose dependent manner. In all studied cancer cell lines increasing concentrations of VPA induced apoptosis and cell cycle arrest in G1/S phase.

Our findings indicate that valproic acid could be regarded as potential agent in lung cancer treatment.

## P6.10

### Evaluation of biological activity of ferrocenyl derivatives in human liver cancer cells

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Cancer still remains the second most common cause of death all over the world despite a significant progress in the diagnosis and the treatment over the past decades. Thus, there is a continuously searching for new compounds with a high specificity to cancer cells and low cytotoxicity against normal cells. One of the most promising compounds in this field are iron containing derivatives ferrocenes – bioorganometallic agents which conjugates are of great importance for medicinal chemistry. It has been suggested that the cytotoxic properties of ferrocenes toward cancer cells might be related to their capability to generate extensive intracellular oxidative stress.

The aim of this study was to investigate whether ferrocene derivatives 15, 15Cl and 18Cl, designed and synthesized in our laboratory, can generate reactive oxygen species (mainly superoxide radical ( $O_2^{\bullet-}$ ) in HepG2 human liver cancer cells that could contribute to the anticancer activity of these compounds. Excessive production of  $O_2^{\bullet-}$  can result in tissue damage, which often involves the generation of highly reactive hydroxyl radical ( $^{\bullet}OH$ ) and other oxidants in the presence of catalytic iron or copper ions. The superoxide indicator dihydroethidium (DHET) was used to assess ROS generation. In cell cytoplasm oxidation-sensitive fluorescence probe dihydroethidium DHET displays blue fluorescence. After oxidation to ethidium, which intercalates DNA and stains the cell nucleus, the fluorescence of the probe switches to the bright red color. Measurement of intensity of both blue and red fluorescences in time enables assessment of the kinetics of ROS production in treated cells. HepG2 cells were exposed to 20, 60 and 100  $\mu M$  of 15, 15Cl and 18Cl. After 0.5, 3 and 6 h of exposure, fluorescence was measured over 0-180 min period.

The results showed that none of investigated ferrocenes caused a statistically significant increase in the level of superoxide oxygen radical ( $O_2^{\bullet-}$ ) in HepG2 line, regardless of the exposure time and the concentration of ferrocene conjugates. This confirms the results by other authors demonstrating that ferrocene exhibit a much larger share in the production of the nonradical hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radicals ( $^{\bullet}OH$ ) (Fenton chemistry).

## P6.11

### Combined treatment with quercetin and imperatorin as a potent strategy for killing T98G cells

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Glioblastoma multiforme (GBM), classified by WHO as a IV grade tumor, represents the most common primary malignant brain tumor. Despite the available multimodality treatment, no effective therapy for it has been developed to date. In population-based studies, most patients diagnosed with GBM survive less than a year despite intensive treatment including surgical resection, radiation, and chemotherapy. Like most cancers, human brain tumors exhibit an unusual level of Hsp27 and Hsp72, implicated in cell proliferation, which leads to chemoresistance and decrease sensitivity of cancer cells to programmed cell death induction.

One of the well known Hsps inhibitor is quercetin (3,3',4',5'-7-pentahydroxyflavone), present in daily diet, characterized by strong antioxidant, anti-inflammatory, and antiproliferative properties. In recent years, quercetin has attracted special attention as a potential anticancer agent inducing apoptosis in numerous types of cancer.

Imperatorin (8-isopentenylxyporalen), a major active furanocoumarin isolated from the root of *Angelica officinalis*, has been reported to possess a wide range of biological activities including antiinflammatory, anticoagulant, and photosensitizing properties. Recent reports have shown pharmacological actions of imperatorin against cancer including oncogene suppression, inhibition of proliferation and induction of apoptosis in various cancers.

Therefore, the aim of our study was to assess the effect of quercetin and imperatorin applied separately or in combinations on programmed cell death induction (apoptosis, autophagy) in the human glioma T98G cell line cultured *in vitro*. In order to determine the molecular mechanism by which apoptosis and autophagy take place in T98G cells, we investigated the expression of marker proteins involved in these processes at the molecular level such as proapoptotic caspases, antiapoptotic Hsp27 and Hsp72, proautophagic beclin-1.

Apoptosis and autophagy were identified using specific fluorochromes (Hoechst 33342 and acridine orange respectively) while the level of proteins expression was measured by western blotting technique. The specific siRNA transfected method was used for blocking of the expression of Hsp27 and Hsp72 genes. Our experiments revealed that quercetin and imperatorin administered simultaneously induced apoptosis more effectively than incubation with single drugs. These results were accompanied with decreased Hsp27 and Hsp72 expression and increased activity of caspases. Autophagy was not observed. Additional blocking of the expression of heat shock proteins Hsp27 and Hsp72 with specific siRNA in T98G cells significantly increased the sensitivity to apoptosis induction upon quercetin and imperatorin treatment.

## P6.12

### Tumor cell lysate vaccine in combination with IL-12 gene therapy inhibits growth of murine melanoma

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Some anticancer drugs kill cancer cells by inducing necrosis. Dying 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 CAMEL-tumor cell lysate vaccine. It has been reported that CAMEL peptide causes mitochondrial swelling and disrupts mitochondrial membrane. Disrupted mitochondrial membrane is followed by a decrease in intracellular ATP and this leads to cell death. CAMEL-treated tumor cell lysates were used to immunize mice that had been challenged with B16-F10 melanoma cells. Our results show that the investigated tumor cell vaccine inhibited tumor growth, both in the prophylactic and therapeutic settings. To still improve the achieved therapeutic effect of CAMEL-tumor cell lysate 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. Our preliminary data suggest that combination therapy involving CAMEL-tumor cell lysate vaccine and IL-12 gene therapy significantly inhibited tumor growth, as compared to mice receiving single-agent therapy. Combination of tumor cell lysate vaccine with IL-12 increased total number of cells present in cervical lymph nodes. The number of CD4+ and CD8+ T cells in lymph nodes from combination-treated mice was augmented as compared to control group or mice treated with either tumor cell lysate or IL-12. This allows us to conclude that combination of CAMEL-tumor cell lysate vaccine and the interleukin-12 gene seems to be a promising approach to eliminate tumors.

