metastatic PC3 and DU145 cells). Co-culture fibroblasts with LNCaP cells resulted in significant down-regulation of expression of glypican-1 and up-regulation of CSPG4/NG2 expression in the fibroblasts, changing overall proteoglycans expression pattern in the cells. Interestingly, CSPG4/NG2 expression is more characteristic for nerve cells and cancer stem cells, and up-regulation of the gene in the cancer cell-exposed fibroblasts may argue for their dedifferentiation. Co-culture fibroblasts with more aggressive DU145 and PC3 resulted in similar results with an additional down-regulation of versican expression. This data show, that prostate cancer cells affect normal fibroblasts by the other way than normal prostate cells and stimulate them to express another set of proteoglycans, which usually are expressed in poor-differentiated or morphologically different cells. Data obtained by immunocytochemical analysis confirmed RT-PCR data and showed specific activation of some proteoglycans at the region of contact between fibroblasts and normal but not cancer prostate cells in co-culture. Taken together, obtained results show that normal prostate epithelial cells affect surrounding fibroblasts and modify transcriptional activities of proteoglycan-coding genes in the cells, while cancer cells change the proteoglycans expression in cancer cells-exposed fibroblasts seems contributing to reprogramming of normal fibroblasts to CAFs. Understanding the mechanisms of this reprogramming may help to develop a new approach for cancer therapy based on the return reprogramming of CAFs to normal fibroblasts and correction of tumour microenvironment.

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A99

The influence of live *Streptococcus pyogenes* on the growth of solid murine tumors

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Ability of *Streptococcus pyogenes* to fight cancer was discovered and clinically tested more than 100 years ago (Coley, 1897), but the mechanisms of these effects are unknown. Recently several murine models demonstrating the anti-cancer activity of Group A Streptococcus (GAS) M49 were established (Maletzki, 2008). The present study is devoted to investigation of anti-cancer activity of GAS M39 strains.

Materials and Methods: Tumor cells were injected subcutaneously in concentration of 200,000 cells per animal. Two solid tumor models were used: hepatoma 22a in C3HA mice and sarcoma 37 in Swiss mice. GAS (wild and mutant strains) were injected into the tumor 2×104 per animal twice with a 5-day-interval. The influence of GAS on tumor growth was evaluated by measurement of tumor diameter and animal survival. Inactivation of M protein (emm) gene was generated by insertional mutagenesis after cloning the fragment of the emm gene in suicidal plasmid pT7ermB.The plasmid was introduced into GAS by electroporation. Insertion had been proved by DNA sequencing.

Results: Intratumoral injections of wild GAS M39 strain did not influence on tumor size in mice bearing hepatoma 22a and sarcoma 37. The second injection of GAS enhanced inhibition of the tumor growth, but increased the death rates. The treatment by emm mutant strain caused the inhibition of tumor growth and showed less mortality rate both tumor models. The average number of completely cured animals was 10%.

Conclusion: Inactivation of emm gene in GAS M39 strain resulted in increased anti-tumor activity and decreased lethality rate. We hypothesized that the absence of anti-phagocytic M protein may increase phagocytosis of GAS by macrophages and thus enhance their antitumor activity.

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P39

Blood cell-surface-bound exosomes as a new source of tumor-specific microRNA

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Background: Microvesicles release by neoplastic cells were found in blood and carrying a large number of tumor distinctive molecules, like miRNA/mRNA, proteins. Considering extensive surface of blood cells we wonder if exosomesare circulated exclusively in plasma or could be associated with surface of blood cells.

Materials and Methods: Microvesicles circulating in blood of healthy women (HW) and breast cancer patients (BCP) were studied. Blood was fractionated into plasma and cellular fractions; in order to release cell surface bound material, blood cells were treated as it was described for cell-surface-bound (CSB) cirDNA (Tamkovich, 2005).Exosomes were pelleted at 100,000 g from 0.1 μm filtered supernatants obtained by plasma and CSB eluates centrifugation at 17,000 g, resuspended in PBS and stored in aliquots at −80 °C. Size distribution of the microparticles was characterized by TEM (Jeol, Japan), anti CD-63, CD-24, CD-9 antibodies (BD Biosciences, USA) were used as exosome markers, protein concentration was measured by NanoOrange Protein Quantitation kit (Molecular Probes, USA), total RNA and miRNA were isolated by mirVana kit, 28S rRNA were quantified by RT-qPCR after reverse transcription with 6-mer random primers, miRNA using TaqMan Small RNA Assays kit (Applied Biosystems, USA). DNA was isolated using "DNA Isolation Kit" (BioSilicaLtd, Russia) and measured by TaqMan multiplex real-time PCR for

