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ANALYSIS OF RAT C6 GLIOMA CELLS STABLY TRANSFECTED WITH β-1,4 N-ACETYLGALACTOSAMINYLTRANSFERASE (GM_2/GD_2 SYNTHASE)

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Gangliosides, sialic acid containing glycosphingolipids, may play several functional roles in cell differentiation and development, and in pathological phenomena, such as neoplastic transformation (1). Gangliosides profile undergoes deep changes during these biological processes and might be playing a role in the uncontrolled growth of tumoral cells. Although the mechanisms regulating ganglioside expression remain unclear, cell type-specific glycosylation may be due to the differential expression of different glycosyltransferases (2). To address this question, cloned genes that determine ganglioside expression are essential tools. In order to understand the relationships between ganglioside pattern and glycosyltransferase activities and the possible effects that eventually early synthetized gangliosides could have on the morphology and behaviour of tumor cells, we transfected C6 glioma cells with the β-1,4 N-Acetylgalactosaminyltransferase (GalNAcT-1) cDNA (3). These cells represent a good experimental model because the only ganglioside they express is GM_3 (both N-Acetyl and N-Glycolyl GM_3). We used a cytomegalovirus derived expression vector, containing the neomycin resistence gene (Neo gene), to subclone the GalNAcT-1 cDNA and reach a stable transfection of C6 glioma cells. We detected no morphological and/or proliferative differentiation of the C6 glioma cells after transfection. On three clones (clone 3, 6 and 7, 54° culture stage), among all the ones isolated, we verified the success of transfection by PCR amplification of Neo and GalNAcT-1 genes on the genomic DNA extracted from these clones. The results show that only the transfected cells, not C6 glioma cells, present equal levels of Neo and GalNAcT-1 genes amplification. Furthermore, we have evalued the level of GalNAcT-1 mRNA by RT-PCR: with this method is possible to have not only qualitative but also quantitative informations about gene expression. At the same time, we amplified a fragment of mouse β-actin (285 bp) as internal standard. The results show good and equal levels of GalNAcT-1 mRNA in all the clones of transfected cells, while it is not present in the C6 glioma cells. The analysis of the ganglioside pattern in the three transfected clones strangely showed no variation as regard to the C6 glioma cells with the exception of clone 6 in which small amounts of GM_2 are detectable. Some appreciable differences between C6 and transfected cells came out with the glycosyltransferase assays. Besides the GalNAcT-1 activity (with GM_3 and GD_3 as substrates), we also tested the following sialyltransferases (SAT): SAT-1, SAT-2, SAT-4 (using GM_1 and aGM_1 as substrates) and the β-1,3 N-Acetylglucosaminyltransferase (GlcNAcT-1) activities. We found that all the sialyltransferase activities are significantly decreased in transfected clones when compared to C6 glioma cells: the activity values are reduced to quite fifty percent. On the contrary, there is an increase of GlcNAcT-1 and of GalNAcT-1 activities (using either GM_3 and GD_3 as substrates), both undetectable in C6 glioma cells. All the enzymes have comparable activities among the three clones. These data could support the hypothesis that GalNAcT-1 activity and, according to it, the ganglioside pattern may be regulated with post-tradutional mechanisms, involving the levels of active protein present in the cells. We need at this point further studies and experiments, one of which could be the detection of the protein with specific antibodies and the analysis of the same cells after *in vivo* grafting, for example subcutaneously.

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