Published in final edited form as:

Biochim Biophys Acta. 2008 March ; 1780(3): 325–346.

Structure and function of glycosphingolipids and sphingolipids: Recollections and future trends

Sen-itiroh Hakomori

Division of Biomembrane Research, Pacific Northwest Research Institute, Seattle, WA 98122, USA; and Departments of Microbiology and Pathobiology, University of Washington, Seattle, WA 98195, USA, email hakomori@u.washington.edu; ph 206-726-1222, fax 206-726-1212

Abstract

Based on development of various methodologies for isolation and characterization of glycosphingolipids (GSLs), we have identified a number of GSLs with globo-series or lacto-series structure. Many of them are tumor-associated or developmentally-regulated antigens. The major question arose, what are their functions in cells and tissues? Various approaches to answer this question were undertaken. While the method is different for each approach, we have continuously studied GSL or glycosyl epitope interaction with functional membrane components, which include tetraspanins, growth factor receptors, integrins, and signal transducer molecules. Often, GSLs were found to interact with other carbohydrates within a specific membrane microdomain termed "glycosynapse", which mediates cell adhesion with concurrent signal transduction. Future trends in GSL and glycosyl epitope research are considered, including stem cell biology and epithelial-mesenchymal transition (EMT) process..

Keywords

glycosyl epitope; glycosphingolipid; ganglio-series; globo-series; lacto-series; tumor-associated antigen; developmentally-regulated antigen; contact inhibition; cell growth; cell motility; cell adhesion; signal transduction; carbohydrate-carbohydrate interaction; microdomain; glycosynapse; sphingosine-dependent kinase; protein kinase C δ ; tetraspanin; growth factor receptor; integrin; epigenetic regulation; stem cell; niche; epithelialmesenchymal transition (EMT)

I. Introduction

Professor Carl G. Gahmberg, the Editor of this specialized volume on membrane biology and glyco-sphingobiology, asked me to write a review article for this volume. I understand that the review is to be based on the topics which I presented at the beginning of the Glyco-sphingobiology symposium organized by Professors Roger A. Laine, Reiji Kannagi, and Yasuyuki Igarashi, at Tokushima, Japan, Feb 26 - Mar 01, 2007. The context of my talk was a retrospective of studies with many colleagues, mainly on glycosphingolipids (GSLs), during the past 50 years, with some comment on future trends in this area.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Glycosphingolipids are abbreviated as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*Biochem J* **171**: 21–35, 1978, Table I); however, the suffix -Ose or -OseCer is omitted. Ganglio-series gangliosides are abbreviated according to the extended version of Svennerholm's list (e.g., Holmgren et al., *Proc Natl Acad Sci USA* **77**: 1947–1950, 1980).

I started my research career (late 1950s) with characterization of blood group antigens with "lipoid nature" [1], which were then highly ambiguous, since it was difficult to purify them and characterize their properties. Struggling in purification and characterization of "lipoid antigens", it was fortunate that many ways were opened through development of new technologies, which are briefly mentioned in Sec. II-ii. "Lipoid antigens" are now identified as complex GSLs with many defined carbohydrate sequences [2]. A large number of GSLs with new structures, including developmentally-regulated and tumor-associated antigens, have been characterized (see Sec. II).

A series of studies initiated by Dr. Shoei Iseki (Gunma Univ., Gunma, Japan) [3], and Drs. Salvador Luria, Phil Robbins, and colleagues (MIT, Boston, MA, USA) [4,5], described dramatic changes in bacterial phenotype (*e.g.*, growth behavior, and colony shape) through structural changes of cell surface lipopolysaccharides by phage-induced lysogenic transformation. It was a great impact for me to learn of a realistic role of cell surface glycans in control of cellular phenotype.

These studies with bacteria provided a new concept that cell surface glycans, even in eukaryotic cells, may define various cellular phenotypes, including oncogenic transformation and development. The first clear evidence for dramatic changes of GSLs in hamster and mouse fibroblasts associated with oncogenic transformation by DNA virus was provided by us [6] and others [7]. These studies, together with various others (see Sec. III), based on dynamic glycosyl response upon cell contact, led to the concept of cell "sociology", as first termed by Herman Kalckar [8]. However, it took close to 40 years to substantiate the functional role of cell surface glycosylation, which causes contact inhibition of cell growth (see Sec. IV-i).

All our studies so far on functional roles of glycosyl epitopes in GSLs and glycoproteins are based on their *cis* or *trans* interaction with membrane proteins, to modulate cell adhesion and signal transduction, which are the central theme of this review.

II. Studies on basic structures of glycosphingolipids

i. Structures of GSLs known up to early 1960s

Isolation and characterization of molecular components present in cells and tissues were the major theme in classic biochemical science at the early stage of development. The same situation applied to GSLs. Only cerebroside (GalCer and GlcCer), sulfatide, LacCer, brain gangliosides, and GSLs with tetrasaccharide composition, later termed "globoside", were known by the early 1960s, through pioneering studies by G. Blix, E. Klenk, T. Yamakawa, and R. Kuhn. Readers will find these classic studies described in a few monographs [9–11]. Major events such as discovery of sialic acid, gangliosides, globoside, and their composition and structures, are summarized in Table 1.

ii. Basic methods developed for determination of glycosyl structures in GSLs and other glycoconjugates

These methods, developed during the early 1960s to 1970s, as listed in Table 2, greatly facilitated our studies.

Methylation analysis for determination of sugar linkages, furanoside vs. pyranoside distinction, and branching points, has a long history. But the methylation reaction *per se* is sluggish, taking a long time to complete, and methods for identification of partially O-methylated sugars were difficult. It was a coincidental occasion that an effective methylation method was developed in 1964 [25], and well-designed gas chromatography-mass spectrometry (GC-MS) equipment was commercially available at that time. Thus, identification of partially O-methylated sugars was performed with great success for neutral

sugar samples [26]. However, yield of aminosugar derivative after permethylation was unsatisfactory, for the reason described in Table 2. This problem was solved by application of acetolysis, followed by hydrolysis and reduction, which greatly enhanced yield of partially O-methylated hexosaminitol [27–29].

Electroimpact MS of entire permethylated GSL was found to be useful for sequence analysis [30]. This was the first example of total MS of permethylated glycan. The approach is widely used nowadays.

Analysis of anomeric linkages and sequences. Well-qualified exoglycosidases such as jackbean β -N-acetylglucosaminidase [31], jackbean β -galactosidase [32], fig α -galactosidase [33,34], α -N-acetylgalactosaminidase of pig liver [35], and many other enzymes are useful for sequential analysis of various types of GSLs and glycosyl residues. As an example, complete anomeric structure and sequence of Gb4, Gb3, and Forssman antigen have been identified by use of these enzymes [36,37].

Release of oligosaccharide from GSL. This was accomplished by oxidation of olefinic double bond of Sph, followed by alkaline β -degradation. Originally, ozonolysis [38] and periodate/osmium tetroxide [39] were used. Finally, a simpler procedure based on [39] with better yield was described [40]. These methods are useful for identification of oligosaccharides, for not only chemical but also immunological or cell biological purposes. A novel ceramidase capable of releasing oligosaccharide from ceramide is available, *e.g.*, [41,42]. These enzymes are highly useful to separate oligosaccharides from GSLs, if carbohydrate chain length is relatively short. There is a limitation if carbohydrate chain length is >6–8 monosaccharides.

iii. Characterization of GSLs with ganglio-, lacto-, and globo-series

Ganglio-series—Brain gangliosides are characterized by the presence of common core structure GalNAc β 4Gal and/or ganglio-N-tetraosyl core (Gal β 3GalNAc β 4Gal β 4Glc) [23], and one or two α 2-3NeuAc linked to internal or terminal Gal, or both [24] (see Table 1). Ganglio-series gangliosides are based on such core structures, and are basically different from globo-series or lacto-series structures as described below. Our lab has not been involved in structural analysis of ganglio-series gangliosides.

Globo-series—Presence of ceramide tetrasaccharide as major component of human erythrocytes [43] was confirmed, and the compound was termed "globoside" since it was obtained as a globular, birefringent precipitate [44]. In contrast to brain gangliosides, carbohydrate composition and sequence of globoside (Gb4) were clearly distinct, and the sequence GalNAc $\rightarrow$ Gal $\rightarrow$ Gal $\rightarrow$ Glc $\rightarrow$ Cer was proposed [45]. Globotriaosylceramide (Gb3), accumulated in Fabry's Disease, was found to have the structure Gal α 1-4Gal β 1-4Glc β 1-Cer, and this structure is the inner core of all globo-series GSLs [34, 36]. Sequence of sugars, and position of sugar linkages in globoside, were determined previously [45,46]. Complete globo structure was identified as GalNAc β 1-3Gal α 1-4Gal β 1-4Glc β 1-Cer, by a combination of enzymatic hydrolysis and NMR spectroscopy [36].

Heterogenetic Forssman antigen had been known as GSL [47], and was found to have similar carbohydrate composition as globoside, but with α -GalNAc in contrast to β -GalNAc [48]. In subsequent studies, structure of Forssman antigen was established as GalNAc α 3GalNAc β 3Gal α 4Gal β 4Glc β 1-Cer [37]. In some cases, the Forssman epitope (GalNAc α 3GalNAc β 3Gal α 4 $\rightarrow$ R) was found to be carried by other structures [49].

Rat renal cell carcinoma has a novel GSL similar to but claimed to antigenically different from globoside ("cytolipin R") [50]. This GSL was characterized as having position of internal α -

Gal different from globoside, i.e., GalNAc β 1-3Gal α 1-3Gal β 1-4Glc β 1-Cer, and was termed "isogloboside" [51,52].

Extended globo-series antigens, i.e., Gal β 1-3, Fuc α 1-2Gal β 1-3, or NeuAc α 2-3Gal β 1-3 linked to terminal GalNAc of Gb4, were isolated and characterized from human embryonal carcinoma as stage-specific embryonic antigen-3 and/or -4 [53,54]. These structures were highly expressed in early embryo or embryonal carcinoma of mouse, primates, and humans, and are assumed to be important functional molecules involved in defining stage of stem cell development [55]. Globo-series structures were all identified as listed in Table 3.

Lacto-series—Gangliosides having glucosamine, and releasing by ozonolysis an oligosaccharide similar to lacto-N-tetraose, were found in bovine erythrocytes and spleen [58]. Ganglioside with NeuAc α 2-3Gal terminus was found as major ganglioside in human erythrocytes, and a neutral GSL having the structure Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc β -Cer was found as cross-reactive with anti-pneumococcal type XIV antibody [59]. Le^a GSL antigen, Gal β 1-3[Fuc α 1-4]GlcNAc β 1-3Gal β 1-4Glc β -Cer [60] and/or Le^x GSL antigen, Gal β 1-4[Fuc α 1-3]GlcNAc β 1-3Gal β 1-4Glc β -Cer [28] were found to be highly accumulated in colorectal, gastric, and non-small cell lung cancer tissues. Some of these structures were characterized by novel ceramide having phytosphingosine and α -hydroxy fatty acid [28]. Later, blood group ABH antigens present in human erythrocytes were found to be carried by common structure Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc β -Cer [2]. These early studies clearly indicated the presence of GSLs with lacto-series type 1 and type 2 chain.

Since then, extensive further studies were directed to isolate a number of branched and unbranched lacto-series GSLs. In total, 15 novel types of lacto-series GSLs were isolated and characterized; some of them are listed in Table 4. Of particular interest is the presence of long, unbranched poly-LacNAc having terminal NeuAc linked to Gal, and internal GlcNAc with α 1-3Fuc, termed "myeloglycan" [61,62], and highly branched poly-LacNAc carrying multiple epitopes [63]. If the epitope is sialyl 2-3Gal at the I-terminus, as found in placenta, it reacts with mAb NUH2. If the epitope is Le^x, the branched glycan could be SSEA-1, presumably having multiple Le^x, although the exact structure of SSEA-1 remains to be elucidated.

IV. Characterization of GSL changes associated with oncogenic transformation, or with developmental processes

i. Blocked or incomplete synthesis, with or without accumulation of precursor

—Some GSLs are highly expressed in normal cells which are reduced or deleted in transformed cells. Typical examples: (a) GM3 highly expressed in BHK cells or chicken embryonic fibroblasts was reduced or deleted upon transformation by polyoma virus, whereby level of LacCer increased several-fold [6]. A more recent observation along this line is that GM3 highly expressed in mouse and chicken fibroblasts was reduced or deleted upon Jun-induced oncogenic transformation. In this case, transformed phenotype was reverted to that of normal cells by enhanced GM3 synthesis through GM3 synthase gene transfection [70]. (b) Higher gangliosides GD1a, GD1b, and GT1b were deleted, and precursor GM3 was enhanced, by SV40 transformation of 3T3 cells [7]. These changes were interpreted to mean that ganglioside synthase gene is blocked by transformation mechanism ("incomplete synthesis" model).

ii. Specific GSLs or glycosyl epitopes expressed in transformed cells through neosynthesis

—Gg3 expressed in normal mouse 3T3 fibroblast is minimal. The level was greatly enhanced by transformation by Kirsten strain murine sarcoma virus (Kmsv). Kmsv tumors grown in Balb/c mice expressed high-level Gg3, but Gg3 expression in various cells and tissues in normal Balb/c mice was highly limited, except for a few sites in spleen and gastrointestinal epithelia [71]. Similarly, Gg3 is highly expressed in murine leukemia L5178Y

grown in DBA/2 mice, and treatment of L5178Y leukemic mice with anti-Gg3 IgG3 mAb completely suppressed leukemia growth [72].

The concept of GSLs as tumor-associated antigens, expressed in experimental tumors as above, was further extended to various human tumors, when mAb approach was applied for characterization of human tumor-associated antigens (e.g., [73]). Gb3, a common core structure of globo-series GSLs, was highly expressed in Burkitt lymphoma, defined by IgM mAb 38-12 [74]. GD3 ganglioside is highly expressed in human melanoma, defined by mAb 4.2 [75] or R24 [76]. Extended Le^a (Le^a-on-Le^a) antigen [68] is highly expressed in gastric and colorectal cancer, defined by mAb ST-421 [69]. Disialyl type 1 chain (disialyl-Lc4) is highly expressed in colorectal cancer, defined by mAb FH9 [77]. These antigens are listed in Table 5. It is difficult, however, to assess whether appearance of these tumor-associated GSL antigens is due to precursor accumulation from incomplete synthesis, or to "neosynthesis" through activation of glycosyltransferase genes.

iii. Developmentally-regulated expression of GSLs or glycosyl epitopes—A few types of antigens expressed highly at a defined stage of embryonic development were identified by specific mAbs, and were termed "stage-specific embryonic antigens" (SSEA) [85,86]. These antigens were clearly identified as GSLs, or as glycosyl epitopes with carrier molecules whose properties are not fully characterized. Their structures and expression patterns at early stage of mouse embryogenesis are shown in Fig. 1.

