Session VI: Various aspects of cancer research – from epigenome regulation to anti-cancer drugs

Lectures

L6.1

Mutations in regulators of the epigenome and their effects on the DNA methylome in cancer

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Keywords: epigenome; methylome; mutation; cancer

Genome-wide profiling for genetic alterations in cancer has identified mutations in genes that are associated with epigenetic programming of genomes for DNA methylation patterns, histone modifications patterns and the positioning of nucleosomes. Here a systematic evaluation of the available cancer genome profiling data established by large international consortia, in order to identify recurrently mutated genes or pathways was described. Using curated list of approximately 700 epigenetic regulators and currently available genome-wide datasets on genetic and epigenetic alterations in cancers, the distribution of alterations in epigenetic regulators was described. Epigenetic genes were classified as potential oncogenic or those with tumor-suppressor function based on the location of mutations relative to functional domains and their frequencies. A panel of 50 epigenetic genes, including: DNMTs, histones (*H3F3A, HIST1H3B*), histone editors (*KDM5C, KDM6A*) and writers (*MLLs*, *SETD2, EZH2, ATM*) that can promote epigenetic changes in cancer was identified. Using correlative analysis of publicly available methylation data with information on deregulated epigenetic driver genes, many identified subtype-specific methylation clusters were correlated with groups of up to 3 epigenetic regulators. This analysis provides a source for the identification and link between methylation groups and deregulated epigenetic genes.

Major cancer specific methylation changes have been observed in promoters and gene bodies. Tissue-specific cancer methylation differences have been located in enhancers and regulatory regions of non-coding RNAs. Based on identified results, the major mechanism of non-coding RNA deregulation in cancer has been investigated on independent data cohort. Using integrative analysis of non-coding RNA in early-onset prostate cancer, non-coding RNAs were classified as tumor-suppressive and oncogenic. About 120 novel prostate cancer specific non-coding RNAs that have been epigenetically deregulated have been identified.

Our study on the defects in regulators of the epigenome will help to understand mechanisms leading to distinct epigenetic patterns and will allow the molecular validation of defined correlations in experimental settings.

L6.2

Adaptor proteins in cancer stem cells signaling

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Keywords: cancer stem cells; adaptor proteins; carcinogenesis

According to a modern concept of basic cancer biology, tumors consist of many cell populations with different characteristics and functions. Recent studies indicate that a subpopulation of tumor cells with tumor-initiating ability (cancer stem cells, CSCs) play an important role in acquired resistance to chemotherapy and radiation, tumor dissemination and metastasis. Adaptor proteins function as important components of signaling networks. Adaptor molecules are not simply links between receptors and their effectors, but are increasingly viewed as regulators of signaling pathways dynamics thereby influencing the specificity of cell responses. Given the important role of adaptor proteins in propagating cellular signals, it is quite likely that their dysfunction may be involved in carcinogenesis. The adaptor protein Ruk/CIN85, containing multiple SH3 domains, was implicated in carcinogenesis by influencing a number of processes such as apoptosis, cell adhesion, motility and invasion. Here we addresses the role of Ruk/CIN85 in CSCs biology using human breast (MCF-7) and lung (A549) adenocarcinoma cells engineered to overexpress Ruk/CIN85. In MCF-7 and A549 cells, Ruk/CIN85 was shown to enhance such stemness characteristics as: the ability to form sheroids; the expression of surface markers (CD44, CD24, CD133); the resistance to doxorubicin, tamoxifen, etoposide and cisplatine; increased expression and activation of ABC transporters; activation of ALDH, Src, mTOR and Akt kinases, and NF-$\varkappa$B transcription factor. Moreover, Ruk/CIN85 knockdown in MCF-7 cells using siRNA technology resulted in reduction of stemness characteristics. The data obtained provide evidence that Ruk/CIN85 is required for the maintenance and propagation of CSCs.

L6.3

The role of CD150 receptor in the regulation of signalling pathways in chronic lymphocytic leukemia B cells

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Keywords: CD150/SLAMF1; chronic lymphocytic leukemia

Cell surface CD150/SLAMF1 expression on chronic lymphocytic leukemia (CLL) B cells is associated with a favourable clinical outcome. However, the role of CD150 in CLL pathogenesis is still unclear. The aim of our work was to study CD150 expression and CD150-mediated signalling pathways in CLL. Cell surface CD150 expression (csCD150+) was observed in 43% of CLL cases. Unexpectedly, in csCD150- CLL cases we detected cytoplasmic expression of CD150 antigen. We showed that exclusive cytoplasmic expression of CD150 in several CLL cases was not associated with endoplasmic reticulum stress and ceramide metabolism. Ligation of CD180, but not of CD40 or BCR, lead to CD150 upregulation on mRNA and the protein level. Moreover, CD150 was colocalized with CD180 on the plasma membrane of CLL cells (R=0.98±0.1). Expression of mCD150, nCD150 and sCD150 isoforms was detected in all tested CLL cases, with significantly higher expression levels of nCD150 (20% CLL cases) and sCD150 (70% CLL cases), compared to normal B cells. Ligation of the CD150 receptor on CLL cells induced Akt, JNK1/2, and p38MAPK phosphorylation. Analysis of the transcription factors profile in CLL revealed that high PU.1 protein expression level positively correlated with CD150 cell surface expression in CLL ($r=0,4$; $p<0,05$). In a half of the studied CLL cases, CD150 ligation downregulated the PU.1 mRNA. Moreover, mRNA expression of PU.1 target genes CCL3 and CCL5 was significantly decreased after CD150 ligation. CD150-mediated downregulation of CCL3 and CCL5 and regulation of PU.1 expression may contribute to a favourable clinical outcome of csCD150+ CLL cases.

L6.4

Modulation of temozolomide action towards glioblastoma cells *in vitro* by its combination with doxorubicin and immobilization with nanoscale polymeric carrier

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

Human glioblastoma is the most common primary brain tumor with poor prognosis. Even with aggressive treatment using surgery, radiation, chemotherapy, median reported survival is less than one year. Alkylating agents, such as temozolomide (TMZ), are among the most effective cytotoxic agents used for malignant gliomas with poor responses.

