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Adhesive and invasive properties of C6 rat glioma cells stably transfected with the human α2,8 sialyltransferase cDNA

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Gangliosides are involved in tumor cells proliferation, adhesion, migration and invasiveness. We investigated the modifications of these parameters in C6 rat glioma cells, stably transfected with the cDNA coding for the human α2,8 sialyltransferase (SAT-II), enzyme involved in the ganglioside metabolism, cloned in the pRc/CMV expression plasmid. Transfected clones were identified by PCR on genomic DNA and SAT-II expression was evaluated by RT-PCR. In clones C6-S1 and -S4, expressing the SAT-II cDNA, the synthesis *ex novo* of GD_3 was clearly observed, while control C6 and clone C6-p13 (transfected with the empty plasmid) only synthesized ganglioside GM_3. *In vitro* assays demonstrated that clone C6-S1 is more invasive and adhesive than controls and *in vivo*, after subcutaneous grafting in nude mice, C6-S1 cells grow more aggressively than C6-p13. Thus, GD_3 seems to modulate interactions of C6 cells among them and with the extracellular matrix. Since a major hallmark of malignant gliomas is their striking neovascularization and GD_3 is a potent angiogenic factor, we are now planning to study *in vitro* and *in vivo* the role of GD_3 in the development of C6 vascularization.

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Isolation of neuronal ligands for the myelin-associated glycoprotein (MAG)

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Myelin-associated-glycoprotein (MAG) has been proposed to be important for the organisation of myelin and to regulate neurite outgrowth. It belongs to the sialoadhesin family and binds to α2,3-linked sialic acids.[1]

For a better understanding of the biological significance of this interaction we performed this study to identify the counter receptors of MAG on the neuroblastoma cell line N_2A. Binding to these cells is sialic acid-dependent and occurs mainly to *N*-glycans of glycoproteins, as shown by experiments with glycosylation inhibitors. Six glycoproteins with molecular weights between 40 and 120 kDa were affinity-purified with MAG from ^{3}H-GlcNAc-labeled N_2A cells. These are likely to represent counter receptors for MAG, since none of them was detected if other adhesion molecules, i.e. NCAM or CD22, were applied. Furthermore, α2,3-sialyllactose completely inhibited the interaction of these glycoproteins with MAG, demonstrating its dependence on sialic acid recognition.

Reference

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Analysis of IgG N-glycans from rheumatic diseases by fluorophore assisted carbohydrate electrophoresis

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A rapid, cost effective test which discriminates between rheumatic diseases in their early stages is still not available to the Rheumatologist. One clinical feature of the rheumatic diseases, which may be exploited in the development of a diagnostic test is the change in glycosylation profile of immunoglobulin G (IgG). IgG contains on average 2.5 nitrogen linked biantennary (1,3 and 1,6 linked) oligosaccharide molecules which have heterogeneity at the non-reducing terminus. Rheumatoid arthritis is associated with hypogalactosylation. Our objective was to develop a fluorophore assisted electrophoresis system which could reproducibly resolve IgG derived biantennary glycans. The glycans from serum IgG were released by PNGase and labelled with 2-amino benzoic acid. They were resolved on 22% polyacrylamide gels using either Tris-TAPS, Tris-Glycine or Tris-Tricine as resolving buffer. The glycans with greatest diagnostic potential are the neutral biantennary glycans which vary in the proportion of galactose (g0, g1, g2). Resolution of glycans was governed by the choice of running buffer. Tris-Glycine resolved the neutral glycans into 4 bands whereas the other buffers resolved them into 3 bands. N-glycans from rheumatoid arthritis (RA), psoriatic arthritis, ankylosing spondylitis, SLE, primary Sjogrens syndrome and normals were analysed by fluorophore assisted carbohydrate electrophoresis. IgG glycans from RA patients were shown to have a decreased level of g2 in accordance with previous studies. Fluorophore assisted electrophoresis maybe a useful technique in the early diagnosis of rheumatoid arthritis.

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Oligosaccharide heterogeneity and functional variation of AGP expressed in the plasma of rheumatoid arthritis patients

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α1-acid glycoprotein (AGP) is a positive acute phase serum glycoprotein with five N-linked complex oligosaccharide chains. It is hypothesised that unique AGP glycoforms of the endothelial cell adhesion molecule E-Selectin thereby inhibiting leucocytic vascular escape to an inflammatory focus. The oligosaccharide fucosylation, branching and sialylation of rheumatoid AGP was examined, in comparison to normal plasma AGP, using high pH anion exchange chromatography and ConA affinity chromatography. RAAGP showed hyperfucosylation and a tendency towards increased oligosaccharide branching. AGP purified from the blood of individual rheumatoid arthritis sufferers was found to block sialyl Lewis X mediated cell binding by immobilised E-Selectin.