Purinergic Signaling Affects Tumor-Spheres Growth in Human Glioblastomas

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Introduction: Gliomas are very aggressive CNS tumors with elevated incidence of recurrence and death. Glioma cell lines, such as U87-MG, produce *tumor-spheres* when in high confluence, which are similar to neuro-spheres, and are enriched with tumor stem cells (TSC). This subpopulation is important for the establishment and maintenance of the tumor mass, and seems to be more resistant to traditional therapies. In gliomas, TSC were identified expressing the protein CD133. It is suggested that the spheres contain stem cells involved in tumor resistance. Purinergic receptors are involved in many biological processes such as proliferation and differentiation, and the degradation of ATP is slow in glioma cells, which results in its accumulation in the extracellular space. **Objetive**: Characterize the TSC population in U87 and the effect of ATP in sphere formation. Analyze the presence of CD133 protein in spheres and monolayer and the expression of purinergic receptors and marker genes of differentiated cells (GFAP, GLAST, GLT, CAMKII, Enolase, NMDA, AMPA) and undifferentiated cells (CD133, Nestin, Oct-4). **Methods and Results**: Spheres were obtained plating the cells on a thin layer of agar 1%. Total RNA was extracted from spheres and monolayer, and the genes of interest were amplified in a RT-PCR. CD133, OCT-4 are more expressed in spheres, while GLAST, NMDA presented higher expression in the monolayer. Among the purinergic receptors, $P2X_4$ expression was observed exclusively in spheres, whereas $P2X_6$ in the monolayer. Cells plated in the presence of ATP 100uM formed 40% less spheres ($P<0.05$) when compared to control. **Conclusion**: Spheres have components of stem cells and the purinergic signaling is involved in important aspects of TSC biology. Financial support: PIBIC/CNPq-UFRGS, FAPERGS.

Key words: ATP, glioblastoma, stem cells, tumor-spheres, tumor stem cells.