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## P6.13

### Effect of GLUT1 down-regulation on the viability of thyroid cancer cells

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Cancer cells are characterized by rapid proliferation and require adaptive metabolic changes to allow continued biosynthesis and cell growth in the setting of hypoxia and low nutrient availability. Tumor cells accelerate glycolysis and glucose uptake in order to compensate the inefficient production of energy from glucose. The enhancement of glucose transport across the plasma membrane is mediated by a family of facilitative glucose transporter proteins named GLUT. In particular, an increased expression of hypoxia-related GLUT1 has been frequently found in a variety of malignancies. Many studies have reported correlation between GLUT1 expression level and aggressive tumor behavior. Therefore, the aim of this study was to determine the association between GLUT1 expression level and viability of follicular (FTC-133) and anaplastic (8305C) thyroid cancer cell lines. Cells were cultured in medium with different glucose concentration (2, 5, 25 mM/l) under normoxia and hypoxia conditions. Hypoxia has been induced by exposure cells to cobalt chloride, a chemical hypoxia mimicking agent. Down-regulation of GLUT1 has been achieved by using specific small interfering RNAs (RNAi) duplexes that target the coding region of human GLUT1 mRNA sequences. The effect of RNAi was assessed by real-time PCR assay and Western blot. Pre-designed siRNAs for GLUT1 effectively reduced the mRNA and subsequently protein level in FTC-133 and 8305C cells. Proliferation and viability of cells has been measured by tetrazolium salt-based colorimetric assay for evaluation of metabolic activity.

The results indicate that GLUT1 expression level in both thyroid cancer cells inversely correlates with glucose availability. We shown significant increase of GLUT1 after hypoxia mimicking agent treatment, however expression of hypoxia-inducible factor 1  $\alpha$  decreased in low glucose medium. Although RNAi caused a significant decrease in GLUT1 protein level, we observed only slightly impact on cells viability. Thus, our study suggest that GLUT1 is involved in the uptake of glucose by thyroid cancer cells, however other glucose transporters may also play significant role in thyroid malignances.

## P6.14

### Investigation of reciprocal influence of renal cancer cell lines and non-malignant cells on cell proliferation and migration rate

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In carcinogenesis and cancer spread, the tumor microenvironment (TME) determines the underlying processes. Hallmarks of cancer, such as constantly activated proliferative signaling, inhibition of growth suppressors and apoptosis, activating invasion and metastasis, deregulation of cell energetics and abrogation of immune destruction are mostly regulated by TME, its cells and factors secreted by these cells. Hence, this study is focused on changes in proliferation and migration rate which are caused by reciprocal influence of cancerous cells and non-malignant ones. Thus, renal cancer cell lines (786-O; Caki-2, RCC6) were incubated with different concentration of conditioned media (10%, 30%, 50%) from healthy lung epithelial NL20 (ATCC® CRL-2503™) and mesothelial cells (Met5a). Healthy cells were incubated with different concentration of conditioned media (10%, 30%, 50%) from cancerous lines as well. Alamar Blue and MTT assays were used for investigation of proliferation rate of the examined cells. Wound Healing assay was used for evaluation of invasion and migration rate. Obtained results do not give conclusive results. Although, in comparison to control (cells cultured without conditioned media) same cell line exhibit slight differences in proliferation and migration rates. Planned studies need to be further investigated.

## P6.15

### EGFR alterations in brain tumors and human cancer cell lines

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Alterations of the epidermal growth factor receptor (EGFR) gene occur frequently in human malignant neoplasms. The most common of these alterations is deletion of the extracellular domain fragment (exons 2-7) that results in the expression of truncated receptor (EGFRvIII). EGFRvIII is responsible for constant signal transduction, despite it cannot bind the EGFR ligands. EGFRvIII expression results in enhanced tumorigenicity, decreased apoptosis and enhanced resistance to radio- and chemotherapy of the tumor cells. Therefore, this EGFR alteration may be considered as an important prognostic factor in some types of cancer.

We examined qualitative EGFRvIII expression at the mRNA level in 88 tumor samples resected from patients with diagnosed tumors of central nervous system (75 primary brain tumors and 13 metastases) and in four human cell lines (TE671 rhabdomyosarcoma, MOG-G-CCM astrocytoma, T98G glioblastoma and SK-N-AS neuroblastoma). EGFRvIII expression was observed in 6 of 34 glioblastoma multiforme cases (9.38%), 1 of 3 of anaplastic astrocytomas, 1 of 4 oligodendrogliomas. One analyzed tumor sample classified as malignant lymphoma showed EGFRvIII expression. 17.95% of astrocytomas showed EGFRvIII expression (7 of 39 analysed tumors). Expression of both wild type EGFR and EGFRvIII was shown in 44.4% tumor samples expressing EGFRvIII. Wild type EGFR and EGFRvIII were expressed in 2 glioblastomas GIV, one oligodendroglioma GIII and one malignant lymphoma tumors. All of analyzed tumor cell lines expressed EGFRvIII. Meningiomas (N=23) GI and GII did not express EGFRvIII variant.

Our findings suggest that EGFRvIII and/or wild type EGFR expression may be considered as a molecular marker of primary malignant brain tumors.

## P6.16

### Expression level changes of *GRHL* genes in human non-melanoma skin cancers

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Grainyhead-like proteins (GRHL) constitute a highly conserved family of transcription factors whose structures have been conserved in the course of evolution of multicellular organisms. In mammals there are three *GRHL* genes present on different chromosomes, which are expressed in a tissue- and spatio-temporally-specific fashion. The GRHL factors are critical for development and homeostasis of the surface epithelium. Many of their target genes (like E-cadherin, desmoglein 1, PTEN, hTERT, PCNA and others) were previously implicated in carcinogenesis. Based on literature data and our preliminary results we hypothesized that reduced expression of the *GRHL* genes may increase susceptibility to epidermal carcinogenesis. Preliminary studies from our laboratory have shown that reduced *Grhl1* expression in mice increases the incidence of DMBA/TPA-induced non-melanoma skin cancers (NMSC). It has also been demonstrated that *Grhl3* knock-out mice are more susceptible to skin lesions upon chemical carcinogenesis. Links to carcinogenesis for *Grhl2* have been shown in *in-vitro* studies.

In human, reduced level of *GRHL3* expression (by 90% in over half of the samples studied) was observed in head and neck NMSC. The aim of our research is to investigate whether various types of human skin cancers derived from epidermal cells are accompanied by changes in the expression levels of *GRHL* genes and to establish the causes of these changes in the genetic and epigenetic context. We collected NMSC samples as well as control healthy tissue samples from 35 Polish patients (in collaboration with The M. Skłodowska-Curie Memorial Cancer Center, Institute of Oncology in Warsaw). In this group of patients we observed downregulation of *GRHL1* and *GRHL3*. Moreover, downregulation of these genes was significantly correlated. To explain changes in *GRHLs* expression levels we decided to search for: specific point mutations, loss of heterozygosity and copy number variation, changes of methylation profile in regulatory sequences, miRNAs specifically regulating *GRHLs* expression. Global changes in transcriptomes of different NMSCs with different *GRHLs* expression are also studied. To detect and identify *GRHL* gene disruptions in skin cancers, we use: New Generation Sequencing, DNA-methylation analysis, Human Gene Expression Microarrays, Lentiviral-based systems with miRNAs. Our findings will provide new molecular insights into the links between the *GRHL* genes and epidermal neoplasia in the human context.

