miR-126, miR-25, miR-205, miR-183, miR-125b) were measured by qRT-PCR and normalized to miR-16.

Concentrations of four miRNAs (miR-19b, miR-21, miR-25, miR-183) were significantly different in lung cancer versus healthy individuals (p<0.05, T-test, two-sided). Two miRNAs were upregulated (miR-19b, miR-21), two were downregulated (miR-25, miR-183) in cancer patients. Four miRNAs (miR-19b, miR-126, miR-25, miR-205) were found to be differentially regulated in SCC patients when compared to healthy controls. In AC patients only two miRNAs (miR-19b, miR-183) were differently expressed. Thus, cancer subtypes have different input into overall picture.

Receiver Operating Characteristic (ROC) curve analysis of differentially expressed miRNAs in total study population and subtype-based groups showed that miR-19b has highest predictive value for total population and SCC patients, while miR-183 was more effective in discriminating patients with AC. All miRNAs except miR-21 showed strong bias towards one of the subtypes. Presumably a combination of miRNAs with opposite bias should provide a more potent diagnostic tool for cancer detection in total population than individual miRNAs or panels of miRNAs that are specific to one cancer subtype. Indeed, stepwise binary logistic regression has identified the combination of miR-19b and miR-183 to be a strong prediction of disease in total population and yielded a solid increase in AUC: 0.990 (miR-19b+miR-183) versus 0.806 (miR-19b) or 0.924 (miR-183). This combination can be used to identify lung cancer with 94.7% sensitivity and 95.2% specificity.

Thus, biological aspects such as tumor genetics and phenotype, stage of disease, response to therapy, and other meaningful tumor properties of such heterogeneous malignancies as LC can interfere with contents and composition of cell-free miRNA pool. Wide profiling of circulating miRNAs will specify biomarkers of LC phenotypes and improve non/less-invasive LC diagnostics.

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A84

Polymorphic markers Arg72Pro and Gln157Lys of TP53 gene in non-small cell lung cancer

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The development of individual medicine is a very important part of the whole health care system and especially of the oncology patients’ treatment. The predictive markers could help to prevent the development of the different diseases. Individual risk assessment is based on the study of polymorphisms in genes specific to different pathologies, especially those with a significant social impact, such as lung cancer.

Like many other cancers, lung cancer is a multi-factorial disease. The tumor suppressor genes are important in its pathogenesis. One such very important gene is TP53, which encodes the p53 tumor suppressor protein. It regulates the activation of specific cellular processes and signaling pathways involved in regulation, recognition of signals inside the cell, coordination of metabolic processes, genome repair, cell division and death (apoptosis), and interactions between cells. Insufficient production or property modification of this protein leads to the development of serious diseases, including lung cancer.

The TP53 gene has a number of polymorphic markers; the Arg72Pro and the Gln157Lys markers are very important in case of lung cancer. They are located in the DNA-binding domain of TP53 at exons that play essential structural and chemical roles in the contact between the p53 protein and specific DNA sequences that constitute the p53 response elements. These mutations result in a significant loss of DNA-binding activity and transactivation capacity (P. Hainaut and M. Hollstein, 2000).

We studied the association of polymorphic markers Arg72Pro and Gln157Lys of TP53 gene with the risk of non-small cell lung cancer (NSCLC) in patients from the Moscow region. Our study included 88 patients with NSCLC, 160 healthy persons as a control for Arg72Pro and 60 healthy persons as a control for Gln157Lys. We used PCR-RLFP analysis to identify the alleles of the polymorphic markers.

We observed higher frequencies of the markers predisposition genotypes in group of patients then in control group. The distribution frequency of Pro/Pro genotype Arg72Pro marker was 0.307 in the group of patients and 0.075 in the control. For the Gln157Lys marker the Lys/Lys genotype was not observed. The frequency of Gln/Lys genotype Gln157Lys marker was 0.377 in patients and 0.106 in the control. We found the association of both markers with the risk of NSCLC development. The genotype Pro/Pro of Arg72Pro marker showed the increasing of NSCLC risk: OR = 5.46, p = 8 × 10^-6. The presence of Gln/Lys genotype of Gln157Lys marker also led to increased risk of cancer development: OR = 5.10, p = 0.002.

Our results suggest the importance of studied polymorphic markers for risk of NSCLC assessment. The status of the Arg72Pro and the Gln157Lys markers of TP53 gene can serve as an independent prognostic indicator in this type of cancer.

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A84a

Role of BRCA1 dysfunction in sporadic triple-negative breast cancer

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Background: There is increasing evidence that BRCA1-related DNA-repair defects determine sensitivity to certain agents, such as platinum-based chemotherapy. There is a lot of evidence about a link between TNBC and BRCA1 deficiency. Many clinical characteristics and molecular features are shared by sporadic triple-negative breast cancer and BRCA1-associated breast cancer. The majority of BRCA1-related breast cancers are of basal-like/triple-negative phenotype. Identification of specific
markers of BRCA1-dysfunction will be essential to translate an understanding of defective DNA repair into targeted treatments for this poor prognosis subtype.