SSEA-1, defined by mAb directed to F9 mouse embryonal carcinoma, is not expressed in 2–4 cell stage embryo, but is maximally expressed at 16–32 cell cleavage stage (morula), which induces Le^x-dependent adhesion in the presence of Ca²⁺, *i.e.*, "compaction", and declines in later stages [85,87]. The SSEA-1 epitope was clearly identified as Le^x glycan [87,88], and Le^x oligosaccharide, but not Le^a oligosaccharide, inhibited the compaction process [88,89]. SSEA-3 (Gb5 and globo-H) [54] or SSEA-4 (sialyl-Gb5) [53], defined by respective mAbs which were originally raised against 4- to 8-cell stage mouse embryo, are highly expressed in human embryonal carcinoma and in human embryonal stem cells [86]. In mouse embryo, SSEA-3 and -4 are maximally expressed at 4-cell stage, and decline later [86]. The functional roles of these GSLs remain to be studied. Preliminary studies indicate that SSEA-3 (Gb5 and globo-H) is involved in adhesion and signal transduction of human embryonal carcinoma cells; it is therefore assumed to be involved in adhesion and induced differentiation of human ES cells [55].

IV. Functional role of GSLs and glycosyl epitopes in control of cell growth, adhesion/ motility, and signal transduction

i. Two approaches used for functional analysis of GSLs and glycosyl epitopes—Two approaches as below have been successfully utilized.

a. Cell surface labeling with galactose oxidase, followed by Na[³H]BH₄: depicts the pattern of surface exposed Gal or GalNAc, and their changes associated with oncogenic transformation or other cellular phenotype [90]. Application of this method to distinguish cell surface profile of hamster fibroblast NIL and its polyoma transformant (NILpy) revealed the presence of a dominant peak (peak a, *i.e.*, "galactoprotein a") in NIL which was absent in NILpy [91]. In contrast, NILpy cells had a dominant peak (peak c, *i.e.*, "galactoprotein b") [91] (Fig. 2A-i). Change of glycoproteins corresponding to galactoprotein a and b in cell extract was further confirmed by slab gel electrophoresis with direct fluorography [92] (Fig. 2A-ii). The same protein, with molecular mass ~220 kDa, was detected by cell surface labeling with ¹²⁵I-lactoperoxidase [93]. A similar component was also found as "fibroblast surface antigen" [94]. These ~220 kDa glycoproteins were later collectively termed "fibronectin".

Subsequent extensive studies indicated that fibronectin has multiple functional domains with specific binding capability to heparin, collagen, cell surface integrin, fibrin, etc. [95] (Fig. 2A-iii). Galactoprotein b in NIL cells was identified based on its gene cloning as integrin $\alpha 3$ by Tsuji et al [96], who also found that the ligand for $\alpha 3$ is laminin-5 [97], originally termed "epiligrin" by Carter et al [98].

Gal oxidase approach for cell surface labeling was also used for GSLs, and showed distinctive differences between NIL and NILpy cells. nLc4Cer was only present, and labeled strongly, in NILpy cells [99]. Gal oxidase labeled GSLs of NIL cells were greatly affected by *Ricinus communis* lectin treatment of glycoprotein, indicating GSL crypticity is affected by organizational framework of membrane [100]. In general, cryptic GSLs such as Gb3Cer or Gg3Cer are greatly influenced by glycoprotein organization in membrane, since both Gb3Cer and Gg3Cer were well labeled by Gal oxidase method. Organizational status of these GSLs has been studied, as related to physiological difference of cell phenotypes [101,102].

b. Exogenous addition of GSLs, inducing phenotypic changes of cells: GSLs have the novel property to interact with cell surface membrane, and incorporate into membrane to affect cellular function and phenotype. The first such experiment was performed by Roger Laine using NIL cells with exogenous addition of globoside (Gb4), although the exact organizational status of GSLs in membrane was unknown at that time [103]. $\sim 8 \times 10^{-4}$ M Gb4 strongly inhibited NIL cell growth, but NILpy cell growth was less inhibited. Growth-inhibited NIL cells showed "contact-oriented" appearance, *i.e.*, cells were aligned in a certain direction, in contrast to randomly-oriented non-growth-inhibited cells. Effect of Gb4 on synchronized cell cycle of NIL cells was determined for cell number, mitotic index, and [3 H]thymidine incorporation. Globoside greatly reduced cell number at mitotic stage. Mitosis was not observed in Gb4-added cells. Therefore, peak of thymidine incorporation at N phase was abolished in Gb4-added cells (Fig. 2B).

This experimental design with exogenous addition of GSL to observe phenotypic changes has been often used subsequently in functional analysis of GSLs. Effects of GSLs are more clearly observable when cells are grown in serum-free condition, *i.e.*, chemically-defined media (*e.g.*, GM3 effect on fibroblast growth factor receptor in BHK cells [104]. Specific targets of GSL have been considered after the microdomain concept was introduced. *E.g.*, GM3 inhibited neuroblastoma cell growth by enhancing translocation of Csk into microdomain GEM, where Csk inhibit cSrc and other Src family kinases (Prinetti, Iwabuchi, & Hakomori [105]. A number of studies along this line have been performed since then, *e.g.*, effect of GSLs on contact inhibition of cell growth (see Sec. IV-ii); effect of gangliosides, particularly GM3, on growth factor (EGF, PDGF, FGF) receptors (see Sec. IV-iii); effect of TSP/ ganglioside complex on growth factor receptors (see Sec. IV-iv); etc.

Enhanced expression of specific GSLs can now be tested by transfection of specific glycosyltransferase genes. Effect of exogenous GSL can be confirmed by depletion of specific GSLs expressed in cells by RNAi approach (see Sec. IV-iv). initial experiments along this line were started by phenotypic changes of NIL cells by exogenous addition of Gb4.

ii. Contact inhibition of cell growth and motility is associated with cell surface glycosylation profile—Many studies indicate that oncogenic transformation is closely associated with incomplete synthesis of GM3, Gb4, or Forssman, in hamster fibroblasts BHK or NIL, or mouse or chicken fibroblasts (see Sec. III-i). This indicates that terminal sugar structure in glycosyl residue at the cell surface may function in maintenance of growth control in normal/ contact-inhibited cells. Loss of terminal sugar residue, associated with loss of contact inhibition in transformed cells, indicates a possibility that terminal sugar could be a

susceptible site for cell recognition. This hypothesis has not been unambiguously clarified, but is strongly supported by the following observations:

Confluent/ contact-inhibited BHK or NIL cells display enhanced synthesis of specific glycosyltransferase such as α -Gal transferase for synthesis of Gb3/ Gb4 is therefore enhanced [106]. Similarly, synthesis of Gb4, GM3, and Forssman was enhanced in non-transformed BHK or NIL cells when growth of cells was arrested at contact-inhibited state. In contrast, transformed cells displayed greatly reduced level of contact-sensitive GSL species such as Gb4, GM3, and Forssman [107–109]. α -Gal transferase response (for Gb3 synthesis) upon increase of cell population density, reflecting degree of cell contact, in contact-inhibitable BHK and NIL cells, and loss of this response in polyoma virus-transformed cells, is shown in Fig. 3. Loss of Gb3, Gb4, and Forssman in transformed NIL cells was also detected by cell surface labeling with galactose oxidase / NaB^3H_4 .

The mechanism by which cell contact induces inhibition of cell growth and motility through change of glycosylation status remains largely unknown. There is a high possibility that GSLs are complexed with TSP at the cell surface, and such complex interacts with growth factor receptors or integrins to induce signal transduction to inhibit cell growth or motility (see Sec. IV-iii).

iii. Tyrosine kinases associated with growth factor receptor (GFR) are inhibited by surrounding gangliosides—Studies described above (Sec. IV-i), which were performed during the early 1970s, suggested that GSLs may interact with unidentified functional membrane components, which in turn may cause changes in cellular phenotype. However, at that time, no realistic information on such functional components was available. It took almost 10 years for development of the current concept of growth factor receptors with tyrosine kinases [110–112], or integrin receptors defining cell adhesion and motility [113, 114]. In addition, for understanding GSL effect on cell growth in culture, basic knowledge on types of growth factors required for culturing specific types of cells was needed. This knowledge was acquired through extensive studies by G. Sato and colleagues, to establish chemically-defined media [115].

a. Fibroblast growth factor receptor: Thus, the first experiment on effect of GSL on BHK cell growth in chemically-defined media was undertaken. BHK cells require fibroblast growth factor (FGF) but not epidermal growth factor (EGF) or platelet-derived growth factor (PDGF). Only cells pre-incubated with GM3 displayed complete refractoriness of growth to FGF stimulation, whereby ^{125}I -labeled FGF was accumulated at the cell surface due to failure of internalization of FGF. Labeled FGF added to control BHK cells without GM3 pre-treatment was well internalized. It was assumed that high GM3 level blocked function of FGFR [104]. However, at that time, we had no knowledge on tyrosine kinase associated with FGFR; studies along this line were not performed until 20 years later [116].

b. PDGF receptor: Growth of mouse 3T3 cells depends on PDGF and its receptor. Therefore, effects of gangliosides on PDGF-dependent growth of 3T3 cells, and on tyrosine kinase associated with PDGFR, were studied. GM1 showed stronger inhibition of PDGF-dependent growth and PDGFR tyrosine kinase than GM3 [117]. Similar studies by Yates and colleagues indicated that GD1a, GD1b, and GT1b had much stronger inhibitory effect than other gangliosides on PDGFR tyrosine kinase [118].

c. EGF receptor: This line of studies was further extended to effects of gangliosides on EGF-dependent A431 cell growth, and on tyrosine kinase associated with EGFR. GM3, but no other GSLs, strongly inhibited EGF-dependent cell growth, and EGFR tyrosine kinase [119]. Since EGFR is highly expressed in various epidermal cancers, and its tyrosine kinase activity is

closely associated with cancer malignancy, a possibility was investigated whether any ganglioside could have better inhibitory effect than GM3 (see below).

d. Effect of lyso-GM3 and de-N-acetyl-GM3 on EGFR: Hanai et al. found that lyso-GM3 showed much stronger inhibitory effect than GM3 on EGFR tyrosine kinase *in vivo* as well as in membrane extract *in vitro*. Furthermore, lyso-GM3 was detected in normal A431 cells [120]. In contrast, exogenously added "de-N-acetyl-GM3" (GM3 having de-N-acetyl sialic acid) strongly promoted EGFR tyrosine kinase and promoted growth and motility of A431 cells [121].

Thus, effect of gangliosides on EGFR tyrosine kinase is more complex than originally considered. *I.e.*, (i) tyrosine kinase is modulated by GM3 when EGFR is activated by EGF under normal conditions; (ii) trace quantity of lyso-GM3 present, which may result from GM3 by de-N-acylation, strongly inhibits receptor function; (iii) de-N-acetylation of GM3 in resting A431 cells may promote cell growth, possibly through a channel different from simple activation of EGFR (see Fig. 4). Exogenous lyso-GM3 is highly cytotoxic, whereas lyso-GM3 dimer is not cytotoxic, but inhibits EGFR tyrosine kinase as strongly as lyso-GM3. Therefore, synthetic lyso-GM3 dimer has been studied for inhibition of EGFR activity and A431 cell growth, for the purpose of developing pharmacologically effective inhibitors of epidermal tumor cell growth [122].

iv. GSL/ tetraspanin complex inhibits integrin-dependent cell motility, and HGF-induced cell motility and growth—Tetraspanins (TSPs) are ubiquitously present in various normal cell membranes [123], and are known to interact with integrins and modulate cell motility [124]. TSP CD9 was found as a motility-modulating protein [125], and TSP CD82 was identified as cell motility inhibitory gene product KAI-1 [126,127]. Their expression is down-regulated in various types of human cancer, and survival of cancers patients is correlated with this reduced expression [127,128]. We now find that these TSPs are complexed with gangliosides, and their function is based on such complex.

a. Loss of GM3/ CD9 complex in Jun-induced oncogenic transformation, and reversion to normal phenotype by restoration of the complex: GM3/ CD9 complex inhibits integrin $\alpha 3$, $\alpha 5$, $\beta 1$ in mouse or chicken fibroblasts. GM3 synthase gene and GM3 levels were greatly reduced when these cells underwent Jun-induced oncogenic transformation. In this process, CD9 complex with GM3 was lost, leading to activation of integrin-dependent signaling to enhance cell motility, and consequent formation of large colonies in soft agar. When the transformed cells were transfected with GM3 synthase gene, and GM3 level was restored, oncogenic phenotype was reverted to normal cell phenotype, *i.e.*, colony formation in soft agar was inhibited as in non-transformed cells [70]. The mechanism of such reversion remains to be studied. The crucial factor in this process is GM3 level, but not CD9 or integrin level. It is hypothesized that GM3/ CD9 complex regulates integrin activity and integrin-dependent signaling. This possibility is supported by studies of a bladder cancer model.

b. Level of GM3/ CD9 complex in bladder cancer cells defines degree of malignancy: Benign, non-invasive bladder cancer cell line KK47 has high level of GM3/ CD9 complex, and displays low cell motility. In contrast, highly invasive bladder cancer cell line YTS-1 has very low level of GM3, and therefore GM3/ CD9 complex is absent. YTS-1 displays high cell motility, particularly when adhered to laminin-5 (LN5)-coated plate, which leads to $\alpha 3$ integrin-induced signaling. Exogenous GM3 added to YTS-1 causes reversion of highly invasive to non-invasive phenotype similar to that of KK47 [129]. GM3 depletion from KK47 by incubation of cells with GlcCer synthase inhibitor P4 causes conversion to YTS-1-like phenotype with high invasiveness. Low GM3 level activates cSrc, whereas high GM3 level causes Csk translocation into "glycosynaptic microdomain", with subsequent inhibition of

cSrc. Inhibition of cSrc by PP2 (the Src inhibitor) reduces motility. Thus, GM3 in microdomain plays a dual role in defining cell motility and invasiveness: (i) modulates interaction of $\alpha 3$ with GM3/ CD9 complex; (ii) activates or inhibits cSrc through translocation of Csk [130].

c. GM2 complexed with CD82 inhibits cell growth and motility: Growth and motility of many epithelial cells, and human cancers, are controlled by "scatter factor" or hepatocyte growth factor (HGF). This process involves HGF-induced activation of Met receptor kinase [131] and its functional interaction with $\alpha 3\beta 1$ integrin receptor [132]. The major difference between normal bladder epithelial HCV29 cells and highly malignant YTS-1 cells is the presence vs. absence of CD82; there is no difference in GM2 or GM3 level. Specific interaction of CD82 with GM2 but not with GM3 was demonstrated based on adsorption of CD82 to GM2-coated polystyrene beads. GM2/ CD82 complex interacts specifically with Met, and inhibits Met kinase. In addition, the complex blocks LN5-induced $\alpha 3\beta 1$ cross-talk with Met [132]. Other GSLs, including GM3, GM1, and Gb3, are incapable of forming a complex with CD82, and therefore have no effect on HGF-induced or $\alpha 3\beta 1$ -dependent signaling leading to cell growth/ motility [132].

