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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, nitroxide 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 TiNi-based alloy scaffold