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Silencing of CA12 by Interfering RNA Sensitizes GBM Cells to Chemotherapy and Reduces Cell Proliferation

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Introduction: Glioblastoma (GBM) is the most common subtype and lethal brain tumor in the human species. The treatment of GBM remains a major challenge for clinicians since these aggressive brain tumors are highly resistant to radio- and chemotherapy. The poor prognosis of GBM patients is in part due to the presence of a heterogeneous population of glioblastoma stem cells (GSC), characterized by invasive behavior and resistance to apoptosis. Hypoxia is a predominant feature in the microenvironment of GBM and is associated with tumor growth. GBM cells express membrane bound carbonic anhydrases (CAs), a family of ubiquitous enzymes that regulate physiological and pathological processes. These enzymes have been shown to regulate environmental acidity and, in particularly, the expression of CA12 in GSCs may directly promote the survival and proliferation of disseminated cancer cells in a hypoxic environment. The presence of two isoforms in human tissue have been indicated and alternatively spliced protein forms have been linked to aggressive behavior of cancer cells and patient prognosis. Then, we propose that CA12 promotes growth of GBM by facilitating proliferation of the GSC population in the tumor and we suggest that CA12 upregulation maybe is correlated with poor prognosis in GBM.

Objective: Investigate the correlation of CA12 expression with GBM subtype and analyze the effects of CA12 knockdown (CA12KD) on proliferation and chemoresistance of GBM cells. In silico, determine the correlation of CA12 with patient survival.

Methodology: The gene and protein expression of CA12 was evaluated in mesenchymal(MES) (GBM9, GBM33), pro-neural(PN) (GBM157) cells and (U87) cells line by conventional PCR, qRT-PCR and Western blot. Cell viability and the effect CA12KD on chemoresistance of U87 cells were analyzing treating cells with conventional glioma therapeutic Temozolomide (100□M, 200□M and 300□M) using a CellTiter-Glo® (Promega).

Results: The results of gene and protein expression identified the presence of two CA12 isoforms (long and short variant) with different expression according to subtype of GBM. However, pro-neural GBM157 cells expressed only the shorter CA12 isoform. CA12 expression was increased under hypoxia 1%. However, CA12KD reduced gene and protein expression in the GBM cells. The CA12KD sensitized U87 cells to treatment with TMZ and reduced cell viability. In addition, in silico results indicated a tendency of high CA12 expression are correlated with worse patient survival.

Discussion: These partial results indicated that GBM cells exhibit two isoforms of CA12 and demonstrated that protein expression increased under hypoxia. In addition, MES cells contained both CA12 isoforms and PN expressed only the shorter CA12 isoform. The effect of CA12KD indicated the important contribution of CA12 in the resistance of GBM cells to chemotherapy. However, additional experiments increasing drug concentration will be necessary to confirm this hypothesis.

Conclusion: This study suggest that CA12 isoforms is expressed on different subtypes of GBM and represent an interesting therapeutic target. In addition, the chemo-sensitizing effect of CA12KD maybe represent an attractive strategy to improve adjuvant therapy for GBM.

Keywords: Glioblastoma stem cell; Hypoxia; Carbonic anhydrase.