V. Cell adhesion and signal transduction mediated by carbohydrate-to-carbohydrate interaction

Major processes of cell adhesion/ recognition have been considered to be mediated by: (i) Interaction between the same protein receptor, such as Ig superfamily protein receptors [133] and cadherin receptors [134], or between different protein receptors, such as integrins and extracellular matrix proteins [113,114], or leukocyte integrins (CD11/ CD18) and intercellular adhesion molecules (ICAM, VCAM) [135]. These mechanisms are basically protein-to-protein interaction (PPI) [133,136]. (ii) Glycosyl epitopes expressed in glycoprotein or GSL are recognized by carbohydrate-binding proteins through carbohydrate-to-protein interaction (CPI). The carbohydrate-binding proteins involved are galectins [137], selectins [138], and siglecs [139,140].

On the other hand, we and others have observed a third mechanism mediating cell adhesion, carbohydrate-to-carbohydrate interaction, as described below.

i. The novelty of carbohydrate-to-carbohydrate interaction (CCI), and two lines of well-studied CCI-based processes—PPI and CPI processes as in (i) and (ii) above are well-established. In contrast, a novel process based on carbohydrate-to-carbohydrate interaction (CCI) has been found as a basis of cell adhesion and, more recently, specific signal transduction. Two lines of studies clearly established the occurrence of CCI mediating adhesion of mouse embryonal cells, by us [87,141,142], and CCI mediating species-specific autoaggregation of sponge cells, by Max Burger's group [143–145]. The former was based on Le^x -to- Le^x interaction; the latter was based on 3-sulf-GlcNAc $\beta 3$ Fuc α or Gal $\beta 4$ GlcNAc $\beta 3$ Fuc α as a unit oligosaccharide linked to proteoglycan. In both Le^x glycan interaction and sponge cell glycan aggregation, the adhesion process requires Ca^{2+} , and is abolished in the presence of EDTA (Fig. 5).

ii. Occurrence of various CCI based on GSL-to-GSL interaction—CCI may occur widely, from primitive marine animals to mammalian cells, although well-established cases are limited, and we assume there are a number of undiscovered cases of CCI. Using purified GSLs isolated from various mammalian tissues and cells, preliminary studies have been conducted regarding possible occurrence of homotypic or heterotypic CCI, besides Le^x -to- Le^x as above. Strong CCI was found between GM3-Gg3 [146,147], and sulfatide-GalCer [148,149]. Interactions between H- Le^y , H-H [150], Gb4-Gb5, Gb5-Gg3, and Gb5-nLc4 [55] have been suggested, although unambiguous chemical/ physical basis, and physiological

significance of such interaction, remain to be studied. Interactions of various CCI based on GSL-to-GSL interaction are summarized in Fig. 6.

iii. GSL interactions with N-linked glycans—More recently, studies were extended to GSL interactions of N-linked glycans of glycoproteins. We found that GM3 bound strongly to a specific complex-type N-linked glycans with 5–6 GlcNAc termini (*e.g.*, "Os. Fr.B"), to a lesser degree to glycans with 3–4 GlcNAc termini, and essentially did not bind to glycans with 2 GlcNAc termini [151]. GM3 interaction with EGFR, which has been known for years (see Sec. VII-ii), is now shown to be based on interaction of GM3 with N-linked glycans having multiple GlcNAc termini, since: (i) GM3 interacts with EGFR, as demonstrated by binding of EGFR to GM3-coated polystyrene beads, and this interaction is inhibited by Os Fr.B; (ii) Os Fr.B blocks the inhibitory effect of GM3 on activation of EGFR; (iii) swainsonine-treated A431 cells, which express more terminal GlcNAc, show enhanced inhibition of GM3 on EGFR activation [152].

iv. Interactions of specific N-linked glycans among themselves—Recently, complex-type N-linked glycans similar to Os Fr.B as above were found to interact among themselves (self-recognition). This interaction was revealed by strong binding of Os Fr.B conjugated to aminoceramide, to plates coated with the same Os Fr.B/ aminoceramide conjugate [153].

VI. Microdomains displaying GSL- or glycosylation-dependent adhesion and signal transduction

The essential function of GSLs in eukaryotic and animal cells is their strong ability to interact with specific functional proteins (see Sec. III and IV). In addition, GSL clusters in membrane are capable of binding among themselves, or to N-linked glycans (see Sec. V). Such property of GSLs is based on their clustering ability (*cis*-CCI), to form microdomains in which TSPs, integrins, growth factor receptors, and signal transducers (such as cSrc, Src family kinases, RhoA, Ras) are also present, and which are involved in GSL- or glycosylation-dependent adhesion, signal transduction, and changes in cellular phenotypes such as cell growth, motility, and differentiation. Such microdomains are distinct from "lipid raft" [154] in the following properties: (i) they are cholesterol-independent, and are often resistant to cholesterol-binding reagents such as nystatin, filipin, and β -cyclodextrin; (ii) they often depend on TSPs, and are solubilized together with TSPs in organic solvents, *i.e.*, lipophilic property depends on TSP rather than cholesterol. These distinctive properties of such microdomains vs. "lipid raft" are summarized in Table 6. Such microdomains showing a close association with glycosylation function were termed "glycosynapse" [155,156], in analogy to "immunological synapse" [157], the microdomain of immunocytes involved in adhesion of T-cells or B-cells to antigen-presenting cells.

VII. Studies on sphingolipids controlling cellular phenotype

Following studies by Bell, Hannun, and Merrill, indicating that sphingosine (Sph) inhibits protein kinase C (*i.e.*, PKC α and β) [158,159], we found that D-*erythro*-N,N-dimethyl-Sph (DMS) displayed much stronger inhibitory effect than Sph on conventional PKC [160]. Sph and DMS also displayed enzyme-specific and substrate-specific inhibition of c- and v-Src kinases determined with α - and β -casein, respectively. Sph, DMS, GM3, and psychosine also inhibited Src kinases [161].

The effect of Sph is unstable, since Sph is converted to ceramide (Cer) or Sph-1-phosphate (Sph-1-P). Therefore, the real Sph effect is often difficult to interpret in terms of biological significance. In contrast, the effects of DMS, and its analogue N,N,N-trimethyl-Sph (TMS), are assumed to be stable, since they are not converted to Cer or Sph-1-P. Sph, DMS, and TMS

inhibit tumor cell metastasis [162–164], as well as inflammatory processes [165,166], because of their strong inhibitory effect on activation of endothelial cells or platelets. Tumor cells or myelocytes secrete lymphokines (*e.g.*, TNF- α), which stimulate endothelial cells or platelets to induce *de novo* synthesis of E-selectin, or to translocate P-selectin to the cell surface. This process leads to adhesion of tumor cells or myelocytes to endothelial cells or platelets through E- or P-selectin, to initiate tumor cell metastasis or inflammatory processes. Since PKC plays an essential role in signal transduction through lymphokines, to activate endothelial cells or platelets, DMS/ TMS inhibit tumor cell metastasis and inflammation. DMS/ TMS were more effective inhibitors of PKC than H-7 or calphostin, the well-established PKC inhibitors [165].

Effects of sphingolipids as modulators of signal transduction were greatly progressed by a series of studies focused on Cer or Sph-1-P, rather than Sph, during the early 1990s. It is generally accepted that Cer inhibits cell growth and induces apoptosis [167–172], whereas Sph-1-P, the first Sph catabolite, promotes cell growth (*e.g.*, for 3T3 fibroblasts), induces differentiation (for endothelial cells), or inhibits motility (for B16 melanoma cells), depending on the type of Sph-1-P receptor [173–178].

Separately from this major trend of sphingolipid studies focused on Cer and Sph-1-P, we performed studies on functional roles of Sph and DMS in activation of various protein kinases. Protein kinases activated only by Sph or DMS, but not by Cer, Sph-1-P, or twelve other sphingolipids, phospholipids, and glycerolipids, were termed "Sph-dependent kinases" (SDKs) [179,180]. Three groups of SDKs have been separated, based on difference of substrate and their specificity.

i. SDK-1. Sph or DMS activates caspase-3 through activation of a "cascade" of caspases. Caspase-3 hydrolyzes full-length PKC δ into regulatory domain (N-terminal half) and kinase domain (C-terminal half). The kinase domain of PKC δ *per se* is SDK-1 [181], which was known to phosphorylate 14-3-3 at interfacing Ser (Ser60 of β , Ser59 of η , and Ser58 of ζ isoform) to induce 14-3-3 dimer formation. 14-3-3 dimer inhibits anti-apoptotic Bcl-2 at mitochondrial membrane (*i.e.*, promotes apoptosis) [180]. SDK-1 effect is summarized in Table 7. Apoptotic stimuli (*e.g.*, exposure of cells to serum-free condition) induce a rapid Sph increase within 30 min, which induces multiple mechanisms, such as translocation of PKC δ to mitochondrial membrane, activation of caspase-3, release of SDK-1 through hydrolysis of PKC δ , 14-3-3 dimer formation through phosphorylation of interfacing 14-3-3, its interaction with mitochondrial membrane, and with cell surface integrin, etc. [181,182]. These mechanisms are summarized schematically in Fig. 7.

ii. SDK-2 phosphorylates calreticulin (50 kDa) and protein disulfide isomerase (PDI) (55 and 58 kDa) [183].

iii. SDK-3 is associated with glucose-regulated proteins (GRPs) and heat shock proteins (HSPs), and is co-purified with them during separation. Purified SDK3 separated on Q-Sepharose column specifically phosphorylates GRP94, GRP105, and HSP86 [183].

Both SDK-2 and SDK-3 are chaperone proteins associated with endoplasmic reticulum. The cell biological significance of SDK-2 and SDK-3 requires extensive further study.

VIII. Future trends

Based on our current knowledge of functional roles of glycosyl epitopes in GSLs and glycoproteins, future trends of studies can be predicted. A few such trends, described below, are simply presented for my own "memorandum", while I doubt that such predictions are well justified.

◆ **Trends of studies on cell recognition through glycosyl epitopes**—The basic function of glycosyl epitopes in GSLs and glycoproteins is cell recognition, which consists of weak cell-cell tethering to strong cell-cell binding or cell-substratum binding, and subsequent signal transduction to alter cell phenotype. The mechanisms of these processes are summarized in Table 8. Among these, item 1, carbohydrate binding by protein (CPI), and item 2, carbohydrate modulating protein receptor function, which is essentially PPI, have been well studied. In contrast, item 3, carbohydrate-to-carbohydrate interaction (CCI) (see Sec. V) has been studied to some extent, but what is known is only a small fragment. A number of unknown processes based on CCI remain to be studied. Regarding Item 4, specific oligosaccharides are known to bind DNA with distinct target sequence, and such oligosaccharides have the ability to interfere with transcription factor function [184]. On the other hand, specific DNA sequences termed "aptamers" bind with high selectivity and affinity to cellobiose, but not to lactose, maltose, or gentiobiose [185]. These are the only studies I know so far, but the results indicate a tremendous future for functional role of carbohydrates in regulation of transcription or other processes.

◆ **Molecular assembly and organization of glycosyl epitopes in membrane microdomains**—All the processes listed in Table 8 take place in membrane microdomains where glycosyl epitopes as GSLs or as glycoproteins are organized with specific membrane receptors, auxiliary protein TSP, or signal transducers. So far, a few microdomains are distinguished, such as (i) "lipid rafts" as signaling platforms which are strongly affected by cholesterol; (ii) "immunosome" for immunocyte adhesion and signaling, typically observed for T-cell receptors in T lymphocytes and antigen-presenting cells; (iii) "glycosynapse" as functional units displaying glycosylation-dependent adhesion and signal transduction. Since the organizational framework of glycosyl epitopes in GSLs and glycoproteins, with various types of membrane receptors and signal transducers, is increasingly important, another major future trend will be detailed studies on membrane microdomains.

Our knowledge of microdomains has not been progressed for the past 15 years, since available methodology to separate different microdomains is highly limited. What we really need is to create new methods to distinguish subtle differences of microdomains. This is possible only through advancement of biophysical approaches.

◆ **Genetic and epigenetic regulation of glycosylation**—Glycosylation is controlled by (a) well-established glycosyltransferase genes, and their transcription factors involved in regulation of glycosyltransferase expression; and (b) less-studied epigenetic mechanisms through (i) methylation or acylation of coding nucleosome DNA, which controls phenotypic expression of mRNA, and (ii) non-coding DNA, which is outside the genomic DNA encoding small non-coding RNA such as "riboswitch" and "microRNA" [186,187].

The area (a) has been extensively studied, and most of the mechanisms of transcription factors are known. Genes for glycosyltransferases, sugar nucleotide synthases and transporters, and sulfotransferases have been extensively studied; the total number of such "glyco-genes" is ~180 so far. In contrast, genes for glycosylhydrolases are also present in large numbers, and many of them remain to be studied.

The area (b-i) is in the early stage of study; further extensive studies are required for epigenetic control mechanisms of each type of glycosyltransferase gene. The area (b-ii), through small non-coding RNA, may affect expression of "glyco-genes". This area is totally unknown, and remains entirely for future study.

◆ **Genetically-defined glycosylation differences that may cause disease susceptibility**—A few cases have been known:

i. Possible expression of incompatible blood group P¹PP_k antigen in gastric cancer of a patient with blood group pp: A blood group *pp* patient with gastric cancer had anti-P¹PP_k antibodies (then termed Tj^a) in serum; mistaken blood transfusion caused disappearance of cancer; the cancer tissue had P¹-like antigen [188].

ii. Nonsecretor has much higher urogenital *E. coli* infection than secretor: Nonsecretor patients expressed sialyl-Gb5 epitope (NeuAc2-3Galβ3GalNAcβ3Galα4Galβ→R) in urogenital epithelia, to which infectious *E. coli* could bind, since it had "adhesin" to recognize this epitope. In contrast, **secretor** patients expressed globo-H and globo-ABO epitope (Fuc1-2Galβ3GalNAcβ3Galα4Galβ→R), to which infectious *E. coli* did not bind. Thus, nonsecretors have 4–5x higher *E. coli* infection in urogenital epithelia, particularly in women [189,190].

iii. Blood group A or B expression in tumors suppresses tumor cell malignancy: Expression of blood group A or B determinants in various tumors is often reduced or deleted, which promotes tumor cell malignancy [191,192]. A group of lung cancer patients with blood group A showed much higher survival rate when A expression in tumor was continued, than those in whom A expression in tumor was deleted [192]. Transfection of A or B gene in colonic cancer cell lines greatly reduced integrin-dependent tumor cell motility and cell growth [193, 194].

iv. Infertility in women is caused by antibodies directed to glycosyl epitope of sperm cells carried by male-specific CD52: Based on long-standing studies, a significant number of infertility cases were found to be associated with presence of anti-sperm antibodies in patient sera, which inhibit sperm cell motility in the presence of complement [195]. Subsequently, human mAb H63C4 was established based on fusion of lymphocytes of a patient (having high anti-sperm titer) with mouse myeloma NS1. The antigen epitope of H63C4 was identified as i, sialyl-i, and sialyl-I [196]. Previously-established anti-sialyl-I mAb NUH2 [197] also inhibited sperm cell motility. The antigen present in sperm cells was recently identified as male-specific CD52, the GPI-anchored glycan through a short peptide to which N-linked glycans, sialyl-i and sialyl-I, are linked [198,199] (Fig. 8).

