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CONFERENCE REPORT

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# MATERIALS OF SYMPOSIUM AND SUMMER SCHOOL "FUNDAMENTAL PRINCIPLES OF CANCER BIOTHERAPY", MAY 21–23, 2018, KYIV, UKRAINE

The International Scientific Symposium "Fundamental Principles of Cancer Biotherapy" was held on May 21–23, 2018 in Kyiv, Ukraine. It was organized by R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) of the National Academy of Sciences of Ukraine (NASU). The symposium was supported by the VACTRAIN project (TWINNING on DNA-based Cancer Vaccines, No 692293) in the frame of the European Union's Horizon 2020 Research and Innovation Program.

At the symposium, the invited speakers and young researchers from Ukraine, Poland, Latvia, India and Sweden presented the latest data of their studies. The main focus of the meeting was cancer biotherapy, including discussion on the new targets and methodologies. More than 70 researchers took part in the work of this conference. At the opening speech, the Director of R.E. Kavetsky IEPOR of NASU, Academician of NASU Vasyl Chekhun gave a brief overview on IEPOR research and collaborations. He also mentioned that the VACTRAIN grant provided the opportunities of the professional growth for the young generation of research fellows. Dr. Chekhun welcomed all the attendees in Kyiv, especially this year, when we celebrate the centenary of the Ukrainian Academy of Sciences. The conference venue, a Great Conference Hall of the NASU, was an obvious choice for this event.

Galina Selivanova (Sweden) presented a new conception, concerning a guardian of a genome — the p53 protein. Galina told about two faces of p53, so called "the p53-healer" and "the p53-killer". It was extremely important to learn about successful clinical trials of the novel anticancer medicine, based on activation of the p53 protein. Liudmyla Drobot (Ukraine) spoke about plasticity of cancer cells, especially about an amoeboid stage of epithelial-to-mesenchymal transition (EMT) that helps tumors cells to migrate and metastasize. Elena Kashuba (Ukraine) continued the EMT topic and elucidated a role of mitochondrial ribosomal protein S18-2 (MRPS18-2) in this process in prostate cancer. During the conference, new directions of cancer therapy were discussed by Volodymyr Shlyakhovenko, Artem Tykhomyrov, Oksana Skachkova and Olha Karaman (all from Ukraine). Andrey Alexeyenko (Sweden) spoke about systems biology — an important part of tumor biology. Philosophy and history of cancer research was discussed by Chacnhal Mitra (India).

The poster sessions with vivid discussions on the different topics related to cancer therapies were held during these two days of the conference (see selected abstracts). Cancer therapy and the problems of personalized cancer therapy are also in the spot of the interest of the society. A journalist from "Health of Ukraine" newspaper was present at the conference and we expect to see an article soon.

## SELECTED ABSTRACTS

### FUNCTIONAL ACTIVITY OF THE NADH-DEPENDENT REDUCTASE SYSTEM IN A LIVER MICROSOMAL FRACTION IN RATS WITH GUERIN'S CARCINOMA UNDER CONDITIONS OF ESSENTIAL NUTRIENTS ADMINISTRATION

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**Background:** The liver monooxygenase system (MOS) plays a major role in the metabolism of important endogenous substrates as well as in biotransformation of xenobiotics. However, the growth of tumor impairs the MOS functioning that results in the increase of the intracellular concentration of the free radical molecules. Free radicals attack important macromolecules, leading to cell damage and homeostatic disruption. It was shown already that essential nutrients, such as retinoids and polyunsaturated fatty acids, play an important role in cancer prevention. These compounds are recognized also by their anti-proliferative and anti-oxidative activity, but it is unknown how they affect MOS in the liver of tumor-bearing animals.

**The aim** of the study was to determine the activity of the NADH-dependent reductase system in the microsomal fraction and the superoxide radical generation by this system under conditions of essential nutrient administration.

**Methods:** As a model of malignant tumor growth, Guerin's carcinoma was employed. ω-3 PUFAs and retinoids were administered by gavage for 4 weeks prior to the carcinoma implantation and then for the entire duration of tumor growth. The daily dose of ω-3 PUFAs was 120 mg/kg of a body mass. Retinoids were administered as the retinyl ester at a dose of 3000 IU. Microsomal fraction was obtained by differential centrifugation. A catalytic activity of NADH-cytochrome b5 reductase (CYB5R), cytochrome b5 (CYB5) and P450 content (CYP), and also the rate of generation of superoxide anion radical by compo-

nents of the reductase chain were assessed in these fraction, using a spectrophotometric assay.

**Results:** NADH-dependent CYB5R activity in the liver microsomal fraction in rats with Guerin's carcinoma increases by 29% in comparison with a control group. The significant increase in the rate of generation of superoxide anion radical by components of the reductase chain (4.6 fold) indicates the change of the electron transport in the reductase chain. CYB5R transmits electrons not to CYB5, but to the oxygen molecule, followed by the formation of reactive species. The high CYB5R activity and the enhanced generation of $O_2^{\cdot}$ lead to a 2-fold decrease in the content of the membrane-associated CYB5. Administration of pharmacological doses of retinoids prior to and post-implantation of the tumor leads to a reduction of functional activity of the NADH-dependent reductase system in the microsomal fraction and decrease of CYP content by 48%. The generation of a large amount of hydroxyl metabolites of retinol further contributes to the damage of liver. The ω-3 PUFAs supplementation, which has anti-oxidative activity and can repair damaged membranes by embedding into them, has some stabilizing effects. However, the complete recovery of the studied activities to the corresponding indicators of control group was not shown.

**Conclusions:** The obtained results indicate that administration of pharmacological doses of the vitamin A prior to grafting carcinoma cells impairs the functional activity of NADH-dependent reductase system in the microsomal fraction of the liver. The ω-3 PUFAs, as components of membranes, help to maintain the functional state of the MOS components in the endoplasmic reticulum.

## TUMOR-INFILTRATING LYMPHOCYTES, HYPOXIA AND CANCER-ASSOCIATED ADIPOCYTES IN PRIMARY TUMORS IN PATIENTS WITH GASTRIC CANCER AND OVERWEIGHT

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**Background:** Obesity has been linked to more aggressive characteristics of the major common cancers, including breast and prostate cancer, colorectal, endometrial, esophageal, pancreatic and renal cell cancer. The growth and metastasizing of solid tumor growth require the interaction of tumor cells with the surrounding tissue. Due to the ubiquitous nature of adipose tissue, many types of solid tumors grow in the close proximity or direct contact with adipocytes and adipose-associated stromal and vascular components. Adipocytes modulate the tumor microenvironment by promoting angiogenesis, affecting immune cells and altering metabolism to support growth and survival of metastatic cancer cells. During their interaction with cancer cells, adipocytes dedifferentiate into pre-adipocytes or are reprogrammed into cancer-associated adipocytes (CAA) that secrete adipokines, stimulating adhesion, migration, and invasion of tumor cells.

At the same time, the stromal components of tumor microenvironment, such as tumor-infiltrating lymphocytes (TILs) and hypoxia are also the crucial factors for tumor growth. However, the very limited data are reported, describing possible associations between these factors and CAA in patients with gastric cancer (GC) and with overweight, in particular.

**The aim:** We wanted to examine the dependence between the number TILs, $CD8^+$ and $CD45RO^+$ T lymphocytes, hypoxia levels, the CAA density and a body mass index (BMI) in GC patients.

**Methods:** Expression of CD8, CD45RO and perilipin (PLIN, a marker for viable adipocytes) was assessed by immunohistochemistry; hypoxia levels in tumor was evaluated by $^{31}$NMR spectroscopy; BMI was calculated. 94 patients with primary GC were diagnosed and treated at the Kyiv City Clinical Oncological Center. No chemotherapy or radiation prior to surgery was conducted. Tumors were classified and staged, according to the 2002 version of the UICC staging system. All patients were thoroughly informed about the study that was approved by the local ethics committee.

**Results:** 36.6; 58.6 and 88.4% of patients with BMI < 25, 25 < BMI < 30, BMI > 30 showed a high CAA density in tumors, respectively. Median number of the CAA was 26.5%. The probability of the high density of CAA in tumors of patients with BMI > 30 was increased by a factor 11 (OR 11.01, $\chi^2$ = 12.9, 95% CI 28.933–5.749, $p < 0.001$) in comparison with patients with BMI < 25. Under severe and moderate hypoxia (PME < 1.4) in tumors the high CAA density was detected in 71.4; 6.8 and 91.7% of patients with BMI < 25, 25 < BMI < 30 and BMI > 30, respectively. 57.1; 42.8 and 16.6% of patients with BMI < 25, 25 < BMI < 30 and BMI > 30 had a high number of TILs in tumor, respectively. The median number of $CD8^+$ and $CD45RO^+$ cells was 34.4 and 36.4%, respectively. When tumors showed the high CAA density, then 43.1; 32.7 and 20.8% of $CD8^+$ T cells and 43.8; 28.7 and 22.9% $CD45RO^+$ T cells was detected in tumors of patients with BMI < 25, 25 < BMI < 30, BMI > 30, respectively. The TILs density decreased significantly in patients with BMI > 30 (OR = 4.51, $\chi^2$ = 4.38, 95% CI 10.486–1.916, $p < 0.05$) as compared with patients with BMI < 30.The probability of the presence of the low number of $CD8^+$ T cells in tumor was increased by the factor of 5.0, when severe and moderate hypoxia was detected ($p = 0.046$).

**Conclusions:** The elevated BMI values were consistently associated with the high CAA number, as well as with decrease of the number of $CD8^+$ and $CD45RO^+$ T cells in GC, but not with hypoxia levels.

## EFFECT OF LACTOFERRIN ON LEVELS OF ESSENTIAL ELEMENTS IN ANIMALS WITH SENSITIVE AND RESISTANT WALKER-256 CARCINOSARCOMA

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**Background:** It is known that the development of hormone-dependent tumors, including breast cancer (BC), is accompanied by an imbalance of homeostasis of microelements (ME), which affects the change in the catalytic activity of enzymes, regulation of hormones, growth factors and the content of transporting proteins. It is shown that the most informative for the estimation of antagonistic and synergistic interactions between ME is not only assessment of their content, but also analysis of their ratio. However, there is practically no data on the correction of mineral metabolism during malignant process with the use of iron and copper-binding protein, lactoferrin (LF).

**The aim:** To investigate the content of Fe, Cu, Zn, Ca and Mg in blood serum (BS) and tumor tissue (TT) of animals with Walker-256 carcinosarcoma with different sensitivity to doxorubicin (DOX) treated with exogenous LF or its combination with DOX.

**Methods:** In BS and TT of experimental animals, we have analyzed ME content after administration of LF at concentrations of 1 and 10 mg/g. The change in ME composition in BS and TT of animals with DOX-resistant carcinosarcoma after administration of 10 mg/g LF, DOX, or LF and DOX combination, was determined. The content of Cu, Zn, Mg, Ca and Fe in BS and TT was determined by the atomic emission spectral method, while Fe content in the BS was analyzed using biochemical method.

**Results:** It was shown that administration of LF in a concentration of 1 mg/g did not change ME content in BS of animals with sensitive carcinosarcoma compared with control, while in a concentration of 10 mg/kg LF caused 1.5–2-fold decrease in the content of Fe, Cu, Ca in the BS and 1.2-fold increase of the content of Mg and Zn. The administration of LF at 1 or 10 mg/kg decreased the Cu/Zn ratio (1.44 and 0.52) and Ca/Mg ratio (3.23 and 2.21) in the BS of treated animals compared to control values (2.04 and 4.10). However, LF in a concentration of 1 mg/g caused 1.5-2-fold decrease in TT content of Fe, Cu, Ca against the background of unchanged Zn and Mg content. The administration of LF in concentrations of 1 and 10 mg/kg caused a decrease in Cu/Zn ratio (0.15 and 0.14) and Ca/Mg ratio (0.23 and 0.20) in TT, compared with the control (0.2 and 0.37, respectively). In BS of animals with DOX-resistant carcinosarcoma, administration of LF in a concentration of 10 mg/g resulted in a 1.2 to 1.5-fold decrease in the ME content, except for Zn, which caused a decrease in Cu/Zn ratio (1.52) and Ca/Mg ratio (4.27) compared with the control values (2.27 and 5.16). Separate administration of DOX caused a decrease in the BS content of all ME by 1.3 times along with a 1.2-fold increase in Fe content. The most pronounced changes in the content of ME in the BS of these animals were detected in the case of a combined action of LF and DOX: 1.5-2-fold decrease of the content of Fe, Cu and Ca at the background of 1.2-fold increase of Zn and Mg content of, which caused a decrease in Cu/Zn ratio (1.50) and Ca/Mg ratio (4.03). Resistant TT showed a similar nature of ME changes, with the most pronounced effect in the case of the combined action of LF and DOX, which was reflected in the maximal decrease in Cu/Zn ratio (0.14) and Ca/Mg ratio (0.21) compared to the control values (0.18 and 0.33).

**Conclusions:** It was shown that the administration of LF in concentrations of 1 and 10 mg/kg to animals with DOX-sensitive Walker-256 carcinosarcoma changed ME composition and ME ratio in BS and TT, with a more pronounced effect in the cases of higher LF dose. It was found that the combined effect of LF and DOX caused the maximal decrease in Cu/Zn and Ca/Mg ratio in BS and TT of animals with DOX-resistant carcinosarcoma, evidencing on inhibition of the activity of matrix metalloproteinases and epithelial-mesenchymal transition.