TMZ dose- and time-dependently inhibited the viability of human glioblastoma T98G and rat brain glioma C6 cell lines. The action of the conjugate of TMZ with a novel polymeric carrier functionalized with phosphatidylcholine (TMZ-PC) was more pronounced than that of free TMZ. TMZ's IC50 was 243 μM for T98G cells, while its immobilization by novel polymeric nanocarrier reduced IC50 to 166 μM. TMZ-PC resulted in approximately 2 times enhancement of anticancer activity of TMZ. TMZ-PC caused apoptosis via the activation of MAPK signaling pathway, Rb protein and inhibition of STAT3 and affected a transition through G2/M phase of cell cycle. Immobilization of TMZ on the PC also increased drug intercalation into DNA. Synergistic effect of Doxorubicine and TMZ was detected when human glioblastoma T98G cells were treated.

Immobilization of TMZ with novel polymeric nanocarrier leads to an increase in cytotoxicity of TMZ and does not change the mechanism of its action. This makes studied TMZ-PC complex perspective for future preclinical investigations.

L6.5

Novel cytotoxic agents in the development of effective drug combinations to treat glioma

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Keywords: complex therapy; glioblastoma; bradykinin antagonists; chitinase 3-like 2; cell cycle regulation

Glial tumors are driven by multiple molecular aberrations that cannot be controlled by a single targeted agent. To find out which drug combinations will enable the development of therapeutic regimens with improved effectiveness and decreased toxicity, the cytotoxic effects of several bradykinin antagonists (BA) were analyzed for different glioblastoma (GB) cell lines.

Among all the BA under investigation, BKM-570 appeared to be the most effective, with IC50 values of 4 μM and 3.3 μM in rat glioma C6 and human glioblastoma U251 cell lines, respectively. BKM-570 suppressed ERK1/2 and AKT1 phosphorylation in U251 cells. Temozolomide (TMZ), the first-line anti-gliomic drug used in clinics, has only a temporary positive effect and severe side effects in GB patients. We showed that the combination of BKM-570 and TMZ led to significant potentiation of TMZ cytotoxicity at sub-therapeutic concentrations.

Recombinant proteins with cytotoxic properties are promising agents for complex therapeutic applications. We revealed that the glioma-associated protein CHI3L2 inhibited the viability of U251 cells more effectively than TMZ. Furthermore, the combination of CHI3L2 and BKM-570 resulted in an additive cytotoxic effect. CHI3L2-mediated decrease of cell viability was associated with a G1/S transition arrest. CHI3L2 provoked the dramatic reduction of pRB phosphorylation and a significant decrease of cyclin D1 expression, as well as a substantial increase in p53 level. In addition to the accumulation of p53, we observed the upregulation of CDK inhibitor p21. Therefore, G1/S arrest in CHI3L2-treated cells could be realized via activation of pRB, down regulation of cyclin D, and activation of p53.

Posters

P6.1

Adaptor protein RUK/CIN85 affects breast cancer cells motility and invasion *in vitro*

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Keywords: adaptor proteins; breast cancer; EMT

Via interaction with its binding partners, the adaptor protein Ruk/CIN85 is involved in signaling networks that control key physiological responses such as cell motility, invasion, adhesion, and apoptosis. Here we studied the Ruk/CIN85 influence on the adhesion, motility, and invasion of human and mouse breast adenocarcinoma cells *in vitro*. We also tried to reveal possible mechanisms that mediate effects of Ruk/CIN85 on these processes.

To investigate Ruk/CIN85 roles in cell adhesion, motility, and invasion, subclones of human MCF-7 and murine 4T1 breast adenocarcinoma cells with different expression levels of Ruk/CIN85 (high, low, or moderate) were generated. Then, adhesiveness to the substrate and extracellular matrices, motility (using scratch test), and invasion (using transwell assay) were studied. To explore the possible mechanisms, by which Ruk/CIN85 modifies cell responses, the expression of cytoskeletal ERM proteins, CD44 antigen and EMT markers were analyzed by Western blotting.

It was investigated that Ruk/CIN85-overexpressing cells are characterized by reduced cell adhesiveness and enhanced cell motility and invasion through Matrigel, collagen type 1, and fibronectin. These changes in cell behavior correlated with EMT markers and CD44 expression levels, but not of ERM proteins.

The data obtained suggest a possible role of Ruk/CIN8 in breast cancer metastasis.

P6.2

Effect of photodynamic reaction on the matrix metalloproteinases 2 and 9 activity and inhibition in selected cancer cell lines

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Keywords: 5-aminolevulinic acid; cancer; cell lines; matrix metalloproteinases; photodynamic therapy; proteolytic activity

5-aminolevulinic acid-based photodynamic therapy (5-ALA-PDT) combines 5-aminolevulinic acid (5-ALA), acting as a photosensitizer, and light in the intracellular photochemical reaction, which produces reactive oxygen species (ROS). ROS-mediated anticancer PDT may damage tumor cells in a non-specific manner, affecting numerous signaling pathways, including those related to the secretion of proteolytic enzymes. Two of these secretory proteases, matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9, respectively) regulate the adhesion and motility of tumor cells, which plays a key role in cancer progression and metastasis. Because ROS participate in the activation of MMP-2 and MMP-9 zymogens, the association between activity of these enzymes and PDT procedure requires additional, more detailed studies.

In this study, Me45 melanoma cell line and SW480 colorectal adenocarcinoma cell line were treated with 5-ALA-PDT procedure and subsequently investigated in terms of the extracellular MMP-2/MMP-9 activity, intra- and extracellular amount of these proteases, and the inhibitory potential against purified MMP-2/MMP-9 enzymes. No significant differences in the extracellular activity of MMP-2 and MMP-9 were observed 18 hours after PDT treatment. Nevertheless, prolonged cultivation (45 and 115 hours) revealed considerable loss of the amount and activity of MMP-2 and MMP-9 proteases in Me45 and SW480 cell lines (respectively), what suggests the long-lasting effect of the PDT procedure. Decrease in the secretory activity was not related to the intracellular accumulation of proteases. The Me45 cell line revealed stronger inhibitory effect against purified MMP-2 and MMP-9 enzymes than the SW480 cell line. In both cases, the effect was reversible.

P6.3

Cancer-specific binary expression system activated in mice by bacteriophage HK022 Integrase

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Keywords: cancer cells eradication; lung cancer mice; site-specific recombination; coliphage HK022 integrase; Luciferase reporter

A binary systems was developed that is intended to eradicate cancer cells. It is based on a site specific recombination reaction that induces the expression of a cytotoxic gene specifically in cancer cells. One element of the binary system is a plasmid that expresses the site-specific integrase (Int) recombinase of coliphage HK022 under the tumor specific *hTERT* promoter. The other element is a second plasmid that carries the luciferase (*luc*) reporter gene silenced by the presence of a transcription terminator (Stop) inserted between the *luc* reading frame and its promoter. Stop is flanked by recombination sites of Int.