The line of these studies as exemplified in items i–iv above will be increasingly important. More effort should be focused to find differences in glycosylation correlated with development of specific diseases, such as diabetes, autoimmune diseases, and diseases caused by degenerative processes with unclear etiology (*e.g.*, arthritis, Alzheimer's, Parkinson's).

♦ Possible role of glycosylation in stem cell biology

Stem cells with multi-differentiation ability are found in early embryo, and display two distinct processes: (i) differentiate into various tissues, organs, and eventually individuals; or (ii) lose differentiation ability to form embryonal carcinoma cells, and grow *in vivo* as malignant cancer to kill the host. Process (i) vs. (ii) is determined by difference in microenvironment of stem cells. The process (i) is provided by contact/ adhesion of stem cells with surrounding "niche", which promotes differentiation with normal cell cycle and growth. Process (ii) fails to provide the same microenvironment as in process (i), presumably due to absence of similar adhesion with "niche" as in process (i), which induces de-differentiation and uncontrolled cell growth.

Cells similar to embryonal stem cells are found in various adult tissues and organs. Properties of such "adult stem cells" are maintained by adhesion with surrounding niche cells, to display restricted, regulated growth as in normal cells in normal tissues. "Niche", a group of cells that maintains stem cell function through their adhesion, activates various signaling molecules such

as Notch, TBGF, Wnt, and BMP (bone morphogenetic protein), although the molecular mechanism of adhesion remains unclear (for review see [200,201]).

A major question at this time is whether glycosylation plays a key role in defining stem cell function, and their adhesion to induce activation of signaling molecules as above. Mouse embryonal stem cells express glycosyl epitopes SSEA-1 [87], -3, and -4 [53,54], which may mediate adhesion of stem cells to induce differentiation. Recent studies indicate that a group of cells in neuronal tissues expressing SSEA-1 is correlated with stem cells [202], and glycosyl expression changes during the 12th through 28th day of differentiation, as defined by many antibodies and lectins [203,204]. However, critical studies on functional role of glycosylation which may mediate adult stem cell adhesion, with subsequent differentiation, have not been performed.

Studies of stem cell processes and their molecular mechanism will provide answers to two basic questions: (i) how cells initiate multiple directions of differentiation, to form specific types of cells with specific functions; (ii) whether cancer cells are derived from adult stem cells when the microenvironment changes, according to the "Cohnheim hypothesis" [205]. Question (ii) needs some explanation. Under this hypothesis, oncogenic phenotype of stem cells can be inhibited when stem cells are "strayed" in tissues and placed in a certain microenvironment. Oncogenic phenotype is "awaken" when the stem cells are exposed to a different environment; see also [201]. According to this hypothesis, genetic mutation, activation of oncogenes, or inhibition of anti-oncogenes are not necessary. Rather, epigenetic changes caused by the microenvironment are sufficient to induce oncogenic phenotype. Cohnheim's view, which was forgotten for over 130 years, is now revived by increasing evidence for epigenetic conversion and reversion of oncogenic phenotype (*e.g.*, [70,130,206–208]). It should be noted that malignancy of mouse embryonal carcinoma cells was completely lost, and cells differentiated into "perfect mice", when embryonal carcinoma cells were placed in blastocyst. This surprising observation, shown by Beatrice Mintz 30 years ago [209,210], is now clearly explained.

♦ Epithelial-mesenchymal transition (EMT): Is there any glycosylation effect?

The EMT, originally discovered by Elizabeth Hay [211], is the major target mechanism of oncogenic transformation and developmental process [212,213]. The majority of tumors are derived from epithelial cells, and the process of oncogenic transformation is regarded as EMT. A number of current studies suggest that cancer cells display properties similar to those of "myofibroblasts" that underlie epithelial cells. "Cancer-activated fibroblasts" result from EMT, and are characterized by down-regulation of E-cadherin, up-regulation of N-cadherin, vimentin, and oncofetal fibronectin, and different susceptibility to various growth factors, particularly TGF- β , chemokines, and ECM-degrading proteases; in many cases, EMT is induced by TGF- β [213,214]. Interestingly, expression of oncofetal fibronectin with change of III-CD is based on site-specific O-glycosylation change [215].

So far, the functional role of glycosylation in EMT processes, according to the EMT definition, has not been systematically studied. Morphology and HGF-dependent growth of normal bladder epithelial cells were converted to HGF-independent, highly malignant cellular phenotype by deletion of TSP CD82 by RNAi, whereby ganglioside GM2 complexed with CD82 was lost, and epithelial morphology was converted to fibroblastic morphology [132]. This oncogenic conversion could be EMT. On the other hand, reversion of tumor cells to normal cells can be observed as discussed in Sec. IV-iii, in which TSP CD9 or CD82 complexed with ganglioside GM3 or GM2 inhibits GFR or integrin receptor. The process is therefore ganglioside-induced inhibition of GFR and integrin.

Acknowledgments

I hereby acknowledge with sincere thanks and appreciation the many scientists with whom I have worked over the years, in the studies cited in this review. This article was written in response to the kind invitation of Professors Carl Gahmberg (Univ. of Helsinki) and Roger Laine (Louisiana State Univ.). Many of the studies cited in this paper were supported by the U.S. National Institutes of Health, and in part by Otsuka Pharmaceutical Co., Japan. I appreciate the help of Steve Anderson in preparation of the manuscript and figures.

References

1. Hakomori S. Blood group lipid from human liver: III. Tohoku J.Exp.Med 1954;60:331–345. [PubMed: 14359303]
2. Hakomori S, Strycharz GB. Investigations on cellular blood group substances: I. Isolation and chemical composition of blood group ABH and Le^b isoantigens of sphingoglycolipid nature. Biochemistry 1968;7:1279–1286. [PubMed: 5677817]
3. Iseki S, Sakai T. Artificial transformation of O antigens in *Salmonella* E group: II. Antigen-transforming factor in bacilli of subgroup E₂. Proc.Japan Acad.Sci 1953;29:127–131.
4. Uetake H, Luria SE, Burrous JW. Conversion of somatic antigens in *Salmonella* by phage infection leading to lysis or lysogeny. Virology 1958;5:68–91. [PubMed: 13519750]
5. Uchida T, Robbins PW, Luria SE. Analysis of the serologic determinant groups of the *Salmonella* E-group O-antigens. Biochemistry 1963;2:663–668. [PubMed: 14075095]
6. Hakomori S, Murakami WT. Glycolipids of hamster fibroblasts and derived malignant-transformed cell lines. Proc.Natl.Acad.Sci.USA 1968;59:254–261. [PubMed: 4298334]
7. Mora PT, Brady RO, Bradley RM, McFarland VW. Gangliosides in DNA virustransformed and spontaneously transformed tumorigenic mouse cell lines. Proc.Natl.Acad.Sci.USA 1969;63:1290–1296. [PubMed: 4311252]
8. Kalckar HM. Galactose metabolism and cell "sociology". Science 1965;150:305–313. [PubMed: 5319435]
9. Thierfelder, H.; Klenk, E. Die Chemie der Cerebroside und Phosphatide. Berlin: Verlag von Julius Springer; 1930.
10. Yamakawa, T. Glycolipids of mammalian red blood cells. In: Schute, E., editor. Lipoide: 16 Colloquium der Gesellschaft Physiologische Chemie. Berlin: Springer-Verlag; 1966. p. 87-111.
11. Hakomori, S. Chemistry of glycosphingolipids. In: Kanfer, JN.; Hakomori, S., editors. Sphingolipid biochemistry. New York: Plenum Press; 1983. p. 1-165.
12. Blix G. Über die Kohlenhydratgruppen des Submaxillarmucins. Hoppe-Seyler's Z.Physiol.Chem 1936;240:43–54.
13. Blix G, Svennerholm L, Werner L. The isolation of chondrosamine from gangliosides from submaxillary mucin. Acta Chemica Scandinavica 1952;6:358–362.
14. Walz E. Über das Vorkommen von Kerasin in der normalen Rindermilz. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 1927;166:210–222.
15. Levene PA, Landsteiner K. On some new lipoids. J.Biol.Chem 1927;75:607–612.
16. Klenk E. Über die Natur der Phosphatide und anderer Lipoide des Gehirns und der Leber bei der Niemann-Pickschen Krankheit. Hoppe-Seyler's Z.Physiol.Chem 1935;235:24–36.
17. Blix G. Einige Beobachtungen über eine hexosaminhaltige Substanz in der Protagonfraktion des Gehirns. Skandinavische Archiv für Physiologie 1938;80:46–51.
18. Klenk E. Neuraminsäure das Spaltprodukt eines neuen Gehirnlipoids. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 1941;268:50–58.
19. Klenk E. Über die Ganglioside des Gehirns bei der infantilen amaurotischen Idiotie vom Typ Tay-Sachs. Berichte der Deutschen Chemischen Gesellschaft 1942;12:1632–1636.
20. Klenk E. Über die Ganglioside, eine neue Gruppe von zuckerhaltigen Gehirnlipoiden. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 1942;273:76–86.
21. Klenk E. Zur Kenntnis der Ganglioside. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 1951;288:216–220.

22. Yamakawa T, Suzuki S. The chemistry of lipids of posthemolytic residue or stroma of erythrocytes: I. Concerning the ether-insoluble lipids of lyophilized horse blood serum. *J.Biochem. (Tokyo)* 1951;38:199–212.
23. Kuhn R, Wiegandt H. Die Konstitutionen der Ganglio-N-tetraose und des Ganglioside GI. *Chem.Bericht* 1963;96:866–880.
24. Kuhn R, Egge H. Über Ergebnisse der Permethylierung der Ganglioside GI und GII. *Chem.Berichte* 1963;96:3338–3348.
25. Hakomori S. A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *J.Biochem. (Tokyo)* 1964;55:205–208. [PubMed: 14135466]
26. Bjorndal H, Hellerqvist CG, Lindberg B, Svensson S. Gas-liquid chromatography and mass spectrometry in methylation analysis of polysaccharides. *Angew.Chem.Intl.Ed.Engl* 1970;9:610–619.
27. Hellerqvist CG, Lindberg AA. Structural studies of the common-core polysaccharide of the cell-wall lipopolysaccharide from *Salmonella typhimurium*. *Carbohydr.Res* 1971;16:39–48.
28. Yang H-J, Hakomori S. A sphingolipid having a novel type of ceramide and lacto-N-fucopentaose III. *J.Biol.Chem* 1971;246:1192–1200. [PubMed: 5545063]
29. Stellner K, Saito H, Hakomori S. Determination of aminosugar linkages in glycolipids by methylation: Aminosugar linkage of ceramide pentasaccharide of rabbit erythrocytes and/or Forssman antigen. *Arch.Biochem.Biophys* 1973;155:464–472. [PubMed: 4735853]
30. Watanabe K, Laine RA, Hakomori S. On neutral fucoglycolipids having long branch carbohydrate chains: H-active glycosphingolipids of human erythrocyte membranes. *Biochemistry* 1975;14:2725–2733. [PubMed: 807238]
31. Li SC, Li YT. Studies on the glycosidases of jack bean meal. 3. Crystallization and properties of beta-N-acetylhexosaminidase. *J Biol Chem* 1970;245:5153–5160. [PubMed: 5506280]
32. Li SC, Mazzotta MY, Chien SF, Li YT. Isolation and characterization of jack bean beta-galactosidase. *J Biol Chem* 1975;250:6786–6791. [PubMed: 239949]
33. Li YT, Li SC. Anomeric configuration of galactose residues in ceramide trihexosides. *J Biol Chem* 1971;246:3769–3771. [PubMed: 5104445]
34. Li YT, Li SC, Dawson G. Anomeric structure of ceramide digalactoside isolated from the kidney of a patient with Fabry's disease. *Biochim Biophys Acta* 1972;260:88–92. [PubMed: 5012456]
35. Weissmann B, Hinrichsen DF. Mammalian alpha-acetylgalactosaminidase. Occurrence, partial purification, and action on linkages in submaxillary mucins. *Biochemistry* 1969;8:2034–2043. [PubMed: 5785223]
36. Hakomori S, Siddiqui B, Li Y-T, Li S-C, Hellerqvist CG. Anomeric structures of "globoside" and ceramide trihexoside of human erythrocytes and "BHK" fibroblasts. *J.Biol.Chem* 1971;246:2271–2277. [PubMed: 5103071]
37. Siddiqui B, Hakomori S. A revised structure for Forssman glycolipid hapten. *J.Biol.Chem* 1971;246:5766–5769. [PubMed: 4328838]
38. Wiegandt H, Baschang G. Die gewinnung des zuckeranteiles der glykosphingolipide durch ozonolyse und fragmentierung [The isolation of the sugar portion of glycosphingolipids by ozonolysis and fragmentation]. *Z Naturforsch B* 1965;20:164–166. [PubMed: 14345165]
39. Hakomori S. Release of carbohydrates from sphingoglycolipid by osmium-catalyzed periodate oxidation, followed by treatment with mild alkali. *J.Lipid Res* 1966;7:789–792. [PubMed: 4291317]
40. MacDonald DL, Patt LM, Hakomori S. Notes on improved procedures for the chemical modification and degradation of glycosphingolipids. *J.Lipid Res* 1980;21:642–645. [PubMed: 7400693]
41. Ito M, Kurita T, Kita K. A novel enzyme that cleaves the N-acyl linkage of ceramides in various glycosphingolipids as well as sphingomyelin to produce their lyso forms. *J Biol Chem* 1995;270:24370–24374. [PubMed: 7592649]
42. Okino N, Tani M, Imayama S, Ito M. Purification and characterization of a novel ceramidase from *Pseudomonas aeruginosa*. *J Biol Chem* 1998;273:14368–14373. [PubMed: 9603946]
43. Klenk E, Lauenstein K. Über die zuckerhaltigen Lipoide der Formbestandteile des menschlichen Blutes. *Hoppe-Seyler's Z.Physiol.Chem* 1951;288:220–228. [PubMed: 14937664]

