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THE INFLUENCE OF CISPLATIN ON TRANSCRIPTIONAL PROFILE OF TUMOR-ASSOCIATED MACROPHAGES OF BREAST CANCER AND COLON CANCER

I.V. Larionova[1,2], L. Tengfei[3], A.M. Kiselev[4], E.V. Denisov[1,2], N.V. Cherdyntseva[1,2], J.G. Kzhyshkowska[1,3,5]

Laboratory of translational cellular and molecular biomedicine, Tomsk State University, Tomsk, Russia[1]
Cancer Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia[2]
University of Heidelberg, Medical Faculty Mannheim, Institute of Transfusion Medicine and Immunology, Mannheim, Germany[3]
Institute of Molecular Biology and Genetics, Almazov National Medical Research Centre, St. Petersburg, Russia[4]
German Red Cross Blood Service Baden-Württemberg–Hessen, Mannheim, Germany[5]

In the present study we demonstrated the whole transcriptome analysis of model tumor-associated macrophages (TAM) of breast and colon cancer with using next-generation sequencing data. We identified the most significantly increased and down-regulated genes as well as differentially expressed genes for breast and cancer TAM. Our results showed that TAM can be reprogrammed by chemotherapeutic agents and obtain mostly pro-inflammatory program.

Keywords: Tumor-associated macrophages, chemotherapy, cisplatin, cancer, next-generation sequencing.

Introduction

The application of chemotherapeutic agents is the most effective approach to the treatment of major oncological diseases [1]. We showed that an increased number of tumor-associated macrophages (TAM) correlated with a poor response to neoadjuvant therapy in breast cancer patients [2]. Identification of the phenotypic and functional characteristics of TAM during therapy is necessary to improve the effectiveness of chemotherapy, identify new mechanisms for poor response to NAC and to develope personalized approaches to the treatment of breast cancer and colon cancers [3, 4]. In the present study we demonstrated the analysis of whole transcriptome sequencing of samples of model breast and colon cancer TAM which indicated the most activated pathways under cisplatin treatment.

Material and Methods

Human primary monocytes-derived macrophages were isolated with using CD14+positive selection and stimulated ex vivo by IL4 and TGFb and supernatants of breast adenocarcinoma cell line MCF-7 and colorectal carcinoma cell line Colo206F to model cancer-specific TAM. Cisplatin treatment was performed on day 6 of macrophage differentiation. The whole transcriptome sequencing was performed. The libraries were prepared with the NEXT flex Rapid Directional qRNA-Seq Kit. Ribosomal RNA was removed from the NEBNext® rRNA Depletion Kit (Human/Mouse/Rat). Prepared libraries were then pooled and sequenced with using Illumina NextSeq500 instrument. The Hallmark, Reactome, KEGG databases were used for the experiment.

Results

The genes were selected according to their functional component and the highest value of differential expression (log2FoldChange values was at least 2, p-value <0.000001). At the transcriptional level, it was significantly increased the expression of genes involved in the inflammatory response,

interferon-dependent pathways, p53-dependent apoptosis, genes responsible for DNA damage, and genes that participate in the response to cisplatin and tamoxifen and in transplant rejection. The expression of genes involved in lipid and fatty acid metabolism, cholesterol homeostasis, glycolysis, oxidative phosphorylation, MTORC1 signaling, NOTCH signaling decreased, that indicates that the metabolism of macrophages changes in the direction of inhibiting certain metabolisms under the influence of the chemotherapeutic agent.

This approach helped to identify differences in the programming effect of chemotherapeutic agents on model TAM of breast adenocarcinoma and colon cancer. The most pronounced differences were cisplatin stimulation of the proto-oncogen KRAS signaling pathway, activation of the IRF7 interferon regulatory factor, DNA repair, and cisplatin suppression of angiogenesis, glycolysis, glucose metabolism, hypoxia, insulin metabolism, platelet activation, epithelial-mesenchymal transition in tumor-associated macrophages of intestinal adenocarcinoma. In the TAM of breast carcinoma it was mostly activated the pathways associated with the G protein-coupled receptor (signal transmission in the cell), the epithelial-mesenchymal transition, the activation of the proto-oncogenic protein MYC, the metabolism of RNA, including mRNA, regulation of translation, regulation of the mitochondrial transport.

Conclusions

The global influence of chemotherapeutic agents on the transcriptional profile of model tumor-associated macrophages was first established. We demonstrated that tumor-associated macrophages can be reprogrammed by chemotherapeutic agents. Macrophage response to cisplatin had a similarity to the antiviral response and can trigger an inflammatory antitumor targeting program.

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MELANOMA CELL GROWTH AND MIGRATION ALTERATIONS UNDER MIR-204-5P INHIBITOR APPLICATION

S.N. Lavrentev, M.B. Aksenenko, N.V. Palkina, A.V. Komina, T.G. Ruksha

Federal State Budget Educational Institution of Higher Education «Krasnoyarsk State Medical University named after Professor V.F. Voyno-Yasenetsky» of the Ministry of Health of the Russian Federation, Krasnoyarsk, Russia

MicroRNAs are regulatory molecules which play a role in melanoma biology as well as in other malignancies. MicroRNAs can affect multiple protein expression as posttranscriptional modulators. Therefore these molecules are considered as plausible therapeutic targets in cancer. The aim of the present study was to determine the effects of microRNA miR-204-5p specific inhibitor on melanoma cell growth and migration capacities.

C57Bl6 mice with transplanted melanoma B16 cells were kept in conditions of natural light without any restrictions on access to water and food. Inhibition of this microRNA we realized by inserting into the neck crease inhibitor LNA 25 mg/kg twice on the 7th and 14th day after the transplantation. After that we produced the observation of animals with measurement of the sizes of tumor node every day. We finished the experiment on the 19th day after the transplantation.

miR-204-5p specific inhibitor application resulted in the increase of melanoma cell proliferation that confirms miR-204-5p action in melanoma as oncosupressor. Besides, melanoma cells showed decreased migration rates under miR-204-5p inhibition.

miR-204-5p may be implicated in melanoma pathogenesis and act as oncosupressor.

Keywords: melanoma, LNA inhibitor, microRNA, miR-204-5p.