When the two plasmids, complexed with a specific carrier (*in vivo*-jet PEI) are tail-injected in lung cancer mice, they are transported into the lungs and Int, specifically expressed in cancer cells, excises the Stop terminator leading to the specific expression of the Luc reporter in the cancer cells.

Experiments are in progress that replace the *luc* gene with *dta*, that expresses the diphtheria toxin, intended to eradicate the cancer cells without affecting normal cells.

P6.4

Influence of EGFR and c-Met inhibitors on melanoma viability

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Keywords: melanoma; invasion; EGFR inhibitors; c-Met inhibitors; cell signaling

Malignant melanoma represents 4% of all cancer cases. For this reason, better understanding of the molecules and pathways involved in melanoma progression can contribute to better prognostic information and identification of possible new therapeutic targets. Our studies focus on agents that block epidermal growth factor receptors (EGFR) and hepatocyte growth factor receptor (c-Met), which could selectively decrease the viability and migration of cancer cells. They prevent further signal transduction through pathways activated by these receptors, which are often disrupted in cancer cells. Moreover, EGFR as well as c-Met are often overexpressed in invasive melanoma cells.

Our goal is to recognize the influence of EGFR and c-Met inhibitors on the viability of human melanoma cell lines, derived from primary (A375, WM35) and secondary (WM9, WM239, HS294T) tumors. Based on the knowledge that both receptors participate in signaling pathways important for cancer invasion, we will also verify the effect of EGFR and c-Met inhibitor combinations on melanoma development.

We used inhibitors independently and in combination (i.e., pairs of anti-EGFR and anti-c-Met agents). We analyzed the cytotoxicity of these inhibitors, and IC10, IC50, IC90 were calculated. We then selected melanoma cell lines best suited for our research and inhibitor concentrations to use further in mixes. Results from the cytotoxicity of agent mixes showed not only an additive effect, but also a synergistic effect in some cases. Our next goal is to establish the influence of selected inhibitors on the formation and activity of invadopodia – invasive structures present in cancer cells.

P6.5

Impact of flavanols from evening primrose (*Oenothera paradoxa*) defatted seeds on expression of cyclooxygenase-2 and nuclear transcriptional factor NF-κB in human colorectal adenocarcinoma cells

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Keywords: colorectal cancer; COX-2; Nf-kappaB; evening primrose

Currently plant polyphenols are shown as nutrients with chemopreventive properties. These compounds prevent several types of cancers, including colorectal cancer.

In this study, we investigated the inhibitory effect of evening primrose flavanol preparation (EPFP) on growth of human colorectal adenocarcinoma cell line SW-480. We also determined the influence of EPFP on both cyclooxygenase-2 (COX-2) gene expression and nuclear transcriptional factor NF-κB which is a central mediator of inflammation.

The results from crystal violet staining assay revealed that EPFP (25-150 μM catechin equivalents/CE) inhibits the growth of SW-480 cells in a concentration-dependent manner. We observed a 50% decrease in the growth of cells treated with 111 μM CE of EPFP when compared to control cells.

In the SW-480 cell line, EPFP inhibited expression of both COX-2 mRNA and protein levels. At a concentration 100 μM CE of EPFP, RT-PCR analysis showed nearly a 40% decrease in COX-2 mRNA expression compared to control cells. Furthermore, EPFP at the same concentration downregulated COX-2 protein expression by 22% and 31% when samples were assessed by Western Blot and enzyme-linked immunosorbent assay (ELISA), respectively.

We also observed a 32% and 58% suppression of NF-κB protein expression after treatment of SW-480 cells with 100 μM CE EPFP using Western Blot analysis and flow cytometry, respectively.

These results suggest that EPFP exhibits anti-inflammatory and cytotoxic activities in SW-480 cells and it can be effective as natural colon cancer chemopreventive agent in the future.

P6.6

Flavanols from Japanese quince (*Chaenomeles japonica*) fruit inhibit expression of cyclooxygenase-2 and nuclear transcriptional factor NF-κB in human colon cancer cells

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Keywords: flavanols; COX-2; NF-kappaB; colon cancer

There is a growing interest in plant polyphenols, which exhibit pleiotropic biological activities, including anti-inflammatory, antioxidant, and anticancer effects.

Our study aimed to determine the influence of polyphenols on the expression of cyclooxygenase-2 (COX-2) and nuclear transcriptional factor NF-κB both of which are involved in inflammation.

Our experimental setup included a flavanol preparation from Japanese quince (*Chaenomeles japonica*) fruit (JQFFP) and the colon cancer cell line SW-480. We assessed the influence of the above-mentioned flavanol preparation on expression of COX-2 mRNA and protein in SW-480 cells by means of quantitative real time RT-PCR and Western Blot, respectively. Additionally, we determined the influence of JQFFP on NF-κB expression using two methods (Western Blot and flow cytometry). Besides, we assessed the influence of JQFFP on the growth of SW-480 cells using a crystal violet staining assay.

First, our results demonstrate that JQFFP (100 μM catechin equivalents (CE)) inhibits COX-2 mRNA expression by 63% and COX-2 protein by 21% when compared to control cells.

Secondly, JQFFP (100 μM CE) inhibits NF-κB expression by 35% and 57% when assessed by Western Blot and flow cytometry, respectively.

Thirdly, JQFFP (100 μM CE) inhibits the growth of SW-480 cells by 25% after 48 hours of incubation.

Taking the above results into consideration, we conclude that JQFFP has anti-inflammatory activity. In the future, JQFFP may be considered as a chemopreventive agent for the treatment of colon cancer.

P6.7

Differentiated impact of digested and non-digested red cabbage extracts on the growth of human colon cancer cell lines

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Keywords: red cabbage; digested red cabbage; cell growth; colon cancer

Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) is one of the most commonly consumed vegetables worldwide. Due to their rich composition, microelements and macroelements, vitamins and polyphenols, red cabbage extracts (RCEs) have been hypothesized to possess antioxidant, anti-inflammatory, and anticancer properties; they may also ensure a healthy digestive tract and cardiovascular system. Studies related to RCEs are mainly performed *in vitro* as the first step of research. *In vitro* models, however, do not reflect real conditions in the human body; the fate of tested extracts and their actual activities inside the organism remain unknown.

