ANALYSIS OF C6 RAT GLIOMA CELLS STABLY TRANSFECTED WITH THE HUMAN β1,4 N-ACETYLGALACTOSAMINYLTRANSFERASE (GalNAcT-I) AND α2,8 SIALYLTRANSFERASE (SAT-II)

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Gangliosides are involved in cellular communication and differentiation and play an important role in proliferation, adhesion, migration and invasiveness of tumor cells (1,2). Gangliosides may participate in tumor progression in different ways: directly, by a specific binding with some components of the extracellular matrix (ECM), or indirectly, by stimulating the synthesis of specific components of the ECM and the release of metalloproteinase and/or angiogenic factors (3,4). Our purpose was to analyze the biochemical, morphological and cellular modifications induced by stable transfection with the human GalNAcT-I and SAT-II cDNAs (5,6) in C6 rat glioma cells. We selected this specific cell line due to its expression of a single ganglioside species (GM_3).

C6 cells were submitted to three different transfections: in the first one, the empty pRc/CMV eukaryotic expression vector was employed; in the second one, the same vector, in which the GalNAcT-I cDNA was previously subcloned, was used; in the third one procedure, cells were cotransfected with both the GalNAcT-I and the SAT-II cDNA constructs. Among the different clones isolated, only three of themwe analyzed: p13, transfected with the empty vector, G1, transfected with GalNAcT-I, and 5F, transfected with both GalNAcT-I and SAT-II. The efficiency of the transfections was evaluated by: 1- Dot Blot and PCR amplification of specific regions contained in the two human cDNAs and in pRc/CMV (Neo gene, coding for antibiotic resistance) on the genomic DNA extracted from the clones; 2- RT-PCR amplification on the total RNA extracted from the same clones. The ganglioside pattern was analyzed by HP-TLC, after lipid extraction and purificatic from the cell pellets. The different species were identified by TLC-immunostaining with monoclonal antibodies (MAbs) or by the b-subunit of Cholera toxin. Cell adhesion was evaluated *in vitro* on reconstituted extracellular matrix (Matrigel) and on some purified components of the ECM: Fibronectin, Laminin, Type IV collagen and Trombospondin. Cell motility and invasiveness were tested *in vitro* using the Boyden chamber, before and after an unspecific chemotactic stimulation.

The ganglioside pattern did not show any significant variation in the clone p13 with respect to the C6 parental cells, while in the clones G1 and 5F the synthesis *ex novo* of the expected species was evidentiated: infact in G1 GM_2 and GM_1 were present, as GM_2 and GD_3 in 5F. In experiments concerning tridimensional growth morphology, adhesion, motility and invasiveness, the C6 wild type and the clones p13 and G1 showed a similar behaviour. Significant differences were evident only in the clone 5F, characterized by the synthesis *ex novo* of GD_3. Particularly, 5F is more adhesive to the ECM and to some purified components of the ECM and has increased motility and invasiveness, expecially after chemotactic stimulation. Moreover this clone presents, *in vitro*, a growth rate significantly higher than the other clones. It has been recently reported that polysialylated gangliosides, such as GD_3, can interact with Fibronectin (FN) (7). Although the gangliosides alone seem to be unable to mediate adhesion, they may be involved in modulating the strenght or specificity of the interaction between FN and its receptor (8). Since adhesion is a crucial step in tumor cells migration and invasion toward surrounding tissues, our data could explain the aggressive behaviour of the clone 5F. Moreover data in the literature show that GD_3 is a potent stimulator of VEGF (vascular endothelial growth factor) release (9) and that an increase in GD_3 content well correlates with the degree of malignancy of gliomas (10). Work are in progress in our laboratory to analyze these clones, both *in vitro* and *in vivo*, to better understand the effect of the synthesis *ex novo* of GD_3 on neo-vascularization, another variable in tumor tissue organization.

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A gratefull aknowledgement is due to the others collaboratos. A special thanks goes to Prof. B. Berra for his precious support.