## P6.17

### Impact of the opioid peptides on the breast cancer progression

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**Introduction:** Breast cancer is the most common female cancer. Current knowledge of the characteristics of this type of cancer showed that the risk of breast cancer is much lower among women who breastfed. Suitable nutrition and lifestyle can prevent cancer, but it also plays a big role in succour treatment.

Particularly noteworthy is the  $\beta$ -casomorphin and casoxin released from the amino acid sequences of proteins in milk and dairy products. They can potentially play a major role in carcinogenesis, as released opioid peptides penetrate into the breast milk during lactation. Given the protective effect of breast-feeding, it is appropriate to determine the effect of opioid peptides on the process of carcinogenesis.

**Aim of the research:** The essence of the experiment was to determine the impact of  $\beta$ -kazomorphin, casoxin and hybrid compounds on the proliferation of cancer cells, expression of  $\mu$ -opioid receptor (MOR) and the level of cytokines secretion into the culture medium.

**Material and methods:** The project has used a cell model of human breast cancer with a standardized MCF-7 cancer cell line. It is important that the selected cell line has the  $\mu$ -opioid receptor, by which opioid peptides affect the human body.

**Results:** The results suggest that the presence of opioid peptides causes a significant change of tumor cell proliferation.  $\beta$ -casomorphin antagonist of  $\mu$ -opioid receptor causes a marked increase in the proliferation of tumor cells. Adding a culture casoxin antagonist of  $\mu$ -opioid receptor inhibits cell growth. The largest inhibitory effect produces a compound hybrid agonist and antagonist. However gene expression of  $\mu$ -opioid receptor was reduced in all the cases studied. Results of the study complement the knowledge on the effects of opioid peptides in the etiology of breast cancer processes.

## P6.18

### Translocated FGF1 and FGF2 inhibit apoptosis

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Fibroblast growth factor 1 and 2 (FGF1 and FGF2) were found to be overexpressed in some tumors and potentially contribute to self-stimulation and survival of cancer cells. Apart from the classic activation pathway via cell-surface receptors, FGF1 and FGF2 have a unique property of receptor-dependent translocation from extracellular space to the cell cytosol and nucleus. The role of both growth factors' intracellular trafficking remains unclear.

We identified new intracellular FGF1- and FGF2-binding proteins which are known to be involved in apoptosis process (p53, sirtuin 1, CDK4, nucleophosmin and UACA) suggesting that the growth factors may affect cell survival. To examine the potential role of intracellular FGF1 and FGF2 in apoptosis we tested the effect of exogenously added recombinant FGF1 and FGF2 on NIH 3T3 cells in different stress conditions. Upon apoptosis induction by different factors (such as serum starvation, staurosporine, NSC348884 and tenovin-6) we observed decreased caspase-3/7 activity and increased cell viability in the presence of FGF1 or FGF2. To eliminate the effect of receptor-based signaling we performed experiments with specific inhibitor of receptor kinase activity (PD 173074). In control experiments we used an efficient inhibitor of FGF1 and FGF2 translocation (bafilomycin A1) and observed no differences in apoptosis level in the presence and absence of the growth factors. Similarly to results obtained for NIH 3T3 cells, we observed reduced caspase activity in staurosporine-treated HEK 293 cells transiently transfected with FGF1 or FGF2 in comparison to control (non-transfected) cells. Altogether our results suggest that the role of translocated FGFs is protection of cells against apoptosis and promotion of cell survival.

## P6.19

New synthetic betulin derivatives elicit anti-tumour activities *in vitro*Sylwia Katarzyna Król<sup>1</sup>, Magdalena Dmoszyńska-Graniczka<sup>1</sup>, Andrzej Stepulak<sup>1,2</sup><sup>1</sup>Medical University of Lublin, The Chair and Department of Biochemistry and Molecular Biology, Lublin, Poland; <sup>2</sup>MSW Hospital, Department of Otolaryngology, Lublin, Poland  
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Betulin (BE), a pentacyclic triterpene has been shown to demonstrate interesting anti-cancer potential towards numerous human cancer cells *in vitro*. Thus, BE has also been attempted to apply as a precursor in the synthesis of new BE derivatives (SBD), as the novel candidates for chemotherapy of several tumours.

In this study, neural (human neuroblastoma SK-N-AS, human rhabdomyosarcoma/medulloblastoma TE671) and glial (human glioblastoma multiforme T98G, rat glioblastoma C6) tumour cells were treated with SBD and evaluated for viability, proliferation and cell cycle progression.

SBD decreased significantly the cells viability and inhibited cell proliferation in a dose- and time-dependent manner. Neuroblastoma cells (SK-N-AS) were the most sensitive to the anti-proliferative and cytotoxic effect of SBD.

Although detailed mechanisms responsible for chemopreventive and anti-tumour potential of SBD still require further investigations, our preliminary *in vitro* results indicated that SBD could be considered as novel chemopreventive and treatment agents for neural and glial tumours.

## P6.20

## Diagnostic potential of JARID1B expression in differentiating between early melanomas and benign human skin lesions

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Differential diagnosis of early melanomas and naevi may be difficult. Numerous studies demonstrated divergent diagnoses reported by different experts and based on examinations of architectural and cytological features of melanocytic skin lesions (for instance [1]). The histone demethylase JARID1B (Jumonji AT-Rich Interactive Domain 1B) plays important role in regulating processes of cellular proliferation and differentiation and its expression was demonstrated in subpopulations of melanoma cells required for a continuous growth of the tumours [2]. Our recent study indicated that human melanomas begin to express the protein early in the disease progression [3].

The present study aimed at assessment of usefulness of JARID1B expression for differentiating between melanomas and benign melanocytic lesions. The diagnostic algorithm was the same as used in our earlier studies [4, 5].

JARID1B was detected immunohistochemically in formalin-fixed paraffin-embedded samples of 30 benign naevi (including 11 dysplastic, Spitz and Reed) and 27 primary melanomas (including 16 early lesions — I and II Clark's infiltration levels). Diagnostic performance of the test was assessed using the ROC (receiver operating characteristics) analysis.

Expression of the JARID1B protein was in melanomas significantly stronger compared to naevi both in central ( $P \approx 10^{-17}$ ) and peripheral ( $P \approx 10^{-16}$ ) regions of the lesions. The differences were significant also for a group of early melanomas (I and II Clark's level) and all the naevi ( $P \approx 10^{-10}$  and  $P \approx 10^{-11}$  for central and border regions, respectively). The diagnostic test based on a determination of percentage fractions of the JARID1B-positive cells allowed for differentiation of melanomas and naevi with high sensitivity and specificity as shown by areas under ROC curves (AUC=0.981±0.019 — center and 0.979±0.020 — periphery of the lesions investigated) and importantly its performance was good even for the early melanomas (AUC=0.975±0.028 — center and 0.975±0.028 — periphery).