LINE1 and α-satellite repeats (Bryzgunova, 2011). Results: TEM with immunogold labeling demonstrate presence of exosomes in 30–100 nm membrane-wrapped particles isolated from both plasma and CSB eluates. TEM, NanoOrange and 28S rRNA RT-qPCR data demonstrate that CSB exosomes constitutes 2/3 of total blood exosomes. Exosomes ranged 50–70 nm prevail in blood of BCP, whereas 30–50 nm exosomes in blood of HW. Exosomal DNA is less than 0.3% of cell-free blood DNA. RNA integrity and specific quantity checked by Bioanalyzer (Agilent Technologies, USA) do not differ in cell-free and CSB exosomes. Preliminary data demonstrate overpresentation of cancer-specific miRNA (miR-103, miR-191, miR-195) in exosomes bound with erythrocyte's as compared with exosomes bound with leukocytes or circulating in plasma.

Conclusion: Exosomal DNA obviously do not have any diagnostic value in contrast to RNA. CSB exosomes represent valuable source of material for small-invasive cancer diagnostics.

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P39a

The characterization of total circulating DNA from blood of healthy donors and cancer patients

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Background: It was shown that circulating DNA could be used as a valuable source of material for cancer diagnostics (Fleischhacker, 2007), but unknown and unpredictable factors frequently dramatically decrease concentration of tumor-specific genetic biomarkers by influencing on generation and circulation in bloodstream of extracellular DNA. Sequencing of total circulating DNA could either provide valuable information regarding such factors or determine novel targets (DNA markers) for cancer diagnostics.

Materials and Methods: We used a Sanger's technology (ABI PRIZM 3110 sequenator) and BLAST analysis of circulating DNA from plasma and cell-surface-bound fraction of healthy individuals and patients with breast cancer. DNA was isolated by guanidine thiocyanate/glass milk method (Tamkovich, 2004) followed by Sau3A and EcoRI hydrolysis. Fragmented DNA was ligated into the pBlueScript II KS(-) vector. Competent XL-Blue (*E. coli*) cells were transformed with the vector by electroporation and after recovery the cells were plated on LB+Amp agar plates. The inserts length estimate by PCR-analysis.

Results: The majority of investigated circulating DNA fragments were about 100–800 bp. Sequence analysis revealed that number of circulating DNA fragments does not depend from the size of paternal chromosome both in normal and pathological state, but fragments from chromosome six were found in the female bloodstream more frequently. It was found, that in cancer patients blood were observed frequency of occurrence of pseudo genes and CpG islands below then in healthy female blood. BLAST analysis demonstrates that 10% of coding DNA fragments circulating in blood of the breast cancer patients is associated with development of this pathology.

Conclusion: Sequencing of circulating DNA demonstrates a variable concentration of different DNA sequences in comparison with genomic DNA. Further characterization of circulating DNA may be beneficial in diagnosis and prognosis and may also contribute to determining the source and function of circulating DNA.

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P30

Genetic aspects of prostate cancer development

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Prostate cancer is presented as the most commonly diagnosed cancer in men. According to statistical reports, every 6th man have the prostate cancer and out of them every 34th man will have the castrate-resistant form of disease. The existing clinical and prognostic factors can explain cancer outcome in small part of patients. The prediction of individual prostate cancer prognosis and anticancer therapy effect are the main goals of the advanced personalized therapy. The modern genetic technologies development (GWAS, genome sequencing) will allow to find the effective prognostic markers of prostate cancer.

During the GWAS performance it was found loci associated with the prostate cancer development. The analysis of on-line base "A Catalog of Published Genome-Wide Association Studies" (www.genome.gov), containing the full information of genome associative investigations as well as 30 studies of prostate cancer, revealed the associations of 159 SNPs in different ethnic groups of patients (Caucasian, Asian and Negroid). 86 SNPs among them located in 77 genes, 65 SNPs – had the intergenic localization. The GWAS performance in prostate cancer Caucasian patients have found the associations of 76 SNPs in 72 genes and 65 SNPs in intergenic regions. SNPs in genes were located in 81.4% of cases in introns, in 5.3% – in other locations as well as missense changes (near 5′-UTR, ncRNA). It was obtained 30 markers related to disease of different ethnic groups of patients in GWAS. 17 SNPs among them (rs10187424, rs6545977, rs7584330, rs10936632, rs651164, rs12155172, rs1512268, rs4242384, rs3123078, rs11199874, rs7127900, rs7130881, rs10875943, rs4775302, rs5759167, rs1327301, rs5919432) had the intergenic localization, 12 SNPs (rs1465618, rs12621278, rs17181170, rs6763931, rs17021918, rs12500426, rs7679673, rs2121875, rs2242652, rs6465657, rs7501939, rs1859962) were located in introns and 1 SNP (rs130067) – in exon.