We used *in vitro* digested red cabbage extract (RCE) and investigated its polyphenol composition, its impact on the growth of colon cancer cell lines (SW480, SW620), and we compared our results with those for parent, non-digested RCE. *In vitro* digestion reproduces the digestive process in the human upper gastrointestinal tract. It involves an initial pepsin/HCl step, which simulates gastric conditions, and bile salts/pancreatin digestion, which simulates small intestine conditions. Colon-available RCE is a result of *in vitro* digestion.

We found that *in vitro* digestion altered the polyphenol composition of RCE relative to the non-digested RCE. However, it did not impair the biological properties of the RCE. In fact, the *in vitro* digested RCE was more effective at inhibiting the viability of colon cancer cells than the parent RCE. The differences in activity of the extracts were especially pronounced in the more aggressive SW620 cell model.

P6.8

Is there any natural alternative to classical chemotherapy treatment?

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Keywords: phytochemicals; chemotherapy; gastrointestinal tumors; anti-cancer drugs

The scientific goal of this project was to verify whether some phytochemicals have the ability to chemoprevention of several human cancer cell lines (liver model cell line HepG2, colon cell line LS 180, pancreatic cell line BxPC3). Selection of these tumor lines, among others, is related to the very low efficiency of treatment, which may occur in postoperative metastasis. At present, many anti-cancer drugs used for treatment of gastrointestinal tumors lead to a large number of side effects. One well-known chemotherapeutic agent is epirubicin.

For centuries, compounds isolated from plants were used as therapeutic agents for many diseases and illnesses; almost half of them are anti-cancer drugs. In recent years, more attention has been dedicated to studying phytochemicals that occur in spices (e.g., curcumin, capsaicin, piperine), daily diet (e.g., phenethyl isothiocyanate, indolo-3-carbinol, p-coumaric acid, lipoic acid), and many fruits and herbs (e.g., ursolic acid) or are synthesized by plants (e.g., berberine, quercetin).

Our recent, unpublished data show that some phytochemicals when added to the culture medium decreased the number of living cells in a manner dependent on concentrations. In some cases, the therapeutic effect of phytochemicals is very similar to that of epirubicin.

Our results suggest that some phytochemicals might be an alternative to drugs used in classical chemotherapy treatment.

P6.9

Anticancer features of new stilbenoid derivatives

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Keywords: stilbenes; oxepines; anticancer activity; cancer cell lines; apoptosis

Stilbenoids, including resveratrol (3,5,4'-trihydroxy-(E)-stilbene), are naturally-occurring phytoalexins. Combretastatins are *cis*-stilbenes that were first isolated from the bark of the African willow tree (*Combretum caffrum*). Combretastatin A-4 is the most extensively-tested component that inhibits tubulin polymerization by binding to β-tubulin at the colchicine site, revealing highly anti-mitotic and anti-angiogenics activity.

The search for new, more potent derivatives is ongoing and stilbenes exhibit anticancer activity. Thus, we synthesized 12 new stilbenoid derivatives, including are five (*E*)-2-hydroxystilbenes (1-5) and seven individually-substituted oxepines (6-12), with a motif of (*Z*)-stilbenes in their skeleton. All of these were in the *trans* isomeric form because of steric stability.

Here, we report that three of five stilbenoids and two of seven oxepines showed anti-cancer activity against two cancerous cell lines, HeLa and U87. The IC50 was about 50 μM after a 24-h treatment, based on an Alamar blue assay. The potential mechanism of action is through induction of apoptosis. Western blot implicated the PARP protein proteolytic cleavage in cells exposed to the test compound. Additionally, mass spectrometry analysis of cancer cells incubated in the presence of selected stilbenoids showed differential protein expression, suggesting breakdown of the tubulin network.

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P6.10

Gene-methylation of DNA in patients with renal cell carcinoma

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Keywords: DNA methylation; renal cancer; cell-free DNA

Clear cell renal cell carcinoma (ccRCC) is an incurable disease for most patients. Hypermethylation of tumor suppressor genes often occurs in the early stages of cancer and it is a promising marker for cancer detection. The purpose of this study was to determine the methylation status of selected tumor suppressor genes in the genomic DNA (gDNA) of a tumor and cell free DNA (cfDNA) of RCC patients. We used methylation-specific real-time PCR using bisulfite converted DNA as a matrix and primers specific to methylated CpG-islands.

Methylation of the LRRC3B gene in tumor gDNA and in respective plasma cfDNA obtained before surgery was detected in 80% (16 of 20) of patients. Methylation of the first CpG-island of RASSF1 gene occured in 93.1% (27 of 29) of patients and methylation of the second CpG-island of RASSF1 gene was present in 51.6% (16 of 31) of the patients. The results revealed the presence of gene-specific methylation in cfDNA obtained from the plasma of patients in the majority of cases. Such a strong correlation suggests that detection of methylation of CpG-islands of LRRC3B and RASSF1 genes could be used to develop test systems for non-invasive diagnostics of malignant tumors in the early stages.

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P6.11

Effects of combination treatment with evening primrose flavanol preparation and cisplatin on the growth of HT29 and Colo205 human colorectal adenocarcinoma cell lines

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Keywords: flavanols; cisplatin; colon cancer; proliferation

Natural plant extracts are known for their properties in cancer prevention and treatment. Extract-derived polyphenols, such as resveratrol or curcumin, show synergistic action with anticancer drugs in inhibiting proliferation, invasion and metastasis, which allows to reduce the doses of chemotherapeutic agents. Evening primrose (*Oenothera paradoxa* Hudziok) seeds are rich in polyphenols, which have antioxidant properties and exert diverse biological effects. Cisplatin (*cis*-diamminedichloroplatinum (II); CDDP) is a chemotherapeutic agent used in the treatment of cancer.

The aim of this study was to investigate the collective action of evening primrose flavanol preparation (EPFP) and CDDP on HT29 and Colo205 colorectal adenocarcinoma cell lines. The inhibition of proliferation was assessed using the MTT assay. We observed the effects after 48 h and 72 h of exposure to EPFP and CDDP. In both cell lines, combination treatment caused greater inhibition of growth than treatment with CDDP alone.

Our results indicate that EPFP inhibits the proliferation of HT29 and Colo205 cells and its effect is concentration-dependent. It is worth emphasizing that EPFP enhances anti-proliferative effects of CDDP.

