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Cell regulatory function of glycosphingolipids: Carbohydrate recognition and biosignaling

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Abstract: The emphasis laid by glycobiologists on cell adhesion in terms of carbohydrate interaction is now shifted to the aspects where the interaction is associated with the regulation of various cellular activities via biosignalling and glycosphingolipids, in particular, gangliosides (sialic acid-containing glycosphingolipids) play an important functional role.

The significance of the carbohydrate-protein interaction was distinctly recognized by discovery of the lectins as the carbohydrate sensor, though blood group substances already suggested the occurrence of such an interaction. Subsequently, the importance of this type of interactions has been widely recognized in cell to cell interactions and recognition not only in multicellular organisms but also in the relationship between animal cells and microbes. As a consequence, the emphasis laid by glycobiologists on the lectins as a sugar probe gradually has shifted onto the aspect of cell adhesion. Thus, tumor cells in metastasis and recently leukocytes in the initiation and promotion of inflammation are intensively studied on the basis of the cell adhesion mediated by carbohydrate–protein interaction. These studies also are aiming at developing carbohydrate - based drugs to inhibit or modulate metastasis and inflammation.

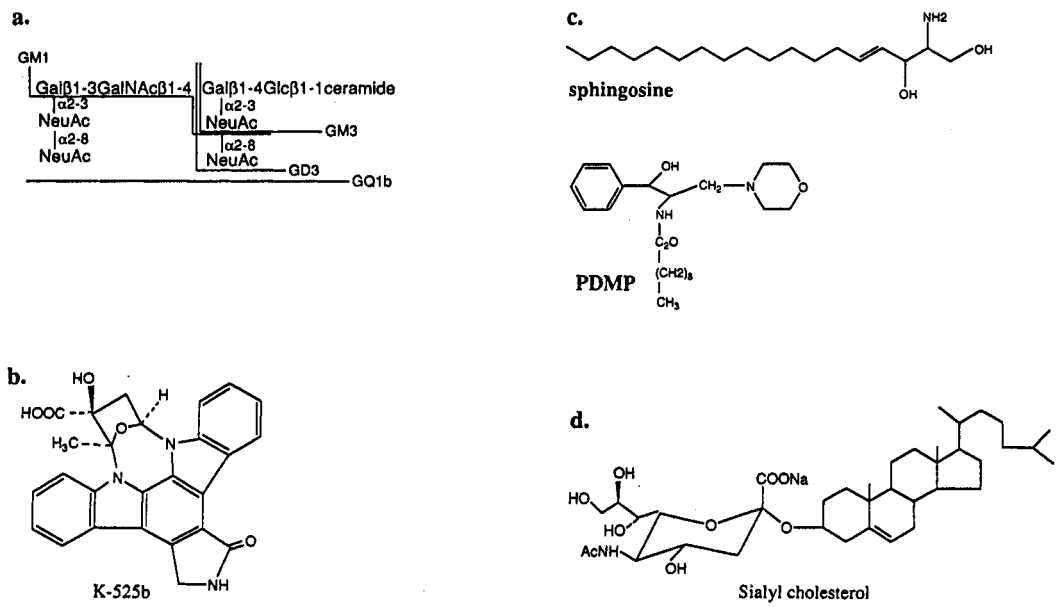

Fig.1

Meanwhile, it soon became clear that the interaction does not remain mere binding of carbohydrate to protein molecules at cell surface, but may evoke a change in cellular activities (Table I). In fact, monoclonal antibody to a ganglioside GD3 (Fig. 1a) was found to reversibly inhibit growth of rat embryonic fibroblastic cells, 3Y1, which were transfected and transformed with adenovirus 12 oncogenic DNA fragment, E1, and thereby developed a new carbohydrate antigen GD3 at cell surface (G in Table I). Contact inhibition of cell growth in primary cultured hepatocytes from adult rats can be mimicked by the *in vitro* interaction between the cells and the plasma membranes prepared from those cells. The inhibition was abolished by treatment of the membranes with either β-galactosidase or endoglycosidase F, indicating involvement of cell surface carbohydrate and its receptor (glycoreceptor) in the contact inhibition of hepatic cell growth (H in Table I). To the contrary, the contact inhibition was not observable among primary cultured hepatocytes prepared from liver tissues of juvenile rats and also between these juvenile cells and their plasma membrane preparation. Sialidase-treated juvenile plasma membranes, however, developed the inhibitory activity, suggesting that sialylation may control the contact inhibition of cell growth in a developmental stage-specific manner.