We suggest that the JARID1B protein may serve as a useful marker in aiding the differential diagnosis of benign naevi and melanomas even at early stages of a progression of the latter.

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## P6.21

### Carbohydrate metabolism in the rat liver-tumor effects in derivatives L-glutamine and L-phenylalanine

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Tumor development is accompanied by changes in the body of biochemical indices indicating its systemic action on almost all tissues of the body. Therefore, relevant studies are aimed at finding compounds that reduce this effect on normal tissues and organs. It is known that glutamine is included in numerous reactions play a key role in the relationship of the carbohydrate, protein and other parts of metabolism. In this connection the research of metabolic status of an organism when administered to a tumor-derived L-glutamine and L-phenylalanine is of an interest.

The purpose is to study the state of carbohydrate metabolism in the liver of rats with tumors PC-1 to identify possible relationships correction tumor - an organism.

Experiments were conducted in rats with tumors RS-1, which 10 to 19 day administered intragastrically derivatives L-glutamine and L-phenylalanine (equimolar doses): L-glutamine aspartilglutamin, AS2-1, AS2-5 and the mixture was 70% phenylacetate and 30% phenylacetylglutamine — composition 1. The animals were decapitated on the 20th day. In liver homogenates were determined activity of hexokinase (HK), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PD) and lactate and glucose.

HA activity was found to decrease in tumor-bearing rat liver RS-1 when administered L-glutamine, AS2-1, AS2-5 Composition 1. LDH (pyruvate-utilizing) significantly reduced when administered L-glutamine and AS2-5. LDH activity (lactate-utilizing) increased with the introduction of AS2-1 and composition 1. G6PD activity — a key enzyme in the pentose phosphate cycle — decreased rat liver tumor-RS-1, AS2-1-treated and the composition 1. Introduction of aspartilglutamin, AS2-5, AS2-1 and composition 1 has led to increased levels of free glucose. Lactic acid content in the liver decreased after application of AS2-5 compositions 1 and AS2-1. These research findings show that in rat liver tumor-RS-1 when administered L-glutamine derivatives, and L-phenylalanine reduced the activity of glycolysis reactions and pentose cycle lactate and also reduced operating time increases the concentration of free glucose in the liver. These changes may contribute to the restoration of impaired energy metabolism in normal tissues of the body and reduce tension compensatory mechanisms that support the consistency of carbohydrate metabolism in tumor-bearing.

## P6.22

### Nuclear maspin inhibits proliferation of breast cancer cells

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Maspin belongs to serine protease inhibitors superfamily and it is classified as class II tumor suppressor [1]. Maspin expression is downregulated in many types of cancer during cancerogenesis, but its function is still not well known. The studies on maspin which have been published so far by others based on model with maspin located in cytoplasm while the subcellular localization of maspin in cancer may be cytoplasmic, nuclear or mixed. Nuclear location of maspin in cancer cells seems to be beneficial for patients survival [2].

Our clinical data obtained by analyses of archived breast cancer samples showed the relationship between maspin localization and protein level with Ki-67 expression and patients survivability. The aim of this study was to analyze the influence of maspin localization on both normal epithelial cells derived from mammary gland and breast cancer cells. We constructed the model system enabling analysis the effect of different maspin localization on cell proliferation. Constructs coding eGFP (control), maspin-eGFP and maspin-NLS-eGFP were transfected into breast cancer cells and normal epithelial cells from breast with targeted cytoplasmic and nuclear location of maspin. We analyzed the effect of maspin location in normal epithelial cell line MCF10A and three breast cancer cell lines — MCF-7, MDA-MB-231 and SKBR-3. The strong correlation between nuclear maspin and decreased in cells number expressing Ki-67 protein in breast cancer cell lines was observed while there was no effect in normal epithelial cells from mammary gland. This effect on cell proliferation of nuclear maspin on breast cancer cells was statistically significant in comparison to cytoplasmic maspin. These data may provide a new insight into mechanisms of cancer development and show maspin significance in cancer progression and potential usage as a prognostic factor.

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## P6.23

### Synthetic resveratrol derivative, 3,3',4,4',5,5'-hexahydroxy-trans-stilbene, accelerates senescence of mesothelial cells and promotes senescent HPMC-dependent growth of colorectal and pancreatic cancers

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3,3',4,4',5,5'-hexahydroxy-*trans*-stilbene (M8) is a synthetic analogue of resveratrol (RVT), highly appreciated for its anti-cancer activity. It has been suggested that several biological properties of M8, e.g. its antioxidative capacity, are much more pronounced than in RVT. Because we have recently observed that RVT delays senescence in human peritoneal mesothelial cells (HPMCs) *in vitro*, in this project we wanted to find out if whether M8 may act on HPMC growth stronger than its natural precursor. To this end, we examined a wide range of parameters associated with cell growth and senescence in primary cultures of omental HPMCs derived from different donors (n=8–12), simultaneously exposed to RVT and M8 at 0.5 and 10 mM.

The results showed that 0.5 mM M8, in contrast to RVT, did not improve replicative lifespan of HPMCs. At the same time, 10 mM of M8 reduced cell growth potential (decreased PCNA level) and prematurely induced cell growth arrest. This was accompanied by increased activity of senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -Gal) and enhanced oxidative DNA damage (8-OH-dG). The senescence-promoting activity of M8 could be related to remarkable induction of ROS release by early-passage cells which was followed, in contrast to RVT, by decreased activity of superoxide dismutase (SOD). In addition, 10 mM M8 increased the percentage of apoptotic cells in late-passage cultures which could also play a role in declined reproducibility of HPMCs *in vitro*. Interestingly, we found that soluble factors released to environment by HPMCs that senesced prematurely under M8 stimulated proliferation of colorectal and pancreatic carcinomas *in vitro*.

Altogether, our results suggest that the pro-senescence effect of M8 towards HPMCs and senescent HPMCs-dependent growth-promoting activity of M8 towards colorectal and pancreatic cancers may strongly affect clinical usefulness of this stilbene, even despite its direct anti-proliferative and pro-apoptotic activity towards different kinds of cancer cells.

#### References:

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## P6.24

### Tetraspanin CD151 is involved in control of stem cell-like phenotype in mammary epithelial cells

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Breast cancer (BCa) is the leading cause of women death worldwide. Human breast ductal carcinoma originates from epithelial hyperproliferation, evolves into *in situ* (DCIS) and invasive carcinomas (IDC), to finally become a metastatic disease. DCIS is a highly heterogeneous precursor of IDC. Recent studies have implicated a number of genes in development of DCIS but so far, there are no markers which would reliably identify the initial steps of its progression into invasive disease.