P6.12

DNA methylation of GPX3 and loss of heterozygosity of RASSF1 and VHL in renal cell carcinoma

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Keywords: DNA methylation; LOH; renal cancer; cfDNA

During recent years, many publications have suggested that cell-free DNA (cfDNA) might be a cancer biomarker. In samples from patients with different types of malignant tumors, genetic and epigenetic changes that are associated with cancer development and progression have been detected on cfDNA. Therefore, we tested markers that are based on epigenetic and genetic changes in order to interrogate aberrant DNA methylation and the loss of heterozygosity (LOH) of distinct genomic regions, respectively. Blood plasma and tumor tissue were obtained from 35 clear cell renal cell carcinoma (ccRCC) patients who received a nephrectomy before surgery. It is well known that deletions involving the short arm of chromosome 3 are typically characteristic of ccRCC development. The highest frequency of LOH (40%) in genomic DNA was found at D3S1038, corresponding to the VHL gene (locus 3p21.3), compared to 14.2% at D3S966 and 31.4% at D3S1568, which corresponded to the RASSF1 gene (locus 3p25–26). Total number of LOH was 65.7%, and in one sample LOH was detected for all three loci. Methylation-specific PCR of the GPX3 gene revealed that 65% of the tumor's genomic DNA was methylated, while in cfDNA this figure was 42%. In summary, the D3S1038 microsatellite marker with the highest level of LOH must be verified on cfDNA in future studies. The GPX3 gene can serve as a potential marker for creating a modern, non-invasive test for ccRCC diagnostic.

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P6.13

Post-synthetic modification of DNA-oligonucleotides with boron clusters using „click chemistry" methodology

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Keywords: click chemistry; EGFR; boron clusters

DNA nanotechnology is a branch of technology that exploits nucleic acids' ability to self-assemble in order to construct nanostructures with specific properties. There are numerous potential applications of DNA nanostructures (e.g. diagnostics and therapeutics for human health) [1].

Based on previous studies using boron clusters to modify nucleic acids [2], we designed conjugates of DNA oligonucleotides and oligo-functionalized boron clusters (*o*-carboranes and metallacarboranes) as potential building blocks for nano-framework construction.

In this communication, we present a new approach to synthesize this type of object using modified Huisgen 1,3-dipolar cycloaddition („click chemistry"). Chemical synthesis of alkyne-functionalized DNA oligonucleotides (5- and 22-mers) was carried out using a phosphoramidite solid-phase method. As a proof of principle example, we chose the antisense oligonucleotide for Epidermal Growth Factor Receptor (EGFR) mRNA.

The modified units 1 and 2 were introduced into the internal position of the antisense oligonucleotide. The resulting oligonucleotides were purified by high performance liquid chromatography (HPLC) and post-synthetically modified with boron clusters linked to alkylazides (3-5) using a cooper-catalyzed azide-alkyne cycloaddition reaction. The proper sequence and purity of the compounds were confirmed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. These conjugates will be used for EGFR-targeted gene silencing. Their activity will be monitored using a dual fluorescence assay in HeLa cells trasfected with pGFP-EGFR and pDsRed-N1 plasmids.

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P6.14

Development of a dual fluorescence assay for monitoring of anti-EGFR silencing activity of chemically modified oligonucleotides; construction of pGFP-EGFR fusion plasmid

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Keywords: oligonucleotides

The aim of the project was to evaluate gene silencing activity of boron clusters containing antisense oligonucleotides [1] directed towards mRNA of the epidermal growth factor receptor (EFGR). This protein is involved in regulating cell growth in breast carcinomas [2]. Here we present an approach for construction of the pGFP-EGFR plasmid coding *gfp-egfr* fusion gene [3]. The mRNA of EGFR was isolated from HeLa cells and reverse-transcribed and amplified to its cDNA. The 330 nucleotides coding fragment, present in four splicing variants of EGFR transcripts, was obtained by the cDNA digestion with *Sac*I and *Eco*RI restriction enzymes, and cloned into the pUC18 plasmid. After transformation to TOP10 strain of *E. coli* the isolated DNA plasmids were sequenced. The proper sequence insert was re-cloned to pEGFP-C1 plasmid and amplified in *E. coli*. The pEGFP-C1 and control pDsRed-N1 plasmids were expressed in HeLa cells to evaluate gene silencing activity of antisense oligonucleotides directed towards mRNA of EGFR.

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P6.15

Semiquantitative proteomics reveals novel cellular targets of potent anticancer and antifungal indolo[2,3-b]quinoline drug, DiMIQ, in *Candida albicans* biofilms

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Keywords: *Candida albicans*; DiMIQ; biofilms; cancer related infections

Infectious diseases caused by pathogenic fungi have begun to emerge as a major threat in cancer patients. The most common fungal pathogen *Candida albicans* typically causes superficial infections in the general immunocompetent population but is more likely to cause life threatening disseminated infections in cancer patients. DiMIQ (5,11-dimethyl-5H-indolo[2,3-b]quinolone) is a synthetic neocryptolepine analog with a broad spectrum of bioactivities. DiMIQ and its derivatives displayed promising anticancer-antifungal characteristics which could aid future anticancer-antimicrobial treatment [1, 2]. DiMIQ is classically believed to intercalate DNA and inhibit DNA topoisomerase II activity, but can also affects natural membranes [3].

DiMIQ substantially modified the proteome, which consisted of 800+ proteins. Functional mapping confirmed the activity of DiMIQ against DNA metabolizing enzymes; however a significant down-regulation of proteins involved in cell cycle, DNA processing, protein synthesis, cell communication/signal transduction, certain aspects of cell rescue/defense/virulence, and cellular transport clusters was also observed. Identified up-regulated proteins played key roles in general metabolism, biogenesis of cellular components, and energy pathways. In particular, DiMIQ targeted the biogenesis of mitochondrion and cell wall components, and those involved in metabolism of lipids/fatty acids/isoprenoids, sugars/glucosides/polyols/carboxylates, amino acids, and vitamins/cofactors/prosthetic groups. In conclusion, we have provided an extensive catalog of DiMIQ-regulated proteins and revealed cellular targets that potentially can facilitate design of novel more selective DiMIQ-based derivatives for future anticancer-antimicrobial treatments.