Several lines of evidence support the concept that glycosphingolipids of cell surface membranes participate in biosignaling of the cells in a particular manner. In Table I, possible regulatory functions of glycosphingolipids in cell signaling are listed up. In the case of F, particular glycosphingolipid molecules specifically as well as stoichiometrically bind to receptor protein molecule to regulate the functional state of the receptor, indicating the importance of lipid microdomain surrounding membrane receptor molecules (Fig. 2). This notion was recently proved in the case of the interaction between nerve growth factor (NGF) receptor and a ganglioside GM1 (Fig. 1a) which has been known to stimulate and enhances the action of

Table 1 Modes of involvement of glycosphingolipids in cellular functions

A. Modulation by parturbation of lipid matrix
Regulation by alteration of physicochemical properties and/or three dimensional membrane architectures
e.g., Permeability, electrical parameters, transporters, etc.

B. Intracellular membrane trafficking, sorting, targetting and shedding.
Sphingolipid segregation into microdomains in membranes
Cytoskeleton-associated glycolipids (CAG)

C. Cell adhesion, guidance, migration and targetting
e.g., Uronic acid-containing glycosphingolipid, etc.

D. Metabotropic signaling
e.g., Sphingosine and its derivatives
Ceramide
De-N-acetylation, etc.

E. Ionotropic signaling
e.g., Ca^{2+} ion channels, etc.

F. Functional modulation of receptor in microdomain of membranes
Alteration of receptor function by annular (boundary) lipid molecules. e.g.,
oligomerization of NGF receptor by GM1 ganglioside.

G. Perturbation or binding by ligand of cell surface carbohydrate chain.
Lectin/Antibody/Toxin interactions with glycoconjugates.
Carbohydrate-carbohydrate interactions.

H. Glycomessage-glycoreceptor interacction (signal recognizing, transducing and responding)
Intracellular (endo-) type signal transduction
Extracellular (ecto-) type signal transduction
e.g., ecto-protein kinase
ecto-protein phosphatase

I Direct transfer of glycomessage to cell nucleus to modulate gene functions
Carbohydrate- mediated modulation of nucleo-protein supramolecular structure
Regulation of gene expression (activation or suppression) by glycosylation
or carbohydrate-binding.

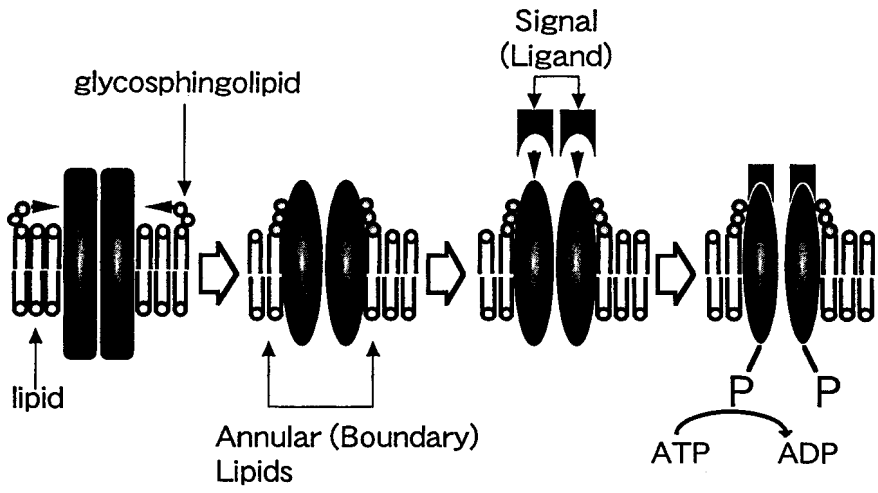

Fig. 2

NGF in terms of neuritogenesis. GM1 was found to directly and tightly associates with Trk, the high-affinity tyrosine kinase-type receptor for NGF, resulting in strong enhancement of neurite outgrowth and neurofilament expression in rat pheochromocytoma PC12 cells elicited by a low dose of NGF that alone is not sufficient to induce neuronal differentiation (Mutoh, T., *et al.*, 1995). The action of GM1 was specific, and interestingly N-glycosylation of Trk protein seemed to be necessary not only for the association of GM1 with Trk protein but also for its tyrosine kinase activity catalyzing autophosphorylation.