44. Yamakawa T, Suzuki S. The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes: III. Globoside, the sugar-containing lipid of human blood stroma. *J.Biochem.(Tokyo)* 1952;39:393–402.
45. Yamakawa T, Yokoyama S, Kiso N. Structure of main globoside of human erythrocytes. *J Biochem (Tokyo)* 1962;52:228–229. [PubMed: 14002302]
46. Yamakawa T, Nishimura S, Kamimura M. The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes: XIII. Further studies on human red cell glycolipids. *Jap.J.Exp.Med* 1965;35:201–207. [PubMed: 5294403]
47. Papirmeister B, Mallette MF. The isolation and some properties of the Forssman hapten from sheep erythrocytes. *Arch.Biochem.Biophys* 1955;57:94–105.
48. Ando, S.; Yamakawa, T. On the oligosaccharide of Forssman-active sheep red cell glycolipid. In: Sweeley, CC., editor. *Chemistry and metabolism of sphingolipids*. Amsterdam: North-Holland Publ. Co.; 1970. p. 91-95.
49. Yamamoto H, Iida-Tanaka N, Kasama T, Ishizuka I, Kushi Y, Handa S. Isolation and characterization of a novel Forssman-active acidic glycosphingolipid with branched isoglobo-, ganglio-, and neolacto-series hybrid sugar chains. *J.Biochem.(Tokyo)* 1999;125:923–930. [PubMed: 10220585]
50. Rapport MM, Graf L. Immunochemical reactions of lipids. *Prog.Allergy* 1969;13:273–331. [PubMed: 4980079]
51. Laine R, Sweeley CC, Li YT, Kisic A, Rapport MM. On the structure of cytolipin R, a ceramide tetrahexoside hapten from rat lymphosarcoma. *J Lipid Res* 1972;13:519–524. [PubMed: 5068000]
52. Siddiqui B, Kawanami J, Li YT, Hakomori S. Structures of ceramide tetrasaccharides from various sources: Uniqueness of rat kidney ceramide tetrasaccharide. *J.Lipid Res* 1972;13:657–662. [PubMed: 4342427]
53. Kannagi R, Cochran NA, Ishigami F, Hakomori S, Andrews PW, Knowles BB, Solter D. Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells. *EMBO J* 1983;2:2355–2361. [PubMed: 6141938]
54. Kannagi R, Lavery SB, Ishigami F, Hakomori S, Shevinsky LH, Knowles BB, Solter D. New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific embryonic antigen 3. *J.Biol.Chem* 1983;258:8934–8942. [PubMed: 6863318]
55. Yu S, Withers DA, Hakomori S. Globoside-dependent adhesion of human embryonal carcinoma cells, based on carbohydrate-carbohydrate interaction, initiates signal transduction and induces enhanced activity of transcription factors AP1 and CREB. *J.Biol.Chem* 1998;273:2517–2525. [PubMed: 9446552]
56. Kawanami J. Lipid of cancer tissues. II. Neutral glycolipids of Nakahara-Fukuoka sarcoma tissue. *J Biochem (Tokyo)* 1967;62:105–117. [PubMed: 6073166]
57. Ito A, Saito S, Masuko T, Oh-eda M, Matsuura T, Satoh M, Nejad FM, Enomoto T, Orikasa S, Hakomori S. Monoclonal antibody (5F3) defining renal cell carcinoma-associated antigen disialosyl globopentaosylceramide (V³NeuAcIV⁶NeuAcGb5), and distribution pattern of the antigen in tumor and normal tissues. *Glycoconj.J* 2001;18:475–485. [PubMed: 12084983]
58. Kuhn R, Wiegandt H. Uber ein glucosaminhaltiges gangliosid. *Z Naturforsch B* 1964;19:80–1. [PubMed: 14134897]
59. Siddiqui B, Hakomori S. A ceramide tetrasaccharide of human erythrocyte membrane reacting with anti-type XIV pneumococcal polysaccharide antiserum. *Biochim.Biophys.Acta* 1973;330:147–155. [PubMed: 4149633]
60. Hakomori S, Koscielak J, Bloch KJ, Jeanloz RW. Immunologic relationship between blood group substances and a fucose-containing glycolipid of human adenocarcinoma. *J.Immunol* 1967;98:31–38. [PubMed: 6018768]
61. Stroud MR, Handa K, Salyan MEK, Ito K, Lavery SB, Hakomori S, Reinhold BB, Reinhold VN. Monosialogangliosides of human myelogenous leukemia HL60 cells and normal human leukocytes. 1. Separation of E-selectin binding from nonbinding gangliosides, and absence of sialosyl-Le^x having tetraosyl to octaosyl core. *Biochemistry* 1996;35:758–769. [PubMed: 8547256]

62. Handa K, Stroud MR, Hakomori S. Sialosyl-fucosyl poly-LacNAc without the sialosyl-Le^x epitope as the physiological myeloid cell ligand in E-selectin-dependent adhesion: Studies under static and dynamic flow conditions. *Biochemistry* 1997;36:12412–12420. [PubMed: 9376344]
63. Lavery SB, Nudelman ED, Salyan MEK, Hakomori S. Novel tri- and tetrasialylpoly-N-acetyllactosaminyl gangliosides of human placenta: Structure determination of pentadeca- and eicosaglycosylceramides by methylation analysis, fast atom bombardment mass spectrometry, and ¹H-NMR spectroscopy. *Biochemistry* 1989;28:7772–7781. [PubMed: 2611213]
64. Fukushima K, Hirota M, Terasaki PI, Wakisaka A, Togashi H, Chia D, Suyama N, Fukushi Y, Nudelman ED, Hakomori S. Characterization of sialosylated Lewis^x as a new tumor-associated antigen. *Cancer Res* 1984;44:5279–5285. [PubMed: 6386148]
65. Magnani JL, Nilsson B, Brockhaus M, Zopf D, Stepkowski Z, Koprowski H, Ginsburg V. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *J.Biol.Chem* 1982;257:14365–14369. [PubMed: 7142214]
66. Hakomori S, Nudelman ED, Lavery SB, Kannagi R. Novel fucolipids accumulating in human adenocarcinoma: I. Glycolipids with di- or trifucosylated type 2 chain. *J.Biol.Chem* 1984;259:4672–4680. [PubMed: 6707026]
67. Fukushi Y, Nudelman ED, Lavery SB, Rauvala H, Hakomori S. Novel fucolipids accumulating in human cancer: III. A hybridoma antibody (FH6) defining a human cancer-associated difucoganglioside (VI³NeuAcV³III³Fuc₂nLc₆). *J.Biol.Chem* 1984;259:10511–10517. [PubMed: 6469974]
68. Stroud MR, Lavery SB, Nudelman ED, Salyan MEK, Towell JA, Roberts CE, Watanabe M, Hakomori S. Extended type 1 chain glycosphingolipids: Dimeric Le^a (III⁴V⁴Fuc₂Lc₆) as human tumor-associated antigen. *J.Biol.Chem* 1991;266:8439–8446. [PubMed: 2022659]
69. Watanabe M, Ohishi T, Kuzuoka M, Nudelman ED, Stroud MR, Kubota T, Kodaira S, Abe O, Hirohashi S, Shimosato Y, Hakomori S. *In vitro* and *in vivo* antitumor effects of murine monoclonal antibody NCC-ST-421 reacting with dimeric Le^a (Le^a/Le^a) epitope. *Cancer Res* 1991;51:2199–2204. [PubMed: 1706961]
70. Miura Y, Kainuma M, Jiang H, Velasco H, Vogt PK, Hakomori S. Reversion of the Jun-induced oncogenic phenotype by enhanced synthesis of sialosyllactosylceramide (GM3 ganglioside). *Proc.Natl.Acad.Sci.USA* 2004;101:16204–16209. [PubMed: 15534203]
71. Rosenfelder G, Young WWJ, Hakomori S. Association of the glycolipid pattern with antigenic alterations in mouse fibroblasts transformed by murine sarcoma virus. *Cancer Res* 1977;37:1333–1339. [PubMed: 192447]
72. Young WWJ, Hakomori S. Therapy of mouse lymphoma with monoclonal antibodies to glycolipid: Selection of low antigenic variants *in vivo*. *Science* 1981;211:487–489. [PubMed: 7455688]
73. Hakomori S. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. *Adv.Cancer Res* 1989;52:257–331. [PubMed: 2662714]
74. Nudelman ED, Kannagi R, Hakomori S, Parsons M, Lipinski M, Wiels J, Fellous M, Tursz T. A glycolipid antigen associated with Burkitt lymphoma defined by a monoclonal antibody. *Science* 1983;220:509–511. [PubMed: 6836295]
75. Nudelman ED, Hakomori S, Kannagi R, Lavery SB, Yeh M-Y, Hellström K-E, Hellström I. Characterization of a human melanoma-associated ganglioside antigen defined by a monoclonal antibody, 4.2. *J.Biol.Chem* 1982;257:12752–12756. [PubMed: 7130176]
76. Pukel CS, Lloyd KO, Travassos LR, Dippold WG, Oettgen HF, Old LJ. GD₃, a prominent ganglioside of human melanoma: Detection and characterization by mouse monoclonal antibody. *J.Exp.Med* 1982;155:1133–1147. [PubMed: 7061953]
77. Fukushi Y, Nudelman ED, Lavery SB, Higuchi T, Hakomori S. A novel disialoganglioside (IV³NeuAcIII⁶NeuAcLc₄) of human adenocarcinoma and the monoclonal antibody (FH9) defining this disialosyl structure. *Biochemistry* 1986;25:2859–2866. [PubMed: 3087411]
78. Wiels J, Fellous M, Tursz T. Monoclonal antibody against a Burkitt lymphoma-associated antigen. *Proc.Natl.Acad.Sci.USA* 1981;78:6485–6488. [PubMed: 7031655]

79. Mènard S, Tagliabue E, Canevari S, Fossati G, Colnaghi MI. Generation of monoclonal antibodies reacting with normal and cancer cells of human breast. *Cancer Res* 1983;43:1295–1300. [PubMed: 6337705]
80. Bremer EG, Lavery SB, Sonnino S, Ghidoni R, Canevari S, Kannagi R, Hakomori S. Characterization of a glycosphingolipid antigen defined by the monoclonal antibody MBr1 expressed in normal and neoplastic epithelial cells of human mammary gland. *J.Biol.Chem* 1984;259:14773–14777. [PubMed: 6501317]
81. Nilsson O, Mansson J-E, Brezicka T, Holmgren J, Lindholm L, Sorenson S, Yngvason F, Svennerholm L. Fucosyl GM₁ - A ganglioside associated with small cell lung carcinomas. *Glycoconj.J* 1984;1:43–49.
82. Vangsted AJ, Clausen H, Kjeldsen TB, White T, Sweeney B, Hakomori S, Drivsholm L, Zeuthen J. Immunochemical detection of a small cell lung cancer-associated ganglioside (FucGM₁) in serum. *Cancer Res* 1991;51:2879–2884. [PubMed: 1851663]
83. Saito S, Lavery SB, Salyan MEK, Goldberg RI, Hakomori S. Common tetrasaccharide epitope NeuAcalpha2-3Galbeta1-3(NeuAcalpha2-6)GalNAc, presented by different carrier glycosylceramides or O-linked peptides, is recognized by different antibodies and ligands having distinct specificities. *J.Biol.Chem* 1994;269:5644–5652. [PubMed: 7509790]
84. Ito A, Lavery SB, Saito S, Satoh M, Hakomori S. A novel ganglioside isolated from renal cell carcinoma. *J.Biol.Chem* 2001;276:16695–16703. [PubMed: 11278988]
85. Solter D, Knowles BB. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc.Natl.Acad.Sci.USA* 1978;75:5565–5569. [PubMed: 281705]
86. Shevinsky LH, Knowles BB, Damjanov I, Solter D. Monoclonal antibody to murine embryos defines a stage-specific embryonic antigen expressed on embryos and human teratocarcinoma cells. *Cell* 1982;30:697–705. [PubMed: 6183004]
87. Fenderson BA, Zehavi U, Hakomori S. A multivalent lacto-N-fucopentaose III-lysyllysine conjugate decompacts preimplantation mouse embryos, while the free oligosaccharide is ineffective. *J.Exp.Med* 1984;160:1591–1596. [PubMed: 6491606]
88. Gooi HC, Feizi T, Kapadia A, Knowles BB, Solter D, Evans MJ. Stage-specific embryonic antigen involves a1-3 fucosylated type 2 blood group chains. *Nature* 1981;292:156–158. [PubMed: 6165896]
89. Bird JM, Kimber SJ. Oligosaccharides containing fucose linked a(1,3) and a(1,4) to N-acetylglucosamine cause decompaction of mouse morulae. *Dev.Biol* 1984;104
90. Gahmberg CG, Hakomori S. External labeling of cell surface galactose and galactosamine in glycolipid and glycoprotein of human erythrocytes. *J.Biol.Chem* 1973;248:4311–4317. [PubMed: 4711609]
91. Gahmberg CG, Hakomori S. Altered growth behavior of malignant cells associated with changes in externally labeled glycoprotein and glycolipids. *Proc.Natl.Acad.Sci.USA* 1973;70:3329–3333. [PubMed: 4357866]
92. Carter WG, Hakomori S. Isolation and partial characterization of "galactoprotein a" (LETS) and "galactoprotein b" from hamster embryo fibroblasts. *Biochem.Biophys.Res.Commun* 1977;76:299–308. [PubMed: 194598]
93. Hynes RO. Alteration of cell surface proteins by viral transformation and proteolysis. *Proc.Natl.Acad.Sci.USA* 1973;70:3170–3174. [PubMed: 4361679]
94. Ruoslahti E, Vaheri A, Kuusela P, Linder E. Fibroblast surface antigen: A new serum protein. *Biochim.Biophys.Acta* 1973;322:352–358. [PubMed: 4203032]
95. Sekiguchi K, Hakomori S. Functional domain structure of fibronectin. *Proc.Natl.Acad.Sci.USA* 1980;77:2661–2665. [PubMed: 6994104]
96. Tsuji T, Yamamoto F, Miura Y, Takio K, Titani K, Pawar S, Osawa T, Hakomori S. Characterization through cDNA cloning of galactoprotein b3 (Gap b3), a cell surface membrane glycoprotein showing enhanced expression on oncogenic transformation: Identification of Gap b3 as a member of the integrin superfamily. *J.Biol.Chem* 1990;265:7016–7021. [PubMed: 1691184]
97. Tsuji T, Kawada Y, Kai-Murozono M, Komatsu S, Han SA, Takeuchi K, Mizushima H, Miyazaki K, Irimura T. Regulation of melanoma cell migration and invasion by laminin-5 and a3b1 integrin (VLA-3). *Clin.Exp.Metastasis* 2002;19:127–134. [PubMed: 11964076]