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P6.16

Synthesis and antioxidant properties of new 1,2-benzothiazine derivatives

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Keywords: antioxidants; 1,2-benzothiazine; EPR; DPPH; synthesis

Antioxidants have received much attention for the beneficial effects on human health especially that can prevent cardiovascular, neurodegenerative and cancer diseases by scavenging free radicals, mainly reactive oxygen species (ROS), produced during cell metabolism.

Therefore, we designed and synthesized two series (A and B) of 1,2-benzothiazine derivatives as potential anti-inflammatory and analgesic agents with antioxidant properties. Series A has propylene linkage between thiazine and piperazine nitrogen while series B 2-oxoethylene. In the synthesis of designed molecules the key starting compound was saccharine. The reaction of saccharine with corresponding 2-bromoacetophenones afforded 2-(p-substituted)-benzoylmethyl-1,1-dioxo-1,2-benzothiazol-3-one derivatives. Then was conducted Gabriel-Colman rearrangement with formation of 1,2-benzothiazine ring. In series A 1,2-benzothiazines were alkylated with 1-(3-chloropropyl)-4-arylpiperazines and in series B with 1-(2-chloro-1-oxoethyl)-4-arylpiperazines.

We applied electron paramagnetic resonance (EPR) - radical scavenging activity (RSA) technique to determine the ability of the antioxidant to scavenge stable free radical with an unpaired electron (DPPH•). Decreasing signal as a function of time for one of analogues 1,2-benzothiazine was observed. Field under the absorption curve was proportional to the amount of stable radicals remaining in the sample. Results for both groups indicated a non-linear relationship between the concentration (DPPH•) to the time and showed slightly different shape. More effective scavenging free radicals in first few minutes were demonstrated for series B than series A. After 15 minutes intensity remain percentage of DPPH• for series B was about 15-35%, while for series A 18-25%.

P6.17

Oat beta-glucan reveals antitumor activity in human epithelial lung cancer

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Keywords: oat beta-glucan; lung cancer; oxidative stress

Beta-glucans are widely used in treatment, cosmetics, and the food industry. Glucans play a significant role in activation of the immune and antioxidant system. They also demonstrate anti-cancer properties. In the current study the antitumor activities of high and low molecular weight beta-glucan derived from oats were investigated in human lung cancer cells (A549) and normal keratinocytes (HaCaT). The effect of beta-glucan from oat was evaluated by cellular viability assessment, lipid peroxidation, manganese superoxide dismutase evaluation and cytoskeleton visualisation. Our results indicate strong anti-tumor properties of new beta-glucan from oat and at the same time no toxicity for normal cells.

P6.18

The influence of metalloestrogens (chromium VI and chromium III) on the mitochondrial activity of estrogen dependent wild type breast adenocarcinoma cells (MCF-7/WT)

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Keywords: estrogen; metalloestrogens; genotoxicity; breast adenocarcinoma

Increasing incidence of estrogen-dependent breast cancer has stimulated research of environmental factors that influence estrogen–dependent processes, especially factors that interact with estrogen's receptor. One of the most important ones is chromium, a metalloestrogen. This metal is widely present in the environment. It's toxicity depends on the valence electrons. Chromium(VI) is classified as a human carcinogen (IARC list), while chromium(III) is used to make glucose tolerance.

The final products of estradiol biotransformation of C-4, C-16 hydroxylation, and C-2 methoxylation are 4-hydroxyestradiol (4-OHE2), 16-a-hydroxyestrone (16α-OHE1), and 2-metoxyestradiol (2-MeOE2), respectively. The role of these metabolites in carcinogenesis is unknown; however, there are some reports of 4-OHE2-adducts in breast cancer tissue. Others have reported anticancer activity of 2-MeOE2.

The aim of this study was to evaluate chromium/estrogen interactions in human breast adenocarcinoma cells and to determine the contribution of oxidative stress to these interactions. We determined the role of 17-β-estradiol (E2), 4-OHE2, 16a-OHE1, and 2-MeOE2 after exposure to metalloestrogens (chromium VI and chromium III). The study used the human breast cancer cell line (MCF-7/WT). The cytotoxicity of each compound was evaluated using the MTT assay. Next, the genotoxicity of estrogen or chromium alone was estimated using the alkaline comet assay. The ultimate goal of this research is to examine the effect of estrogen/chromium VI or estrogen/chromium III interactions on the genotoxicity of MCF-7/WT cells.

Our results from the mitochondrial activity assay and genotoxicity evaluation indicated an important role for estradiol and its metabolites in MCF-7/WT cells after exposure to metalloestrogens.

P6.19

The combination of bleomycin and betulinic acid with nanosecond pulsed electric fields (nsPEFs) in human cancer and normal cell lines

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Keywords: nsPEF; electroporation; bleomycin, betulinic acid

Nanosecond pulsed electric field (nsPEF) is the application of ultra-short electrical pulses and high voltage of electrical field intensity to induce cell membrane permeabilization. It can be used in the effective elimination of tumor cells or as a support in standard chemotherapeutic (CT) procedures.

In the following study, we analyzed the effect of nsPEF alone or in combination with therapeutic procedures on cancer cell viability. We studied this effect in human gastric adenocarcinoma cells (EPG85-257P and EPG85-257RDB), human melanoma cells (Me45), human epidermoid carcinoma cells (A431) and normal keratinocytes (HaCaT). The in vitro therapeutic procedures were used with bleomycin and betulinic acid. For combination therapy (nsPEF-CT), ultra-short pulses were applied for a duration of 10 ns and a time rise of 2 ns. The electric field was applied in the range of 0÷100k V/cm. A field intensity 12.5 kV/cm was applied in all selected cell lines to enhance drug transport. The effect of each procedure (CT, nsPEF, nsPEF-CT) was evaluated by the measurement of oxidoreductive mitochondrial potential (MTT assay). The permeability of cell membranes was visualized with a DHCC fluorescent marker. Our results indicated that nsPEF alone can be applied to effectively decrease cancer cell viability. Additionally, nsPEF improves intracellular drug transport and can enhance pharmacological treatment, specifically betulinic acid and bleomycin. Moreover, the nsPEF procedure takes only 10 min and 2 sec. long and is minimally invasive

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P6.20

Evaluating the effectiveness of electrochemotherapy with leucovorin in pancreatic cancer — *in vitro* study

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Keywords: pancreatic cancer; leucovorin calcium; electroporation; folic acid; electrochemotherapy