Increasing evidence suggests that tetraspanin CD151, one of the best characterized members of the transmembrane protein family, is involved in invasiveness of human cancers. Up-regulation of CD151 expression correlates with poor prognosis and shorter overall survival of breast cancer patients. Recently, a crucial role of CD151 in DCIS was demonstrated both *in vitro* and *in vivo*. It was found that this tetraspanin promoted intraductal proliferation of mammary epithelial cells. Conversely, depletion of CD151 suppressed neoplastic cell growth and contributed to lumen formation. Such changes were observed in mammary epithelial HB2 cell line developing *in vivo* DCIS-like lesions. CD44+/CD24- phenotype is thought to characterize a subpopulation enriched in cancer stem cells in BCa. The aim of the study was to establish a potential relationship between CD151 and induction of stem cell characteristics in HB2 cell line, using flow cytometry and real time quantitative PCR analyses. We demonstrated that: 1) wild type (HB2/CD151+) cells were CD24+/CD44+; 2) knocking down of CD151 significantly decreased expression of CD24 without affecting level of CD44 expression; 3) CD151 inhibited expression of Slug and Sox9 – transcription factors associated with cell stemness. Therefore we conclude that CD151 is involved in governing of stem cell-like phenotype which might have implication for breast cancer progression.

## P6.25

### The role of *HOXA4*, *HOXA5* and *MEIS1* promoter DNA methylation and expression in acute myeloid leukemia patients

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*HOX* genes encode transcription factors (TFs) crucial for embryogenesis and tissues' differentiation. Members of *HOXA* cluster play a role in early stages of hematopoiesis. Aberrations in *HOXA* and their cofactor *MEIS1* are found in human neoplasms, including acute myeloid leukemia (AML).

The role of *HOXA4*, *HOXA5* and *MEIS1* promoter DNA methylation and mRNA expression in AML was assessed in this study.

Study involved 78 AML patients and 12 normal bone marrow (BM) samples. DNA from Ficoll-isolated mononuclear cells was bisulfite converted and DNA methylation level of promoter regions was determined by quantitative methylation specific PCR (qMSP). Relative expression level was measured using qRT-PCR.

39.4% and 27.5% of patients revealed high methylation level of *HOXA4* and *HOXA5*, respectively, compared to control samples. Correlation between these genes' methylation levels was found ( $R=0.3019$ ,  $p=0.008$ ). *MEIS1* promoter was unmethylated.

Association between promoter methylation and low genes' expression levels was not observed within entire group of patients. An inverse correlation of *HOXA4* methylation and expression was found in a group of patients with normal karyotype (NK AML) (Spearman correlation  $R=-0.362$ ,  $p=0.049$ ).

Correlation between *MEIS1* and *HOXA* genes expression levels were observed ( $p=0.0395$ ,  $R=0.256$ ;  $p<0.0001$ ,  $R=0.7909$ , *HOXA4* and *HOXA5*, respectively).

DNA methylation/expression levels were related to some of clinical/demographical characteristics: *HOXA4*, *HOXA5* and *MEIS1* expression levels correlated with patients age ( $p=0.0116$ ,  $R=0.296$ ;  $p=0.042$ ,  $R=0.242$  and  $p=0.0035$ ,  $R=0.337$ , respectively), *HOXA4* methylation as well as *HOXA5* and *MEIS1* expression levels were correlated with white blood cell (WBC) count ( $p=0.018$ ,  $R=0.278$ ;  $p=0.005$ ,  $R=0.334$ ;  $p=0.015$ ,  $R=0.278$ , respectively).

Patients with favorable cytogenetic risk showed decreased *HOXA5* and *MEIS1* expression level compared to intermediate and bad risk patients ( $p$

NK AML patients, classified as intermediate risk, showed the highest *HOXA4* methylation level and both *HOXA* genes and *MEIS1* expression level. *NPM1* mutations carriers exhibited elevated *HOXA4* methylation and *HOXA5*, *MEIS1* expression values comparing to *NPM1* wild-type patients ( $p=0.0178$ ,  $p=0.004$ ,  $p=0.0001$ , respectively).

Aberrant promoter methylation/expression of *HOXA4*, *HOXA5* and *MEIS1* is found in a group of AML patients, mainly with NK correlating to certain demographical and clinical parameters of patients characteristics and risk classification.

## P6.26

### Effect of cytoplasmic actin isoforms overexpression on actin cytoskeleton organization and migration of human colon cancer cells

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Actins are eukaryotic proteins, which are involved in diverse cellular functions including muscle contraction, cell motility, cell adhesion, cell division and maintenance of cell shape. Two cytoplasmic actin isoforms —  $\beta$  and  $\gamma$  differ only by four amino acids located at positions 1–3 and 10.  $\beta$  actin contains Asp-Asp-Asp at the N-terminus and Val at position 10 of polypeptide chain, whereas  $\gamma$  actin possesses N-terminal tripeptide Glu-Glu-Glu and Ile at position 10. The proportion of cytoplasmic actins varies and depends on the cell type. Cytoplasmic actin isoforms —  $\beta$  and  $\gamma$  are ubiquitously expressed and essential for cell functioning. However, their unique contributions are not very well understood. Because of that we decided to trigger overexpression of  $\beta$  or  $\gamma$  actin isoform in the human colon cancer cell line BE, representing mesenchymal mode of motility and to observe its effects on cell migration and invasion abilities. The cells were transfected with plasmids pAcGFP-C1- $\beta$  actin or pAcGFP-C1- $\gamma$  actin, containing cDNA for  $\beta$  or  $\gamma$  actin isoforms. Both type of transfectants were characterized by the increased migration and invasion abilities. Furthermore the migration velocity of pAcGFP- $\beta$  actin and pAcGFP- $\gamma$  actin transfected cells demonstrated higher migration velocity in comparison to control cells. This difference is statistically significant only in case of  $\gamma$  isoform overexpressing cells. Actin overexpressing cells presented also the elevated filamentous to monomeric actin ratio. This was accompanied by a distinct cytoskeletal actin rearrangements observed under laser scanning confocal microscope. Overexpressed actins were observed mainly in filamentous form and localized at the submembranous region of the cell body, especially near to the leading edge and on the tips of pseudopodia. The area occupied by these protrusions is statistically significantly larger in cells overexpressing  $\beta$ - or  $\gamma$ -actin than in control cells.

In conclusion, the elevated level of  $\beta$  or  $\gamma$  actin leads to actin cytoskeletal remodeling followed by an increase in migration and invasion capacity of human colon BE cells. Our research clearly suggests that both cytoplasmic actin isoforms are involved in cell migration and invasion.

## P6.27

## Analysis of circulating immune complexes from serum of lung cancer patients

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**Introduction:** Determination of the presence of circulating immune complexes (CIC) and the identification of atypical proteins may be of importance in the diagnosis of cancer [1, 3].

**Aim:** The aim of the study was to analyze the level of circulating immune complexes and to characterize proteins within these complexes in sera obtained from patients with lung cancer. The occurrence of heat shock proteins (HSP70, HSP90) in protein fractions of immune complexes was also characterized.