Materials and methods: Twenty-two patients with triple-negative breast cancer (TNBC) were treated with neoadjuvant platinum-based chemotherapy followed by surgery. Pathologic treatment response was assessed in correlation with biomarkers of BRCA1-dysfunction. Pathological response was evaluated according to the Chevallier classification (Ch). All patients were screened for germline mutations in BRCA1 (185delAG, 5382insC, 4153delA, 4158A/G, C61G) and BRCA2 (6174delT). All samples negative for germline mutations in BRCA1 and BRCA2 then submitted to BRCA1-dysfunction screening. Biomarkers of BRCA1-dysfunction included: BRCA1 somatic mutations (C61G, 185delAG, 5382insC), promoter methylation of BRCA1, low BRCA1 mRNA expression, high ID4 mRNA expression, PTEN (T4721C) mutation, p53 (Arg72Pro, Pro72Pro) mutations. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP), methylation-specific PCR were used for BRCA1-dysfunction analysis.

Results: Twelve patients (54.5%) achieved a pathological complete response (pCR) (Ch1+ Ch2). Ten patients (45.5%) had a residual disease (Ch3+ Ch4). BRCA1 mRNA expression was absent in 16/22 (72.7%) patients, low in 5/22 (22.7%). 11/21 (52.4%) patients with other than normal BRCA1 mRNA expression achieved a pCR. BRCA1 promoter methylation was detected in 9/22 (40.9%). 6/9 patients with BRCA1 promoter methylation achieved a pCR. RAD51 mRNA levels were low in 14/22 (63.6%), high in 1/22 (4.5%). 8/14 (57.1%) patients with other than normal RAD51 mRNA expression achieved a pCR.

High ID4 mRNA levels were determined in 5/22 (22.7%). 3/5 (60%) patients with high ID4 mRNA expression achieved a pCR. p53 (Arg72Pro, Pro72Pro) mutations were identified in 12/22 (54.5%). No patient had PTEN (T4721C) mutation. No statistically significant correlation was found between the BRCA1 mRNA expression, BRCA1 promoter methylation, RAD51 mRNA expression, high ID4 mRNA levels, p53 (Arg72Pro, Pro72Pro) mutations and pCR to neoadjuvant platinum-based chemotherapy (p > 0.05). Eleven patients had BRCA1 somatic mutations: 6/22 (27.3%) – BRCA1 5382insC, 4/22 (18.2%) – BRCA1 185delAG, 3/22 (13.6%) – BRCA1 C61G. 3/4 patients with BRCA1 185delAG mutation had pCR. RAD51 mRNA levels were low in 14/22 (63.6%), high in 1/22 (4.5%). 8/14 (57.1%) patients with other than normal RAD51 mRNA expression achieved a pCR.

Conclusion: BRCA1 5382insC somatic mutation is associated with good response to neoadjuvant platinum-based chemotherapy (p = 0.01). BRCA1 185delAG and BRCA1 C61G mutations in the BRCA1 RING domain may predict resistance to neoadjuvant platinum-based chemotherapy (0.04 and 0.1, accordingly).

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P148

CD68 expression in inflammatory cell infiltration of nonspecific invasive breast cancer

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Background: Tumor-associated macrophages play a main role in tumor progression and dissemination. Taking into account the high heterogeneity of tumor the different clinical impact of macrophages, infiltrating different sites of tumor, could be expected. The aim was to detect the level of CD68+ cells (macrophages) in the different site of stroma in breast tumor in comparison to clinical course.

Materials and methods: One thirty-six women with nonspecific invasive breast cancer T1-4N0-3M0, who were treated in General Oncology Department of Tomsk Cancer Research Institute (Tomsk, Russia), were included in the present study. Patients did not receive preoperative treatment. The material was fixed in 10–12% neutral formalin. Preparation of the histological material was carried out according to standard procedures. Morphological examination of the surgical specimens was performed by the standard method using a light microscope "Carl Zeiss Axio Lab. A1" (Germany) and slidescanner "MiraxMidIZeiss" (Germany). Metastatic lesion was detected in regional lymph nodes. Immunohistochemical study was performed according to standard procedures. Cytoplastic expression of these markers was determined in the inflammatory cell infiltrate of different tumor segments: (1) in areas with soft fibrous stroma; (2) in areas with coarse fibrous stroma; (3) in the areas of the so-called “maximum stromal-and-parenchymal relationship” where the individual tumor cells, short strands and groups of tumor cells arranged in soft fibrous stroma; (4) among parenchymal elements; (5) in gaps of ductal tumor structures. Double-stained immunofluorescence was performed according to standard procedures using Leica TCS SP2 laser-scanning spectral confocal microscope (Germany). The following primary antibodies were used: mouse monoclonal anti-human CD68 (BD Biosciences) and rabbit polyclonal anti-stabilin-1 or RS1 (marker of M2 macrophages).

Methods: The highest expression of CD68 in the inflammatory cell infiltrate was detected more frequently in areas with soft fibrous stroma (54%) or the so-called “maximum stromal-and-parenchymal relationship” (79%) in patients with breast cancer. The lowest expression of CD68 was observed in areas with coarse fiber stroma (23%). The CD68-positive cells of the inflammatory infiltrate were located between parenchymal elements of tumor (88%). Inverse correlation (R = –0.67; p = 0.02) observed between tumor size and the expression of CD68 in the cells of the inflammatory infiltrate in gaps of tubular tumor structures. The CD68 expression in cells of the inflammatory tumor infiltrate was correlated with the presence of metastatic regional lymph nodes. It was found that in the case of the lymph node metastases the average score of CD68 expression in cells of ductal gaps tumor structures was lower (1.4 ± 0.5) in comparison with the negative lymph nodes case (3.1 ± 1.0; F = 10.9; p = 0.007). Same time no correlation between the CD68 expression in the inflammatory cell tumor infiltrate and the rate of tumor malignancy was found.

CD68 expression in inflammatory cell infiltration of nonspecific invasive breast cancer

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