Pancreatic ductal adenocarcinoma (PDAC) is renowned for the malignant nature and resistance to treatment. Despite considerable progress in cancer regimens, it is still among the deadliest cancers with fatal predictions of survival. As a consequence, there is growing importance of experimental therapy in PDAC treatment. An example is electroporation, a modern technique that enables permeabilization of cell membranes, thereby enhancing drug effectiveness. Leucovorin calcium is a reduced form of folic acid. In a similar manner to the chemotherapeutic 5-fluorouracil, it affects thymidylate synthase, limiting DNA synthesis in rapidly proliferating cancer cells. Additionally, it acts as a chemoprotectant for normal pancreatic cells. Therefore, combining leucovorin with electroporation represents a novel approach of treating this disease by means of low toxic and cost-effective substances. Our investigation aims to determine the influence of therapy with leucovorin calcium combined with electroporation on PDAC cells. Material for the research was a human PDAC cell line resistant to daunorubicin (EPP85-181RD). In order to measure cell viability, MTT assays were performed. The extent of necrosis and apoptosis was evaluated via comet assays. Additionally, the activity of glutathione S-transferase (GSTπ) and low-voltage-activated T-type calcium channels (α1H) was assessed using immunocytochemical staining. The results obtained support the hypothesis that electroporation enhances drug delivery into cells. Advantageous results were obtained using relatively low concentrations of leucovorin calcium combined with an electroporation current intensity of 800 V/cm. However, to fully understand the mechanism of leucovorin action in PDAC, more studies on different cell lines, including primary cell cultures, need to be conducted.

P6.21

Reversible electroporation of human breast cancer cells for the support of photodynamic reaction — an *in vitro* study

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Keywords: electroporation; electro-photodynamic reaction; breast cancer

Reversible electroporation (EP) uses a pulsed electric field of high intensity to create temporal disorders in the structure of a cell membrane. Because it can improve cellular drug delivery, EP has been combined with chemotherapy in a clinical procedure called electrochemotherapy. The aim of our study was to evaluate the potential of using reversible EP to improve delivery of photosensitive compounds to breast cancer cells, to enhance the effectiveness of the photodynamic reaction (PDR) *in vitro*.

The experiments were performed on two human breast adenocarcinoma cell lines: sensitive (MCF-7/WT) and resistant to doxorubicin (MCF-7/DX). Photofrin® and the cyanine dye IR-775 were used as the photosensitive compounds. Confocal microscopy and bright-field microscopy were used to observe the cellular morphology 24 hours after EP or electrophotodynamic reaction (EP-PDR). Changes in the cellular mitochondrial activity were evaluated using the XTT assay. A cell death pathway was evaluated using the TUNEL assay. EP and PDR alone were not toxic to the cells. However, application of EP resulted in an increase in PDR effectiveness in drug-sensitive and drug-resistant types of breast cancer cells *in vitro*.

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P6.22

microRNAs in formalin-fixed paraffin-embedded samples from stereotactic brain biopsies as potential diagnostic biomarkers of primary central nervous system lymphoma

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Keywords: lymphoma; PCNSL; microRNA; FFPE; diagnosis

Introduction: Primary central nervous system lymphomas (PCNSL) are an aggressive, extra nodal form of non-Hodgkin lymphomas. Early and precise diagnosis of PCNSL is important for proper treatment, but this remains challenging. Despite numerous diagnostic possibilities, histological examination of stereotactic biopsy material is the most commonly used approach.

Aim: To examine the value of miR-9, miR-let-7b, miR-125b, miR-155 and miR-196b assessment in formalin-fixed paraffin-embedded (FFPE) samples from stereotactic brain biopsies for the differential diagnosis of PCNSL vs. non-neoplastic neurological diseases.

Material and Methods: Leftover FFPE samples from stereotactic brain biopsies collected for the routine diagnostic purposes from patients with CNS lesions suspected of PCNSL consulted at the Memorial Cancer Center and Institute of Oncology in Warsaw. Expression levels of microRNAs were measured by RT-qPCR using TaqMan® MicroRNA assays.

Results and Conclusions: We found significantly ($p<0.01$) higher levels of miR-155 and miR-196b and significantly ($p<0.01$) lower levels of miR-let-7b, miR-125b and miR-9 in FFPE brain biopsies from patients with PCNSL compared to patients with non-neoplastic neurological diseases. We propose that this set of five microRNAs as promising biomarkers in the diagnosis of PCNSL.

P6.23

microRNAs in the cerebrospinal fluid of differential diagnosis of primary central nervous system lymphoma

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Keywords: microRNA; cerebrospinal fluid; RT-qPCR; PCNSL; diagnosis

Introduction: Precise differential diagnosis of primary central nervous system lymphoma (PCNSL) and non-neoplastic diseases of the central nervous system (CNS) is a prerequisite for proper treatment. Diagnosis of PCNSL remains challenging, despite the use of brain imaging techniques, histopathological examination of brain biopsies, cytological and flow cytometric examination of cerebrospinal fluid (CSF). Furthermore, brain biopsy carries a risk of neurological complications, and sometimes cannot be performed due to inaccessible lesion location.

Aim: Evaluation of the utility of selected microRNAs, including: miR-21, miR-19b and miR-92a in CSF for the differential diagnosis of PCNSL vs. non-neoplastic diseases of the CNS.

Material and Methods: Leftover CSF samples collected for routine diagnostic purposes from patients with CNS lesions suspected of PCNSL consulted at the Memorial Cancer Center and Institute of Oncology, Warsaw. Expression levels of microRNAs were evaluated by RT-qPCR.

Results and Conclusions: We found a distinct differences in the expression profile of miR-21, miR-19b and miR-92a in the CSF from patients with PCNSL compared to non-neoplastic diseases of the CNS. Our preliminary data on the expression levels of other microRNAs implicated in B-cell lymphoma pathogenesis or in brain physiology (including: miR-9, miR-9*, miR-155, miR-125b, miR-196b) suggest their potential utility in differential diagnosis of PCNSL.

P6.24

Evaluation of spectral and photosensitizing properties of cyanine complexes on human squamous lung cancer cells A549

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Keywords: cyanine dyes; photodynamic reaction

Cyanine dyes are compounds that are considered to be potential photosensitizers that could be used in photodynamic therapy to kill cancer cells. To test this, the spectral properties and localization in A549 human squamous lung cancer cells of three cyanine dyes, 1610 15A, RR340 and UMK25A 15, were evaluated. The cells were exposed to photodynamic reaction with a maximum wavelength of 470 nm. The proliferative activity of cells was examined by an MTT assay. It was observed that cyanine dyes were efficiently taken up by cells and was distributed in mitochondria, which was visible by fluorescent microscopy as intensely glowing grains. They cyanine dye RR340 was found to have the best properties as a photosensitizer, as it reduced cell survival after irradiation nearly two times. We can conclude that all cyanine dyes tested in our study have the potential to be candidates for use as photosensitizers in photodynamic diagnosis and therapy of cancer.

