Vascular endothelial growth factor (VEGF) modulates functional activity of murine peritoneal macrophages in vitro

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Recruitment of mononuclear phagocytes from the blood into tissues is considered to be a crucial process during inflammatory reactions, wound healing and tumor growth. Macrophages are known to reveal high plasticity and may change under the influence of microenvironment. The aim of the study was to evaluate the changes of macrophage functional activity under the influence of Vascular endothelial growth factor (VEGF) in vitro. This factor is known to be the main angiogenic factor but also possesses several immunomodulatory properties. Here we report that VEGF revealed a dose-dependent effects on cultured freshly isolated murine resident peritoneal macrophages: modulated iNOS mRNA expression, nitrooxide and superoxide anion production, decreased 5'-nucleotidase (5'-N) activity, but had no influence on fluid-phase pinocytosis. Moreover, VEGF increased expression of its own mRNA via autocrine pathway as well as of VEGF protein expression. VEGF also induced up-regulation of extracellular matrix protein thrombospondin-1 (TSP-1) mRNA, which is considered as a part of macrophage activation phenotype. Production of cytokines and chemokines by macrophages was screened with the help of Multi-analyte ELISArray kits. It was found that incubation of macrophages in the presence of VEGF increased the production of angiogenic cytokines – TNF-α and IL-6 as well as several monocyte and leukocyte chemoattractants such as MCP-1, RANTES and MIP-1β. Therefore, we suggest that locally established VEGF gradient may influence inflammatory phenotype of tissue macrophages as well as potentiate their pro-angiogenic properties. This work was supported by Russian Foundation for Basic Research – Russia, Grant No. 15-04-06150.

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Epigenetic changes in human cervical carcinomas associated with viral induced pathogenesis


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Epigenetics investigates mechanisms that control inheritance of gene expression program during somatic cell divisions. These mechanisms include regulation by DNA methylation, histone post-translational modifications and nucleosome positioning, functioning of regulatory non-coding RNAs, control of alternative splicing of mRNA precursors and high-order chromatin organization. Genome-wide loss of epigenetic stability and increased epigenetic plasticity are common features of all tumor types. In normal tissues epigenetic plasticity allow cells to response on environment signals. Thus, in tumor cells its constitutive activation leads to epigenetic heterogeneity that are the additional hallmark of the most of the classical cancers. Cervical cancers are one of the most interesting models for the analysis of the role of epigenetic changes in tumor progression. These types of tumors are associated with infection of human papilloma viruses of so-called high-risk group (HR-HPV) and characterized by well-defined stages of malignant conversion from intraepithelial neoplasias to carcinomas. The viral DNA can persist in episomal form or integrates into the host-cell genome.

Cellular genomes encode genetic information in their linear sequence, but appropriate gene expression requires chromosomes to fold into dynamic complex three-dimensional structures. Scaffold/matrix attachment regions (S/MARs) are specialized genomic DNA sequences that take part in organization of these structures. We demonstrated that methylation of S/MARs was required for their attachment to nuclear matrix and that methylation status of S/MARs was changed in cervical cancer cell compared to normal cells.

DNA methylation plays an important role in the regulation of gene expression. We found that methylation of the regulatory sequences in the HPV16 genome specifically changes in transformed compared to the normal cervical epithelial cells. Next, we showed that methylation of the transcription factor binding sites modulates the viral oncogene expression. These data suggest that the HPV16 genome methylation may represent an important mechanism that initiates the development of HPV-associated tumors.

Using next generation sequencing, we identified pattern of differentially expressed microRNAs in clinical samples of the cervical lesions. We confirmed expression of microRNAs that have been described previously as well as identified new microRNAs that can be potentially involved in the development and progression of cervical cancer. Spectrum of differentially expressed microRNAs includes microRNAs targeting tumor-suppressor genes as well as oncogenes.

Telomerase is a key regulator of cell proliferation. This enzyme is silent in normal cells and activated in most of the tumors. Few forms of RNA (hTERT), encoded by telomerase gene were detected in different tumor cells and among them three forms (alfa, beta and gamma) are most well pronounced. We found that in cervical tumors expression of all three forms are significantly increased. In some cases, we also observe higher level of hTERT expression in neighboring “normal tissue”. The correlation between expression levels of these three forms varied on different stages of the disease (three stages on intraepithelial neoplasias and carcinomas). The function of these three hTERT forms is still not well understood.

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Antitumor activity of allogeneic bone marrow cells immobilized in porous-permeable TiN-based alloy scaffold

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The most effective method in treatment of inoperable cancer is currently miniallogeneic transplantation. This type of treatment focuses on reaction of “graft-versus-tumor”. According to preliminary data, such treatment leads to long-term remission in patients with metastatic cancer, who did not respond to previous therapy. Anti-tumor immune response after transplant may be extended or enhanced by additional infusion of donor lymphocytes. Research has shown that introduction of donor lymphocytes achieves complete remission of the tumor, even in case of relapse after allogeneic transplantation from the same donor, but these effects are unstable and not so long because of rapid elimination of donor lymphocytes [1]. The main problem is the preservation of cell transplantation viability and functional activity of the cells in patient’s body, and protecting them from recipient’s immune system. In recent years, most suitable for this purpose are the three-dimensional porous biomaterials (3D-scaffold).