Very high biological activity of glycosphingolipids was found first in a minor component of brain gangliosides in association with neuritogenesis (Tsuji, S., Arita, M., and Nagai, Y., 1983). A tetrasialoganglioside GQ1b (Fig. 1a) strongly promoted neurite outgrowth of cultured human neuroblastoma cells, GOTO and NB-1, at a few nanomolar concentrations. The analysis of the structure and activity relationship showed that the oligosaccharide structure of GQ1b is very strictly required. Later studies revealed that the carbohydrate signal of GQ1b is recognized first by GQ1b-specific glycoreceptor at cell surface and then is transmitted by a unique, cell surface localized signal transduction pathway which is catalyzed by the unique protein kinase(s) of a cell surface type (ecto-type signal transduction) (H in Table I) (Nagai, Y., 1995). The ecto-protein kinase catalyzes phosphorylation of the extracellular domain of cell surface membrane proteins. In the case of GQ1b three surface proteins, (54, 60 and 64 kDa) were principally phosphorylated at serine or threonine residue but not at threonine. The precise molecular mechanism of the interaction between GQ1b and its glycoreceptor, the coupling of glycoreceptor with ecto-kinase and subsequent neuritogenic signaling are yet to be elucidated. However, cell membrane-impermeable protein kinase inhibitor K-252b (Fig. 1b) abolished ecto-phosphorylation as well as neurite outgrowth of living cells in the presence of GQ1b, indicating that both events are closely coupled with each other.

Meanwhile, cDNA encoding an α2,8-sialyltransferase (SAT-II) responsible for GD3 biosynthesis was cloned and was found to also catalyze α2,8-sialylation of ganglioside GT1b, precursor for GQ1b biosynthesis (SAT-V) (Fig. 1a; Fig. 3). This cDNA was introduced into a subline of mouse neuroblastoma cell lines, Neuro 2a cells, which has GM3 ganglioside alone, but neither GD3 nor GQ1b. In this transfected cells now GD3 and GQ1b were expressed at cell surface and subsequently the cells started neuritogensis, providing evidence that endogenously generated GQ1b is physiologically functinal as well (Fig. 4).

Exogenous GQ1b but not other gangliosides also acted to specifically stimulate functional synapse formation associated with spontaneous synchronous oscillations of intracellular Ca^{2+} in a long-term primary cultured rat cerebral cortical neurons, of which glycosphingolipids were depleted to a large extent on addition of D-PDMP (D-*threo*-1-*phenyl*-2-*decanoylamino*-3-*morpholino*-1-*propanol*, D-PDMP), a specific inhibitor of glucosylceramide synthase, that constitutes the initial step of glycosphingolipid biosynthesis (Fig.3c and 3)(Mizutani *et al.* 1996). On the other hand, L-*threo*-PDMP, the enantiomeric form of D-PDMP markedly stimulated GQ1b synthesis and also the functional synapse formation measured by synchronized socillatory activity of intracellular Ca^{2+} between the neurons in the same cultured cell system, again suggesting the necessity of endogenous GQ1b in the functional synapse