98. Xia Y, Gil SG, Carter WG. Anchorage mediated by integrin $\alpha 6 \beta 4$ to laminin 5 (epiligrin) regulates tyrosine phosphorylation of a membrane-associated 80-kD protein. *J.Cell Biol* 1996;132:727–740. [PubMed: 8647901]
99. Gahmberg CG, Hakomori S. Surface carbohydrates of hamster fibroblasts: I. Chemical characterization of surface-labeled glycosphingolipids and a specific ceramide tetrasaccharide for transformants. *J.Biol.Chem* 1975;250:2438–2446. [PubMed: 164440]
100. Gahmberg CG, Hakomori S. Surface carbohydrates of hamster fibroblasts: II. Interaction of hamster NIL cell surface with *Ricinus communis* lectin and Concanavalin A as revealed by surface galactosyl label. *J.Biol.Chem* 1975;250:2447–2451. [PubMed: 1168186]
101. Urdal DL, Hakomori S. Characterization of tumor-associated ganglio-N-triaosylceramide in mouse lymphoma and the dependence of its exposure and antigenicity on the sialosyl residues of a second glycoconjugate. *J.Biol.Chem* 1983;258:6869–6874. [PubMed: 6189830]
102. Wiels J, Holmes EH, Cochran N, Tursz T, Hakomori S. Enzymatic and organizational difference in expression of a Burkitt lymphoma-associated antigen (globotriaosylceramide) in Burkitt lymphoma and lymphoblastoid cell lines. *J.Biol.Chem* 1984;259:14783–14787. [PubMed: 6438103]
103. Laine RA, Hakomori S. Incorporation of exogenous glycosphingolipids in plasma membranes of cultured hamster cells and concurrent change of growth behavior. *Biochem.Biophys.Res.Commun* 1973;54:1039–1045. [PubMed: 4753182]
104. Bremer EG, Hakomori S. GM₃ ganglioside induces hamster fibroblast growth inhibition in chemically-defined medium: Ganglioside may regulate growth factor receptor function. *Biochem.Biophys.Res.Commun* 1982;106:711–718. [PubMed: 6288031]
105. Prinetti A, Iwabuchi K, Hakomori S. Glycosphingolipid-enriched signaling domain in mouse neuroblastoma Neuro2a cells: Mechanism of ganglioside-dependent neuritogenesis. *J.Biol.Chem* 1999;274:20916–20924. [PubMed: 10409636]
106. Kijimoto S, Hakomori S. Enhanced glycolipid: a-galactosyltransferase activity in contact-inhibited hamster cells and loss of this response in polyoma transformants. *Biochem.Biophys.Res.Commun* 1971;44:557–563. [PubMed: 4330775]
107. Hakomori S. Cell density-dependent changes in glycolipid concentrations in fibroblasts, and loss of this response in virus-transformed cells. *Proc.Natl.Acad.Sci.USA* 1970;67:1741–1747. [PubMed: 4321344]
108. Sakiyama H, Gross SK, Robbins PW. Glycolipid synthesis in normal and virus-transformed hamster cell lines. *Proc.Natl.Acad.Sci.USA* 1972;69:872–876. [PubMed: 4337244]
109. Hakomori S, Kijimoto S. Forssman reactivity and cell contacts in cultured hamster cells. *Nat New Biol* 1972;239:87–88. [PubMed: 4349668]
110. Cohen S, Carpenter G, King L. Epidermal growth factor receptor-protein kinase interactions: Co-purification of receptor and epidermal growth factor-enhanced phosphorylation activity. *J.Biol.Chem* 1980;255:4834–4842. [PubMed: 6246084]
111. Cohen S, Fava RA, Sawyer ST. Purification and characterization of epidermal growth factor receptor/protein kinase from normal mouse liver. *Proc Natl Acad Sci U S A* 1982;79:6237–6241. [PubMed: 6983068]
112. Hunter T, Cooper JA. Epidermal growth factor induces rapid tyrosine phosphorylation of proteins in A431 human tumor cells. *Cell* 1981;24:741–752. [PubMed: 6166387]
113. Hynes RO. Integrins: A family of cell surface receptors. *Cell* 1987;48:549–554. [PubMed: 3028640]
114. Ruoslahti E. Integrins. *J.Clin.Invest* 1991;87:1–5. [PubMed: 1985087]
115. Barnes D, Sato G. Methods for growth of cultured cells in serum-free medium. *Anal.Biochem* 1980;102:255–270. [PubMed: 6999941]
116. Toledo MS, Suzuki E, Handa K, Hakomori S. Cell growth regulation through GM3-enriched microdomain (glycosynapse) in human lung embryonal fibroblast WI38 and its oncogenic transformant VA13. *J.Biol.Chem* 2004;279:34655–34664. [PubMed: 15143068]
117. Bremer EG, Hakomori S, Bowen-Pope DF, Raines EW, Ross R. Ganglioside-mediated modulation of cell growth, growth factor binding, and receptor phosphorylation. *J.Biol.Chem* 1984;259:6818–6825. [PubMed: 6327695]
118. Yates AJ, VanBrocklyn J, Saqr HE, Guan Z, Stokes BT, O'Dorisio MS. Mechanisms through which gangliosides inhibit PDGF-stimulated mitogenesis in intact Swiss 3T3 cells: Receptor tyrosine

phosphorylation, intracellular calcium, and receptor binding. *Exp.Cell Res* 1993;204:38–45. [PubMed: 8416794]

119. Bremer EG, Schlessinger J, Hakomori S. Ganglioside-mediated modulation of cell growth: Specific effects of GM₃ on tyrosine phosphorylation of the epidermal growth factor receptor. *J.Biol.Chem* 1986;261:2434–2440. [PubMed: 2418024]
120. Hanai N, Nores GA, MacLeod C, Torres-Mendez C-R, Hakomori S. Ganglioside-mediated modulation of cell growth: Specific effects of GM₃ and lyso-GM₃ in tyrosine phosphorylation of the epidermal growth factor receptor. *J.Biol.Chem* 1988;263:10915–10921. [PubMed: 2839511]
121. Hanai N, Dohi T, Nores GA, Hakomori S. A novel ganglioside, de-*N*-acetyl-GM₃ (II³NeuNH₂LacCer), acting as a strong promoter for epidermal growth factor receptor kinase and as a stimulator for cell growth. *J.Biol.Chem* 1988;263:6296–6301. [PubMed: 2834372]
122. Murozuka Y, Watanabe N, Hatanaka K, Hakomori S. Lyso-GM₃, its dimer, and multimer: Their synthesis, and their effect on epidermal growth factor-induced receptor tyrosine kinase. *Glycoconj.J.* 2007in press
123. Maecker HT, Todd SC, Levy S. The tetraspanin superfamily: Molecular facilitators. *FASEB J* 1997;11:428–442. [PubMed: 9194523]
124. Mannion BA, Berditchevski F, Kraeft S-K, Chen LB, Hemler ME. Transmembrane-4 superfamily proteins CD81 (TAPA-1), CD82, CD63, and CD53 specifically associate with integrin α4β1 (CD94d/CD29). *J.Immunol* 1996;157:2039–2047. [PubMed: 8757325]
125. Miyake M, Koyama M, Seno M, Ikeyama S. Identification of the motility-related protein (MRP-1), recognized by monoclonal antibody M31-15, which inhibits cell motility. *J.Exp.Med* 1991;174:1347–1354. [PubMed: 1720807]
126. Dong J, T, Lamb PW, Rinker-Schaeffer CW, Vukanovic J, Ichikawa T, Isaacs JT, Barrett JC. *KAI1*, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science* 1995;268:884–886. [PubMed: 7754374]
127. Dong JT, Suzuki H, Pin SS, Bova S, Schalken JA, Isaacs WB, Barrett JC, Isaacs JT. Down-regulation of the *KAI1* metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. *Cancer Res* 1996;56:4387–4390. [PubMed: 8813131]
128. Cajot J-F, Sordat I, Silvestre T, Sordat B. Differential display cloning identifies motility-related protein (MRP1/CD9) as highly expressed in primary compared to metastatic human colon carcinoma cells. *Cancer Res* 1997;57:2593–2597. [PubMed: 9205061]
129. Satoh M, Ito A, Nojiri H, Handa K, Numahata K, Ohyama C, Saito S, Hoshi S, Hakomori S. Enhanced GM₃ expression, associated with decreased invasiveness, is induced by brefeldin A in bladder cancer cells. *Int.J.Oncol* 2001;19:723–731. [PubMed: 11562747]
130. Mitsuzuka K, Handa K, Satoh M, Arai Y, Hakomori S. A specific microdomain ("glycosynapse 3") controls phenotypic conversion and reversion of bladder cancer cells through GM₃-mediated interaction of α3β1 integrin with CD9. *J Biol Chem* 2005;280:35545–35553. [PubMed: 16103120]
131. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;4:915–925. [PubMed: 14685170]
132. Todeschini AR, Dos Santos JN, Handa K, Hakomori S. Ganglioside GM₂-tetraspanin CD82 complex inhibits Met and its cross-talk with integrins, providing a basis for control of cell motility through glycosynapse. *J Biol Chem* 2007;282:8123–8133. [PubMed: 17215249]
133. Edelman GM, Crossin KL. Cell adhesion molecules: Implications for a molecular histology. *Annu.Rev.Biochem* 1991;60:155–190. [PubMed: 1883195]
134. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 1991;251:1451–1455. [PubMed: 2006419]
135. Gahmberg CG. Leukocyte adhesion: CD11/CD18 integrins and intercellular adhesion molecules. *Curr Opin Cell Biol* 1997;9:643–650. [PubMed: 9330867]
136. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* 1992;69:11–25. [PubMed: 1555235]
137. Perillo NL, Marcus ME, Baum LG. Galectins: Versatile modulators of cell adhesion, cell proliferation, and cell death. *J.Mol.Med* 1998;76:402–412. [PubMed: 9625297]

138. Varki A. Selectin ligands. *Proc.Natl.Acad.Sci.USA* 1994;91:7390–7397. [PubMed: 7519775]
139. Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Varki A. Siglecs: A family of sialic acid-binding lectins. *Glycobiology* 1998;8:v–vi. [PubMed: 9498912]
140. Crocker, PR.; Floyd, H.; Ferguson, DJP.; Nitschke, L. Sialic acid-dependent cell-cell interactions mediated by siglecs: Roles of sialoadhesin and CD22 in macrophage adhesion and B cell homing to the bone marrow. In: Inoue, Y.; Lee, YC.; Troy, FA., editors. *Sialobiology and other novel forms of glycosylation*. Osaka, Japan: Gakushin Publ. Co.; 1999. p. 111-120.
141. Eggens I, Fenderson BA, Toyokuni T, Dean B, Stroud MR, Hakomori S. Specific interaction between Le^x and Le^x determinants: A possible basis for cell recognition in preimplantation embryos and in embryonal carcinoma cells. *J.Biol.Chem* 1989;264:9476–9484. [PubMed: 2470757]
142. Kojima N, Fenderson BA, Stroud MR, Goldberg RI, Habermann R, Toyokuni T, Hakomori S. Further studies on cell adhesion based on Le^x-Le^x interaction, with new approaches: Embryoglycan aggregation of F9 teratocarcinoma cells, and adhesion of various tumour cells based on Le^x expression. *Glycoconj.J* 1994;11:238–248. [PubMed: 7841799]
143. Misevic GN, Burger MM. The species-specific cell-binding site of the aggregation factor from the sponge *Microciona prolifera* is a highly repetitive novel glycan containing glucuronic acid, fucose, and mannose. *J.Biol.Chem* 1990;265:20577–20584. [PubMed: 2243104]
144. Misevic GN, Burger MM. Carbohydrate-carbohydrate interactions of a novel acidic glycan can mediate sponge cell adhesion. *J.Biol.Chem* 1993;268:4922–4929. [PubMed: 7680344]
145. Bucior I, Scheuring S, Engel A, Burger MM. Carbohydrate-carbohydrate interaction provides adhesion force and specificity for cellular recognition. *J.Cell Biol* 2004;165:529–537. [PubMed: 15148309]
146. Kojima N, Hakomori S. Specific interaction between gangliosylceramide (Gg3) and sialosylactosylceramide (GM3) as a basis for specific cellular recognition between lymphoma and melanoma cells. *J.Biol.Chem* 1989;264:20159–20162. [PubMed: 2584211]
147. Kojima N, Hakomori S. Cell adhesion, spreading, and motility of GM₃-expressing cells based on glycolipid-glycolipid interaction. *J.Biol.Chem* 1991;266:17552–17558. [PubMed: 1894638]
148. Hakomori S, Igarashi Y, Kojima N, Okoshi H, Handa K, Fenderson B. Functional role of cell surface carbohydrates in ontogenesis and oncogenesis [Abstract 8.2]. *Glycoconj.J* 1991;8:178.
149. Stewart RJ, Boggs JM. A carbohydrate-carbohydrate interaction between galactosylceramide-containing liposomes and cerebroside sulfate-containing liposomes: Dependence on the glycolipid ceramide composition. *Biochemistry* 1993;32:10666–10674. [PubMed: 8399212]
150. Zhu Z, Kojima N, Stroud MR, Hakomori S, Fenderson BA. Monoclonal antibody directed to Le^y oligosaccharide inhibits implantation in the mouse. *Biol.Repro* 1995;52:903–912.
151. Yoon S, Nakayama K, Takahashi N, Yagi H, Utkina N, Wang HY, Kato K, Sadilek M, Hakomori S. Interaction of N-linked glycans, having multivalent GlcNAc termini, with GM3 ganglioside. *Glycoconj.J* 2006;23:639–649. [PubMed: 17115280]
152. Yoon S, Nakayama K, Hikita T, Handa K, Hakomori S. Epidermal growth factor receptor tyrosine kinase is modulated by GM3 interaction with N-linked GlcNAc termini of the receptor. *Proc.Natl.Acad.Sci.USA* 2006;103:18987–18991. [PubMed: 17142315]
153. Yoon S, Ikeda S, Sadilek M, Hakomori S, Ishida H, Kiso M. Self-recognition of N-linked glycans with multivalent GlcNAc, determined as ceramide mimetic conjugate. *Glycobiology Advance* Access published on July 2, 2007. 2007
154. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* 1997;387:569–572. [PubMed: 9177342]
155. Hakomori S. The glycosynapse. *Proc.Natl.Acad.Sci.USA* 2002;99:225–232. [PubMed: 11773621]
156. Hakomori S. Glycosynapses: Microdomains controlling carbohydrate-dependent cell adhesion and signaling. *Ann Braz Acad Sci* 2004;76:553–572.
157. Krummel MF, Davis MM. Dynamics of the immunological synapse: Finding, establishing and solidifying a connection. *Curr.Opin.Immunol* 2002;14:66–74. [PubMed: 11790534]
158. Hannun YA, Loomis CR, Merrill AHJ, Bell RM. Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding *in vitro* and in human platelets. *J.Biol.Chem* 1986;261:12604–12609. [PubMed: 3462188]
159. Hannun, YA. Sphingolipid-mediated signal transduction. Austin, TX: R.G. Landes Co.; 1997.