**Material and methods:** The serum samples were obtained from 38 patients with lung cancer. The levels of immune complexes were estimated by means of polyethylene glycol (PEG-6000) precipitation test [2]. The molecular weights of these CIC proteins were studied by electrophoresis (SDS – PAGE). The proteins present in CIC were stained with Coomassie Brilliant Blue in conjunction with a sensitive silver method. The presence of heat shock proteins was confirmed by Dot-Blot.

**Results:** The sera of patients with lung cancer contained elevated levels of circulating immune complexes in comparison to the group of healthy individuals. Differences in electrophoretic separation of the protein profiles between patients and controls were clearly seen. In all samples of lung cancer patients positive reaction indicating the presence of heat shock proteins (HSP70, HSP90) was obtained.

**Conclusion:** The levels of CIC seem to be related to the course of the disease. Although the results are promising in the context of a diagnostic application, studies on a larger group of patients are required.

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## P6.28

## Determination of thioredoxin reductase activity inhibition, in hPca-bearing mice blood, after oral administration of a novel organoselenium compound — Selol 5%

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Several epidemiological and clinical studies showed that selenium (Se) is a trace element essential to human health, playing an important role as an antioxidant and anticancer agent [1]. Apart from its role as an antioxidant and cancer prevention agent, selenium also exhibits anticancer properties. Its supplementation together with drugs and radiation can increase the effectiveness of anticancer treatment, where Se acts as a prooxidant rather than an antioxidant inducing apoptosis through oxidative stress pathway [2]. The Se compounds with the highest activity as free radical scavengers and the greatest anticancer potency contain selenium at the +4 oxidation state [3, 4]. Selol, a mixture of selenitetriglycerides, is a novel organoselenium (Se+4) compound. It reveals lower potential of toxicity than sodium selenite and does not exhibit mutagenic activity. Its antioxidant and anticancer properties including overcoming cancer cell resistance to standard therapy of the drug were proven [5]. Since a recent study showed that thioredoxin reductase (THRR) protein is overexpressed in many cancers and its activity increases significantly in tumor cells, stimulating its growth and reducing apoptosis, specific inhibitors of THRR activity have been searched for. The aim of this work was to examine the effect of Selol 5% supplementation on the activity of the thioredoxin reductase activity in the blood of the healthy mice and hPca-bearing mice. NSG mice were randomly divided into 2 main study groups — healthy mice and mice with grafted androgen-sensitive human prostatic adenocarcinoma cell line (LN-CaP). In the main groups we selected 2 subgroups — study groups supplemented with Selol 5% and control groups supplemented with placebo (vegetable oil). Selol 5% at the dose 17 mg Se kg<sup>-1</sup> body mass was administered orally for 3 weeks to all study groups. Starting 2 weeks after tumor induction, it was supplemented, to the hPca-bearing mice. Thioredoxin reductase (THRR) activity, in the plasma and erythrocytes, was measured spectrophotometrically at the wavelength of 412 nm, using Hill *et al* amendments [6]. We found significant decrease, of THRR activity in the plasma ( $p=0.0047$ ) and erythrocytes ( $p=0.0058$ ) of hPca-bearing mice supplemented with Selol 5%, in comparison to hPca-bearing mice control group. No significant changes in THRR activities were observed in the plasma and erythrocytes of healthy mice, supplemented with Selol 5% and with placebo. Furthermore the THRR activity in plasma, was significant higher, in the hPca-bearing mice control group, than in the healthy control group ( $p=0.0300$ ). Selol 5% is a potential inhibitor of thioredoxin reductase activity *in vivo* in tumor induced subjects, with no action in healthy subjects, what can be important in anticancer therapy.

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## P6.29

### Determining of the secondary structure of human brain-specific BC200 RNA

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Brain cytoplasmic RNA (BC200) is a primate-specific, non-coding RNA almost exclusively expressed in neurons. Increasing evidences suggest that dendritic protein synthesis is instrumental in the implementation of long-term plastic changes at the synapse and that BC200 RNA are involved in such local translational control. Recently it has been suggested that differential expression of dendritic BC200 may be involved in processes like aging and Alzheimer disease (AD). Additionally, it has been reported that the normally neuron-specific transcript BC200 RNA, is atypically expressed in various tumors of non-neuronal origin, including cervix, oesophagus, lung, ovary, parotid, tongue and mammary carcinomas. Moreover expression profiling studies performed in our laboratory revealed that the level of BC200 was altered in glioblastoma, and that downregulation was most prominent in high grade tumors. Glioblastoma multiforme was classified as the most frequent and malignant brain tumors with extremely poor prognosis. Although there are some proposed treatments that can partially alleviate or retard symptoms of glioma, there is a need to improve our understanding of its pathogenesis to enable development of disease-modifying treatments based on innovative technology.

There are many non-coding RNA molecules whose regulation and functionality require formation of the correct structure. Additionally, current studies suggests that a subset of RNA functions may depend more directly on secondary structural motifs than on global folds. The main goal of this study was to determine the secondary structure of primate 200-nucleotide long, by partial hydrolysis with nucleases T1, V1, S1 and lead-induced cleavage. We also examined the availabilities of putative single-stranded regions and base pairing interactions via specific DNAszymes. Structure mapping experiments visualized by RNAscape program demonstrate that secondary structures of BC200 RNA consist of three asymmetric internal loop, where loop I and II are linked by an internal double-stranded segment. Interestingly, our secondary structure model of BC200 RNA based on experimental methods, revealed high similarity between 5' domain of BC200 RNA model and the structure of *Alu* portion of 7SL RNA (presence of an internal asymmetric loop I with hairpin I and II as well as presence of hairpin III). The presence of conserved structural motifs of BC200 RNA confirm the importance of its function in specific protein recognition and ribonucleoprotein complex formation which has a regulatory role in dendritic protein synthesis. Although exact composition and function of BC200 RNP in the dendrites are still unclear, complete structure information about BC200 RNA is a basic requirement for understanding mechanism of its functional roles. It also seems to be critical component to understanding involvement of deregulation of BC200 expression in tumorigenesis and AD.

