20 miRNAs in complex showed that decreased expression was observed much less frequently in the samples with a later RCC stage than in samples with early RCC stage. For example, in a tumor sample of a patient with stage IV (T3aN2M1) expression was reduced only for miR-129 and miR-375 in 10 times for each miRNA, while the expression of miR-9 was increased. In samples with stage III expression was reduced in 2 or 4 out of the selected 20 miRNAs, in samples with stage I expression decrease was observed for 9 out of 20 studied miRNAs. Since kidney tissue from the same patient was taken as the control, it could be assumed that the absence of expression decrease in studied 20 miRNAs in tumors was due to a decrease in their expression in the surrounding tissues, which eliminated the difference in the quantity of miRNAs in tumors and surrounding tissues. An additional experiment, in which kidney tissue from healthy donors with no history of cancer was taken as a control, showed decreased expression of miR-125b, miR-124a, miR-127, miR-203, miR-34c-3p, miR-9, miR-129, miR- 34b in histologically normal tissue of patients with RCC. There was identified a group of miRNAs out of studied 20 ones which may be involved in the progression of RCC, they were at least miR-129, miR-375 and miR-9. Expression characteristics of miRNAs require further study and may be used as biomarkers.

This work was supported by Russian Foundation for Basic Research grant 13–04-00828.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.079

P113

The possible association of novel functional SNP of D-glucuronyl C5-epimerase (GLCE) gene with breast cancer in Siberia region

T. Prudnikova[a], N. Litvyakov[b], N. Domanitskaya[a], N. Cherdyntseva[b], V. Belyavskaya[c,*]. [a] *Institute of Molecular Biology and Biophysics, Novosibirsk, Russian Federation,* [b] *Tomsk Cancer Research Institute, Tomsk, Russian Federation,* [c] *State Research Center of Virology and Biotechnology (Vector), Koltsovo, Novosibirsk region, Russian Federation* * Corresponding author.

Background: Heparansulfate (HS) is a glycosaminoglycan present on the cell surface and in the extracellular matrix, which interacts with diverse signal molecules and is essential for many physiological processes including embryonic development, cell growth, inflammation, and blood coagulation. D-glucuronyl C5-epimerase (GLCE) is a crucial enzyme in HS synthesis, converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA) to increase HS flexibility. Aberrant modification may result in wrong structure of polysaccharide chains of HS and defects of microenvironment associated with malignant transformation.We previously experimentally identified the GLCE polymorphism Ile597Val. Its localization close to the activity center of the enzyme and different physical parameters of the involved amino acids suggest that the polymorphism is functional. So, three different variants of GLCE dimers with different enzymatic activity may exist in heterozygous carriers. Bioinformatics' search (PubMed resource) revealed interracial variations in allele frequency distribution. Unusual high frequency of allele G was shown for black race (45%) compared with white race (17%). Taking into account the increased resistance of negroid race to breast cancer, we assume a potential involvement of the GLCE polymorphism in breast cancer.

Aim: The estimation of effects of GLCE functional polymorphism A2017G (Ile597Val) on the gene expression levels in normal and breast cancer cells and LOH in breast tumors.

Materials and methods: Breast cancer patients (n = 144.) had histologically verified diagnoses. Blood and breast cancer tissue samples as well as matched control tissues were collected from each patient during surgery. Genomic DNA was isolated by phenol extraction. Total RNA was isolated by TRIZol, RNA quantity was accessed by Qubit instrument with appropriate reagents and cDNA was obtained using First Strand cDNA Synthesis kit. SNP A2017G (rs3865014) was analyzed by Custom Real-Time SNP Array and GLCE expression levels were determined using Taq-Man-based Real-Time PCR (Applied Biosystems). Statistical analysis was carried out using a Statistika 9.0 software.

Results: AA genotype carriers had a 2-fold increase in GLCE mRNA levels in tumors compared with control surrounding tissues (0.37 ± 0.77 versus 0.17 ± 0.16, respectively, $p < 0.05$). Oppositely, AG genotype carriers had a 1.5-fold decrease in GLCE mRNA levels in tumors compared with control surrounding tissues (0.39 ± 0.29 versus 0.58 ± 0.33, respectively, $p < 0.05$). However, in any case,the GLCE expression in both normal tissues and breast tumors was more active in AG genotype carriers than in AA carriers. It is known that LOH is often associated with molecular mechanisms of carcinogenesis, we studied this process for the same patients. According our results, LOH was detected in about 10% of cases (5/52 patients), among which G was lost in 3 patients and A was lost in 2 patients.

Conclusion: The obtained data show a possible association between GLCE Ile597Val polymorphism and breast cancer, although the nature of the association remains ambiguous.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.080

P142

Glioma-derived osteopontin and lactadherin shape tumor microenvironment and immune response in rat model of glioma

D. Pszczółkowska*, A. Ellert-Miklaszewska, A. Gieryng, M. Kijewska, P. Wiśniewski, B. Kamińska. *Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland* * Corresponding author.