## DEVELOPMENT, ENGINEERING AND PHYSICAL PROPERTIES OF A LIPOSOMAL FORM OF THE CYTOTOXIC LECTIN FROM *B. subtilis* B-7025

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**Background:** Methods of antitumor biotherapy are widely used for a standard treatment of cancer patients. An important part of biotherapy is antitumor vaccines, which are often included in treatment regimens to prevent disease recurrence and metastasizing. In our previous studies, we have shown that the use of antitumor vaccines, produced from tumor cells with the addition of the cytotoxic lectin produced by *B. subtilis* B-7025 increased medium life expectancy of experimental animals inhibiting the growth of the primary tumor node and decreasing metastasizing. Recently, it was demonstrated that the use of pharmaceutical drugs in liposomal forms changed dramatically their pharmacokinetics and therapeutic efficacy. We hypothesize that the liposomal form of antitumor vaccine could significantly increase the immunogenicity of the antigenic component of the vaccine. This might be due to unique properties of liposomes, protecting the components of antitumor vaccines from biodegradation. So, it should be easier to create a vaccine depot at the injection site and deliver it to the peripheral lymph nodes.

**The aim:** To synthesize the liposomal form of the cytotoxic lectin isolated from *B. subtilis* B-7025 and characterize its physical properties.

**Methods:** The liposomal form of the cytotoxic lectin from *B. subtilis* B-7025 was synthesized by the liposome hydration method [1]. Physical characteristics of synthesized liposomes are defined on Zetasizer Nano ZS (Malvern Panalytical). The efficiency of lectin encapsulation into liposomes was determined by the concentration of free protein in a supernatant at a wavelength of 280 nm using NanoDrop™ 1000 (described in [1]).

**Results:** A median size of unloaded liposomes was 151.7 ± 55.2 nm, the Z-potential was −20.5 mV and the conductivity was 14.1 mS/cm. The average size of liposomes loaded with the cytotoxic lectin was 314.8 ± 113.0 nm, the Z-potential had three peaks: 29.8 mV (49.1% of liposomes), −41.3 mV (32% of liposomes), and −108 mV (16.7% of liposomes), and conductivity was 25.4 mS/cm. The effectiveness of encapsulation of the cytotoxic lectin at the moment of synthesis was 75.89%. The leakage factor on the 1-, 4-, 7-, and 15-day after synthesis acquired negative values: −5.89%, −24.56%, −19.16% and −25.84%, respectively. We suppose that the reduced concentration of the free lectin could be explained by the ability of lectin to form inter-protein complexes.

**Conclusions:** The liposomal form of the cytotoxic lectin from *B. subtilis* B-7025 was synthesized and its physical properties were characterized.

**REFERENCE**

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## BURDEN OF LUNG METASTASES IN AN EXPERIMENTAL MODEL OF BREAST CARCINOMA IN MICE

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**Introduction:** The 4T1 breast carcinoma is a highly tumorigenic and invasive transplantable tumor cell line resembling human triple negative breast cancer. It can spontaneously metastasize from the primary tumor to multiple distant sites including lymph nodes, liver, lung, brain, and bone (Pulaski *et al.*, 2001). Thus, 4T1 is a relevant tumor model including the general field of immunization studies in oncology as well as lung metastases, in particular.

**The aim** of the present study was to characterize capacity of breast carcinoma cells 4T1 to metastasize to the lung by tumor burden evaluation after experimental introduction of different HIV genes in cell line and/or immunization.

**Methods:** Lung samples (n = 32) were analysed from mice transplanted with 4T1luc2 adenocarcinoma cells expressing variants of HIV-1 FSU_A enzyme with and without drug resistance mutations. Twenty four mice were transplanted with 4T1luc2 expressing proteases (PR) (PR-DNA immunized 15, naïve 9). Controls (n = 8) received parental 4T1luc2 adenocarcinoma cells. Metastases were diagnosed and evaluated in formalin-fixed, paraplast-embedded liver tissues. For each mouse, the area of tumor metastases was quantified in five high power (400x) microscope fields of haematoxylin-eosin-stained slides by computer-assisted morphometry using specialized NIS-Elements software (Nikon, Tokyo, Japan). IBM SPSSv23 was applied for statistical analysis, including descriptive assessment as detection of mean values, standard deviation (SD) and 95% confidence interval (CI).

**Results:** Lung metastases were found in lungs of 1/8 PR(Ai) (12.5%; CI 2.2–47.0); 1/8 PR(A2mut) (125%; CI 2.2–47.0); 2/8 PR(A3mut) implanted mice (22.2%; CI 6.3–54.7); and 3/8 in control group (37.%; CI 13.6–69.4). PR(Ai) immunized mice developed metastasis in 1/8 (12.5%; CI 2.2–47.0); PR(A2mut) immunized, in 0/8 (0%; CI 0–32.4); and PR(A3mut) immunized, in 2/8 (22.2%; CI 6.3–54.7) examined cases. The mean size of PR(Ai) metastases was 0.03 $mm^2$ (SD ± 0.02), 0.03 $mm^2$ (SD ± 0.02) of PR(Ai) immunized and 0 $mm^2$ of naïve; PR(A2mut), 0.02 $mm^2$, 0 $mm^2$ of PR(A2mut) immunized and 0.02 $mm^2$ of naïve mice metastases; PR(A3mut), 0.03 $mm^2$ (SD ± 0.02), 0.03 $mm^2$ (SD ± 0.02) of PR(A3mut) immunized and 0 $mm^2$ of naïve; and 0.01 $mm^2$ (SD ± 0.01) in naïve 4T1luc2 implanted mice.

**Conclusions:** Number of subjects with metastases among HIV DNA-immunized mice implanted with HIV-expressing tumors was insignificantly higher than among naïve animals (3/15 *vs* 1/9, $p < 1.0$). DNA-immunization with PR does not protect against lung metastases. Further histological evaluation of lung metastases needs to be done.

## TUMOR-ASSOCIATED ADIPOCYTES AND MINIMAL RESIDUAL DISEASE IN GASTRIC CANCER

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**Background:** It is known that some tumors (gastric cancer (GC), breast, colon and ovarian tu-

mors) develop at sites of anatomical accumulation of an adipose tissue. A modified redox state of the tumor is a factor that damages the mitochondria of adipocytes and re-programs them into tumor-associated adipocytes (TAAs). The latter are characterized by pro-oncogenic properties. TAAs are an important source of adipocytes and energy for the tumor; hence, studying the mechanisms of cell and metabolic symbiosis of adipocytes and tumor cells opens up the new therapeutic and diagnostic possibilities. In particular, the prediction of the course of micro-metastasizing, taking into account the number of TAAs, will allow an individualized anti-tumor therapy for patients with overweight and with a minimal residual disease (MRD).

**The aim:** To characterize the relationship between the number of TAAs and body mass index (BMI), clinical and pathological characteristics, the rate of generation of superoxide radicals (SR), an activity of gelatinases (matrix metalloproteinases-2 and -9) in tumor adipose tissue (TAT), the number of disseminated tumor cells in the bone marrow and the survival of patients with GC.

**Methods:** Immunohistochemistry, zymography, EPR spectroscopy, statistical (Student t-test, Spearman correlation analysis, Kaplan — Mayer survival analysis).

**Results:** A reliable correlation was found between the number of TAAs in the tumor and the BMI of the patients with GC (rho = 0.41; $p = 0.032$). The content of TAAs increases with the growth of tumor size and in patients with the category of pT4 — this value was 1.3 fold ($p < 0.05$) higher than in the tumors of pT1 patients. In patients with both regional and distant metastases, number of TAAs in tumors was higher than in patients without metastases. A large number of TAAs in the tumor is also associated with a high rate of SR generation and a higher activity of gelatinases. On the other hand, the high number of TAAs is associated with the presence of the distant metastases of GC. For M1 patients, the higher rates of SR and the total activity of gelatinases in TATs were detected (in 83 and 64%, respectively). It makes 30% increase in the TAA number in the tumor compared with M0 patients. Patients with less than 26.5% of TATs in the tumor live reliably longer and have a 2.9-fold lower risk of adverse illness, compared with patients with TAT number exceeding 26.5%. For the group of patients with the existing duodenal ulcer, no reliable association between survival and TAT number was detected ($p = 0.6203$). However, we found such association in patients without micro-metastases ($p = 0.005$).

**Conclusions:** Dysfunctional adipose tissue, one of the hallmarks of the imbalance of the redox state, is a modifier of the tumor microenvironment, involved in formation of its aggressive phenotype. The number of TATs is associated with tumor growth and metastasizing. Hence, it can be used as a marker for control of MRD formation and the course of GC in overweight patients.

## EXPRESSION PROFILING OF PROSTATE TUMOR SPECIFIC GENES

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**Background:** Prostate cancer is one of the most common cancers in men worldwide. It is known as a very heterogeneous and complicated cancer type. Many genetic, epigenetic, transcriptomic and metabolomics alterations have oncogenic effect on prostate carcinogenesis. Therefore, the molecular characteristics of individual cancers are necessary for both, a diagnosis and prognosis and also for the development of various cancer therapies. We have selected 30 genes that are associated with the epithelial-mesenchymal transition, and also genes, encoding several hormonal receptors and metabolic enzymes, related to prostate carcinogenesis. We determined alterations in a gene expression pattern and also the presence of the TMPRSS2: ERG fusion in prostate tumors.

**The aim** was to analyze the expression patterns of the cancer-associated genes in benign and malignant prostate tumors, compared with conventionally normal tissues (CNT). We wanted to relate these patterns to PTEN expression levels, presence or absence of the TMPRSS2/ERG fusion, and also clinical and pathological characteristics of tumors.

**Methods:** The relative expression levels (RE) of 47 prostate cancer-associated genes were analyzed in 37 freshly frozen samples of prostate cancer tissues of different tumor stages and Gleason scores, compared with the 37 paired CNTs and 20 samples of prostate adenomas, using quantitative PCR.

**Results:** We have found that 13 genes were expressed differently in adenomas and malignant prostate tumors (*AR, PTEN, VIM, MMP9, KRT18, PCA3, HOTAIR, SCHLAP1, ESR1, GCR, PRLR, SRD5A2, VDR*). We have shown that *PTEN* RE decreased in an adenocarcinoma group as well as in CNTs, compared with adenomas. 54.0% of adenocarcinomas, 48.6% of CNTs and 25.0% of adenomas were characterized by the low *PTEN* RE. We have found that 9 genes differentially expressed in groups with the different levels of *PTEN*, namely *ESR1, GCR, KRT18, MMP2, MMP9, SRD5A2, VIM, PCA3* and *HOTAIR*. We have shown that 9 genes (*AR, ESR1, KRT18, MMP9, PRLR, SRD5A2, PCA3, HOTAIR* and *SCHLAP1*) expressed differently in adenomas, CNTs and adenocarcinomas, depending on the presence or the absence of the TMPRSS2/ERG fusion. We have identified 14 differentially expressed genes in groups of various tumor stages (*AR, KRT18, MMP9, VIM, PCA3, HOTAIR, SCHLAP1, ESR1, GCR, INSR A, IGFR tr, PRLR, VDR, SRD5A2*) and 11 genes

that were expressed differently in tumors of various Gleason score (*AR, CDH1, KRT18, MMP9, OCLN, PCA3, HOTAIR, SCHLAP1, ESR1, VDR, SRD5A2*).

**Conclusions:** We have identified alterations in expression of cancer-associated genes in prostate tumors, depending on the tumor stage, Gleason score, levels of *PTEN* and the presence of the TMPRSS2: ERG fusion. These findings would help to characterize the molecular profiles of prostate tumors with the aim to improve the anti-tumor therapy.

## FREEZING CONDITIONS DETERMINE PRESERVATION OF ANTIGENIC CHARACTERISTICS OF CANCER CELLS

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**Background:** Testing of new antitumor drugs, the creation of vaccines as immune therapeutic agents for the treatment of oncological diseases requires the development of adequate methods to store the tumor cells. There is always a possibility that expression of markers in tumor samples after cryopreservation could be altered.

**The aim** was to evaluate a spectrum of antigens of Ehrlich carcinoma (EC) cells after the serial freeze-thawing, using a variety of cryopreserving media.

**Methods:** The experiments were performed, using Balb/C mice. The primary culture of EC was grown for 7 days in a peritoneal cavity (PC) of mice. Afterwards, the cells were removed from the PC, washed and after centrifugation were frozen down: first to –80 °C, at a rate of 1 °C/min and then cells were plunged into liquid nitrogen, using several cryopreserving media, i.e. Ringer phosphate buffer (RPB), ascites and DMEM with 10% DMSO. Immediately after thawing, the number of cells in the sample was counted as well as their viability was assessed by staining with propidium iodide. A sub-population composition was determined, using monoclonal antibodies against CD44 and CD24 (BD Biosciences, USA) and a FACS Calibur flow cytometer (BD, USA). Cells not subjected to freeze-thawing served as a control.

**Results:** We found that the selected cooling rate provided the preservation of the cells in the sample at the control level, regardless of the freezing medium. The viability of cryopreserved samples in RPB was 42.05 ± 4.12%, in ascites 65.42 ± 2.67% and in DMEM + 10% DMSO 89 ± 3.76%. Cryopreservation of EC cells using RPB and ascitic fluid led to a decrease in the content of $CD44^+$ subpopulations, and an increase in $CD24^+$ positive subpopulations. The use of DMEM + 10% DMSO ensured the safety of all the detectable subpopulations at the control level, including CD44-high cells, the main candidates for cancer stem cells.