160. Igarashi Y, Hakomori S, Toyokuni T, Dean B, Fujita S, Sugimoto M, Ogawa T, El-Ghendy K, Racker E. Effect of chemically well-defined sphingosine and its *N*-methyl derivatives on protein kinase C and src kinase activities. *Biochemistry* 1989;28:6796–6800. [PubMed: 2479412]
161. Abdel-Ghany M, Osusky M, Igarashi Y, Hakomori S, Shalloway D, Racker E. Substrate-specific modulation of *Src*-mediated phosphorylation of Ras and caseins by sphingosines and other substrate modulators. *Biochim.Biophys.Acta* 1992;1137:349–355. [PubMed: 1280164]
162. Okoshi H, Hakomori S, Nisar M, Zhou Q, Kimura S, Tashiro K, Igarashi Y. Cell membrane signaling as target in cancer therapy II: Inhibitory effect of *N,N,N*-trimethylsphingosine on metastatic potential of murine B16 melanoma cell line through blocking of tumor cell-dependent platelet aggregation. *Cancer Res* 1991;51:6019–6024. [PubMed: 1657377]
163. Endo K, Igarashi Y, Nisar M, Zhou Q, Hakomori S. Cell membrane signaling as target in cancer therapy: Inhibitory effect of *N,N*-dimethyl and *N,N,N*-trimethyl sphingosine derivatives on *in vitro* and *in vivo* growth of human tumor cells in nude mice. *Cancer Res* 1991;51:1613–1618. [PubMed: 1998952]
164. Park YS, Hakomori S, Kawa S, Ruan F, Igarashi Y. Liposomal *N,N,N*-trimethylsphingosine (TMS) as an inhibitor of B16 melanoma cell growth and metastasis with reduced toxicity and enhanced drug efficacy compared to free TMS: Cell membrane signaling as a target in cancer therapy III. *Cancer Res* 1994;54:2213–2217. [PubMed: 8174128]
165. Handa K, Igarashi Y, Nisar M, Hakomori S. Down-regulation of GMP-140 (CD62 or PADGEM) expression on platelets by *N,N*-dimethyl and *N,N,N*-trimethyl derivatives of sphingosine. *Biochemistry* 1991;30:11682–11686. [PubMed: 1721534]
166. Kimura S, Kawa S, Ruan F, Nisar M, Sadahira Y, Hakomori S, Igarashi Y. Effect of sphingosine and its *N*-methyl derivatives on oxidative burst, phagokinetic activity, and trans-endothelial migration of human neutrophils. *Biochem.Pharmacol* 1992;44:1585–1595. [PubMed: 1329769]
167. Kolesnick RN, Kronke M. Regulation of ceramide production and apoptosis. *Annu.Rev.Physiol* 1998;60:643–665. [PubMed: 9558480]
168. Pettus BJ, Chalfant CE, Hannun YA. Ceramide in apoptosis: An overview and current perspectives. *Biochim.Biophys.Acta* 2002;1585:114–125. [PubMed: 12531544]
169. Kolesnick RN. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J.Clin.Invest* 2002;110:3–8. [PubMed: 12093880]
170. Venkataraman K, Riebeling C, Bodennec J, Riezman H, Allegood JC, Sullards MC, Merrill AHJ, Futerman AH. Upstream of growth and differentiation factor 1 (uog1), a mammalian homolog of the yeast longevity assurance gene 1 (LAG1), regulates N-stearoyl-sphinganine (C18-(dihydro) ceramide) synthesis in a fumonisin B1-independent manner in mammalian cells. *J.Biol.Chem* 2002;277:35642–35649. [PubMed: 12105227]
171. Smith WL, Merrill AHJ. Sphingolipid metabolism and signaling. *J.Biol.Chem* 2002;277:25841–25842. [PubMed: 12048220]
172. Sato M, Markiewicz M, Yamanaka M, Bielawska A, Mao C, Obeid LM, Hannun YA, M T. Modulation of transforming growth factor-beta (TGF-beta) signaling by endogenous sphingolipid mediators. *J.Biol.Chem* 2003;278:9276–9282. [PubMed: 12515830]
173. Maceyka M, Payne SG, Milstien S, Spiegel S. Sphingosine kinase, sphingosine-1-phosphate, and apoptosis. *Biochim.Biophys.Acta* 2002;1585:193–201. [PubMed: 12531554]
174. Spiegel S, English D, Milstien S. Sphingosine 1-phosphate signaling: Providing cells with a sense of direction. *Trends Cell Biol* 2002;12:236–242. [PubMed: 12062172]
175. Payne SG, Milstien S, Spiegel S. Sphingosine-1-phosphate: Dual messenger functions. *FEBS Lett* 2002;531:54–57. [PubMed: 12401202]
176. Sano T, Baker D, Virag T, Wada A, Yatomi Y, Kobayashi T, Igarashi Y, Tigyi G. Multiple mechanisms linked to platelet activation result in lysophosphatidic acid and sphingosine 1-phosphate generation in blood. *J.Biol.Chem* 2002;277:21197–21206. [PubMed: 11929870]
177. Kihara A, Ikeda M, Kariya Y, Lee E-Y, Lee Y-M, Igarashi Y. Sphingosine-1-phosphate lyase is involved in the differentiation of F9 embryonal carcinoma cells to primitive endoderm. *J.Biol.Chem* 2003;278:14578–14585. [PubMed: 12584204]

178. Ogawa C, Kihara A, Gokoh M, Igarashi Y. Identification and characterization of a novel human sphingosine-1-phosphate phosphohydrolase, hSPP2. *J.Biol.Chem* 2003;278:1268–1272. [PubMed: 12411432]
179. Megidish T, White T, Takio K, Titani K, Igarashi Y, Hakomori S. The signal modulator protein 14-3-3 is a target of sphingosine- or *N,N*-dimethylsphingosine-dependent kinase in 3T3(A31) cells. *Biochem.Biophys.Res.Comm* 1995;216:739–747. [PubMed: 7488188]
180. Megidish T, Cooper J, Zhang L, Fu H, Hakomori S. A novel sphingosine-dependent protein kinase (SDK1) specifically phosphorylates certain isoforms of 14-3-3 protein. *J.Biol.Chem* 1998;273:21834–21845. [PubMed: 9705322]
181. Hamaguchi A, Suzuki E, Murayama K, Fujimura T, Hikita T, Iwabuchi K, Handa K, Withers DA, Masters SC, Fu H, Hakomori S. Sphingosine-dependent protein kinase-1, directed to 14-3-3, is identified as the kinase domain of protein kinase C delta. *J.Biol.Chem* 2003;278:41557–41565. [PubMed: 12855683]
182. Suzuki E, Handa K, Toledo MS, Hakomori S. Sphingosine-dependent apoptosis: a unified concept based on multiple mechanisms operating in concert. *Proc Natl Acad Sci U S A* 2004;101:14788–14793. [PubMed: 15466700]
183. Megidish T, Takio K, Titani K, Iwabuchi K, Hamaguchi A, Igarashi Y, Hakomori S. Endogenous substrates of sphingosine-dependent kinases (SDKs) are chaperone proteins: Heat shock proteins, glucose-regulated proteins, protein disulfide isomerase, and calreticulin. *Biochemistry* 1999;38:3369–3378. [PubMed: 10079081]
184. Liu C, Smith BM, Ajito K, Komatsu H, Gomez-Paloma L, Li T, Theodorakis EA, Nicolaou KC, Vogt PK. Sequence-selective carbohydrate-DNA interaction: dimeric and monomeric forms of the calicheamicin oligosaccharide interfere with transcription factor function. *Proc Natl Acad Sci U S A* 1996;93:940–944. [PubMed: 8570664]
185. Yang Q, Goldstein IJ, Mei HY, Engelke DR. DNA ligands that bind tightly and selectively to cellobiose. *Proc Natl Acad Sci U S A* 1998;95:5462–5467. [PubMed: 9576904]
186. Gibbs WW. The unseen genome: Gems among the junk. *Sci.Am* 2003;289:46–53.
187. Gibbs WW. The unseen genome: Beyond DNA. *Sci.Am* 2003;289:106–113. [PubMed: 14631738]
188. Kannagi R, Levine P, Watanabe K, Hakomori S. Recent studies of glycolipid and glycoprotein profiles and characterization of the major glycolipid antigen in gastric cancer of a patient of blood group genotype *pp* (T_j^{a-}) first studied in 1951. *Cancer Res* 1982;42:5249–5254. [PubMed: 7139617]
189. Stapleton A, Nudelman ED, Clausen H, Hakomori S, Stamm WE. Binding of uropathogenic *Escherichia coli* R45 to glycolipids extracted from vaginal epithelial cells is dependent on histo-blood group secretor status. *J.Clin.Invest* 1992;90:965–972. [PubMed: 1522244]
190. Stapleton AE, Stroud MR, Hakomori S, Stamm WE. The globo-series glycosphingolipid sialosyl galactosyl globoside is found in urinary tract tissues and is a preferred binding receptor in vitro for uropathogenic *Escherichia coli* expressing papen-coded adhesins. *Infect.Immun* 1998;66:3856–3861. [PubMed: 9673272]
191. Davidsohn I, Stejskal R. Tissue antigens A, B and H in health and disease. *Haematologia* 1972;6:177–184. [PubMed: 5063612]
192. Lee JS, Ro JY, Sahin AA, Hong WK, Brown BW, Mountain CF, Hittelman WN. Expression of blood-group antigen A: A favorable prognostic factor in nonsmall-cell lung cancer. *N.Engl.J.Med* 1991;324:1084–1090. [PubMed: 1848917]
193. Ichikawa D, Handa K, Withers DA, Hakomori S. Histo-blood group A/B *versus* H status of human carcinoma cells as correlated with haptotactic cell motility: Approach with A and B gene transfection. *Cancer Res* 1997;57:3092–3096. [PubMed: 9242430]
194. Ichikawa D, Handa K, Hakomori S. Histo-blood group A/B antigen deletion/reduction *vs.* continuous expression in human tumor cells as correlated with their malignancy. *Int.J.Cancer* 1998;76:284–289. [PubMed: 9537592]
195. Isojima S, Li TS, Ashitaka Y. Immunological analysis of sperm-immobilizing factor found in sera of women with unexplained sterility. *Am.J.Obstet.Gynecol* 1968;101:677–683.
196. Tsuji Y, Clausen H, Nudelman E, Kaizu T, Hakomori S, Isojima S. Human sperm carbohydrate antigens defined by an antisperm human monoclonal antibody derived from an infertile women bearing antisperm antibodies in her serum. *J.Exp.Med* 1988;168:343–356. [PubMed: 3294333]

197. Nudelman ED, Mandel U, Lavery SB, Kaizu T, Hakomori S. A series of disialogangliosides with binary 2-3 sialosylactosamine structure, defined by monoclonal antibody NUH2, are oncodevelopmentally regulated antigens. *J.Biol.Chem* 1989;264:18719–18725. [PubMed: 2509467]
198. Schroter S, Derr P, Conradt HS, Nimtz M, Hale G, Kirchhoff C. Male-specific modification of human CD52. *J.Biol.Chem* 1999;274:29862–29873. [PubMed: 10514467]
199. Diekmann AB, Norton EJ, Westbrook VA, Klotz KL, Naaby-Hansen S, Herr JC. Anti-sperm antibodies from infertile patients and their cognate sperm antigens: A review. Identity between SAGA-1, the H6-3C4 antigen, and CD52. *Am.J.Reprod.Immunol* 2000;43:134–143. [PubMed: 10735589]
200. Li L, Neaves WB. Normal stem cells and cancer stem cells: the niche matters. *Cancer Res* 2006;66:4553–4557. [PubMed: 16651403]
201. Polyak K, Hahn WC. Roots and stems: Stem cells in cancer. *Nat Med* 2006;12:296–300. [PubMed: 16520777]
202. Yanagisawa M, Yu RK. The expression and functions of glycoconjugates in neural stem cells. *Glycobiology* 2007;17:57R–74R.
203. Wearne KA, Winter HC, O'Shea K, Goldstein IJ. Use of lectins for probing differentiated human embryonic stem cells for carbohydrates. *Glycobiology* 2006;16:981–990. [PubMed: 16809438]
204. Wearne KA, Winter HC, Goldstein IJ. Temporal changes in the carbohydrates expressed on BG01 human embryonic stem cells during differentiation as embryoid bodies. *Glycoconj.J.* 2007;in press
205. Cohnheim V. Congenitales, quergestreiftes Muskelsarkom der Nieren. *Virchows Archiv fur pathologische Anatomie und Physiologie und fur klinische Medizin* 1875;65:64–69.
206. Wang F, Hansen RK, Radisky D, Yoneda T, Barcellos-Hoff MH, Petersen OW, Turley EA, Bissell MJ. Phenotypic reversion or death of cancer cells by altering signaling pathways in three-dimensional contexts. *J Natl Cancer Inst* 2002;94:1494–1503. [PubMed: 12359858]
207. Wang F, Weaver VM, Petersen OW, Larabell CA, Dedhar S, Briand P, Lupu R, Bissell MJ. Reciprocal interactions between beta 1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: A different perspective in epithelial biology. *Proc.Natl.Acad.Sci.USA* 1998;95:14821–14826. [PubMed: 9843973]
208. Kenny PA, Bissell MJ. Tumor reversion: Correction of malignant behavior by microenvironmental cues. *Int.J.Cancer* 2003;107:688–695. [PubMed: 14566816]
209. Mintz B, Illmensee K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc.Natl.Acad.Sci.USA* 1975;72:3585–3589. [PubMed: 1059147]
210. Illmensee K, Mintz B. Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocysts. *Proc.Natl.Acad.Sci.USA* 1976;73:549–553. [PubMed: 1061157]
211. Hay ED. Theory for epithelial-mesenchymal transformation based on the "fixed cortex" cell motility model. *Cell Motil Cytoskeleton* 1989;14:455–457. [PubMed: 2696597]
212. Hay ED. The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. *Dev Dyn* 2005;233:706–720. [PubMed: 15937929]
213. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006;172:973–981. [PubMed: 16567498]
214. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer* 2006;6:392–401. [PubMed: 16572188]
215. Matsuura H, Takio K, Titani K, Greene T, Lavery SB, Salyan MEK, Hakomori S. The oncofetal structure of human fibronectin defined by monoclonal antibody FDC-6: Unique structural requirement for the antigenic specificity provided by a glycosylhexapeptide. *J.Biol.Chem* 1988;263:3314–3322. [PubMed: 2449438]
216. Kannagi R, Nudelman ED, Lavery SB, Hakomori S. A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. *J.Biol.Chem* 1982;257:14865–14874. [PubMed: 6129244]
217. Sekiguchi K, Hakomori S. Domain structure of human plasma fibronectin. Differences and similarities between human and hamster fibronectins. *J Biol Chem* 1983;258:3967–3973. [PubMed: 6833239]

218. Hakomori, S.; Fukuda, M.; Sekiguchi, K.; Carter, WG. Fibronectin, laminin, and other extracellular glycoproteins. In: Piez, KA.; Reddi, AH., editors. Extracellular matrix biochemistry. New York: Elsevier; 1984. p. 229-275.
219. Ushiro H, Cohen S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A431 cell membranes. J.Biol.Chem 1980;255:8363–8365. [PubMed: 6157683]
220. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990;61:203–212. [PubMed: 2158859]

Abbreviations

CCI, carbohydrate-to-carbohydrate interaction
Csk, C-terminal Src kinase
DMS, *D-erythro-N,N*-dimethyl-Sph
EGF, epidermal growth factor
EGFR, epidermal growth factor receptor
EMT, epithelial-mesenchymal transition
FGF, fibroblast growth factor
FGFR, fibroblast growth factor receptor
FN, fibronectin
GEM, glycolipid-enriched microdomain
GSL, glycosphingolipid
LN, laminin
MS, mass spectrometry
PDGF, platelet-derived growth factor
PDGFR, platelet-derived growth factor receptor
PKC, protein kinase C
Sph, sphingosine
Sph-1-P, sphingosine-1-phosphate
SSEA, stage-specific embryonic antigen
TMS, *D-erythro-N,N,N*-trimethyl-Sph
TSP, tetraspanin

Fig. 1. Stage-specific transition of glycosyl epitopes from globo- to lacto-, and to ganglio-series, during early mouse embryogenesis

Le^x epitope carried by SSEA-1 is not expressed at 4-cell stage, but is maximally expressed at 1.5 – 2 days (8–32 cell; morula stage), and induces Le^x-mediated adhesion (compaction).