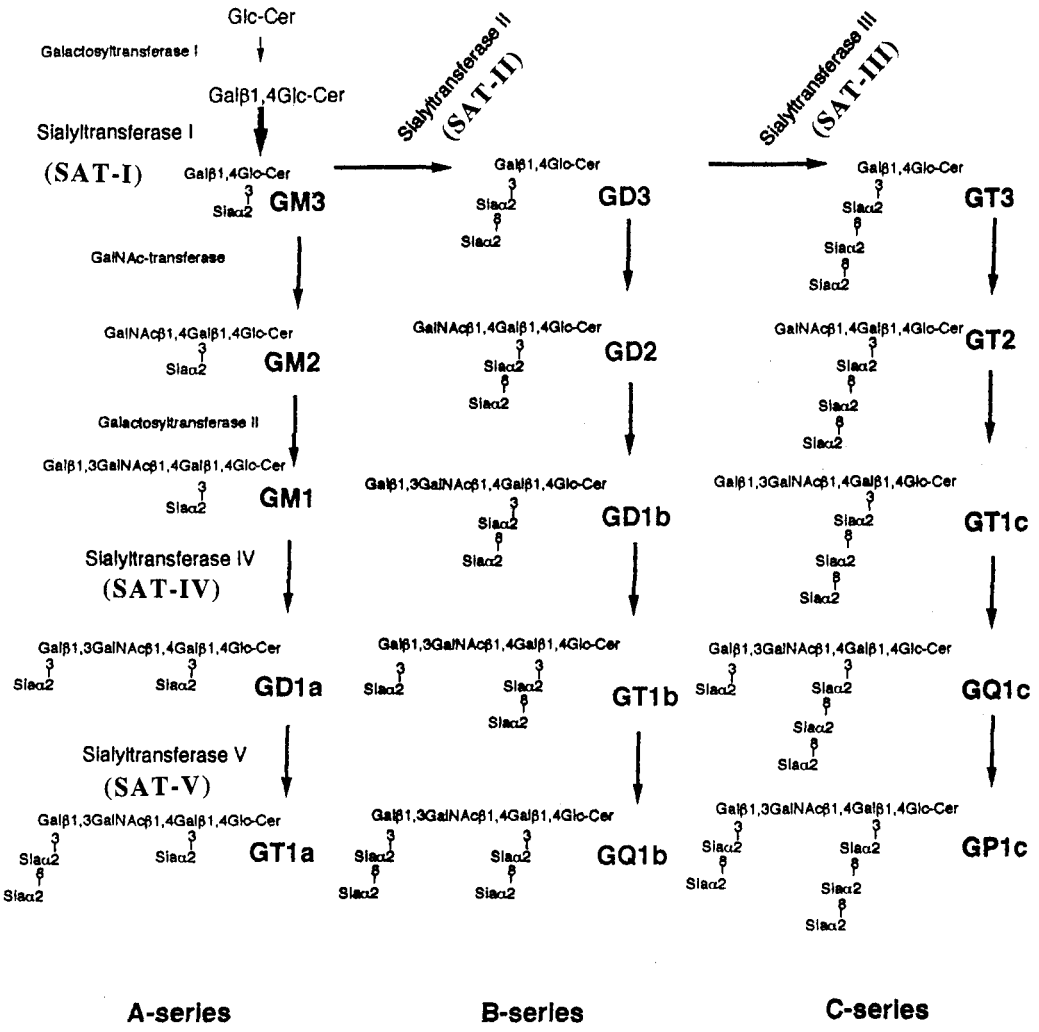

Fig. 3 Biosynthetic Pathway for Gangliosides

formation (Inokuchi J., *et al.* 1997, unpublished). Thus, the effect of L-PDMP is opposite to that of D-PDMP. The ecto-phosphorylation system highly develops in neuronal cells but not so in glial cells and the other cells of extracerebral tissues. Interestingly, the above-mentioned inhibitor, K-252b, distinctly suppressed spontaneous synchronized oscillation of intracellular Ca^{2+} which is associated with functional synapse formation (Muramoto, K. *et al.*, 1988).

Synthetic α- or β-sialyl cholesterol (Fig. 1d) was found to stimulate neuritogenesis in Neuro 2a cells. A large part of sialyl cholesterol uptaken into the cells unexpectedly was found to be transported into the cell nuclei, resulting in two-fold inerease in transcriptional activity, presumably being associated with neuritogenesis (Yamashita, T., Tsuji, S. and Nagai, Y., 1991). This may represent occurrence of another type of glycolipid-mediated biosignaling (I in Table I).

It is now evident that glycosphingolipids are no more mere structural elements of cell membranes but are importantly involved in cell signaling to regulate various cellular functions where the carbohydrate-protein interaction plays a principal role. From a view point of the carbohydrate-protein interactions, brain function, in particular, neuronal network formation and synaptic plasticity may be one of the most interesting targets of glycobiology research. Such functions seem to escape from the deterministic regulation of genes, while enormous varieties of glycoconjugate molecular species also seem to escape

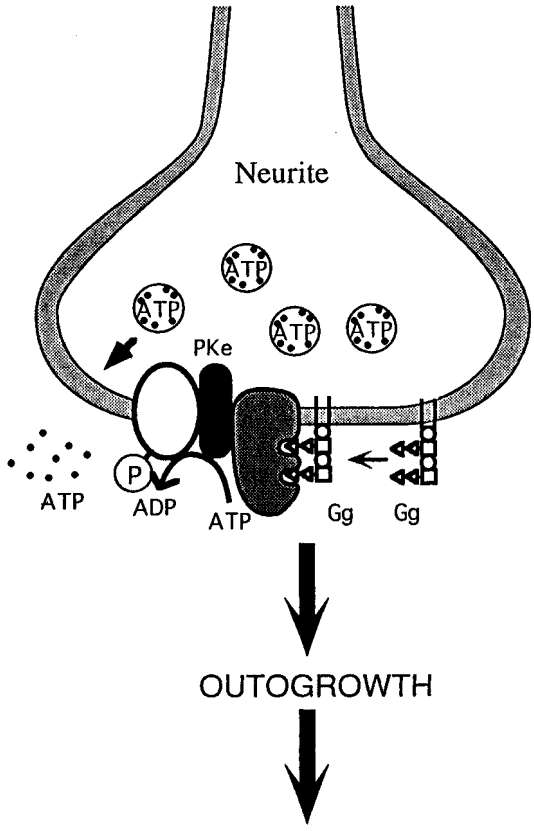

Fig. 4

from rigorously controlled template-mediated biosynthetic mechanism as seen in those cases of protein and nucleic acid biosynthesis. Synapses are known to be enriched with glycoconjugates but little is known about chemistry and biochemistry of these glycoconjugates.

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