**Conclusions:** The obtained data emphasize the need of a careful selection of preservation conditions, determining the preservation of the antigen spectrum of tumor cells during their long-term storage.

## CD150/SLAMF1 RECEPTOR IS INVOLVED IN REGULATION OF CHRONIC LYMPHOCYTIC LEUKEMIA PATHOBIOLOGY

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**Background:** Despite the homogeneous CD150/SLAMF1 expression on subpopulations of normal B cells it is differentially expressed on the cell surface of malignant B cells including chronic lymphocytic leukemia (CLL) B cells. High cell surface CD150 (csCD150) expression level on CLL B cells is associated with favourable prognosis for patients. However, the role of CD150 in the pathobiology of CLL is still unclear.

**The aim** of our study was to explore CD150 signalling properties in CLL B cells.

**Methods:** flow cytometry, qPCR, Western blot analysis, *in vitro* stimulation assay, confocal microscopy.

**Results:** We found that $csCD150^+$ CLL B cells expressed CD180, CD95, CD20, CD22, CD48 and HLA-DR on significantly higher levels than csCD150- CLL B cells. In $csCD150^+$ CLL cases the basal level of tyrosine phosphorylation and phosphorylation of serine/threonine specific motifs that are substrates for AMPK, Akt, PKA, PKC, CDK kinases was higher compared to that in $csCD150^-$ CLL B cells. CD150 ligation on CLL B cells led to activation of pro-survival Akt, mTOR, ERK1/2, JNK1/2 and p38MAPK signalling pathways. However, simultaneous crosslinking of CD150 and CD180, which showed the highest level of colocalisation on the cell surface of CLL B cells, displayed inhibitory effect on activation of Akt/mTOR signalling pathway, as well as MAPK pathways. It should be noted that profile of CD150 and CD180 expression is similar in majority types of B-cell leukemia and lymphoma, with highest expression level in CLL B cells. Moreover, our analysis of CD150 and CD180 expression on malignant B cell lines revealed coexpression of these receptors only in Burkitt lymphoma cell line BJAB. In contrast to CLL B cells, CD150 and CD180 on BJAB cell line were not involved in regulation of Akt, ERK1/2 or p38MAPK pathways.

**Conclusions:** CD150 and CD180 in case of their coexpression and coactivation could block propagation of pro-survival pathways in CLL B cells leading to disease attenuation.

## CREATING GENETIC CONSTRUCTS FOR DETERMINING SPATIO-TEMPORAL DISTRIBUTION OF CORTACTIN AND THE PH DOMAIN OF BCR IN MAMMALIAN CELLS

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**Background:** Translocation between $9^{th}$ and $22^{nd}$ chromosomes leads to generation of a *BCR-ABL* fu-

sion gene. During the translocation, breakpoints are different in a *BCR* part and identical in an *ABL* part. As a result, generated chimeric proteins differ in presence or absence of certain domains of BCR. Previously, 23 potential interaction partners of a PH domain of BCR were identified by mass-spectrometry. They are involved in various important cellular functions, such as regulation of proliferation, signaling and cytoskeleton re-organization. These data, however, require further validation by more reliable methods. For our study, we selected cortactin (CTTN) as it is known to regulate actin branching and membrane remodeling during clathrin-mediated endocytosis through interaction with dynamin that anchors to membrane by its own PH domain. Recently, we have discovered that CTTN, the PH domain and clathrin co-localize in specific regions of a cell, around the nucleus. This may indicate that the PH domain of BCR acts similarly to the PH domain of dynamin in clathrin-mediated endocytosis.

**Aim:** To further investigate this phenomenon, we decided to determine localization of these proteins on a sub-diffraction level, using stimulated emission depletion (STED) microscopy. We also plan to determine their temporal distribution inside the cell by utilizing a fluorescent timing phenomenon.

**Methods:** To accomplish these tasks, we amplified the DNA sequence, corresponding to a coding region of CTTN and the PH domain, by PCR. Obtained fragments were ligated to the pBluescript II SK+ vector. After verification of inserts they were sub-cloned to vectors, designed for super-resolution imaging and fluorescent timing. The PH domain of BCR was sub-cloned to the vector mEos3.2-C1 for fluorescent timing and photo-activation localization microscopy (PALM). CTTN was sub-cloned to the vectors pSlow-FT-N1, pMediumFT-N1 and pFast-FT-N1 for fluorescent timing. For STED imaging, the pEGFP-PH vector, previously created in our department, was used to transfect HEK293T cells to further investigate intracellular distribution of the PH domain of BCR on the sub-diffraction level.

**Results:** We have created and verified genetic constructs for the fluorescent timing of CTTN and the PH domain. We obtained super-resolution images of intracellular distribution of the PH domain of BCR on Leica TCS SP8 STED microscope.

**Conclusions:** Derived genetic constructs may be used for determining spatio-temporal distribution of the PH domain of BCR and cortactin. STED microscopy demonstrates that the PH domain of BCR clusters in structures that resemble clathrin-coated vesicles by size and shape, however, this hypothesis requires further validation by counter-staining of clathrin-coated vesicles.

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## TUMOR-ASSOCIATED ABNORMALITIES IN A MICRO-RNA EXPRESSION SIGNATURE CAN CONTRIBUTE TO THE DISRUPTION OF EPITHELIAL TISSUE INTEGRITY

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**Background:** Normal epithelial cells form a well-ordered sheet where the cells are tightly bound to each other and to basement membrane and undergo contact inhibition of proliferation and movement. On the contrary, impaired adhesion, detachment from the neighboring environment, anoikis resistance, epithelial-mesenchymal transition (EMT), aberrant orientation of mitotic spindle, loss of apical-basal polarity and overcoming of contact inhibition are typical features of cancer cells, contributing to uncontrolled cell proliferation and expansion.

**The aim:** As overexpression and downregulation of microRNAs (miRNAs) are necessary for cancer cells to grow and survive, this research aims to identify how the shifts in miRNA expression profile can promote the disruption of epithelial tissue integrity.

**Methods:** miRNA targets within gene transcripts were predicted *in silico* using the TargetScan software.

**Results:** Overexpressed miRNAs miR-18, miR-19, miR-21, miR-23, miR-27, miR-29, miR-155, miR-181, miR-210, miR-221/222 and miR-375 can silence genes that encode key molecules responsible for cell-cell adhesion — E-cadherin (encoded by *CDH1* gene), claudin 1 (CLDN1), junctional adhesion molecules JAM-A and JAM-C (F11R and JAM3), tight junction proteins ZO-1 and ZO-2 (TJP1/2), occludin (OCLN), cingulin (CGN), nectins 1 and 3 (PVRL1/3), nectin-like molecules 1 and 2 (CADM3/1), alpha-catenin (CTNNA1), p120-catenin (CTNND1), alpha-actinins (ACTN1/2), tropomyosin 1 (TPM1) and vinculin (VCL). In addition, hyper-expressed miRNAs can suppress *CRB1*, *MPP5* and *INADL*, *DLGL1* and *PARD3* genes encoding CRB1/PALS1/PATJ, DLG and PAR3 — main components of, respectively, CRB, SCRIB and PAR complexes responsible for the establishment of epithelial cell polarity. Some overexpressed miRNAs can silence also genes involved in mitotic spindle orientation — *VHL*, *APC*, *PROX1*. Moreover, overexpressed miRNAs can affect the Hippo pathway by silencing of *WWC1* (KIBRA), *LATS2*, *STK3/4* (MST2/1), *LATS1* and *SAV1* genes. At the same time, downregulation of miR-15/16 and miR-205 allows hyper-expression of YAP1 and, respectively, WWTR1 (TAZ) genes which transcripts carry targets of these miRNAs. The Hippo pathway failure allows entry of YAP1/TAZ transcription factors into the nucleus. In addition, hyper-expressed miRNAs can silence *PTK2* (FAK), *SPRY2*, *ARHGEF7* (PIXB) and *PAK1* genes that encode components of the FAK/Src kinase pathway. Transcripts of *SNAI1* (Snail), *SNAI2* (Slug), *ZEB1/2*, *TWIST1*, *KLF8*, *TCF4*, *SIX1*, *FOXC2* and *LEF1* genes carry targets of downregulated miRNAs miR-15/16,

miR-22, miR-31, miR-34, miR-101, miR-124, miR-125, miR-137, miR-140, miR-143, miR-145, miR-148/152, miR-199, miR-200, miR-203, miR-204 and miR-205. Down-regulation of these miRNAs is characteristic to the cancer cells and allows reactivation and hyper-expression of above genes encoding the key transcription factors responsible for the EMT. Moreover, targets of down-regulated miRNAs are revealed in transcripts of *VIM*, *FN1*, *CDH2* and *CDH11* genes encoding mesenchymal cell markers vimentin, fibronectin, N- and OB-cadherin, expression of which is the sign of EMT.

**Conclusions:** Tumor-associated abnormalities in miRNA expression signature can contribute to loss of E-cadherin and silencing of many other epithelial junction and polarity genes as well as to de-repression of genes, responsible for the EMT, mesenchymal phenotype and stem-like properties. In addition, this leads to randomized mitotic spindle orientation and to symmetric division of cells, despite the normal asymmetric division. Silencing of Hippo cascade and FAK/Src pathway leads to attenuation and nullification of the contact inhibition signals as well as reduces or prevents their generation. As a result, transformed cells form irregular multi-layer conglomerates. These events disrupt the epithelial tissue integrity, facilitate cell detachment, proliferation, movement and invasiveness, underlying the tumor progression and metastasis.

## EVALUATING THE INFLUENCE OF MULTIPOTENT MESENCHYMAL STROMAL CELLS DERIVATES ON AN EARLY STAGE OF BREAST CANCER

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**Background:** Tumor microenvironment plays a decisive role in cancer development and metastasis, and affects the therapeutic effectiveness of anticancer drugs. Multipotent mesenchymal stromal cells (MMSCs) are important elements of tumor stroma, but the effect of MMSCs on cancer cells development has not yet completely understood. MMSCs can both stimulate and inhibit tumor progression, depending on the components of microenvironment, genesis and stage of cell differentiation. Special attention is focused on the paracrine effect of products secreted by MMSCs.

**The aim:** Authors wanted to characterize the influence of derivates from human bone marrow MMSCs on proliferation, survival, receptor profile of MCF-7 in 2D and 3D cell cultures *in vitro*.

**Methods:** The monolayer MCF-7 cell culture was cultured in standard conditions (37 °C, 5% $CO_2$, humidity 95%), in DMEM nutrient medium (Sigma, USA), with 2 mM L-glutamine (Sigma, USA), 40 μg/ml Gentamicin (Biopharma, Ukraine). The initial density of inoculated MCF-7 cells was $2 \cdot 10^4$ cells/cm$^2$. Human bone marrow MMSCs were used in this work. To assess the effect of MMSCs derivates on proliferative activity and adhesion properties of tumor population, the MCF-7 cells were incubated in full nutrient conditioned media from MMSCs in 1:1 ratio. For the initial generation of spheroids the DMEM nutrient medium with 2% carboxymethyl cellulose (Bio-Rad, USA) was used. Plates with spheroids were being incubated on an orbital shaker PSU-10i, (Biosan, Latvia) at 80 rpm for 3–5 hours. The spheroid culture was maintained for 7 days. Cell viability was evaluated by MTT assay. The Stemi2000 software Axio Vision Red 4.7 (Zeiss, Germany) was used for processing the images. The volume of aggregates was calculated by Bjerkvig formula after 7 days of cultivation. Markers were detected by applying IHC method with primary monoclonal antibodies Ck (clone AE1/AE3, IS053, Dako, USA), vim (Clone V9, IS630, Dako, USA), EpCAM (Sigma, HPA026761, USA).

**Results:** The impact of MMSC derivatives on breast cancer cells *in vitro* and *in vivo* was estimated. The significant cytostatic effect of the products of human bone marrow MMSCs on tumor population was detected.

**Conclusions:** MMSC derivates inhibited migration of tumor cells to suspension fraction and promoted an increase of expression of tumor associated markers — cytokeratins and EpCAM in 2D and 3D cell cultures, but only in 3D culture expression of vimentin was increased.

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## IRE1 INHIBITION MODIFIES THE EXPRESSION OF TUMOR GROWTH RELATED GENES IN GLIOMA CELLS

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**Background:** We have studied the effect of inhibition of the IRE1 signaling enzyme as well as hypoxia on the expression of genes, encoding important tumor growth related proteins (BRCA1, DEK, BCL2L1, COL6A1, TPD52, HOMER3, and GNPDA1) in U87 glioma cells. Therefore, hypoxia affected the expression level of numerous genes and the effect of low oxygen condition on most hypoxia responsive genes expression is dependent on IRE1 functional activity. Several genes have already been correlated with glioblastoma multiforme, but mechanisms of their regulation by hypoxia and IRE1 signaling pathway are not clarified yet.

**The aim:** We wanted to investigate the effect of hypoxia as well as IRE1 inhibition on the expression of *BRCA1*, *DEK*, *BCL2L1*, *COL6A1*, *TPD52*, *HOMER3*, and *GNPDA1* genes in U87 glioma cells with hopes

of elucidating its mechanistic part in the development and progression of glioblastoma and the contribution to endoplasmic reticulum stress.

**Methods:** Exposure of glioma cells under conditions of hypoxia, RNA isolation, reverse transcription and quantitative polymerase chain reaction in real time analysis.