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## P6.30

### Selenitetriglycerides — redox therapy agents for prostate cancer treatment

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Human prostate cancer (hPCa) is the second most common cancer worldwide. Some cancer prevention trials link selenium (Se) to a significant reduction in deaths from hPCa. The effect of Se depends on the form being ingested and differs according to genotype. Se (IV) has better bioavailability and anticancer activity than Se(II). Organic compounds of Se(IV) have lower toxicity and better bioavailability than inorganic compounds (sodium selenite). The aim of the study was to monitor the effect of SELOL by using gamma-glutamyltransferase (GGT) to determine oxidative stress in selected organs of hPCa-bearing mice. SELOL, a new semisynthetic, organic derivative of Se (IV), is a mixture of selenitetriglycerides with chemopreventive and chemosensitizing properties proven *in vitro* on various cancer cell lines. GGT is important in preventing oxidative stress by metabolizing extracellular glutathione, and can provide GSH precursors back to cells. The intensity of oxidative stress in mice organs in the course hPCa was assessed in 2 variants: without SELOL and after treatment with SELOL. For studying hPCa, we used androgen-sensitive LNCaP cell line xenografts growing in immunodeficient NSG mice. SELOL was administered orally for 2 weeks, starting 4 weeks after tumor induction. After 6 weeks, mice were anaesthetized; tumors and organs were isolated from: control (C), experimental with hPCa (*Ex*), C+SELOL (*C+S*) and *Ex*+SELOL (*Ex+S*) animals. Tumor developed in implantation places, its size was proportional to quantities of grafted LNCaP cells. The activity of GGT in tumors was inversely proportional to its size. 2 weeks of daily administration of SELOL results in a 46% decrease in cancer mass. SELOL treatment induced oxidative stress which caused increase in intracellular redox state in tumors and protein thiolation (there was almost no free glutathione in tumors treated with SELOL); efflux of glutathione was reduced and GGT activity in tumor was normalized. Our study has proven that acting as prooxidant, SELOL shows the necessary activities to fight androgen-dependent prostate cancer. It can normalize redox signaling in key organs, previously disturbed by the disease. Modification of intracellular redox state and the redox state of protein and non-protein thiols is an important aspect of SELOL's anticancer activity, which facilitates the observed reduction of PCa tumor mass.

## P6.31

### Plasma concentration of antioxidative vitamins in patients with non-small cell lung cancer undergoing chemotherapy — the effects of simultaneous introduction of kinesitherapy and vitamin C supplementation

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Continuing the research on the disorders of oxidoreductive balance in non-small cell lung cancer (NSCLC), of which the first stage we have presented at the 46th Meeting of the Polish Biochemical Society, next to the factors already included we have introduced an additional one - respiratory kinesitherapy (RK) — which is recommended for patients with this type of cancer. The aim of the following study was thus the estimation of vitamin A, C and E plasma concentration in patients with NSCLC after chemotherapy supplemented with RK, and also after a chemotherapy supplemented with RK and vitamin C, and a comparison of the outcomes of the two variants of therapy. Material for the study consisted of the plasma of two groups of patients of both sexes: I (61.9±7.8 years, n=24) — treated with standard chemotherapy (2 cycles cisplatin + vinorelbine for 6 weeks) supplemented with RK; II (62.0±7.2 years, n=26) — undergoing the same chemotherapy and RK, additionally receiving vitamin C (3×200 mg/day). Blood samples were collected from all those patients before and after the aforementioned therapy. Concentrations of A, C and E vitamins were marked through spectrophotometry [1] using spectrophotometer T60V (PG Instruments). At the start of the research we found that concentrations of the C and E vitamins for both groups were below the norm, though the concentrations of A vitamin were within the norm. At the end of the aforementioned treatment in group I concentrations of vit. C fell by 42.1%, vit. E by 32.7%, vit. A by 7.29%, whereas in group II concentrations of those vitamins rose respectively by 62.0%, 34.2% and 15.7%. These outcomes show the lowering of the concentrations of C and E vitamins in NSCLC, which is worsened by administration of cytostatics and RK, contrasted with a significant rise in concentrations of those vitamins caused by taking of vit. C — a probable effect of a cooperation between this vitamin and vitamins E and A that has already been noted by many researchers. One should thus conclude that introduction of vitamin C next to chemotherapy and RK is fully rational. It normalizes the concentrations of all those vitamins in NSCLC patients which are lowered by the standard therapy used in this type of cancer. It was also noticed that additionally this supplementation rises the tolerance for the therapy and the general life quality of patients. One can also expect it to counter the oxidative stress which is increased in most cancer types.

#### Acknowledgements:

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## P6.32

### The level and localization of LC3 protein in cancer cells with overexpressed PKCεA/E

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Protein kinase C epsilon (PKCε), a novel PKC isotype is characterized as a calcium-independent and phorbol ester/diacylglycerol 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. Its oncogenic activity seems to be exerted through affects on the ras-signaling cascade at the level of Raf-1 activation. This protein seems to participate in tumor development, tumor invasion and metastasis.

The huge amount of work is devoted to research into finding an effective way to cause death of cancer cells without affecting the way normal cells. There are several kinds of death: necrosis, mitotic catastrophe, autophagy, apoptosis and aging. Autophagy (from the Greek words, *auto* "self" and *phagein* "to eat") is an essential, conserved catabolic mechanism that involves cell degradation. Autophagy as a II type of programmed cell death, is independent of caspases and associated with the appearance of autophagosomes and depends on autophagy proteins. LC3 (rat microtubule-associated protein 1 light chain 3) is the first mammalian protein identified that specifically associates with autophagosome membranes. Conversion protein LC3-I to LC3-II is a proof of the formation of autophagosomes in the cell. The amount of LC3-II is correlated with the extent of autophagosomes.

In the present study the technique of electrophoresis, Western Blot, immunoidentification and microscopy fluorescence with appropriate antibodies were used to shown LC3 protein level and localization in cancer cells over-expressed constitutively active PKCεA/E. Moreover, we estimated the influence of autophagy modulators (rapamycin and 3-MA) and PKC inhibitor (BIM) on the level of LC3 protein.

We observed the lower level of LC3-I and LC3-II protein in HeLa PKCεA/E cells compared to cells without doxycycline-induced PKCεA/E expression (control). In control cells treated with autophagy activator (rapamycin) or inhibitor (3-MA) the level of LC3-II protein increased or decreased, respectively. Moreover the results revealed that rapamycin in HeLa PKCεA/E cells increased the level of LC3-II which was not modified by inhibitor of autophagy. In HeLa PKCεA/E cells simultaneously treated with BIM and rapamycin an increase of autophagy, indicated by LC3-II protein, was observed. This results were confirmed by the assessment of autophagosomes accumulation.

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## P6.33

### Stromal COX-2 expression as a marker of human breast cancer invasiveness

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**Objective:** Data on COX-2 expression and its prospective role as a marker of breast cancer progression remain conflicting. The vast majority of studies on this issue focused on epithelial COX-2 expression. There is, however, a great deal of evidence that points to the breast tumour stroma as active promoter of its progression. It has been proved that stromal cells may, for instance, promote the invasiveness of adjacent tumor cells. The function of stromal COX-2 expression in supporting the process of breast cancer progression remains elusive.

**The aim** of this study was to examine both levels and spatial patterns of COX-2 expression in malignant and benign breast lesions in order to establish a potential role of COX-2 as a marker of breast cancer progression.

**Material and Methods:** COX-2 expression was assessed by immunohistochemistry in standard histopathologic sections using a primary polyclonal antibody and two monoclonal antibodies targeting different epitopes. The study consisted of 52 primary breast cancers (including 10 DCIS), 15 lymph node metastases, 11 benign dysplasias and 10 normal breast tissue samples obtained from breast cancer patients.