Malignant gliomas are fast-growing, heterogeneous and invasive brain tumors strongly infiltrated by non-tumor cells. Glioma attracts variety of immune cells, in particular microglia/macrophages and re-program these cells into immunosuppressive, tumor-supporting cells. Factors responsible for pro-invasive macrophage polarization and shaping tumor microenvironment in tumor-supporting manner are poorly known. We analyzed glioma secretome using proteomical approach and identified lactadherin (Mfge8) and osteopontin

(Spp1) in microglia-activating fractions. Both osteopontin and lactadherin are $\alpha v\beta 3/\alpha v\beta 5$ integrin ligands able to interact with receptors present on microglia and macrophages and thus could be involved in pro-invasive polarization of microglia/macrophages. Moreover, both Spp1 and Mfge8 are overexpressed in glioma cells, but not in non-transformed astrocytes. C6 glioma cells stably expressing shRNA specific to lactadherin (shMfge8), osteopontin (shSpp1) and negative shRNA (shNeg) were implanted into striatum of Wistar rats. There was no difference in proliferation and viability of C6 glioma cells, cells stably expressing shRNA specific to lactadherin, ostopontin and negative shRNA in vitro, that demonstrates the negligible effect of autocrine production of both protein on tumor cell growth. Knockdown of Spp1 and Mfge8 resulted in significant reduction of tumor volume in rat model of glioma. Immunochemical analysis of brain sections revealed similar numbers of infiltrating microglia/macrophages (Iba1 staining), but the reduced number of ameboid, arginase 1 expressing cells in Mfge8 – depleted tumor. Treatment of endothelial cells with rhMFGE8 revealed significant effect of that protein on angiogenesis in vitro, however lactadherin-depleted tumors do not exhibit reduced blood vessel density in rat glioma model. FACS analysis showed that silencing of Spp1 does not affect total number of CD11b-positive cells, but strongly modulates microenvironment by leading to significant changes in percentage of Tc and Treg cells infiltrating tumor-bearing hemisphere. Our results suggest that glioma-derived integrin ligands are important factor in polarization of glioma infiltrating microglia/macrophages into the pro-invasive phenotype and its targeting could be a new therapeutic strategy.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.081

A23

Serum cytokine profile in Wistar rat with experimental breast cancer

T. Raiter[a,*], O. Kazakov[a], D. Strunkin[b], A. Poveshcenko[a], A. Lykov[a], T. Miller[a], A. Kabakov[a]. [a] *Scientific Institute of Clinical and Experimental Lymphology, Novosibirsk, Russian Federation,* [b] *Scientific Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation* * Corresponding author.

It's well documented that cytokines in patients with breast cancer are indicators of survival, assessing the risk of recurrence and mortality. However, the obtained data about serum levels of cytokines are contradictory. The problem of participation of cytokines in the growth and spread of tumors requires further study.

The purpose was the determination of serum level of cytokine in experimental model of breast cancer in Wistar rat. Material and methods: Breast cancer was induced by intramammalirian injection of N-methyl-N-nitrosourea. Some of the rats were subjected to only surgery or only polychemotherapy (cyclophosphamide, methotrexate, 5-fluorouracil).Serum levels of cytokines: Bio-Plex Pro Rat Cytokines 24-Plex Assay (Bio-Rad, USA) were used.

Results: It was found that the serum levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-13, IL-17A, IFN -γ, MIP-1α, MIP-3α, RANTES, TNF-α in rats with experimental breast cancer were significantly higher compared with intact rats. We obtained that pro-inflammatory cytokines are associated with progression of breast cancer in rats. The serum levels of IL-1α, IL-2, IL-4, IL-12, IL-18, G-CSF, GM-CSF, GRO/KC, IFN-γ, MIP-1α, RANTES in rats after surgical treatment were significantly higher compared to those in rats without surgical treatment. It was also observed that in rats after surgical treatment,the serum levels of IL-1α, IL-2, IL-4, IL-12, IL-18, G-CSF, GM-CSF, GRO/KC, IFN-γ, MIP-1α, RANTES were decreased. We suppose that these data may indicate that tumor cells can also produce cytokines. Moreover, we obtained that in intact rats, serum levels of IL-1α, IL-18, EPO, G-CSF, GRO/KC, MIP-1α were higher compared to those in rats with breast cancer treated with surgery. However, the serum levels of IL-6 and IL-1β (markers of pro- inflammation and tumor metastasis) were higher in surgically treated rats compared to those in intact rats. It has been proved that the removal of primary tumors (without regional lymph node removal and subsequent polychemotherapy) can lead to accelerated growth of metastases. It was found that the polychemotherapy resulted in a significant decrease in the serum levels of IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17A, IL-18. Polychemotherapy also leads to suppression in serum levels RANTES, VEGF, MCP-1. We found that the serum levels of IL-2, IL-5, IL-7, IL-13, IL-17A, IL-18, G-CSF were significantly higher in rats after combination of surgery and polychemotherapy.

Conclusion: The serum level of cytokines in rats with experimental breast cancer depends on the type of treatment.

http://dx.doi.org/10.1016/j.ejcsup.2015.08.082

T40

Morphologic evaluation of prognostic factors in colon adenocarcinoma

G. Raskin[a,b,*], S. Petrov[c], K. Pozharissky[a], R. Orlova[a,b]. [a] *Russian Research Center for Radiology and Surgical Technologies, St. Petersburg, Russian Federation,* [b] *St. Petersburg State University, St. Petersburg, Russian Federation,* [c] *Kazan State Medical University, Kazan, Russian Federation* * Corresponding author.

Colon adenocarcinoma is one of the most wide spreading malignant tumors in the world, mortality of which remains high. Estimation of cancer on late stages, absence of prognostic and predictive factors, poor understanding of progression mechanism may lead to elevation of mortality.

The aim of investigation was to identify morphologic prognostic factors and their combination for colon adenocarcinoma.

For this purpose 776 patients with colon adenocarcinoma with the median age 57.6 years were selected. Receptors for chemokine (CCR10, CXCR4), stem cell marker (ALDH1), ki-67, MSH2, MSH6, MLH1, PMS2 were investigated by immunohistochemistry (IHC). Results of IHC were compared with clinical data. Analysis of 217 colon adenocarcinomas by Ki-67 showed that 86% of cases had high proliferative level (Ki-67>30%), among them 39% of cases had very high level of Ki-67 (>70%). We also analyzed proliferation of stem cells using double IHC stain for ALDH1 and Ki-67. It was