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# Chemotherapeutic agent cisplatin suppresses stabilin-1-mediated clearance of EGF by tumor-associated macrophage

*Irina Larionova, Elena Kazakova, Artem Kiselev, Marina Patysheva, Julia Kzhyshkowska*

Laboratory of translational cellular and molecular biomedicine, Tomsk State University, Tomsk, Russia

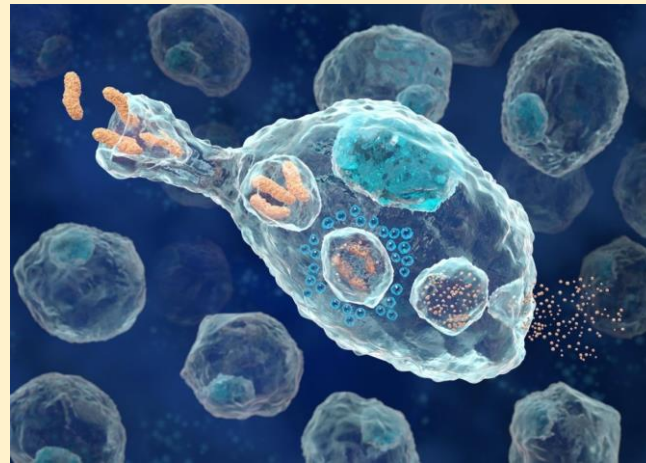
Cancer Research Institute, Tomsk National Research Medical Center, Tomsk, Russia

University of Heidelberg, Medical Faculty Mannheim, Institute of Transfusion Medicine and Immunology, Mannheim, Germany

Almazov National Medical Research Centre, Saint Petersburg, Russia.

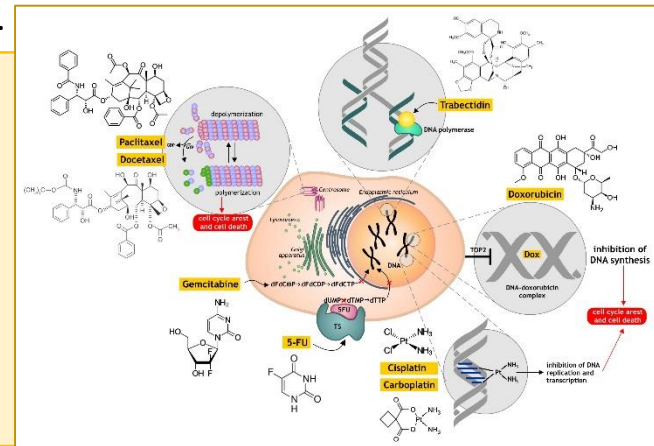
## Motivation

Tumor is a complex system of transformed cells interacting with the surrounding heterogeneous cellular and molecular microenvironment. Tumor microenvironment affects key activities of cancer cells and contributes to the tumor response to chemotherapy. Tumor-associated macrophages (TAMs) are a major component of innate immunity supporting primary tumor growth and metastasis. Numerous evidence indicate that TAMs can accumulate in tumors after chemotherapy and contribute to chemoresistance. In this way, it is important to investigate how the functional program of TAMs changes under chemotherapy treatment, and what are the possible mechanisms of the modulating effect of TAMs on the treatment effectiveness.



## Aim

Earlier we have shown that cisplatin may affect the transcriptional program of TAMs, and that in tumors of different localization the effect of the CT agent on the macrophage functions may be different. The aim of this study was to identify the thinner mechanisms of the interaction of TAMs and cisplatin.



## Methods

TAMs was generated from human CD14+ monocytes differentiated in vitro in the presence of IL4 and supernatants from tumor cell lines of MCF-7 (breast adenocarcinoma) and Colo206F (colorectal cancer). Full-transcriptome next-generation sequencing (NGS) of modeled TAMs was performed. For the endocytic uptake of EGF we used flow cytometry analysis. Confocal microscopy was used for the analysis of stabilin-1-mediated internalization and endocytic trafficking of EGF in CHO (Chinese hamster ovary) cells and in modeled TAMs. Validation of sequencing data by real-time PCR was performed for selected genes implicated in the endocytic uptake: DNM3, STX8, DENND1A and EHD1.