**Results:** COX-2 expression in tumours was significantly stronger than in normal tissue and non-cancerous breast lesions. COX-2 expression in epithelial tumour cells was not associated with any clinico-pathological features of breast cancer. On the contrary, enhanced expression of COX-2 protein in primary tumour stroma was significantly associated with lymph node metastases, regardless of the type of antibodies used for COX-2 detection.

**Conclusion:** Enhanced COX-2 expression in stromal cells may stimulate breast cancer progression by increasing metastatic capacity of tumour cells. Our findings indicate that the stromal COX-2 expression may be considered a prognostic marker for human breast cancer.

## P6.34

### Sulphoraphene, a promising anticancer phytochemical from cruciferous plants

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Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer deaths among females worldwide [1]. Standard treatments combining surgery, radiation therapy, chemotherapy and hormone therapy are often poorly effective. Intrinsic (innate) and acquired (adaptive) resistance critically limits the outcome of cancer treatments. Moreover, anticancer therapies have severe side effects that reduce their efficacy. These problems might be solved by application of supportive treatment. Isothiocyanates present in cruciferous vegetables are considered to be promising chemopreventive and anticancer phytochemicals. Numerous studies have proved that isothiocyanates such as sulforaphane or erucin cause: inhibition of phase I carcinogen-activating enzymes, induction of phase II carcinogen detoxification, induction of the cell cycle arrest and apoptosis [2-4]. Molecular mechanism of action of structurally related sulforaphene remains largely unknown. The purpose of our study was to investigate the effect of sulforaphene on cell proliferation and induction of apoptosis in three phenotypically different breast cancer cells that differ markedly in the level of receptors for estrogen, progesterone and HER-2: T47D [ER (+), PR (+), HER-2 (-)], SKBR-3 [ER (-), PR (-), HER-2 (+)] and MDA-MB-231 [ER (-), PR (-), HER-2 (-)]. Our results indicate that sulforaphene inhibits cell viability in a dose-dependent manner. Estimated IC<sub>50</sub> values are similar for all cell lines: 8.2 μM for T47D, 7.3 μM for SKBR-3 and 9.7 μM for MDA-MB-231. Anticancer activity of sulforaphene is connected with induction of apoptosis, as we observe increased PARP cleavage in sulforaphene-treated cells.

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## P6.35

## Betaglycan polymorphisms in endometrial cancer

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Betaglycan is the first identified accessory receptor of TGF $\beta$  cascade. Betaglycan deprived of any enzymatic domain is responsible for TGF $\beta$  ligands presentation to their dedicated canonical receptors TGF $\beta$  type I and type II. Although betaglycan was identified in the mid '80 of the last century its role in the cancer transformation is still unknown, particularly in endometrial cancer. Our previous studies have indicated that betaglycan mRNA level is significantly downregulated in this tumor type (Zakrzewski *et al.* 2011). Betaglycan transcript decrease seems to be an early event during the cancer development and gradual decline is observed in more advanced cancer stages (G1 vs G3).

The aim of the current study was the evaluation of three single nucleotide polymorphisms (SNP) in *TGFBR3* gene coding betaglycan and its association with endometrial cancer development and progression. Analyzed SNP are located in promoter/regulatory region (*rs11466543*) and coding sequence (*rs12566180*, *rs6680463*). SNP genotyping was performed using commercially available fluorescent TaqMan probes. The study group consists of 127 cases of endometrial adenocarcinoma specimens vs healthy controls. All samples were obtained from menopausal and postmenopausal women.

Obtained results indicate that presence of the genotype CT and CG in polymorphic sites *rs12566180* and *rs6680463*, respectively are correlated with higher endometrial adenocarcinoma occurrence (OR 1.62, 95% CI 1.05–2.49,  $p=0.03$ ; OR 1.59, 95% CI 1.03–2.45,  $p=0.03$ ).

In conclusion we suggest that polymorphisms in *TGFBR3* might be regarded as predictive factor for endometrial cancer development in Polish women population.

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## Discovery of polymorphisms responsible for radiosensitivity — preliminary analysis

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**Abstract:** The aim of the study was to develop the data analysis strategy for discovery of genetic background of radiosensitivity in GWAS type of study. Identification of polymorphisms and genes responsible for organism's radiosensitivity may allow for better understanding the process of carcinogenesis and impact of radiotherapy.

**Materials and methods:** The population under investigation is composed of fraternal twins, monozygotic twins and non-related persons, and the analysed sample consists of 130 randomly chosen individuals in total (66, 30 and 34 respectively). Two type of data were collected, first was the result of genotyping of 567096 polymorphisms, the second one includes 9 gene qPCR expressions measured after the irradiation of 2Gy and in normal conditions, accompanied by activity of  $\gamma$ H2AX test on radiosensitivity. Standardized fold changes per each gene were calculated. The first step of the analysis was focused on the subpopulation of non-related persons. To each of SNP, basic statistics were calculated. Also three different models of *SNP-gene expression* interaction (genotype, recessive and dominant) were investigated and the criterion of minimum p-value was used to choose the most suitable one per each SNP-gene pair. From the characteristic p-value to each SNP only those, which show statistic significance were taken to further analysis. The validation of the obtained set of candidate markers was done with the use of fraternal twins, allowing for the discrimination some of the SNPs not directly related to the radiosensitivity. The final verification was performed on monozygotic twins.

**Results:** During the first step, the analysis of interaction between every SNP and radiosensitivity test and gene expression results was performed among the non-related persons. The hypothesis on normality of gene expression distribution within each subpopulation was verified by Lilliefors test, and after the correction on multiple testing, the hypothesis could not be rejected at 0.05 significance level. ANOVA algorithm was applied to investigate the genotype model of interaction and *t*-test to dominant and recessive ones. Having a set of three of p-values obtained for each model, the best one with minimum p-value was chosen. The exemplary distribution of the final models of genetic interaction between SNPs and  $\gamma$ H2AX look as follow: to genotype model belong 1868 SNPs (FDR 7371), to dominant model belong 203875 SNPs (FDR 13950), to genotype model belong 243247 SNPs (FDR 15910) and total number is 448990 SNPs (FDR 22450). One can notice that only 0.41 percent of results are the genotype type of interactions, the most frequent are dominant and recessive models. The final set of 55289 ( $p<0.05$ ), where FDR is 22450, candidate radiosensitivity markers was validated with the use of heterozygotic twins and above 95% were filtered out. The final step of marker verification among monozygotic twins is going to be performed soon.

**Conclusions:** Preliminary analysis shows that different models of interaction must be included in the investigation of the genetic background of biological phenomena.

Looking at allelic frequencies and genotyping results only would not allow for finding the candidate biomarkers.

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