**Results:** It was shown that the expression level of breast cancer 1 early onset (BRCA1) and tumor protein D52 (TPD52) mRNAs is strongly up-regulated in U87 glioma cells by inhibition of IRE1 in comparison with the control cells. At the same time, the expression level of collagen, type VI, alpha 1 (COL6A1), DEK oncogene (DEK), glucosamine-6-phosphate deaminase 1 (GNPDA1) and homer homolog 3 (HOMER3) is significantly down-regulated in glioma cells at this experimental condition. It was also shown that hypoxia up-regulated the expression level of COL6A1 and TPD52 mRNAs and down-regulated — BRCA1, DEK, and GNPDA1 mRNAs in control glioma cells and that inhibition of IRE1, which control cell proliferation and tumor growth, modifies the effect of hypoxia on the expression of *COL6A1*, *DEK*, *BCL2L1*, *HOMER3*, and *GNPDA1* genes.

**Conclusions:** The present study demonstrates that hypoxia affects the expression of most studied genes in IRE1-dependent manner, but several aspects of this regulation warrant further investigation.

## KNOCKDOWN OF THE ADAPTOR PROTEIN RUK/CIN85 IN 4T1 AND LLC ADENOCARCINOMA CELLS RESULTS IN INCREASED EXPRESSION LEVELS AND ACTIVITIES OF MMP-2 AND MMP-9, ASSOCIATED WITH ELEVATED PRODUCTION OF ANGIOSTATINS AND SUPRESSION OF INVASION POTENTIAL

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**Background:** The degradation of extracellular matrix (ECM) by matrix metalloproteinases (MMPs), mainly by MMP-2 and MMP-9, has been consistently correlated with migration, invasion as well as angiogenesis in many cancer subtypes including breast and lung cancer. However, an increasing number of publications are accumulated demonstrating that up-regulation of certain MMPs in tumor cells provides a beneficial and protective effect in the course of tumor progression while broad spectrum small molecular MMPs inhibitors proved to be ineffective in clinical trials. There is also evidence suggesting the role of MMP-2/MMP-9 in plasminogen digestion resulting in generation of angiostatins (kringle-containing fragments of plasminogen) that could function as inhibitors of angiogenesis and tumor growth in different *in vitro* and *in vivo* experimental models. Interestingly, according to our preliminary data, overexpression of adaptor protein Ruk/CIN85 in mouse breast adenocarcinoma 4T1 cells resulted in the development of highly aggressive phenotype associated with decreased expression of MMP-2 and MMP-9.

**The aim:** To study the interplay between Ruk/CIN85 knockdown, MMPs expression, production of angiostatins and invasion potential of tumor cells using as the models of mouse breast adenocarcinoma 4T1 cells and Lewis lung carcinoma (LLC) cells.

**Methods:** To down-regulate Ruk/CIN85, 4T1 and LLC cells were stably infected with lentivirus encoding Ruk/CIN85-specific shRNA. Protein expression levels were assessed by qRT-PCR and Western-blotting. Gelatin zymography was used to study enzymatic activity of MMPs. Cancer cells invasiveness was studied using Boyden chamber assay.

**Results:** It was demonstrated that considerably increased expression levels and gelanolytic activities of both MMP-2 and MMP-9 were observed in conditioned medium of Ruk/CIN85 knockdown 4T1 and LLC cells in comparison with control ones. Up-regulation of MMPs was shown to correlate with increased generation of angiostatins and suppression of invasion potential. MMPs inhibitor GM6001 at concentration $1.3 \cdot 10^{-6}$ M restored the invasiveness of Ruk/CIN85 knockdown cells to the values characteristic for corresponding control cells.

**Conclusions:** Based on our findings, it could be assumed that adaptor protein Ruk/CIN85 is a concentration-dependent regulatory component of signaling networks responsible for the control of MMPs expression and thus angiostatins production and modulation of invasion.

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## CD150 AND CD180 ARE INVOLVED IN REGULATION OF PU.1 TRANSCRIPTION FACTOR EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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**Background:** Transcription factor (TF) PU.1 is an important regulator of normal B-cell development. However, PU.1 expression is deregulated in numerous of B-cell malignancies. Downregulation of PU.1 was found in CD38 and ZAP-70 positive chronic lymphocytic leukemia (CLL) cases. This fact leads to consider PU.1 expression as a surrogate prognostic marker of CLL outcome. The question rises whether it is possible to regulate PU.1 expression in malignant B cells? Cell surface receptors with signaling properties are among potential regulators of TF expression and activity.

That is why **the aim** of our work was to explore the regulation of PU.1 expression via CD150 and CD180 cell surface receptors in CLL B cells.

**Methods:** flow cytometry, qRT-PCR, Western blot analysis, *in vitro* stimulation assay.

**Results:** Our results showed that all tested CLL cases were characterized by a lower level of PU.1 mRNA compared to normal B-cell subpopulations ($p \leqslant 0.02$). The PU.1 protein was heterogeneously expressed between CLL cases with significantly highest level in csCD150$^+$ than in csCD150$^-$ CLL cases ($p = 0.002$). Moreover, expression level of PU.1 target gene CD20 was elevated in csCD150$^+$ CLL B cells. Stimulation of CLL B cells *via* CD150 or CD180 receptors resulted in increasing PU.1 mRNA level in CLL B cells with an additive effect of CD150 and CD180 coligation.

**Conclusions:** CLL B cells are characterized by decreased PU.1 mRNA level compared to normal peripheral blood B cells. For the first time we found positive correlation between CD150 and PU.1 protein expression in CLL B cells. Both CD150 and CD180 receptors are involved in regulation of PU.1 mRNA that potentially may lead to transcriptional program modulation in CLL B cells.

## ANALYSIS OF PARP1 AND HISTONE MODIFICATIONS BY CHIP-SEQ MAY ALLOW TO PREDICT GENES TRANSCRIPTIONALLY CONTROLLED BY PARP1

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**Background:** Although the primary role of PARP1 in a cell is still ascribed to its DNA safeguard potential, the last two decades have shed the light on the control of gene transcription by this protein. The new methodological approaches and state of the art technologies, such as chromatin immunoprecipitation followed by a new generation sequencing and bioinformatic analysis uncover the genome-wide association of PARP1 with the chromatin. The search for PARP1 distribution in the genome of human breast cancer cells revealed the relatively strong correlation between PARP1 and POLR2A responsible for gene transcription. Furthermore, PARP1 co-occured with an estrogen receptor (ER) that enhances ER-sensitive gene expression in a hormone-dependent manner. PARP1 was found at genomic regions characterized by acetylation of H3K27 and strongly correlated with histone acetyltransferase. This enzyme activated both gene enhancers as well as proximal and distal gene promoters. Among histone metylation, the H3K9me3 and H3K4me1 showed co-distrubution with PARP1.

**The aim:** to find out whether PARP1 appears to regulate gene transcription by acting within these regulatory regions, bearing in mind that gene enhancers are simultaneously characterized by POLR2A occurrence, H3K4me1 and H3K27ac (the latter is most frequently inserted by EP300).

**Methods:** various bioinformatic softwares.

**Results:** The subsequent analysis aiming to estimate the distance between particular modifications and proteins and a gene transcription start site revealed that they all occurred in proximity or spanned the region, where transcription pre-initiation complex assembled.

**Conclusions:** Further analysis of chromatin interaction (such as 3C) and PARP1 silencing, followed by RNA-Seq are required to confirm PARP1 contribution to defining expression of genes predicted with bioinformatic tools to be controlled by identified enhancers and promoters.

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## PLATELETS ARE ABLE TO CONVERSION OF ENDOGENOUS PLASMINOGEN TO FRAGMENTS AND TO SORT THEM

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**Background:** Platelets play an important role in the process of tumor angiogenesis. The onset of tumor angiogenesis involves a net change in balance between angiogenesis stimulators and inhibitors in favor of the former. Platelets are a rich source of proangiogenic factors. They also store and release angiogenesis inhibitors. Platelets express surface growth factor receptors, which may regulate the process of angiogenesis. Activated platelets serve as pro-coagulant surfaces amplifying the coagulation reactions.

The elucidation of regulation of the platelet functioning by plasminogen/plasmin system is one of the priority areas of our research. Plasminogen/plasmin molecule proteolysis in the organism leads to the formation of kringle-containing fragments (K 1–3, K 1–4, K 5 *etc.*) — angiostatins, that exhibit an anti-angiogenic effect. It has been shown that angiostatins are involved in signaling mechanisms that underlie many normal and pathophysiological processes in the organism, such as cell migration, angiogenesis, metastasis, tissue remodeling, wound healing, axon germination, and others. Thus, the interaction of angiostatins with targets on the plasma membrane of endothelial cell (ATP synthase, integrin αVβ3, c-met receptor of HGF etc.) leads to suppression of proliferative activity of cells and their ability to move and migrate. For some time, it was believed that angiostatine is produced in the organism by some types of tumors, and indeed, an increase of their generation is observed in tumor growth. However, it has recently been established

that angiostatin is found in the organism and under normal conditions, thus, being involved in physiological processes. To date, only a few types of cells that are capable of generating angiostatin in the norm are identified, including monocytes and macrophages.

**The aim:** To determine the role of platelets in angiogenesis.

**Methods:** Detection of angiostatins was carried out by western blotting assay, using polyclonal antibodies monospecific to K 1–3 and K 5, which have been obtained by us for this purpose.

**Results:** We are investigating ability of platelets to generate plasminogen fragments — angiostatins, internalize and secrete formed angiostatins by native and activated platelets. We obtained preliminary data on the interaction of isolated K 1–3 and K 5 plasminogen fragments (angiostatins) with the platelet surface. After previous incubation of platelets with K 1–3 and K 5, the fragments were detected in isolated plasma membranes and absent in cell lysates. The antibodies to K 1–3 revealed plasminogen and 51 kDa angiostatin-like fragment on membranes and in lysates, whereas antibodies to K 5 revealed miniplasminogen on membranes, and a microplasminogen in the inner medium of the cells, indicating the ability of platelets to conversion of endogenous plasminogen and to sorting plasminogen fragments.

**Conclusions:** Most probably, platelets can convert endogenous plasminogen and sort plasminogen fragments.

## EFFECT OF CANCER VACCINES ON MANIFESTATION OF PARANEOPLASTIC ANEMIA AND LEVELS OF ANGIOGENESIS-ASSOCIATED CYTOKINES IN MICE WITH A TRANSPLANTED HIGH-ANGIOGENIC VARIANT OF LEWIS LUNG CANCINOMA

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**Background:** The correction of cancer-associated anemia (CAA), the formation of which is directly associated with an increased level of proangiogenic cytokines, is an important task of clinical oncology. It is known that CAA results in an unfavorable prognosis, worsens the condition of patients and, in the case of hemoglobin < 90 g/l, is a direct contraindication to a special antitumor treatment. One of the experimental tumor models for the study of CAA is a highly angiogenic variant of the Lewis lung carcinoma (LLC/R9), the growth of which is accompanied by the development of anemia, splenomegaly, and thymus involution. Previously, we have investigated the possibility of anemia correction in LLC/R9 mice using cancer vaccines (CVs) prepared on the basis of LLC/R9 cell antigens (CV-LLC/R9) or LLC cell antigens (CV-LLC), as well as anti-tumor and immunological effects of such CVs. It has been shown that both vaccines did not prevent the development of CAA and splenomegaly, and CV-LLC/R9 even exacerbated the manifestations of anemia. However, both vaccines increased the survival of mice with transplanted LLC/R9. The administration of CV-LLC significantly reduced the number and volume of metastases, increased the cytotoxic activity of natural killer cells, and prevented the negative effect of the tumor on the specific cytotoxic activity of splenocytes and macrophages in the presence of autologous blood serum. It is known that the activation of immune cells is accompanied by the production of humoral factors, each of which can be involved in the processes of forming paraneoplastic syndrome.

**The aim** of the study was to investigate the effects of CVs prepared on the basis of antigens of high (LLC/R9) or low-angiogenic LLC strains on the level of proangiogenic (IL-10, IL-1, TNF-α) and antiangiogenic (IFN-γ, IL-4) cytokines in mice with transplanted LLC/R9.

**Methods:** The study was conducted on male C57Bl/6 mice with transplanted LLC/R9 carcinoma. CV was prepared from LLC cells (CV-LLC) or LLC/R9 (CV-LLC/R9); as an adjuvant, a protein-containing metabolite of *B. subtilis* B-7025 was used. The introduction of CV (4 subcutaneous injections) was initiated on the 12$^{th}$ day of tumor growth. At the 33$^{rd}$ day after tumor transplantation, supernatants of splenocytes and blood serum of experimental animals were analyzed for IFN-γ, IL-1, TNF-α, IL-4, and IL-10 levels, using the BD Biosciences test system (USA). The statistical analysis was performed, using the descriptive statistics and the Student's t-test (StatSoft STATISTICA 7.0).

**Results:** In LLC/R9 control mice, we observed a marked tendency to a decreased IL-10 serum level, compared to intact animals. Also, splenocytes of control mice were characterized by the significantly diminished IL-1 level (by 79%, $p < 0.05$) and a marked tendency to increased IL-4 production, compared to intact mice. The introduction of CV-LLC resulted in a significant increase in the serum TNF-α levels (which, due to the high variability of the index, was close to the trend), which could partially contribute to the anti-metastatic action of the vaccine. The use of the CV-LLC/R9 vaccine resulted in a statistically significant increase in serum IL-1 levels (by 501%, $p < 0.05$), compared to unvaccinated tumor-bearing mice. A high level of a proangiogenic cytokine IL-1 can lead to more severe anemia in LLC/R9 mice after vaccination with CV-LLC/R9. The level of production of investigated cytokines by splenocytes of vaccinated animals did not differ significantly from the corresponding indices of control animals.