## Notes

Cisplatin (together with oxaliplatin, carboplatin) is platinum-based alkylating agent, which forms DNA crosslinks both between and within DNA strands (known as interstrand and intrastrand cross-linkages). Epidermal growth factor (EGF) is a key growth factor stimulating progression of breast cancer and colorectal cancer (metastatic spread, epithelial-mesenchymal transition, invasion). Stabilin-1 is a M2 marker; it is a scavenger receptor and plays important roles in the clearance of “unwanted” self-substances.

## Results

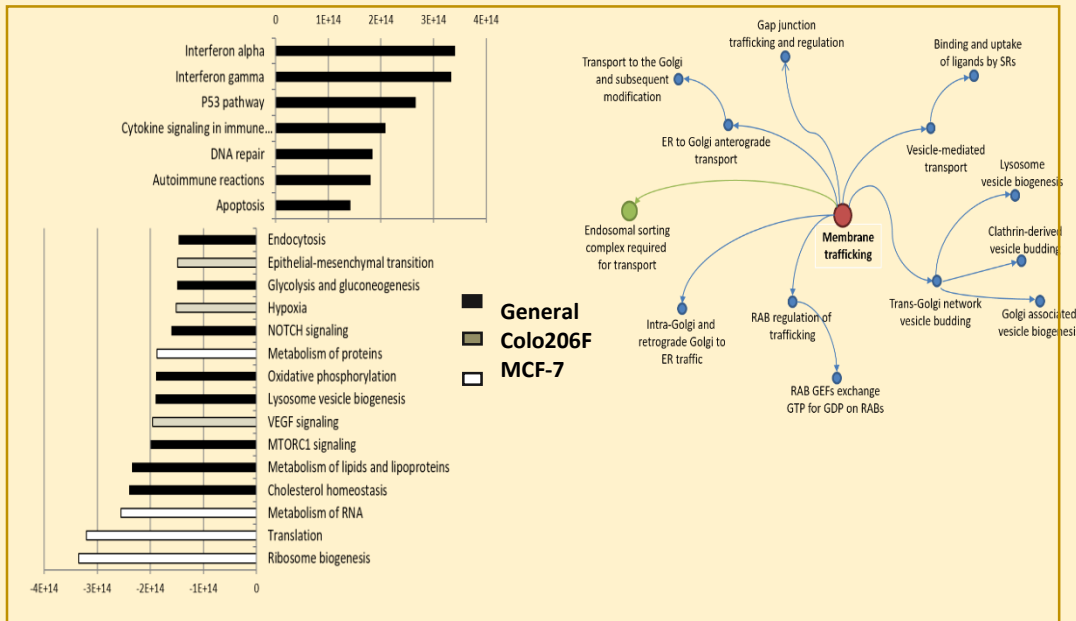


Figure 1: Top signaling pathways activated and inhibited under cisplatin treatment.

