Matrix metalloproteinases (MMPs) – zinc containing endopeptidases which cleave the different components of the extracellular matrix. However, not all biological mechanisms and the effects of these endopeptidases are well-established. Previously it was believed that MMP functions only as proteolytic enzymes, but it was found later that MMPs could act as regulators of protein kinase signal transduction pathways by modifying receptors and signaling molecules.

One of the most important substrates for MMP is transforming growth factor-β (TGF-β), which is a receptor for the ligand TGF-β. Signal transduction pathway comprises a network of protein kinase pathways responsible for many of the processes of tumor progression, including epithelial mesenchymal transformation (EMT). Despite the fact that melanoma cells are not of epithelial origin, they express the epithelial E-cadherin that is necessary for their interaction with the basal layers of the epidermis. The degradation of the extracellular matrix and loss of expression of E-cadherin are major changes needed to start the processes of melanoma invasion. One of the markers of EMT is a transcription factor twist1. Twist1 activates the processes of epithelial-mesenchymal transformation and increases the invasion of melanoma cells by increasing transcription of MMP. The aim of this study was to evaluate expression levels TGF-β, twist1 under selective inhibition of MMP-9 and combined inhibition of MMP-9 and-13 in melanoma model in vivo; to determine the role of MMPs as possible regulators of TGF-β-way signal transduction, as well as associated with TGF-β EMT process.

The study was approved by the local Ethic Committee (protocol No. 144/2012 of 15.11.2012). Melanoma B16-bearing mice (control group consisted of 7 animals, MMP-9 inhibitor treatment group – 7, MMP-9/13 inhibitor treatment group – 7 animals) underwent experimental treatment by MMP inhibitor (Calbiochem, USA) by daily application within 7 days. Control group consisted of animals without treatment. Efficacy of MMP-9 inhibition was evaluated by gelatinize zymography. TGF-β1 and twist 1 transcription factor expression was determined by PCR real-time using StepOne System (Applied Biosystems, USA). Statistical analysis was done by Kruskal–Wallis test and Multiple Comparisons. The P values lower 0.05 were considered as significant.

It was found that the selective inhibition of MMP-9 did not induce changes in expression levels of TGF-β and twist1, however, the combined inhibition of MMP-9 and MMP-13 significantly reduced the expression levels of the transcription factor twist1 and TGF-β. The results show that MMP-9 and MMP-13 act as activators of TGF-β-signal transduction pathway and could be considered as potential molecular targets for experimental therapy for cancer.
KRAS is component of Ras/MAPK signaling cascade that regulates cell proliferation and cell survival. Somatic mutations in KRAS gene are often found in tumors and affect the sensitivity of tumors to target therapy. Mutations in codons 12 or 13 of KRAS gene in colorectal cancer (CRC) are associated with resistance to anti-EGFR antibodies Cetuximab and Panitumumab. The objectives of this work were to develop PCR tests for detection mutations in KRAS gene and analyze the frequency of mutations in KRAS gene in CRC in Russia.

DNA sequencing by Sanger is the most common method for mutation analysis. However, the method has a sensitivity of 20% mutant allele, which is often not sufficient for the analysis of somatic mutations in tumors.

One of the most sensitive mutation analysis methods is allelem specific real-time PCR. This method allows to detect 1% of mutated DNA in the sample. This sensitivity is sufficient for analysis of mutations in tumor samples containing 2–5% or more of tumor cells in normal tissue.

In this study we developed and compared 3 new KRAS assays (1) real-time PCR with allele-specific primers; (2) real-time wild-type blocking PCR with LNA (locked nucleic acid) blocker; and (3) Sanger sequencing with LNA-blocker. First assay is a PCR test with seven reactions using allele-specific primers for detection and genotyping 7 mutations in 12 and 13 codons of KRAS gene. Second assay is real-time PCR with only a single pair of primers and LNA oligonucleotide blocker. LNA-blocker is an oligonucleotide which has a wild-type sequence of codons 12 and 13 of KRAS gene. LNA-blocker binds strongly to wild-type KRAS DNA and suppresses its amplification, but does not block amplification of mutant DNA. The real-time PCR with LNA-blocker can detect mutant DNA but does not genotype mutation. Such assay can be used as a simple and sensitive screening test for mutant KRAS cases if exact genotyping of mutation is not required. We also used LNA-blocker to increase sensitivity of Sanger sequencing. To evaluate sensitivity and specificity of new tests DNA standards were prepared with different ratios of normal and mutant alleles using normal human DNA without mutation and recombinant plasmids with mutations in KRAS (G12C, G12S, G12R, G12V, G12D, G12A, G13D). After optimization all three assays had sensitivity 5% of mutant alleles for the detection of mutations in KRAS gene, using 2.5–40 ng of human DNA.

Performance of new assays for KRAS mutations was compared using 81 colorectal tumor samples. Before analysis relative content of tumor cells in the samples was evaluated by pathologist. If tumor content was less than 20% in the sample then regions with a maximum number of tumor cells were manually macrodissected before the DNA extraction. DNA was purified from formalin fixed paraffin embedded (FFPE) tissue using “FFPE-DNA Kit” (Biolink). All three assays had high sensitivity (95–100%) and specificity (100%) for detection of KRAS mutations in clinical tumor samples. Mutations of the KRAS gene were found in 37 of 81 cases (46%) of CRC including 12 cases of mutation G13D, 11 cases of G12D, 5 cases of mutation G12V, 4 cases G12C, 3 cases of mutation G12A, 2 cases G12S, 1 case G13R. A single case with mutation G13R was missed by allele-specific PCR but was detected by real-time PCR with LNA-blocker and confirmed by sequencing.

New assays have high sensitivity and specificity and are suitable for detection of KRAS mutations in clinical FFPE tumor samples.

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**Dynamic changes of circulating microRNA expression in response to the lung cancer combined therapy**

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Background: The analysis of circulating tumor nucleic acids (DNAs and RNAs) in the blood seems to be a promising approach for the development of the low-invasive methods of tumor detection, valuable for clinical practice. Detection of oncogenic and tumor suppressor miRNAs in the blood plasma/serum is evidence of their participation in pathogenesis and suggest the possibility of their use as tumor markers and targets for therapy. Thus, the determination of expression level of tumor-associated miRNAs in plasma can lead to significant progress in understanding the tumor development and treatment.

**Aim:** Estimation the changes in expression level of miRNAs (miR-19b, miR-25, miR-125b, miR-126, miR-205) in blood plasma from lung cancer patients during combined therapy and to estimate their value as disease monitoring markers.

**Materials and methods:** Blood samples were taken from patients (n = 23) with non-small cell lung cancer treated at the Tomsk Cancer Research Institute. These samples were stabilized and fractionated into plasma and blood cells. MicroRNA was isolated from blood plasma using single-phase phenol-free extraction protocol and purified on silica-based spin columns (BioSilica Ltd, Novosibirsk, Russia). Concentration of five above mentioned miRNAs was measured by quantitative RT-PCR and normalized to miR-16 using dCt method.

**Results:** In this study we analyzed the dynamic expression changes of circulating DNA in blood plasma from lung cancer patients during the combined therapy. Circulating miRNAs were isolated from plasma samples of non-small cell lung cancer patients before treatment, within 30 days after completing chemotherapy and 15 days after surgery, by using developed methodological approach. In case of miR-19b and miR-125b analysis was found that the miRNA expression level correlates with clinical response to chemotherapy and surgery. Increasing level of miR-19b and decreasing level of miR-125b were associated with therapeutic response. Using Repeated measures ANOVA analysis we demonstrated that the miR-19b and miR-125b expression levels changes throughout three check-up points during the