**Conclusions:** The use of CV-LLC at the background of LLC/R9 growth led to the formation of more balanced content of the examined cytokines in the blood serum of animals (in particular, the increase of TNF-α and the prevention of hyper-production of IL-1), which most

likely predetermined the preservation of functional activity of cellular antitumor resistance effects, provided anti-metastatic action and lengthened the survival time of the animals. The administration of CV-LLC/R9 resulted in the accumulation of pro-angiogenic cytokines (in particular, IL-1) in blood serum and decreased level of antiangiogenic cytokines (IFN-γ), leading to more severe tumor-associated anemia.

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## FATTY-ACID COMPOSITION IN THE GUERIN'S CARCINOMA MITOCHONDRIAL FRACTION OF RATS UNDER CONDITIONS OF Ω-3 POLYUNSATURATED FATTY ACIDS ADMINISTRATION

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**Background:** Despite the progress in cancer therapy, conventional cytotoxic therapies lead to unsatisfactory long-term survival, mainly related to the development of drug resistance in tumor cells and toxicity towards normal cells. The ω-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can exert anti-neoplastic activity by inducing apoptotic cell death in cancer cells either alone or in combination with conventional therapies. The ω-3 PUFAs potentially increase the sensitivity of tumor cells to conventional therapies, possibly improving their efficacy, especially in case of cancer cells resistance to treatment. Moreover, in contrast to traditional therapies, ω-3 PUFAs appear to cause selective cytotoxicity towards cancer cells with little or no toxicity to the normal cells. The influence of ω-3 PUFAs supplementation on lipid profiles and metabolism of subcellular organelles such as the mitochondria remains poorly understood. Mitochondria play key roles in activating apoptosis in mammalian cells. Mitochondrial cytochrome c (cyt c) has been found to have dual functions in controlling both cellular energy metabolism and apoptosis.

**The aim** of this study was to assess the modification of fatty acid profiles in the Guerin's carcinoma mitochondrial fraction of rats under conditions of ω-3 PUFAs administration.

**Methods:** The animals were administered ω-3 PUFAs for 4 weeks prior to the carcinoma implantation and then for the entire duration of tumor growth. Daily dose was 120 mg of ω-3 PUFAs per kg of body mass. The content of fatty acids in the mitochondrial fraction was determined by using HRGC 5300 gas chromatographer in glass column with Chromosorb W/HP sorbent in 10% Silar 5CP liquid phase.

**Results:** The results of the research show that in the Guerin's carcinoma mitochondrial fraction of rats during the intensive growth of the tumor (14 days, which corresponds to the logarithmic phase of tumor growth) the levels of ω-6 PUFAs were: 9.5% of arachidonic acid (AA), 7.3% of linoleic acid (LA), 1.8% of docosatetranoic acid (DTA). At the same time the levels of ω-3 PUFAs were: 4.4% of DHA, 0.1% of alpha-linolenic acids (ALA). The ω-3 PUFAs administration prior to and post-implantation of tumor leads to increased levels of AA (20%), DHA (5.5%) and EPA (1.7%) if compared to tumor-bearing rats that didn't receive the lipophilic nutrients. The high level of AA in tumor mitochondrial fraction of rats may indicate a decrease in their metabolism as a result of competition with ω-3 PUFAs for enzymes. The derivatives of ω-3 PUFAs induce apoptotic cell death in the Guerin's carcinoma via activating mitochondrial apoptotic pathways. The change of ω-3/ω-6 has been found to be significant in membrane structure and mitochondrial functioning. The ω-3 PUFAs supplementation before and after implantation of the Guerin's carcinoma resulted in a decrease of cyt c level in mitochondrial fraction and in an increase of the cytosolic cyt c. Through interaction with apoptotic protease activating factors (Apaf), cyt c can initiate the activation cascade of caspases once it is released into the cytosol. The mechanism of such action of the lipophilic nutrients under investigation can be realized through oxygenated active metabolites of ω-3 PUFAs (epoxyeicosatetraenoic acid, epoxydocosapentaenoic acid, resolvin D1).

**Conclusions:** The obtained results indicate that the ratio of ω-6/ω-3 PUFAs plays an important role in the metabolism of PUFAs of the tumor and may be considered as a potential marker for prognosis of tumorigenesis. Therefore, DHA and EPA are potential anticancer agents that might be used for adjuvant therapy or combination therapy with conventional anti-cancer drugs for the treatment of some forms of cancer with minimal toxicity.

## SOMATIC REARRANGEMENTS IN HLA CHROMOSOMAL REGION IN SOLID OVARY TUMORS

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**Background:** HLA (human leukocyte antigens) chromosomal region on short arm of chromosome 6 encodes cell surface molecules of human Major Histocompatibility Complex (MHC) specialized to present antigenic peptides to the T-cell receptor on T cells. The HLA-genes are organized into 3 chromosome regions according to the functions of expressed antigens (class I–III). The major function of the HLA-antigens is to induce and regulate the immune responses.

Lymphocytes that express CD8 react with HLA class I antigens that activate the cytotoxic function, requiring them to be capable of recognizing an infected cell. HLA class III genes encode molecules acting in inflammation; they express complement components C2, C4, and factor B; TNF-alpha and lymphotoxin. It is known that the increased rate of somatic rearrangements in HLA genes is one of the possible mechanisms of immune evasion in the development and progression of some types of cancers.

**The aim** of our study is to analyze the frequency and range of somatic deletions and duplications in the HLA locus in the ovarian tumors of affected patients.

**Methods:** The study of genomic rearrangements in the HLA region was performed using LOH analysis of 2 STR-markers located in HLA chromosome region: D6S2678 (class I) and D6S2925 (class III). The fragment analysis of the STR-markers was carried out by electrophoretic separation of the PCR products in a polyacrylamide gel on an automatic laser analyzer.

**Results:** In 26.1% of the ovarian tumors (6 out of 23), partial deletions/duplications of the HLA class I chromosomal region were detected. In 40.1% of samples (9 out of 22), somatic rearrangements were detected in the chromosomal region of the HLA class III.

**Conclusions:** The LOH analysis using the D6S2678 (Class I) and D6S2925 (class III) STR markers allows us to determine the somatic rearrangements of the HLA region in malignant ovarian tumors, which could be used to predict the immunotherapy effectiveness and treatment outcome in ovarian cancer patients.

## THE INFLUENCE OF TRANSPLANTED ALLOGENIC BONE MARROW AND ADIPOSE DERIVED MESENCHYMAL STEM CELLS ON THE IMMUNE ORGANS OF C57BL/6 MICE

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**Background:** Previously, we have shown that mesenchymal stem cells (MSCs) from a bone marrow and adipose tissue differ in expression of cytoplasmic, nuclear and membrane proteins. These parameters play a role in a proliferative activity, migration properties, and apoptosis level of MSCs during long-term cultivation. Hence, a question arises how MSCs isolated from different primary materials affect functional state of the organs of the immune system.

**The aim** of the present work was to determine the effect of transplanted allogeneic MSCs of bone marrow and adipose tissue on the thymus and spleen of C57Bl/6 mice.

**Methods:** The studies were conducted on 2–3-months-old males of C57Bl/6 mice, weighing 20–24 g. MSCs were isolated and cultivated in a sterile laminar box with compliance to the requirements of asepsis and antiseptics. MSCs were cultured in a $CO_2$ incubator at 37 °C and 5% $CO_2$ in DMEM with 10–15% of fetal bovine serum, 1% of antibiotic-antimycotic solution (Sigma-Aldrich, USA). The following groups of animals were formed: group 1 — intact (control group); group 2 — animals, to whom 0.5 ml of 0.9% NaCl solution (placebo) were injected (into the caudal vein); group 3 — animals, to whom $10^4$ of allogenic bone marrow MSCs (bmMSCs) in 0.5 ml of PBS were injected; group 4 — animals, to whom $10^4$ of allogenic adipose derived MSCs (aMSCs) in 0.5 ml of PBS were injected. Indicators of the weight of peripheral lymphoid organs relative to the body weight (weight index), evaluation of cellularity of the thymus and spleen of animals were estimated at 7, 18 and 25 days after the administration of MSCs.

**Results:** The administered bmMSCs have a stimulating effect on the proliferative activity of thymocytes *in vivo*, as indicated by an increase in thymic cellularity at 7 and 18 days of study, compared with the control and placebo by 79 and 32%, respectively. On the 25th day after the administration of MSC, the content of lymphoid cells in the thymus was reduced, compared to the 18th day, but was significantly higher, than in intact animals and after administration of 0.89% NaCl. A reliable ($p < 0.05$) positive correlation of thymus cellularity with weight index was observed after administration of bmMSCs.

On the 7th day after administration of bmMSCs, cellularity of spleen was significantly higher — by 26%, compared with control animals and by 17%, compared to animals, which were administered with the placebo. On the day 18th, the spleen lymphoid cell content was also significantly higher than in groups 1 and 2, but was less than on the 7th day. On the 25th day the number of lymphoid cells in the spleen was significantly reduced (by 42%), compared with the control animals and by 32%, compared to the animals which were administered with the placebo. The content of lymphoid cells in spleen on the 25th day correlates with the weight index of spleen, indicating its involution. The weight index of the spleen directly correlates with the content of the lymphoid cells.

After the administration of allogeneic aMSCs on the 7th and 18th day, the content of lymphoid cells in the thymus increased significantly, compared with control animals and placebo — by 93 and 42%, respectively. On the 25th day the content of lymphoid cells in thymus also was higher: by 53 and 86%, respectively. A positive correlation between cellularity of thymus and its weight index was established along the entire length of observation after the administration of aMSCs. After

the administration of aMSC, the content of lymphoid cells in the spleen exceeded significantly that in the spleen of intact animals. The number of lymphoid cells increased significantly by 33 and 24%, compared to intact animals and the placebo group on the 7th day. On the 18th day, the cellularity of spleen in the animals with administered aMSCs was significantly higher — by 18 and 14%, respectively. On the 25th day, the lymphoid cell count was higher by 7 and 15%. The weight index of the spleen increased significantly until the 18th day of the experiment. On the 25th day the weight index of spleen did not differ from that in experimental group of animals and placebo.

On the 18th day in group 3 the cellularity of thymus was significantly higher, compared to the group 4: 2.93 ± 0.10 • $10^6$/mg and 2.17 ± 0.16 • $10^6$/mg ($p < 0.05$), respectively. On the 7th and 25th days, there was no difference in thymus cellularity. On the 18th day, the thymus weight index in the group 3 was higher, compared to the group 4: 0.22 ± 0.01 ($p < 0.05$) against 0.16 ± 0.02. On the 7th and 25th days, there was no reliable difference of thymus weight index between these groups of animals.

On the 7th and 18th days after administration of bmMSCs and aMSCs, the cellularity of spleen in groups 3 and 4 did not differ significantly. On the 25th day after administration of bmMSCs, the cellularity of spleen was significantly lower, compared to administration of aMSCs: 1.90 ± 0.17 • $10^6$/mg and 2.90 ± 0.06 • $10^6$/mg ($p < 0.05$), respectively.

On the 7th and 18th days after administration of MSCs, the spleen weight index in group 3 was significantly lower, compared to animals of group 4 (the day 7: 0.65 ± 0.02 and 0.79 ± 0.04 ($p < 0.05$), respectively; the day: 0.62 ± 0.02 and 0.79 ± 0.04 ($p < 0.05$), respectively).

**Conclusions:** Transplantation of allogenic bone marrow and adipose derived MSCs stimulates immune cell in the thymus and spleen to proliferate, which is confirmed by increasing cellularity of these organs and their weight indices.

## THE INFLUENCE OF ONCOLYTIC NEWCASTLE DISEASE VIRUS ON CELLS OF NON-SMALL-CELL LUNG CANCER AND NORMAL KIDNEY CELLS

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**Background:** The virotherapy started to be used for complex treatment of cancer when the ability of some viruses to kill cancer cells had been discovered. In particular, Newcastle disease virus (NDV) is used for complex treatment of cancer because the virus is cytotoxic for malignantly transformed cells and can stimulate production of some cytokines (IFN and TNF) with anti-tumor activity. This virus has a low-level pathogenicity for human organism and can cause mild flu-like symptoms, conjunctivitis, and laryngitis. According to the literature data cells have various sensitivity to the virus *in vitro* which depends on NDV strain and types of cells.

**The aim:** To study the influence of NDV on the cancer and normal cells and to compare the viability of the cancer and normal cells infected with NDV in vitro.

**Methods:** The study was performed on non-small-cell lung cancer cells (A549 cell line), porcine embryo kidney cells (SPEV cell line) and NDV (4 • $10^6$ PFU). Cell culture and virological methods have been used in this study.

**Results:** We revealed a higher sensitivity of malignant A549 cells compared with normal cells of kidney SPEV. The death of more than 50% of cells was noted at 1/40 virus dilution in SPEV cells and 1/320 dilution in A549 cells. The cytotoxic effect of NDV on tested cells was evaluated in 24–72 h after their incubation with virus.

**Conclusions:** 1) NDV is more cytotoxic for cells of non-small-cell lung cancer than for normal kidney cells. 2) This results can become a base for further investigations of interaction between tumor and normal cells with NDV and for creating of alternative ways for cancer biotherapy.