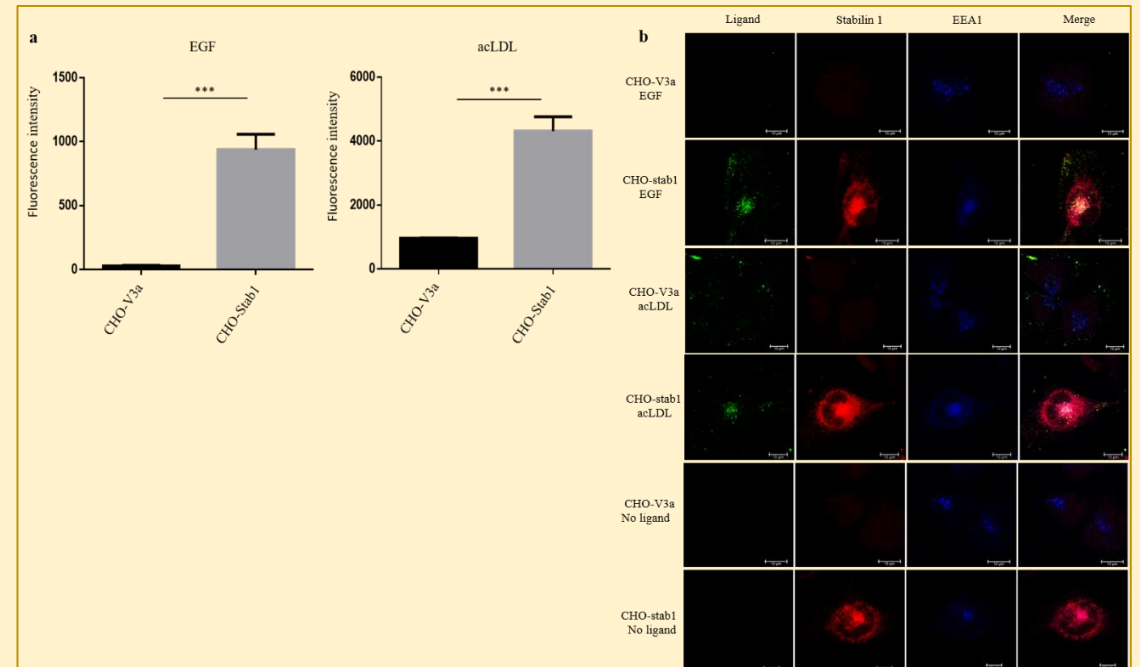


Figure 2: A and B – Stabilin-1 mediates endocytosis of EGF and its trafficking to the endosomal pathway in stably transfected CHO cells.

# Results

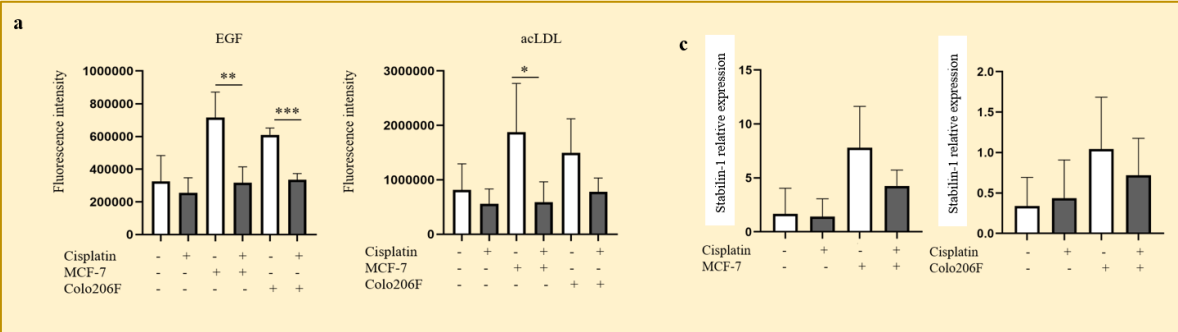


Figure 3: A - Cisplatin decreases the uptake of acLDL and EGF in the modeled TAMs, but does not significantly affect gene expression of stablin-1.

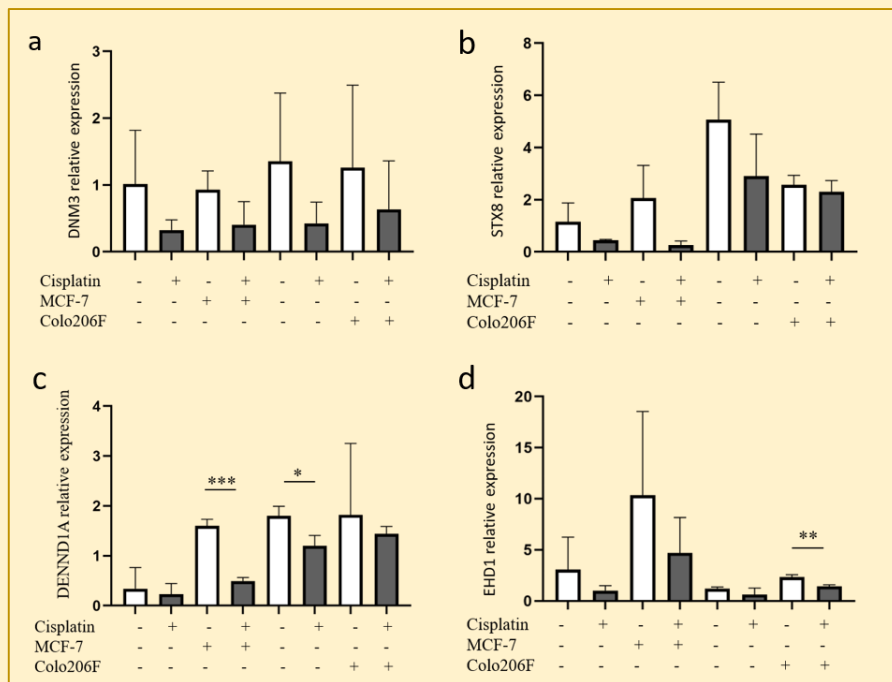


Figure 5: RT-PCR analysis confirmed that cisplatin suppresses expression of genes that controls endocytosis and vesicular transport.