Le^x disappears after compaction, but is expressed at inner cell mass of blastocyst (3.5 days).

Extended globo-series SSEA-3 and -4 (yellow background) are expressed at 0.5 – 1 day, before SSEA-1 appears. Ganglio-series is not expressed until 7 days, when neural crest appears. Data for developmental pattern based on [85–87]. Structure for SSEA-1 [87,88,216]; structures for SSEA-3 and -4 [53,54].

Fig. 2. Two approaches used for functional analysis of cell surface glycosyl epitopes and GSLs
Panel A. Analysis of Gal oxidase surface-labeled component by SDS-PAGE column (A-i), or by slab gel electrophoresis with fluorography (A-ii). Samples from NIL cells: A-i, left; A-ii, lanes 1 and 2. Samples from NILpy cells: A-i, right; A-ii, lanes 3 and 4. Note that peak "a" (galactoprotein a) in NIL is lost in NILpy, while peak "c" (galactoprotein b) is greatly enhanced in NILpy. Lane 1, total protein of surface-labeled NIL cells. Lane 2, fluorography

of lane 1. Lane 3, surface-labeled NILpy cells, extracted with empigin BB (detergent), purified by RCA (*Ricinus communis* lectin) and slab gel electrophoresis. Lane 4, fluorography of lane 3. Empigin BB extract contained mainly galactoprotein b (~125 kDa), which is present mainly in NILpy cells. **A-iii**: Functional domain structure of human plasma fibronectin [217]; for review see [218].

Panel B. Exogenous addition of Gb4 to NIL cells induced growth-inhibited and "contact-oriented" appearance (**B-i-b**), whereas cells without Gb4 addition grew randomly, with partial overlapping (**B-i-a**).

Synchronized NIL cells were subjected to: determination of cell number increase at G2 phase (**B-ii-a**), mitotic index (**B-ii-b**), and thymidine incorporation (**B-ii-c**). Note that Gb4-added cells did not show cell number increase, and had minimal mitosis and DNA synthesis.

Fig. 3. α -Gal transferase response (for Gb3 synthesis) upon cell contact in contact-inhibitable BHK and NIL cells, and loss of this response in transformed cells

UDP-Gal: LacCer α -Gal transferase (Gb3 synthase) (○-○) increased significantly with increase of cell population density in both non-transformed BHK and NIL cells (left panels). This response was lost in polyoma virus-transformed BHK and NIL cells (right panels). UDP-Gal: GlcCer β -Gal transferase (LacCer synthase) (●-●) did not increase with increase of cell population density in either BHK or NIL cells, or their transformants. The figure is based on data summarized from [106] and [109].

Fig. 4. Effects of GM3, lyso-GM3, and de-N-acetyl-GM3 on EGFR in A431 cells

When epidermal growth factor (EGF) binds to its receptor (EGFR), cytoplasmic tyrosine kinase is activated [110,112,219], possibly through receptor-receptor interaction or some other conformational change of EGFR [220]. EGF-induced receptor tyrosine kinase activity was inhibited by ganglioside GM3 [119], presumably through surrounding EGFR in membrane microdomain. However, lyso-GM3, derived from GM3 by ceramidase, inhibited EGFR tyrosine kinase more strongly than GM3 [120]. De-N-acetyl-GM3, derived from GM3 by de-N-acetylase, strongly promoted receptor kinase [121]. The scheme shown is based on these results.

Fig. 5. Two well-established carbohydrate-to-carbohydrate interactions that mediate homotypic cell adhesion

Top: self-recognition of 3-O-sulfated $\text{GlcNAc}\beta 3\text{Fuc}\alpha\text{-O-Ser/Thr}$ linked to proteoglycan, that mediates species-specific sponge cell adhesion and autoaggregation. **Bottom:** self-recognition of $\text{Gal}\beta 4[\text{Fuc}\alpha 3]\text{GlcNAc}\beta 3\text{Gal}$ (Le^x epitope) carried by SSEA-1 glycan, that mediates homotypic adhesion of embryonal stem cells or embryonal carcinoma cells, to induce compaction or autoaggregation. In both cases, presence of Ca^{2+} , which promotes interaction of glycan, is essential.

Le^x/ Le^y/ H interaction

GM3 interaction

Gb4 (globoside) interaction

sulfatide interaction

Fig. 6. GSL structures displaying specific CCI

GSL interactions were determined by binding of liposomes containing specific GSL and labeled with ^3H -cholesterol to specific GSL coated on polystyrene plate [141,146,147]. Strong, weak, or no interaction are indicated by different lines.

Fig. 7. Multiple Sph-induced mechanisms leading to apoptosis

Apoptotic stimulation causes (i) higher Cer level, followed by release of Sph by ceramidase; and (ii) an unknown mechanism causing translocation of PKCδ and its possible accumulation in mitochondrial membrane. These two events are considered to trigger multiple Sph-induced channels for apoptosis processes as below.

- Sph activates a "cascade of caspases" leading to caspase-3 activation (**process 1**).
- Activated caspase-3 cleaves PKCδ to produce PKCδ KD (SDK1) (**process 2**).
- Sph activates SDK1 to phosphorylate 14-3-3 ($\rightarrow 14-3-3-P$) (**process 3**).
- 14-3-3-P inhibits 14-3-3 dimer formation (**process 4**), which in turn inhibits binding of 14-3-3 to pro-apoptotic BAD/BAX, promoting their pro-apoptotic effect (**process 5**).
- Sph inhibits integrin-dependent survival signal, e.g., Akt (**process 6**), leading to apoptosis (**process 7**).
- Sph inhibits BCL-2 gene expression (**process 8**), thereby inhibiting anti-apoptotic BCL-2/ BCL-X (**process 9**).
- Processes 5, 7, and 9 cause release of cytochrome c (**process 10**), which contributes to caspase-3 activation (**process 11**).
- Activated caspase-3 releases PKCδ KD (SDK1), promoting a "vicious cycle" of Sph-induced apoptosis (**process 12**).

The overall process is based on enhanced Sph level, and translocation of PKCδ. The higher the Sph level, the greater the effect of the cycle through released caspase-3 and released PKCδ KD. Susceptibility of adherent tumor cells to Sph-induced apoptosis is less than that of

non-adherent cells, because adherent cells require inhibition of integrin-dependent survival signal by Sph (**process 6**). Which Sph-induced channel is dominant may vary depending on type of cell. From [182].

Fig. 8. Infertility in women is caused by the presence of antibodies directed to sperm antigens sialyl-i (SA-i) and sialyl-I (SA-I), carried by male-specific CD52

Analysis of gangliosides present in human sperm, and thin-layer chromatography (TLC) immunostaining data with mAb H6-3C4, indicate that the male-specific epitope is sialyl-i, i, or sialyl-I, carried by GPI-anchored CD52 [198,199] which consists of a short peptide (12 a.a.) with bulky N-linked glycan as shown (sialyl-i, top; i, middle; sialyl-I, bottom). Since H6-3C4 showed stronger reactivity with sialyl 2-6 lactonorhexaosylceramide than with sialyl 2-3 lactonorhexaosylceramide, the sialyl epitope could be a 2-6, 2-3 mixture. Data are explained in the text. From [196].

Table 1

Classic studies on glycosphingolipids up to the early 1960s.

Topic	Ref.
Polyhydroxy carboxylic acid isolated from submaxillary mucin, named "sialic acid" (gave characteristic color reaction)	[12,13]
Presence of lipid containing "sialic acid"	[14,15]
(a) "Substance X", a lipid compound showing the same color reaction as sialic acid, accumulated in brain of patient with Tay-Sachs disease	[16]
(b) Presence of a similar compound in normal brain	[17]
(c) Isolation of a crystalline "sialic acid-like" compound from Substance X, termed "neuraminic acid"	[18,19]
Composition and possible structure of globoside	[10]
Composition of purified lipid from brain and spleen, claimed to contain sphingosine, fatty acid, hexose, and neuraminic acid in ratio 1: 1: 3: 1, termed "ganglioside"	[20]
(a) Galactosamine was identified in aqueous hydrolysate of brain ganglioside	[13]
(b) Identified galactosamine in brain ganglioside	[21]
Ganglioside containing Glc, GalCer, and sialic acid found as major component in horse erythrocytes, termed "hematoside"	[22]
Complete structural characterization of brain gangliosides, based on gangliotetraosylceramide	[23]
Further confirmation of brain ganglioside structures with sialyl derivatives by methylation analysis	[24]

Table 2

Methods for structural analysis of glycosyl residues

- ◆ **Positions of sugar linkages and branching points**
 i. Methylation with sodium hydride/ methyl iodide in DMSO [25]
 ii. Hydrolysis of glycosidic linkage of permethylated sugar
a. For neutral sugars: hydrolytic yield of partially O-methylated hexitol acetate is high; no problem [26]. **b.** For aminosugars: hydrolysis of glycosidic linkage is hampered if NAc is not protected

The problem is solved by application of acetolysis, which inhibits de-N-acetylation and promotes glycosidic hydrolysis to get quantitative yield of partially O-methylated hexosaminitol acetate [27–29].

- ◆ **Anomeric linkage and sequence analysis**
 Step-by-step application of α or β exoglycosidases with defined specificity gives information on anomeric linkages of each sugar, and their sequences. Examples of enzymes used, and elucidation of structures: see text.
- ◆ **Release of oligosaccharide from GSL for characterization**
 Release of oligosaccharide from GSL by oxidation of olefinic double bond of Sph, followed by β -elimination; see text.

Table 3

Globo-series GSL structures

Gal α 4Gal β 4Glc β 1Cer	GB3 [36]
Gal α 3Gal β 4Glc β 1Cer	iso-Gb3 [52,56]
GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	Gb4 [36]
GalNAc β 3Gal α 3Gal β 4Glc β 1Cer	iso-Gb4 [51,21]
GalNAc α 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	Forssman [37]
Gal β 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	Gb5 (SSEA-3a) [54]
Fuc α 2Gal β 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	globo-H (SSEA-3b) [54]
SA α 3Gal β 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	monosialyl-Gb5 [53] (SSEA-4)
SA α 3Gal β 3GalNAc β 3Gal α 4Gal β 4Glc β 1Cer	disialyl-Gb5 [57]
SA α 2 ₆	

Lacto-series GSL structures

Biochim Biophys Acta. Author manuscript; available in PMC 2009 March 1.

Table 5

Human tumor-associated GSL antigens

sialyl-Le ^a	Galβ3GlcNβ3Galβ4GlcCer SA α Fuc α	[65] GI, pancrease	CA19-9
sialyl-Le ^x	Galβ4GlcNβ3Galβ4GlcCer SA α Fuc α	[64] GI, breast, lung	CSLX
SA-Le ^x -Le ^x	SAα3Galβ4GlcNβ3Galβ4GlcNβ3Galβ4GlcCer Fuc α Fuc α	[67] GI, colorectal	FH6
dimeric-Le ^a (Le ^a -Le ^a)	Galβ3GlcNβ3Galβ3GlcNβ3Galβ4GlcCer Fuc α Fuc α	[68,69] gastric, colorectal	ST-421
Gb3	Galα4Galβ4GlcCer	[74,78] Burkitt lymphoma, ovary	38-13
globo-H	Fucα2Galβ3GalNβ3Galα4Galβ4GlcCer	[79,80] breast, prostate	MBr1
GD3	SAα8SAα3Galβ4GlcCer	[75,76] melanoma	R24 4.2
fucosyl-GM1	Galβ3GalNβ4Galβ4GlcCer Fuc α SA α	discovery and chemical characterization: [81] mAb production: [82]	TKH5
GalNAcβ4 disialyl-Lc ₃	GalNβ4Galβ3GlcNβ3Galβ1→R SA α SA α	small cell lung cancer [83,84] renal cell Ca, prostate Ca	RM2

Table 6

Contrasting properties of "lipid raft" vs. "glycosynapse" or TCR synapse (immunological synapse)

Lipid raft		Glycosyn./ Immunosyn.	
♦	1% Triton X-100 insoluble	♦	1% Triton X-100 soluble 0.5% Brij95 insoluble
♦	cholesterol-dependent (disrupted by chol.-binding reagent)	♦	cholesterol-independent (resistant to chol.-binding reagent)
♦	tetraspanin (TSP)-independent	♦	tetraspanin-dependent
♦	diameter 10 nm - <100 nm	♦	diameter >100 nm; usually 500–1000 nm
♦	highly mobile	♦	less mobile, or non-mobile
♦	not involved in cell adhesion	♦	involved in cell adhesion with concurrent signaling

Sphingosine-dependent kinases (SDKs)

Biochim Biophys Acta. Author manuscript; available in PMC 2009 March 1.

Table 8
Trends in cell recognition through glycosyl epitopes

1.	Chydr recognition by protein (chydr-protein interaction; CPI) <ul style="list-style-type: none">• selectins, galectins, siglecs, and their ligands• many other chydr-binding proteins, their ligands
2.	Chydrs that modulate protein receptor function <p>Example:</p> <pre>graph LR ECM --> Itgr GF --> GFR Itgr <--> NGlycosylation GFR <--> NGlycosylation NGlycosylation <--> GSLsGangliosides GFR <--> GSLsGangliosides</pre>
3.	Chydr-chydr interaction (CCI) <ul style="list-style-type: none">• Homotypic: <i>e.g.</i>, self-recognition of Le^x or SO₃-GlcNAcβ4Fuca (see Fig. 5)• Heterotypic: <i>e.g.</i>, H-to-Le^y, Os Fr.B-to-GM3 (see Fig. 6)• faster reaction, higher specificity than CPI or PPI
4.	Chydr-nucleic acid interaction (CNI)