## IRE1-DEPENDENT EXPRESSION OF SPECIFIC TO IGFBP miRNA IN GLIOMA CELLS

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**Background:** We have studied the level of microRNA miR-7 and miR-182 expressions in U87 glioma cells when inositol requiring enzyme 1 (IRE1) are inhibited. MicroRNAs are small, single-stranded noncoding RNA molecules approximately 22 nucleotides in length, which are involved in the post-transcriptional regulation of gene expression through interaction with the 3'UTR region of target mRNA and following degradation of mRNA. Recent research detected an abnormal expression of the miRNAs in glioma, which could be useful biomarkers.

**Methods:** We analyzed total RNA from U87 glioma cells transfected by empty vector pcDNA3.1 (control) and cells without IRE1 signaling enzyme function (transfected by dnIRE1). For quantification of micro RNA we used quantitative polymerase chain reaction in real time. Firstly, we add a poly(A) tail to the miRNA in total RNA sample using poly(A)-polymerase. Next, we reverse transcribe the polyadenylated miRNA to generate first-strand cDNA for quantitative polymerase chain reaction in real time using universal RT primer. For amplification of the miRNA hsa-miR-7 and hsa-miR-182-5p we used forward primers specific for each miRNA and universal reverse primer.

**Results:** It was shown that the level of miR-7 expression in glioma cells without IRE1 signaling enzyme

function is strongly increased (+113%) compared to control glioma cells measured by quantitative polymerase chain reaction in real time. The level of miR-182-5p expression in cells without IRE1 signaling enzyme function is increased by 38% compared to control glioma cells. Also we have analyzed data for IGF1, IGF2, IGFBP1, IGFBP2, IGFBP3 and have found that 3'UTR region of these mRNAs have specific binding sites for different microRNAs and that some from these mRNAs can specifically bind miR-7 and miR-182-5p. Thus, the changes in the expression level of miR-7 and miR-182-5p in glioma cells without IRE1 signaling enzyme function can possibly contribute to the decreased proliferation rate of these glioma cells mediated via IGF/IGFBP system.

**Conclusions:** We detected the miRNA binding sites in 3'-region of IGF/IGFBP mRNA family. Using quantitative polymerase chain reaction in real time we have shown that the expression level of microRNA miR-7 and miR-182-5p is increased in cells without IRE1 signaling enzyme function compared to control glioma cells that possibly affects the activity of IGF/IGFBP system.

## 4-THIAZOLIDINONE DERIVATIVES RESCUE OSTEOBLAST DIFFERENTIATION FROM NEGATIVE EFFECT OF TNF-α

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**Background:** Malignant tumors development and progression cause destruction of surrounding healthy tissues and in a case of solid tumors also a formation of necrotic masses. Such destructive processes induce a massive chronic inflammation. A growing body of epidemiological and clinical data supports the concept that chronic inflammation promotes tumor development and progression. As a major pro-inflammatory cytokine, tumor necrosis factor (TNF-α) is able to act as an endogenous tumor promoter to bridge inflammation and carcinogenesis (Wang *et al.*, 2008). On the other hand, the negative interaction between pro-inflammatory signals and skeletogenic pathways (BMP and Wnt) which occurs at the sites of inflammation can lead to bone tumors development.

**The aim:** To evaluate anti-inflammatory activity of novel 4-thiazolidinone-based derivatives towards TNF-α-induced inflammatory processes during osteoblast differentiation in mouse mesenchymal precursor cells.

**Methods:** We performed *in vitro* evaluation of functional effect of multifunctional heterocyclic 4-thiazolidinone derivatives (compounds Les-4368, Les-4370, Les-3882 and Les-3288) at different doses (1 μM, 0.3 μM and 0.1 μM) on TNF-α induced inhibition of bone formation by mouse mesenchymal precursor cells of C2C12. These cells were induced to differentiate into osteoblasts by different BMPs. Western blot analysis was used to elucidate a mechanism of anti-inflammatory effects.

**Results:** We found that treatment of C2C12 cells with TNFα completely inhibited the myoblast differentiation, as well as strongly inhibited BMP-induced osteogenesis. Treatment of these cells with investigated compounds allowed in case of Les-4368 and Les-3882 to rescue the osteogenic differentiation from negative effect of TNF-α, and even to convert it from inhibitor into potentiator of osteogenesis compared with control. Possible involvement of NF-κB modulation as a key mechanism mediating anti-inflammatory effects was validated by immunoblot assays.

**Conclusions:** Novel 4-thiazolidinone derivatives, Les-4368 and Les-3882, rescue osteogenesis from negative control of inflammation. The best effect was shown by compound Les-3882 that stimulated osteoblast differentiation at low dose (0.1 μM), presumably via modulation of NF-κB pathway.

## PREDICTING MODEL OF REGULATORY RELATIONSHIPS BETWEEN LONG NON-CODING RNAs BC200/XIST AND DISEASE-ASSOCIATED microRNAs

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**Background:** Long non-coding RNAs (lncRNAs) are defined as a class of important heterogeneous ncRNAs with the length more than 200 nucleotides. The recent study suggested that lncRNAs bc200 and XIST participate in development of several unisex tumors via binding disease-specific microRNAs.

**The aim** of research is to predict and identify miR-lncRNA (XIST and bc200) interactions in several selected cancers — breast cancer, glioblastoma, neuroblastoma.

**Methods:** We used LncRNADisease, Lnc2Cancer, Diana Tools to analyze present expression data of lncRNAs ANRIL and XIST for selected diseases *in silico*. We used HMDD v2.0 and Mir2Disease databases to find possible involvement of target microRNAs in various diseases.

**Results:** Using computational analysis of disease-associated microRNAs (Diana Tools), we predicted potential microRNAs for the sponge activity of bc200 (miR-15, -125, -30 families) and XIST (miR-30, -15 families). Then, we used the HMDD v2.0 and Mir2Disease databases to find possible involvement of these microRNAs in various cancers.

**Conclusions:** The miR 15 family is predicted to bind to XIST and bc200 lncRNAs that can suggest the potential link between these lncRNA in various cancers. We create our prediction model of potential

regulatory network of lncRNAs bc200 and XIST that gives an opportunity to see a more systematic picture of epigenetic regulation.

## TUMOR MARKERS FROM LIQUID BIOPSIES. BENEFITS AND LIMITATIONS

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**Background:** The early diagnosis, prognosis of tumor development and choice of an appropriate target for cancer therapy are the most important topics in a modern oncology research. The most common cases of tumor diagnosis are when tumor is already symptomatic and/or detectable by ultrasound or other physical methods, and such late time of the diagnosis does not contribute to an effective treatment. Investigating tumor features, using tissue biopsy, we study only a spatially and temporally limited sample, which cannot give the whole picture of tumor characteristics. Applying the approach of detection of tumor markers in biological fluids (liquid biopsies) can be a good solution for such problems. Nevertheless, a work with bio-fluids also has certain limitations.

**The aim** of our work was the detection of putative markers of prostate and bladder cancers in biological fluids of patients and evaluation of their potential use in clinics, as diagnostic or prognostic markers for these cancers.

**Methods:** We collected samples of urine and blood from patients with prostate and bladder cancers. We isolated DNA by a standard phenol-chloroform method from whole urine with preliminary CTAB precipitation. We isolated RNA, using Trizol from whole urine (for non-coding RNAs detection) from patients with bladder cancer and cell sediment from urine and plasma of the patients with prostate cancer. For detection of the aberrant DNA methylation, we provided bisulfite treatment of DNA, followed by PCR with primers to methylated and un-methylated templates and an agarose gel electrophoresis. We used Real-Time PCR for detection of mRNAs and lncRNAs, which can be potential markers of prostate and bladder tumors.

**Results:** We found that *PTEN, NKX3.1* and *RASSF* were methylated in 71.4% of samples, and the most frequently methylated gene was *PTEN* (87.5%). For bladder cancer, *NKX3.1* was methylated in 100% of all cases, the *RASSF1A* gene — in 35 out of 36 cases. The results of relative expression of *AurA*, *AurB*, *AurC, BRAF, EGF* and *YWHAZ* in samples from patients with prostate cancer demonstrated that differential expression of all 6 genes could be identified in both, urine and plasma. We observed positive correlation between expression of the *AurC* and *BRAF* genes (rs = 0.688, $p = 0.01$), the *BRAF* and *EGF* genes (rs = 0.719, $p = 0.01$) and the *YWHAZ* and *AurC* genes (rs = 0.591, $p = 0.01$). The results also demonstrated that the relatively high levels of lncRNAs *PANDAR* and *BC200* can be identified in urine samples from people with bladder cancer.

**Conclusions:** Markers, based on detection of methylation of the tumor suppressor genes in biofluids, can tell about even an early stage of carcinogenesis. However, it is difficult to apply them for the choice of treatment. Expression of oncogenes is quantitative, but these quantities do not show the picture of their expression in tumor itself. However, if the high expression of the specific oncogene is observed, this information can be used for the diagnosis and selection of the target for therapy. It is important, that they can be identified in plasma, because for any other tumors, except urological the blood sample can be more useful, than urine (probably). In addition, finally, the specific markers, such as lncRNAs can be used as a tool for diagnosis and prognosis of tumor development.

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## THE TGF-BETA-SMAD AND IL2-STAT5 PATHWAYS ARE INACTIVATED IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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**Background:** Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Europe and USA (about 30%). Most if not all CLL cases, are preceded by the monoclonal B-cell (MBC) lymphocytosis, which occurs in 5–10% of people over the age of 40 and progresses to CLL with a frequency of about 1% per year. Cells of CLL, despite being non-proliferating, express a set of cytokine receptors. Among the most important are receptors for IL2 and TGFB. In lymphoid cells, the active canonical TGFB pathway leads to apoptosis. IL2 is one of the main inducers of T-cell activation and differentiation.

**The aim:** We asked a question what is the status of the IL2 and TGFB pathways in CLL cells, with an aim to uncover the molecular details of appearance of these cells.

**Objects and methods**: peripheral blood samples of CLL patients, immunofluorescent analysis, western blotting, bioinformatics analysis of publicly available data bases on expression.

**Results:** We have shown that the TGFB-SMAD canonical pathway is not active in CLL cells. SMAD-responsive genes, such as BCL2L1 (BCL-XL), CCND2 (Cyclin D2) and MYC are down-regulated in CLL cells, compared with B-cells of peripheral blood of healthy donors. Also, the

IL2-induced JAK-STAT5 pathway is largely inactivated in CLL cells. Despite elevated expression of STAT2 and STAT5 genes at the mRNA level, STAT5-responsive genes, such as BCL2L1 (BCL-XL), CCND2 (Cyclin D2), HIF1A, ID1, MCL1 and MYC are downregulated in CLL-cells, compared to peripheral blood B-cells of healthy individuals. Moreover, we have found that SMAD2 is almost not expressed in CLL cells. No nuclear heterodimers of SMAD4 and SMAD3 (-2) were detected. Importantly, a phosphorylated from of the STAT5 protein was detected; however, no nuclear signal of this form was observed.

**Conclusions:** The TGFB-mediated signaling is not active in CLL cells, due to low (or absent) expression of SMAD4. The phosphorylation status of SMAD2 and -3 should be further elucidated. The inactivation of the JAK-STAT5 pathway could be explained by the high levels of soluble IL2RA, as was reported earlier. Another possibility could be inhibition of STAT2 phosphorylation, leading to inability to form the active transcriptionally protein heterodimers. The phosphorylation status of STAT proteins in CLL cells should be further illuminated. Also, expression of proteins, regulating nuclear export/import should be studied.

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## BURDEN OF LIVER METASTASES IN MODIFIED MURINE BREAST CARCINOMA

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**Background:** Although the prognosis of metastatic liver disease has recently improved, it still remains a treatment challenge. The 4T1_luc2 is a highly tumorigenic cell line, which can spontaneously metastasize from the primary tumor to multiple distant sites including lymph nodes, liver, lung, brain, and bone. Thus could be used as a relevant tumor model including the general field of immunization studies in oncology as well as liver metastases in particular.

**The aim:** To characterize the burden of liver metastases in immunized and naïve individuals using experimental model of breast carcinoma in mice.

**Methods:** Liver samples (n = 31) were analyzed from mice transplanted with 4T1luc2 adenocarcinoma cells expressing variant of HIV-1 FSU_A enzyme with and without drug resistance mutations. 23 mice were transplanted with 4T1luc2 expressing proteases (PR) (PR-DNA immunized 15, naïve 8), from which 7 PR_Ai, 8 PR_A2mut and 8 PR_A3mut. Controls (n = 8) received parental 4T1luc2 adenocarcinoma cells. Metastases were diagnosed and evaluated in formalin-fixed, paraplast-embedded liver tissues. For each mouse, the area of tumor metastases was quantified in 25 high power (×400) microscope fields of haematoxylin-eosin-stained slides by computer-assisted morphometry using specialized NIS-Elements software (Nikon, Tokyo, Japan). IBM SPSSv23 was applied for statistical analysis, including descriptive assessment as detection of mean values and standard deviation (SD).