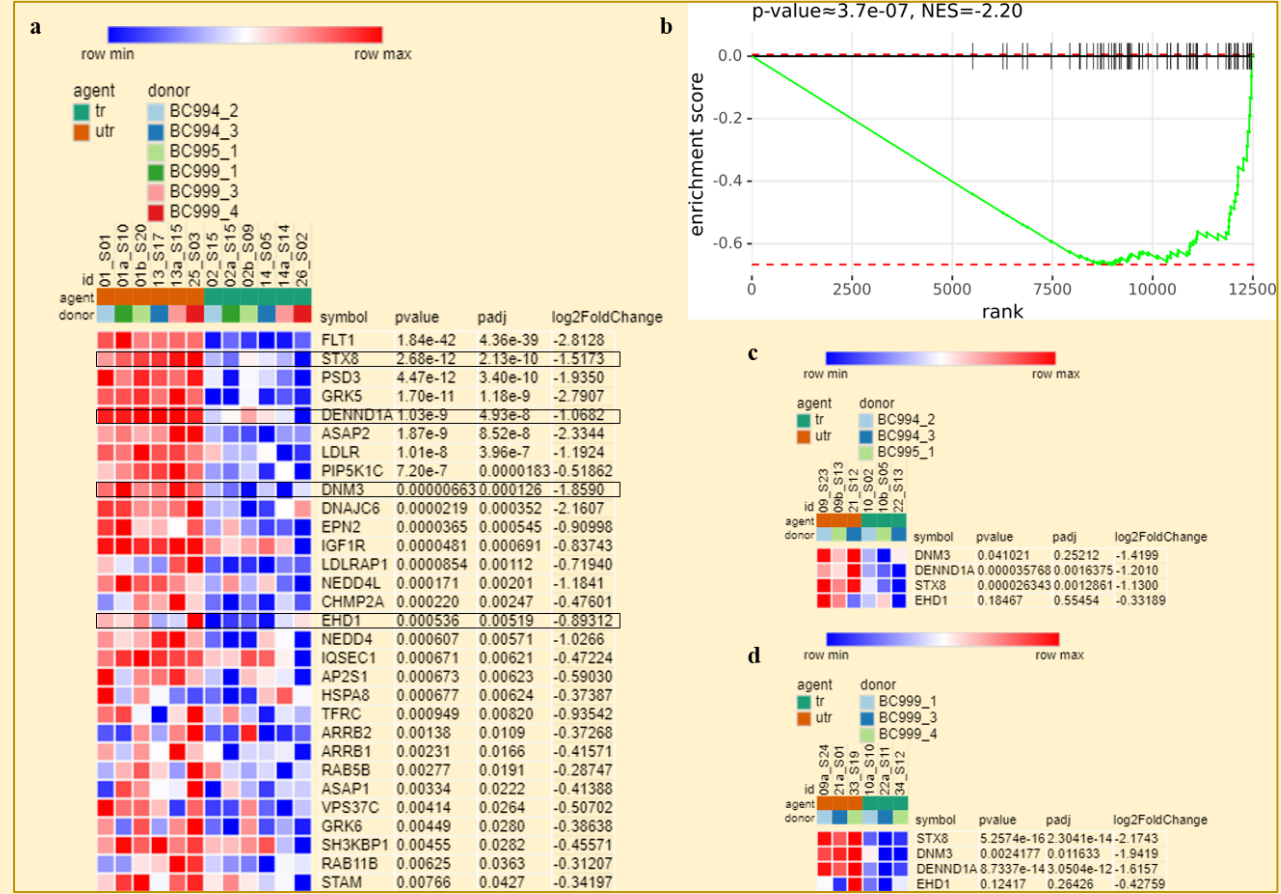


Figure 4: Next-generation sequencing identified that cisplatin decreases the expression of genes, involved in different steps of endocytosis.

## Conclusions

We suggested that suppression of receptor-mediated clearance of tumor-supportive factors by chemotherapeutic drugs may lead to the imbalance in tumor microenvironment and as a result to the chemoresistance. This research was funded by the Russian Science Foundation, grant number #14-15-00350.