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Investigation of the glioblastoma stem-like cells resistance mechanisms to auranofin by the genome-wide knockout CRISPR screening

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Glioblastoma (GBM) is a brain tumor characterized by aggressiveness and tolerance to therapy, largely due to increased resistance to oxidative stress. Auranofin, an inhibitor of the thioredoxin reductase antioxidant system, is highly toxic to GBM cells and is a promising drug for therapy. However, we have previously shown that GBM cells are heterogeneous in their sensitivity to auranofin. The aim of this work was to study the mechanisms of resistance to auranofin in GBM cells using genome-wide CRISPR screening.

For the study, we used 2 cultures of stem-like GBM cells obtained from postoperative patients' material earlier; the cultures differed in their resistance to auranofin. For CRISPR screening, the cells were transduced with viral particles carrying the Toronto KnockOut CRISPR Library V3 [1]. After puromycin selection, the cells were divided into 2 parts, to the first part auranofin was added at an inhibitory concentration of IC20 for negative selection to determine the genes of resistance to the drug, or auranofin at a concentration of IC90 for positive selection to search for genes of sensitivity to the drug, to the second part (control) the appropriate amount of DMSO was added. The cells were cultured in the presence of the drug, then the genomic DNA isolated from the cells was sequenced. Then bioinformatics data processing was performed.

As a result, a number of genes were identified, the knockout of which led to sensitization to auranofin, including genes encoding components of antioxidant systems, proteins of transport and detoxification of xenobiotics, proteins of cellular signaling, components of mitochondria, proteins of repair, replication, splicing, autophagy.

Identification of the mechanisms of cell resistance to auranofin will allow developing approaches to overcome it. The results of the study may also be useful for understanding the mechanisms of tumor resistance to redox therapeutic drugs and chemotherapy in general.

References

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