P6.25

Enhancement of chlorin e6 anti-tumour photodynamic activity by its conjugation with gold nanoparticles, stabilized by a dextran-polyacrylamide copolymer

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Keywords: nanocomposite photosensitizer; gold nanoparticles; chlorin e6; photodynamic therapy

Among various nanotechnology products, gold nanoparticles (GNPs) generate significant interest among biomedical researchers. GNPs act not only as effective therapeutic agent transporters but also as components of nanocomposites for anti-tumour targeted photodynamic therapy due to the following features: the large amount of delivered drug molecules, because of the large nanoparticle surface area; increased accumulation of nanoparticles loaded with photosensitizer in the tumour, because of enhanced permission and retention effects; prevention of drug degradation in the living biological environment, and aggregation of hydrophobic photosensitizer molecules. To this end, the use of GNPs as nanocarriers for photosensitizers seems quite reasonable.

The objective of the study was to produce the GNP-based nanocomposite in the water-soluble polymer and photosensitizer chlorin e6 and to study its anti-tumour photodynamic activity *in vitro* and *in vivo*.

The GNPs were synthesized in a copolymer dextran-graft-polyacrylamide matrix. The size distribution of nanoparticles in solution was established by Dynamic Light Scattering and transmission electron microscopy, and their UV-visible spectra were characterized. Using the fabricated solutions as a nanocarrier system for chlorin e6, singlet oxygen generation of the composite photosensitizer was demonstrated (with a specific sensor) in model solutions. In *in vitro* experiments on the malignant human lymphocyte cell lines MT-4 and Namalwa, the photodynamic activity of the nanocomposite proved to be two-fold higher than the activity of the free photosensitizer. Enhanced nanocomposite anti-tumour photodynamic activity was confirmed in tests on C57Bl/6 mice with implanted lung Lewis carcinoma.

P6.26

Arginine deprivation and radiation in HNSCC: the molecular mechanisms of response in 2-D vs. 3-D models

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Keywords: HNSCC; arginine deprivation; ER stress; radiosensitization

Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignant tumors in the world with relatively low overall survival rate and poor sensitivity to radiation therapy and chemotherapeutic drugs. Metabolic anticancer therapy based on arginine deprivation, currently, is under extensive study as a perspective approach to several solid tumors, including HNSCC.

Responder (SAS) and non-responder (FaDu) to arginine deprivation and irradiation HNSCC cell lines were used in this study. Here, we showed for the first time that one of the responses of HNSCC to single arginine starvation is the activation of ER stress. Our data strongly suggest that arginine deprivation triggers a massive ER stress much more prevalent in SAS cells mainly via IRE1α and PERK signalling pathways. It was shown that prolonged arginine deficiency impairs the main pro-survival signaling pathways and triggers apoptosis only in SAS cells, both in 2-D and 3-D models. However, arginine deprivation-induced SAS cell death does not mainly associate with triggered ER stress and arginine depletion less effectively activated ER stress in 3-D model. In addition, we found that arginine deprivation induces ER stress, which sensitizes SAS cells to single dose irradiation. We also hypothesized that marginal activation of UPR signalling pathways in non-responder FaDu cells can be maintained ER homeostasis during arginine deprivation stress.

Taken together, this study provides a new insight of role ER stress induction in observed arginine deprivation effects in HNSCC models and can be a novel target to develop more efficient metabolic anti-cancer approaches.

P6.27

Colocalization analysis shows the role of PH domain of BCR in the proteasomal degradation and clathrin-mediated endocytosis

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Keywords: PH domain; BCR; clathrin; endocytosis

Chromosomal translocation t(9;22)(q34;q11) leads to generation of different types of Bcr-Abl fusion protein. These fusion proteins are designated as p190, p210, p230 and associated with acute lymphoblastic leukemia (ALL), chronic myelogenous (CML) and chronic neutrophilic leukemia (CNL) respectively. PH domain of BCR is absent in Bcr-Ablp190 and present in Bcr-Ablp210. Earlier research determined 23 potential interaction partners of PH domain of BCR by mass-spectrometry. These proteins are involved in various cellular functions that may determine the differences between CML and ALL progression.

Cortactin (CTTN) is involved in actin branching during clathrin-mediated endocytosis. Altering of this process by possible interaction with BCR-ABL may impair cellular signaling by disrupting the vesicular transport and receptor internalization.

Ubiquitin specific protease 1 (USP1) is deubiquitination enzyme. Possibly BCR-ABL oncoprotein omits proteasomal degradation due to its deubiquitination by USP1. Its accumulation may lead to progression of myeloproliferative disorder.

Methods. HEK293T cells were transfected with vectors coding corresponding sequences tagged with fluorescent proteins using cationic polymer transfection. Transfected cells were evaluated with confocal fluorescent microscopy. Image processing and statistical analysis of colocalization were done in ImageJ software.

Results. Colocalizations of ECFP-CTTN with mKatePH, mCherry-clathrin with ECFP-CTTN, ECFP-CTTN with mKate-PH, and ECFP-USP1 with mCitrinePH were statistically significant according to Pearson and Manders coefficients.

Conclusions. Colocalization of PH domain of BCR with clathrin and CTTN unravels its role in clathrin-mediated endocytosis. Colocalization of PH domain of BCR and USP1 occurs in the nucleus which may have important role in determining spatial resolution of regulation of proteasomal degradation of BCR-ABL.

P6.28

Hypoxia in gene therapy

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Hypoxia is a common phenomenon among tumors. It occurs due to several factors, such as intense proliferation of cancer cells, insufficient angiogenesis within tumors or deregulation of hypoxia-related gene expression patterns. HIF-1α, a subunit of transcriptional factor complex, plays a key role in regulation of hypoxic-stress response and adaptation of malignant cells to the low oxygen tension. Expression of HIF-1α target genes takes place due to hypoxia responsible elements (HREs) in the promoter region of these genes. Gene therapy coupling mechanisms similar to the ones of the regulatory mechanisms existing in HIF-1α pathways may increase specificity of the therapeutic approach.