DOI 10.18699/SBB-2022-602

Targeting urokinase receptor uPAR in cancer: high expectations, modest achievements

Semina E.[1, 2]*, Rysenkova K.[1, 2], Klimovich P.[1, 2], Shmakova A.[1, 2], Karagyaur M.[2], Rubina K.[2]

[1] *Institute of Experimental Cardiology, National Cardiology Research Center of the Ministry of Health of the Russian Federation, Moscow, Russia*

[2] *Faculty of Medicine, Lomonosov Moscow State University, Moscow, Russia*

** e-semina@yandex.ru*

Key words: urokinase receptor, uPAR, urokinase, uPA, neuroblastoma, chemoresistance, apoptosis, CRISPR/Cas9

Elevated levels of urokinase (uPA) and its receptor (uPAR) are detected in various malignancies and urokinase system is considered to be a potential therapeutic target in cancer. Binding of uPA to uPAR triggers the activation of proteinases, thus mediating matrix degradation and facilitating cancer cell invasion/metastasis and neoangiogenesis. In our previous studies using CRISPR/Cas9 technology in Neuro2a neuroblastoma cells, we have demonstrated that uPAR-knockout results in the reduced proliferation, suppressed uPA-dependent invasion and in the activation of caspase-dependent apoptosis. Strikingly, uPAR-deficient cells exhibit increased migration which is independent of the urokinase uPA. In search for the mechanisms of this phenomenon, we have found that urokinase in these cells can penetrate into the nucleus and can affect the expression of oncogenes (such as NF-κB, p53) triggering the onset of the epithelial-mesenchymal transition and selection/proliferation of chemoresistant cancer cells. Although underlying mechanisms are yet to be fully elucidated, we surmise that a decrease in uPAR expression in certain cancer cells can lead to the selection of low-proliferating cells that have a higher migratory potential and are insensitive to antitumor therapy, possibly resulting in the formation of dormant metastases.

The increased attention of scientists to the urokinase system and its role in carcinogenesis seems to be quite justified, since a significant amount of data has been accumulated to date, convincingly proving the direct involvement of the urokinase system in tumor progression. Urokinase uPA and urokinase receptor uPAR in tumor tissue promote extracellular matrix remodeling, invasion, migration and metastasis of tumor cells, tumor angiogenesis, they support the proliferation and survival of tumor cells. High expression of uPA and uPAR is often associated with tumor progression, with a poor prognosis of the course of the disease, and with reduced survival, and targeted suppression of the components of the urokinase system has been effective in a number of experimental models *in vitro* and *in vivo*. Despite this, therapeutic approaches aimed at the uPA/uPAR system did not live up to expectations and did not give convincing results in clinical trials, which left the question of the functional significance of the uPA/uPAR system in oncogenesis unresolved. In addition to insufficient knowledge, the relevance of research in this area is determined by the high prevalence of neoplasms in the population, which also applies to neuroblastoma, the tumor studied in this study.

Our data postulate that suppression of uPAR reduces ERK-dependent proliferation of Neuro2a neuroblastoma cells *in vitro* and tumor growth *in vivo*. At the same time, we

found that uPAR expression is important for maintaining the epithelial phenotype in Neuro2a cells and that uPAR suppression promotes the epithelial-mesenchymal transition, increased cell migration *in vitro*, and metastasis *in vivo*. It turned out that the suppression of uPAR leads to an increase in the expression of mRNA of *Snai1*, which is a transcription factor that directly represses E-cadherin, and to an increase in the expression of IL-6, a cytokine that stimulates cell migration and maintains the mesenchymal phenotype of tumors, which triggers the epithelial-mesenchymal transition in neuroblastoma cells. In addition, the suppression of uPAR in Neuro2a neuroblastoma cells led to the activation of p38 kinase, which inhibits cell proliferation, and an increase in the expression of p21, an inhibitor of cyclin-dependent kinase 1A, which indicated the dormant phenotype of Neuro2a neuroblastoma cells.

Our study is the first to show that uPAR-deficient cells are less sensitive to chemotherapy and demonstrate lower activation of the p53 protein, the main protein that regulates the cell's response to DNA damage and cell death/survival. Finally, by analyzing transcriptome data from human neuroblastomas, we showed for the first time that initially high *PLAUR* expression predicts poor survival in human neuroblastoma, but relapsed neuroblastomas have significantly reduced *PLAUR* expression. From the analysis of scientific sources, it can be concluded that this is the first study that demonstrated the pathological role of uPAR-deficient tumor cells with a chemoresistant and mobile phenotype.

Mining Transcriptome Data for Neuroblastoma. The microarray-based gene expression data for the Versteeg cohort (NCBI GEO accession GSE16476) were downloaded and analyzed in the R2 database (http://r2.amc.nl, accessed on 12 July 2021). The microarray-based gene expression data for primary and relapsed neuroblastoma, published by Schramm et al. (NCBI GEO accession GSE65303), were downloaded and compared using the R2 database (http://r2.amc.nl, accessed on 12 July 2021).

Acknowledgements: This research was supported by the Russian Foundation of Basic Research (Project No. 20-015-00186).