DIFFERENTIAL EXPRESSION OF CHEMORESISTANCE-RELATED GENES UNDER THE EFFECT OF ADENOSINE A3 RECEPTOR ANTAGONIST IN GLIOBLASTOMA STEM-LIKE CELLS

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INTRODUCTION

Glioblastoma (GB) is the most aggressive and common primary malignant tumor of the brain and central nervous system (1). Without treatment, the average patient survival time is about six months, which can be extended to fifteen months with multimodal therapies (2). The chemoresistance observed in GB is, in part, attributed to the presence of a subpopulation of glioblastoma-like stem cells (GSCs) that are characterized by heightened tumorigenic capacity and chemoresistance (3).

OBJECTIVE

To create an alternative treatment therapy for Glioblastoma using the A3 adenosine receptor.

MATERIALS AND METHODS

Different analytical approaches were applied for functional genomics: DAVID (Database for Annotation, Visualization, and Integrated Discovery). The Venn diagram tool was used to calculate the intersection(s) of list of elements. Ensemble, a genome browser for vertebrate genomes, was used to predict regulatory functions. Panther provides comprehensive information about the evolution of protein-coding gene families. INSECT 2.0 (IN-silico SEarchfor Co-occurring Transcription factors) is a web server for biologists analyzing genomic sequence data for in silico cis-regulatory module prediction and analysis. Glio-Vis was used as the data visualization tool for brain tumor datasets. To validate the RNAseq results, we performed RNA extraction with TRIzol RNA and subsequently NanoDrop quantified the samples. Reverse transcription was performed with 1 µg of RNA with MMLV (Promega, Madison, WI, USA). The relative quantification of **RT-**qPCR was performed with the

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2−ΔΔCt method, and the β-actin gene was used as a normalizer (Annex 1). All data were recorded in biologic triplicate**.**

RESULTS AND DISCUSSION

GSCs are situated in hypoxic tumor niches, where they sustain and promote the stem-like phenotype and have also been correlated with high chemoresistance. GSCs have the particularity of generating high levels of extracellular adenosine (ADO), which causes the activation of the A3 adenosine receptor (A3AR) with a consequent increase in the expression and activity of genes related to chemoresistance. Therefore, targeting its components is a promising alternative for treating GB. This analysis determined genes that were up- and downregulated due to A3AR blockades under both normoxic and hypoxic conditions. In addition, possible candidates associated with chemoresistance that were positively regulated by hypoxia and negatively regulated by A3AR blockades in the same condition were analyzed. We detected three potential candidate genes that were regulated by the A3AR antagonist MRS1220 under hypoxic conditions: LIMD1, TRIB2, and TGFB1. (Annex 2). Finally, the selected markers were correlated with hypoxia-inducible genes and with the expression of adenosine-producing ectonucleotidases

CONCLUSION

Hypoxic conditions generate extensive differential gene expression in GSCs, increasing the expression of genes associated with chemoresistance. Furthermore, we observed that MRS1220 could regulate the expression of LIMD1, TRIB2, and TGFB1, which are involved in chemoresistance and correlate with a poor prognosis, hypoxia, and purinergic signaling.

Key words: glioblastoma; epidemiology; incidence; survival; prognosis

BIBLIOGRAFÍA

1. Thakkar, J.P. et al. Cancer Epidemiol. Biomark. Prev. 2014, 23, 1985–1996.
2. Grochans, S. et al. Cancers 2022, 14, 2412.
3. Zhao, Y. et al. Signal Transduct. Target. Ther. 2020, 5, 44.

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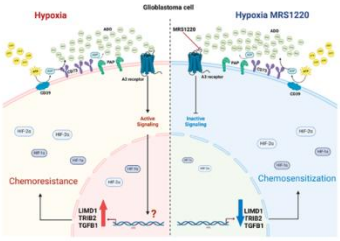
ANNEX 1

Table 1. Molecular functions, biological processes, and pathways related to the down- and upregulated genes after MRS1220 treatment in hypoxic conditions.

	Molecular Functions	Biological Processes	Pathways
29 down regulated genes	Binding Catalytic activity Molecular function regulator Structural molecule activity Transcription regulator activity Transporter activity	Biological Regulation Cellular process Developmental Process Localization Locomotion Metabolic process Response to stimulus Signaling	CCKR signaling map Cytoskeletal regulation by Rho GTPase Integrin signaling pathway PDGF signaling pathway Synaptic vesicle trafficking
34 upregulated genes	ATP-dependent activity Binding Catalytic activity Molecular adaptor activity Molecular function regulator Molecular transducer activity Structural molecule activity Transcription regulator activity	Biological process involved in interspecies interaction between organisms Biological regulation Cellular process Developmental process Localization Locomotion Metabolic process Multicellular organismal process Response to stimulus Signaling	Alzheimer disease: presenilin pathway Angiogenesis EGF receptor signaling pathway FGF signaling pathway Gonadotropin-releasing hormone receptor pathway Inflammation mediated by chemokine and cytokine signaling pathway Notch signaling pathway PDGF signaling pathway

ANNEX 2



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