Specific pore space does not allow immunity effectors quickly destroy the cells in scaffold [2,3]. Research Institute of medical materials and implants with shape memory at the Tomsk State University created a porous-permeable incubators (scaffold) of TiNi-based shape memory alloy (TiNi). The material has unique properties: permeable porous structure by open interconnected pores, characterized by high degree of wettability with tissue media and nanoporous inner surface of the pore walls, exhibits high adhesion to various cell types, so all mentioned meet requirements of the bio-chemical and biomechanical compatibility. Porous TiNi-based scaffolds allow continually to keep the functionality cells and prolong their action [4,5].

The purpose of this study was to investigate possibility of modulation of antitumor response in allogeneic bone marrow transplantation in porous-permeable incubator of TiNi. Results: Intraportalional injection of allogeneic bone marrow (BM) reduces metastasis by 30% and has easy 10%-antitumor effect. At the same time, the implantation of bone marrow cells on incubator of TiNi (BM + TiNi) leads to more pronounced antitumor (25%) and significant antimetastatic effects (45%). Life span of animals with tumors and implanted bone marrow cells on incubator TiNi is increased by 60%

Since bone marrow cells do not have direct antiproliferative effect on tumor target cells in vitro, it is assumed that one of the mechanisms effecting the bone marrow transplantation on neoplastic process is the stimulation of endogenous effectors of antitumor immunity.

The study of morphological parameters immunocompetent organs showed that administration of allogeneic bone marrow cells can reduce thymic regression, decrease splenomegaly at animals with transplanted tumors.

Conclusion: Allogeneic transplantation of bone marrow cells on porous incubator was shown to prolong and enhance antitumor and antimetastatic action compared to injecting the cells. This effect is directly due to the stimulation of antitumor immunity, that is described by the study of immunity factors. The results show perspectives of porous TiNi-based scaffolds in complex treatment of neoplastic diseases.

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Correlation between expression of antigens on tumor cells and recurrent genetic abnormalities in chronic lymphocytic leukemia

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Background: Tumor cells in chronic lymphocytic leukemia (CLL) usually have the “classic” immunophenotype CD19+, CD5+, CD23+, SmIgdim, CD20dim, FMC7-, CD79b-/dim, CD22-/dim. However, sometimes some deviations in antigenic profile CLL cells are observed such as enhanced expression of CD79b, CD22, SmIg and CD20. The most frequent cytogenetic abnormalities in CLL are trisomy 12 and deletions of 13q14.3, 13q34, 6q, 11q, and 17p. It is noted that immunophenotypic deviations in CLL often correlate with some of these cytogenetic anomalies. The investigation of the links between the antigens expression and caryotype of CLL cells leads to differential diagnostics and prognosis in CLL. The goal of the investigation is search for the links between immunophenotypic characteristics of CLL cells and their karyotype.

Material and methods: Bone marrow and blood cells from 35 CLL patients were investigated. Immunophenotyping was made using flow cytometer “Cytomics F500” (Beckman Coulter) with the use of monoclonal antibodies CD3/CD19/CD45, SmIgk/SmIgL/CD19, CD5/CD23/CD19, FMC7/CD38/CD19, CD22/CD79b/CD19, CD10/CD20/CD19. For karyotype determination the standard cytogenetic methods and FISH were used.

Results: The most frequent deletion in CLL patients was del(13)(q14.3), it was sometimes observed together with (13)(q34) and was found in 18 patients (51% of all examined patients). 56% (10/18) of patients in this group had CLL cells phenotype CD19+, CD5+, CD23+, SmIgdim, CD20dim, FMC7-, CD79b-, CD22- and CD38-. Weak (dim) expression of CD79b antigen (in 24–92% of cells) was found in 39% (7/18) of patients with del(13)(q14.3), and expression of CD38 (in 22–86% of cells) – in 17% (3/18).

The same phenotype was observed in 2 patients (6%) having only del(13)(q34), in one patient (3%) with (13)(q34) trisomy and in 6 patients (17%) with normal karyotype 46, XY.

Trisomy 12 was found in 14% (5/35) of explored CLL patients. The immunophenotype of CLL cells in this group was very different with the previous group. All patients (100%) with trisomy 12 had CLL cells with expression of CD79b (94.2% of cells), CD22 (54–94.6% of cells), CD38 (31.1–99.3% of cells). Additionally 2 of 5 patients (40%) had CLL cells with more bright (dim to mod) CD20 expression and 1 patient (20%) had cells with bright...