**Results:** Liver micrometastases were found in livers of 18/23 of 4T1luc2_PR implanted mice. PR immunized mice developed metastasis in 10/15 mice and naïve in 8/8 examined cases, as well as metastasis were found in all control mice. In 4T1luc2_PR_Ai group 165 metastases were found with mean size 636.08 μm$^2$ (SD ± 519.15), from which 112 (21.6 metastases per mouse (SD ± 5.94; mean size 682.02 μm$^2$ (SD ± 581)) in immunized and 53 (23.5 metastases per mouse (SD ± 10.6) with mean size 538.99 μm$^2$ (SD ± 339.51)) in naïve animals. In 4T1_luc2-PR_A2mut group 148 metastases were detected, with mean size 999.9 μm$^2$ (SD ± 1408.66), 128 (23.8 per mouse (SD ± 10.4) metastases with mean size 1029.15 μm$^2$ (SD ± 1506.25) in immunized and 20 (9.5 metastases per mouse (SD ± 6.36) with mean size 812.64 μm$^2$ (SD ± 380.52) in naïve mice. In 4Tluc_2_PR_A3mut 65 metastases (21.67 per mouse (SD ± 8.62) with mean size 843.87 μm$^2$ (SD ± 877.19), from which none were found in immunized mice. In control mice 27 metastases (3.37 per mouse (SD ± 3.2) with mean size 699.35 μm$^2$ (SD ± 280.52) were detected. Inflammatory infiltrates consisting of neutrophils and lymphocytes were found in all groups.

**Conclusions:** Number of metastases per mouse among HIV_DNA-immunized mice implanted with HIV-PR expressing tumors tend to be lower than among naïve animals, but higher compared to control group. Only DNA-immunization with PR_A3mut tend to protect against liver metastases as none were found in immunized animals, compared to other groups. However, the morphology suggests complex tumor-host interaction. Furthermore, 4T1luc2_PR tumors tend to form larger metastases compared to control group.

## METHYLTRANSFERASE ACTIVITY UNDER THE INFLUENCE OF INTERACTION "CORE" 2'-5'OLIGORIBOADENILATES AND THEIR DERIVATIVES

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**Background:** Disturbance in the balance of methylation /demethylation of DNA, which in turn serves as a key

event in epigenetic deregulation in carcinogenesis, was studied. At the moment, a number of inhibitors of DNA-methyltransferases are known, but their mutagenic and toxic effects are a significant disadvantage of these compounds. Natural and synthetic oligoadenylates, which can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction with regulatory domains, can be used as safe analogues.

**The aim:** Study of the influence of natural and synthetic oligonucleotides on the activity of model DNA-methyltransferase *in silico* and *in vitro*.

**Methods:** "HyperChem" — modeling the structure of oligonucleotides "AutodockVina" — molecular docking "Way2drug" — simulation of toxicity and expression of mRNA "String" — cluster analysis of hypothetical expression of mRNA gel electrophoresis.

**Results:** It is shown that the most effective binding of oligonucleotides to methyltransferase occurs with 3 and 5 monomeric aptamer. Toxicity analysis showed that LD50 of oligoriboadenylates is in the range of 200–1500 mg/kg. The study of individual oligonucleotides in the DIGEP program showed their ability to activate the antioxidant and immune system with the simultaneous inhibition of methyltransferase activity. The electrophoretic distribution showed the ability of oligonucleotides to inhibit the methyltransferase reaction in a wide range of micromolar concentrations.

**Conclusions:** Thus, the analysis of molecular docking suggests that the oligoriboadenylates possess the highest energy of binding with the model DNA-methyltransferase EcoRI.

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## MOLECULAR-GENETIC MODELS FOR PROGNOSIS OF DEVELOPMENT OF TUMORS OF BREAST AND OVARIAN CANCER IN WOMEN WITH A FAMILY HISTORY OF CANCER

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**Background:** Among the current problems of clinical oncology (screening, prevention, early diagnosis, personalized treatment), one of the topical issues — the individual risk of malignant neoplasms — is of particular interest, although for most of them, including cancer of the female reproductive organs (FRO), risk factors are mostly already defined [1–4].

**The aim:** To develop a prognostic molecular genetic model for assessing the risk of development of benign and malignant tumors of FRO in patients from cancer-affected families.

**Methods:** The work presents the data on a comprehensive clinical examination of 210 women (90 patients with FRO cancer with aggregation of tumor pathology in families, 65 patients with benign pathology of FRO from cancer-affected families, 55 women — control group of healthy women without family history of cancer). Clinical genealogical analysis, morphological examination of tumors and molecular genetic studies of genomic DNA from peripheral blood and tumors was carried out.

**Results:** It was established that in the families of patients with benign and malignant pathology of FRO, malignant tumors associated with Lynch II syndrome are observed. Based on the analysis of detected ESR-1, Cyp2D6*4 and mutations in *BRCA1/2* genes in cancer patients and in patients with benign pathology, molecular genetic models have been developed to assess the individual risk of development of benign and malignant tumors of FRO. It has been established that these molecular genetic models and combinations of gene mutations and gene polymorphisms (SNP) by the intergene interaction that was analyzed, were found to be reliable in assessing the risk of benign and malignant pathology of the mammary gland and ovary.

**Conclusions:** The model, which included the polymorphic variants of the T397ESR1/Cyp2D6*4 genes was of the best predictive accuracy for the evaluation of the risk of benign tumors of the FRO (71.68%) and the highest reliability ($p < 0.001$). At the same time, all identified models of intergene interaction in the development of malignant pathology of FRO were reliable, prognostically significant with high reproduction and almost identical accuracy (65.00–68.23%). The obtained results indicate a high informativeness of such molecular genetic indices as the polymorphism of ESR1 and Cyp2D6*4 genes and mutations in *BRCA1/2* genes to assess the risk of benign or malignant tumors of FRO in families of patients with family history of cancer.

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## COMPETITIVE INHIBITION — NEW PERSPECTIVE ON *Klebsiella spp.* AS A TRIGGER OF CARCINOMATOSIS

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**Background:** *Klebsiella* is referred to endemic diseases of Western regions of Ukraine, including Transcarpathia. Despite the frequent isolation of their pathogens from the nasal microbiome (especially the *Klebsiella pneumoniae* strain), the role of the carcinogenesis trigger remains uncertain.

**The aim:** Research of antagonistic and inhibitory properties of a non-pathogenic strain to prevent the occurrence of cancer conditions.

**Methods and results:** After a bacteriological study of 47 patients with a diagnosis of oropharyngeal cancer, laryngeal cancer, oral cancer, cancer of the tongue root, larynx cancer with rhinophyma, 5 strains of *Klebsiella rhinoscleromatis* and 2 strains of *Klebsiella ozaenae*, 3 — *K. pneumoniae* were isolated. Numerous studies describe the mechanism of "avoidance" of the immune response by bacteria, which persist in tumors of the gastrointestinal tract and uterus, since the tumor is intact and has an imperfect system of blood supply. It is known that these species lead to chronic inflammation, atrophic or hyperplastic changes in the epithelium. So can they lead to malignancy in people with a genetic predisposition or against a background of precancerous conditions?

We also evaluated the functional activity of follicular, epithelial dendritic cells and peripheral blood cells in response to contact with Commensal, as well as a we made comparative analysis of the cytokine profile of *K. pneumoniae S* (wild, pathogenic strain), *R* (the mutant strain was obtained by culturing in the presence of crystals with nitrosoguanidine) and *B. subtilis* 090 *in vitro*. We found weak immunosuppressive activity in the pathogenic strain, moderate synthesis of IL-6 bacilli, and sufficient synthesis of pro-inflammatory IL-10, -12 and TNF-α nonpathogenic strain.

The antagonistic properties of the mutant strain were also evaluated by prolonged cultivation with pathogenic strain. Expressive suppression of the virulent strain was observed after 48 h *in vitro*.

**Conclusions:** Since the mutant strain is not pathogenic and has the ability to initiate a systemic immune response rather than a local one, it can be further recommended as a promising anti-*Klebsiella* vaccine preparation. And one of the directions of targeted therapy is expedient to consider the introduction into the body of noncapsular mutant strains of *K. pneumoniae S* for competitive inhibition of pathogenic strains, increasing the activity of dendritic cells and combating resistance to antitumor drugs.

## PARP-1 INHIBITORS MODULATE CYTOKINE TRANSCRIPTION IN A THP-1 MODEL OF ACUTE MONOCYTIC LEUKEMIA

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**Background:** Acute monocytic leukemia (AML) belongs to a malignant bone marrow neoplasm, associated with hyperleukocytosis and coagulation abnormalities. The most frequently encountered form is observed in young children under 2 years old. In AML, more than 80% of leukemic cells belong to monocyte lineages such as promonocytes and monocytes. THP-1 cells represent the immortalized monocyte-like cells and are derived from a 1-year infant with AML. These cells resemble healthy monocytes, but maintain capacity to proliferate. THP-1 cells, similarly to immune response cells, produce a variety of pro-inflammatory cytokines. In AML a strong response is an undesirable effect, nonetheless, during the cell contact with the high dose of bacterial endotoxin — lipopolysaccharide (LPS) the process of immunotolerance occurs. That phenomenon is associated with cell inability to produce cytokines (e.g. TNF-α) that in consequence impairs the functioning of immune system. There are much data that show the role of PARP1 protein in many intracellular processes even in regulation of gene expression, which is engaged in pro-inflammatory response.

**The aim:** To confirm the first obtained data suggested that the presence of PARP1 on the chromatin is involved in inhibition of immune tolerance.

**Methods:** The process of immune tolerance in THP-1 cells was induced with the high dose of LPS. To observe the role of PARP-1 in blocking of immunotolerance, cells were preincubated with the inhibitor of PARP-1 — Olaparib — which is responsible for PARP1 maintaining on the chromatin.

**Results:** The first data showed that in presence of PARP1 inhibitor the process of immune tolerance did not occur. Moreover, after differentiation of THP-1 cells with PMA (phorbol ester), the presence of inhibitor did not prevent the development of immunotolerance process. Those results suggested differences in the PARP-1 contribution to immunotolerance induction in differentiated and undifferentiated cells. Inhibitors of PARP-1, which are tested and utilized in cancer therapy, can stop the process of immunotolerance in AML.

**Conclusions**: In case of AML, the PARP-1 inhibitors can stop the process of immunotolerance. Even if this is an undesirable process, nevertheless, the indication of basis of this process can be useful in immunotolerance prevention in healthy monocytes and/or macrophages.

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## CANCER ENZYMOTHERAPY: RIBONUCLEASES IN CANCER TREATMENT

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**Background:** Ribonucleases (RNase) catalyse the breakdown of RNA into smaller components. The cytotoxic effects of RNase include the RNA cleavage leading to the inhibition of the protein synthesis and the induction of apoptosis. In some cases, they become a source of micro-RNA. Attractive idea is to provide enzymes that selectively deplete certain types of RNA in tumor cells.

There are two types of RNases — endoribonucleases and exoribonucleases. Endoribonucleases are RNase A, RNase P, RNase H, RNase III, RNase T1, RNase T2, RNase U2, RNase V1, RNase I, RNase, PhyM and RNase V. Exoribonucleases include Polynucleotide Phosphorylase (PNPase), RNase PH, RNase II, RNase R, RNase D, RNase T, oligoribonuclease, exoribonuclease I and exoribonuclease II. Different RNases such as onconases, bovine seminal RNase, RNase T1, Sarcin, RNase P, actibind and RNaseT2 have recently been studied for the treatment of different type of cancers. Ribonucleoside 3'-phosphate could serve as a pro-moiety in Ribonuclease-Activated Cancer Prodrug because it increases the hydrophilicity of a cancer therapeutic agent. RNases uniquely influence several functions in the tumor cell simultaneously and demonstrated the ability to overcome multidrug resistance and to enhance the cytotoxicity of a variety of anticancer agents. Bovine seminal RNase (BS-RNase) is a unique member of the RNase A family which exist as dimer of RNase A like subunits which are linked by the disulfide like bridges. Dimeric form is able to evade ribonuclease inhibitor but not the monomeric form. Onconases were found to induce the caspase-9 — caspase-3 cascade, which is correlated with the release of cytochrome C from the mitochondria. The use of a novel approach based on the catalytic RNA subunit of RNase P can act as sequence-specific endonucleases and can exclusively cleave target RNA that forms a base pair with the guide sequence (GS) to destroy RNA specifically the tumor-specific fusion genes. The sources of bioactive RNases may be of different origin. We hope that this advancement provides a new therapeutic tool for the treatment of cancer and holds some promise for more selective, non-toxic cancer therapy in the future.

**Conclusions:** This therapy promises to be an effective strategy for the treatment of the cancer.

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## THE EFFECT OF VITAMINE E AS DIFFERENTIATION-LIKE FACTOR IN K562 LEUKEMIC CELL LINE

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**Background:** Chronic myelogenous leukemia (CML) is a clonal hematopoietic stem cell disorder associated with the activity of *BCR-ABL* fusion oncogene. The molecular events underlying the transition from the chronic phase to the blast crisis are still poorly understood. The transcription factor C/EBP-alpha (CCAAT/enhancer-binding protein α) implicated as an inhibitor of cell proliferation and a regulator of differentiation plays a critical role in granulocytic differentiation. The progression of CML to blast crisis is correlated with down-modulation of C/EBP-alpha. Tavor *et al.* (2003) first shown that the restoration of C/EBP-alpha expression in BCR-ABL-positive KCL22 blast cell line transfected with *C/EBPα plasmide vector (*pMT*α)* triggered a proliferative arrest, a block in the G2/M phase of the cell cycle and a gradual increase in apoptosis. Therefore, C/EBP-alpha may be considered as a putative target in differentiation therapies in myeloid leukemias.

**The aim** of the study was to assess the potential of vitamin E as the possible inducer of C/EBP-alpha expression in BCR-ABL-positive CML K562 cells.

**Methods:** Total RNA was extracted using TRIzol (Invitrogen, Gaithersburg, MD) according to the manufacturer's instructions. RNA was converted to cDNA using the Qiagen's QuantiTect Rev. Transcription Kit (Qiagen, Hilden, Germany). C/EBP-alpha and G-CSFR mRNA expression was quantified by real-time RT-PCR using SYBR Green protocol. RT-PCR reactions were carried out using HotStarTaq DNA polymerase (Qiagen), 50 ng of cDNA and SYBR Green in a 1:60,000 dilution in triplicate. PCR conditions were: a 95 °C initial activation for 15 min was followed by 45 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 30 s on an Bio-Rad Real-time PCR Detection System IQ5, CA.

**Results:** We have not found detectable expression of C/EBP-alpha in K562 cells. Upon 48-h culture with vitamin E at a dose of 100 μM, K562 cells expressed both C/EBP-alpha and G-CSFR (granulocyte colony-stimulating factor receptor).

**Conclusion:** Vitamin E restored the expression of C/EBP-alpha mRNA in CML K562 cells. In this setting, G-CSFR expression in vitamin E treated K562 cells seems to point out to the activation of at least one component relevant to granulocytic differentiation. It should be further elucidated whether such effects of vitamin E on C/EBP-alpha transcription factor are direct or mediated *via* antioxidant properties of vitamin E.

## THE EFFECTS OF IMMUNIZATION WITH XENOGENEIC EMBRYO PROTEINS AFTER LEWIS LUNG CARCINOMA SURGICAL REMOVAL

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**Background:** Xenogeneic cancer vaccines utilize xenogeneic homologous proteins or genes as immunogens to break immune tolerance towards tumor-associated antigens. Homologous immunogens can be derived from a broad number of different species. R.E. Kavetsky Institute is conducting research on elab-

oration of a xenogeneic vaccine based on chicken embryo proteins (CEP). In the preliminary experiments, immunization with CEP after surgery prevented a Lewis lung carcinoma (LLC) metastases spread.

**The aim** of this research was to examine the effects of CEP on immune reaction of mice immunized after LLC surgical resection.

**Methods:** LLC cells ($2.5 \cdot 10^5$ cells/mouse) were injected into a hind pad. The tumor was removed on day 17 after LLC cells transplantation. Thereafter, immunization was performed on day 1, 8 and 15. The cytotoxic activity (CTA) of immune cells was determined by the MTT assay. Lymphocytes proliferation was studied by *in vitro* blast-transformation assay (LBTA). IL-4, IFN-γ, IgG level in blood serum was analyzed by ELIZA.

**Results:** Since day 21 until day 38 after the tumor resection, metastases were detected in 6/10 control mice and in 0/11 immunized mice. On day 7 and 14 after tumor resection, the control mice showed features of surgically induced immunosuppression, namely decrease in NK CTA (by 2 and 2.5 times, $0.05 < p < 0.1$ and $p < 0.05$, respectively compared to the intact mice), and increase in IL-4 level (by 1.6 and 1.8 times compared to the intact mice on day 7 and 14, respectively). On the contrary, in the immunized group NK CTA suppression was seen only on day 14; IL-4 production did not differ from the intact mice. Moreover, in this group spontaneous LBTA significantly increased on day 7. The level of IFN-γ and IgG in the immunized group did not differ from the intact mice, while increase in these indices in the control group correlated tightly with volume of metastases.

**Conclusions:** Overall, it is shown that protection from surgically imposed immune suppression may underlie CEP anti-metastatic activity. Further elaboration of xenogeneic CEP-based cancer vaccine is warranted.

## *IN SILICO* STUDY OF THE CYTOKINE WITH ANTITUMOR ACTIVITY AIMP1/P43 POSSIBLE INTERACTION WITH A NOVEL MOLECULAR PARTNER

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**Background:** Multifunctional protein 1 (AIMP1/p43) is an auxiliary component of higher eukaryote aminoacyl-tRNA synthetase complex (MARS). Being outside of the complex it demonstrates noncanonical pleiotropic cytokine activities: modulates the proliferation of different types of cells, stimulates apoptosis and inflammation, suppresses angiogenesis and growth of tumors [1]. Recently it was shown that the AIMP1/p43 protein enhanced mediated immune responses via the up-regulation of Toll-like receptor 2 (TLR2) expression in mouse dendritic cells. These results indicate that AIMP1/p43 may enhance the immune responses by regulating the TLR2 expression [2]. It is known that TLRs not only play an important role in immune responses but recognize molecular partners, which are associated with cancer as well.

**The aim:** To reveal possible features of structural topography of these proteins underlying their interaction.

**Methods:** Bacterial expression of AIMP1/p43, fluorescence spectroscopy, *in silico* search of AIMP1/p43's molecular partners, prediction and analysis of coiled coils and natively disordered regions with use of BioGRID 3.4, PRABI and PrDos bioinformatical tools.

**Results:** Our research has shown the key role of disordered and coiled regions in the formation of interaction interfaces of both molecules under study. Additional hydrophobic regions exposed on the AIMP1/p43 protein surface as a consequence of conformational changes might contribute to the multiple functional activity of the AIMP1/p43 protein.

**Conclusions:** Features of AIMP1/p43 structural organization may be essential for the binding with the TLR2 partner molecule during recognition process.

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## IRE1 INHIBITION LEADS TO A TRANSCRIPTIONAL RE-PROGRAMMING OF U87 GLIOMA CELLS

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**Background:** Inhibition of a key sensory-signaling enzyme of the unfolded protein response pathway IRE1 (inositol-requiring signaling 1) leads to significant down-regulation of glioma growth. Understanding the mechanisms of this phenomenon is crucial for further research on glioma treatment strategies. In the department of molecular biology of Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine we accumulated a substantial amount of data on the differential gene expression in U87 glioma cells upon inhibition of IRE1 signaling enzyme.

**The aim:** To perform a data meta-analysis.

**Methods:** Gene ontology analysis using PANTHER classification system (http://pantherdb.org/) [1].

**Results:** The expression of total 185 genes was studied in U87 glioma cells with IRE1 dominant-negative knockdown. The expression levels of 14 genes (7.5%) remained unchanged as compared to control U87 glioma cells with functional IRE1, whereas the rest of 171 genes (92.5%) were differentially expressed. The mRNA levels of 85 (46%) genes were changed more than 2-fold (either up- or down-regulated) in IRE1-deficient glioma cells as compared to control. 20 genes exhibited more than a 5-fold change in their relative mRNA levels in IRE1-deficient glioma

cells. On the generated data sets (N1 = 185, N2 = 85, N3 = 20) we performed a PANTHER Overrepresentation test using default settings. Selected results were as following:

GO:0009966 — regulation of signal transduction: N1 — 88 genes (47.6%), N2 — 39 (45.9%), N3 — 12 (60.0%);

GO:0042127 — regulation of cell proliferation: N1 — 68 (36.8%), N2 — 37 (43.5%), N3 — 11 (55.0%);

GO:0010628 — positive regulation of gene expression: N1 — 56 (30.3%), N2 — 27 (31.8%), N3 — 9 (45.0%);

GO:0030154 — cell differentiation: N1 — 64 (34.6%), N2 — 34 (40.0%), N3 — 13 (65.0%).

(*Note*: one gene can belong to different groups at once).

**Conclusions:** Inhibition of IRE1 signaling enzyme in U87 glioma cells leads to profound changes in the transcription of numerous groups of genes, to genome reprogramming. Our analysis has shown that the most prominent changes are characteristic for the genes implicated in such processes as cell differentiation, regulation of signal transduction, cell proliferation and gene expression.

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## DOES LYS-PLASMINOGEN MAKE PLATELETS ANTI-ANGIOGENIC?

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**Background:** Angiogenesis is a key process to promote cancer, and anti-angiogenic treatment represents an established therapeutic strategy of cancer. Malignant cells (breast cancer, glioblastoma multiforme, and others) have been shown to recruit and stimulate platelets resulting in release of platelet-stored growth factors, which activate angiogenesis and support tumor development. The endogenous angiogenic inhibitors, angiostatins (AS), which are derived by limited proteolysis of plasminogen (PG), reduce both angiogenesis and vascular permeability. Truncated PG form, Lys-PG, has been recently shown to inhibit platelet responses to agonists.

**The aim:** This study was designed to clarify if PG can affect platelet degranulation and secretion of vascular endothelial growth factor (VEGF), as well as participate in generation of AS by PG fragmentation.

**Methods:** Washed platelets were obtained from fresh human plasma by gel-filtration on Sepharose-2B. Flow cytometry was used for the assessment of Lys-PG (1.2 μM final concentration) effects on agonist-induced P-selectin as an alpha-granule secretion marker and surface actin exposition. PG was conjugated with a fluorophore probe, FITC, to study PG binding with platelet membrane. Secreted VEGF and PG cleaved products (AS-like polypeptides) were detected by western blot.

**Results:** Cytometry analysis indicates that Lys-Pg is able to inhibit agonist-induced alpha-granule release and P-selectin exposition on the platelet membrane. Lack of exposed P-selectin is known to destabilize platelet aggregates and contributes to reduced platelet rolling on endotheliocytes. Interfered platelet secretion is also confirmed by the results of western blot demonstrating that Lys-PG-pretreated platelets became less sensitive to agonist action and released few amounts of angiogenic molecule, VEGF, compared to untreated cells. Platelets stimulated by thrombin or collagen were shown to expose increased levels of cytoskeletal protein, actin, on their surface. Results of the binding assay demonstrated that surface-exposed actin is involved in PG interaction with platelets. Incubation of platelets with either Glu- or Lys-PG resulted in generation of cleaved products, angiogenic inhibitors AS. Anti-actin antibodies partially blocked binding of PG with platelets and prevented its further proteolysis. It can be assumed that plasmin, which can be converted from Lys-PG on the platelet surface, is responsible for the loss of platelet sensitivity to the agonist stimuli by receptor's desensitization and is the key proteinase to provide AS formation.

**Conclusions:** Collectively, our data demonstrate for the first time that Lys-PG displays anti-platelet activities and restricts pro-angiogenic potential of platelets by inhibiting VEGF release and enhancing AS generation. With respect to involvement of platelets in tumor-induced angiogenesis, effects of Lys-PG platelet targeting may open new challenges to optimizing anti-angiogenic therapy of cancer.

## STUDY ON LECTINS FROM THE ALBUMEN GLAND AND MUCUS OF *Helix pomatia* AS BUILDING BLOCKS FOR A PUTATIVE TEST-SYSTEM TO PREDICT IMMUNE RESPONSE UPON TUMOR GROWTH

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**Background:** Lectins represent a big group of proteins and glycoproteins, which are capable to bind selectively glycans and glycan determinants on biopolymers. Almost all groups of organisms contain lectins in different organs and tissues [1], and have a high potential for cancer diagnostic and therapy [2]. Different types of lectins are used as markers for screening of tumor process, as the therapy for oncological pathology [3], and for development of the anticancer vaccines [4]. For example, agglutinin (lectin) of *Helix pomatia* is used for histochemical diagnostic to predict metastasizing of breast cancer [5]. It was shown experimentally that expression of agglutinin of *Helix pomatia* is associated with a high risk of metastasizing and the unfavorable prognosis [6].

**The aim:** To isolate lectins from an albumen gland and mucus of *Helix pomatia* and to investigate their potential for creation of an anticancer vaccine or a test-system for monitoring the efficacy of anticancer chemo- and/or immunotherapy.

**Methods:** A protein from the Albumen gland homogenate and mucus of *Helix pomatia* was salted out. We have got the protein with the concentration of the 3.11 mg/ml of the Albumen gland and 4.79 mg/ml of the mucus. 15 different proteins (11–150 kDa) were detected from the Albumen gland, and 12 (17–146 kDa) — from the mucus, respectively by western blotting. Lectins from the Albumen gland and mucus showed high agglutination properties with rabbit red blood cells. A cross-reactivity of lectins was studied using ELISA.

**Results:** An cytotoxic effect was assessed *in vitro* using the culture of Erlich carcinoma. We did not observe the cytotoxic effect of lectins on tumor cells. The antitumor activity of lectins was studied *in vivo* in the female Wistar rats with transplanted Walker's carcinosarcoma. Rats got injection of lectin in the doses of 1 mg/kg of body weight on days 3, 4, 9 and 15 after transplantation. Lectins did not demonstrate antitumor activity.

For detection of a cross-reactivity between lectins of *Helix pomatia* and antigens of Walker carcinosarcoma, we used blood serum of rats, at the different time of the tumor growth, and intact rats as a control. We observed the cross-reaction of lectins with the blood serum of all rats. For Western-Blot analysis of protein from Albumen gland and mucus, we used antibody from blood serum, which we used for ELISA. We found that the cross-reacting protein has a molecular weight of approximately 82 kDa in a pool of proteins isolated from the Albumen gland. The cross-reacting protein of a pool of the mucus proteins has the molecular weight about 68 kDa.

**Conclusions:** We isolated two pools of lectins from the Albumen gland and mucus of *Helix pomatia*. These lectins showed the high agglutination activity with rabbit erythrocytes. Neither pool demonstrated cytotoxic effect on tumor *in vivo* and i*n vitro*. Lectins from the Albumen gland and from mucus showed cross-reactivity with antigens from the blood serum of rats with transplanted Walker carcino-sarcoma and with blood serum of intact rats. Probably, these lectins can be used as a vehicle for tumor vaccines.

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