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Perspectives in Cancer Research

Aberrant Glycosylation in Cancer Cell Membranes as Focused on Glycolipids: Overview and Perspectives[1]

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Introduction: Glycosylation as a Modulatory Mechanism of Cellular Function and Cell-Social Interaction

A current trend in cell biology is to study the mechanisms of regulatory cellular function through specific structural and functional molecules, *i.e.*, growth factors, their receptors, membrane transporters, cytoskeletal systems, surface membrane and pericellular components, etc., rather than classical enzymes. The key molecules involved in such functional regulation are modified by 2 major chemical processes, phosphorylation and glycosylation. A greal deal of interest has been focused on phosphorylation as a ubiquitous and essential modulatory mechanism of cellular function. This idea has been greatly reinforced by the fact that many transforming genes (cellular as well as viral oncogenes) encode phosphoproteins, which by themselves are protein kinases, although the functions of many of their substrates have not been identified (see, for a review, Ref. 9).

Less attention has been paid to the functional significance of glycosylation than to that of phosphorylation; nevertheless, the changes in glycosylation occur as quickly and dramatically as the process of phosphorylation at various stages of development, differentiation, and oncogenesis (see, for a review, Ref. 38). While phosphorylation patterns are few, the patterns of glycosylation are numerous; over 100 types of glycosylated structures have been characterized among those bound to lipids (ceramides), *i.e.*, glycolipids (see, for a review, Ref. 39). Similar variation of glycosylation can also be found in the peripheral region of carbohydrates in glycoproteins, in addition to the structural variation in the core region of carbohydrate chains bound to proteins through *N*- or *O*-glycosides (see, for reviews, Refs. 42 and 65).

In contrast to phosphorylation, which directly modulates protein function, glycosylation affects the conformation, localization, and organization of functional and structural proteins; thus, glycosylation determines the turnover and organizational framework of proteins within cytoplasm, membranes, and the pericellular matrix (see, for reviews, Refs. 42 and 87). Lipid glycosylation in membranes, particularly cell surface membranes, offers cell recognition sites for cell-social events and may regulate the function of membrane proteins such as receptors and adhesive proteins (38, 115) (Table 1). With the functional significance of glycosylation in mind, this article will present: (*a*) the structural pattern of aberrant glycosylation in tumor cells and its relationship to formation of tumor-associated antigens; (*b*) the cell biological and immunological significance of aberrant glycosylation; and (*c*) prospects, application, and future trends of research in this area. The first item has been reviewed recently (40, 43), and only a brief summary is given.

Our current structural concept of cancer-associated aberrant glycosylation is based on the study of glycolipid epitopes, although the same epitopes are also found in glycoproteins. This is due to the technical fact that glycolipids can be purified to homogeneity, their structures can be elucidated, and a purified glycolipid maintains antigenicity, while oligosaccharide chains in glycoproteins are extremely heterogeneous and difficult to isolate to homogeneity, and antigenicity can be lost in isolated oligosaccharides. There have been tumor-associated glycoprotein epitopes defined by specific antibodies (4, 7, 8, 79) that do not cross-react with glycolipids. The chemical nature of such epitopes is essentially unknown.

Structural Pattern of Tumor-associated Glycosylation

Types of Aberrant Glycosylation in Tumor Cells: Tumor Cell Chemotype. Essentially all tumor cells display different profiles and structures of cell surface carbohydrates from those of nontransformed progenitor cells. Two chemically distinct groups of carbohydrates show drastic changes in tumor cells: (*a*) carbohydrates bound to ceramides (glycosphingolipids or glycolipids) inserted in the lipid bilayer; and (*b*) carbohydrates bound to cell surface proteins (glycoproteins). During the past 15 years, extensive biochemical studies have been focused on the elucidation of the changes of glycoconjugates in tumor cells as compared to their progenitors. The results of these studies are summarized in Table 2. Tentatively, 10 types of chemical changes in glycolipids can be listed. Types 1 to 5 are caused by incomplete synthesis of normally existing carbohydrate chains and accompanying precursor accumulation; types 6 to 10 are due to neosynthesis through activation of new glycosyltransferases that are characteristic of tumor cells and are absent, or present only in small quantities, in normal cells (see, for reviews, Refs. 40 and 43).

Recently, essentially the same changes that have been found in glycolipids, *i.e.*, neosynthesis and incomplete synthesis, have also been detected in the peripheral region of glycoprotein carbohydrates (17, 74, 116, 117). Nevertheless, the major common

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Table 1

Biological significance of glycosylation (Summarized from reviews in Refs. 38, 42, and 87)

Lipid glycosylation
1. Confers membrane rigidity
2. Regulates membrane protein function (receptors, adhesive proteins, etc.)
3. Determines cell social events, interaction with various ligands (antibodies, toxins, bacterial, viral, and cellular lectins)

Protein glycosylation
1. Maintains protein conformation
2. Affects the rate of protein degradation (regulates turnover)
3. Determines the localization, mobility, and organization of proteins within cells
4. Determines cell social events (the same as lipid glycosylation, but more indirectly)

Table 2

Chemotypes of aberrant glycosylation in tumor cells (summarized from results reviewed in Refs. 40 and 43)

A. In glycolipids
  a. Incomplete synthesis with or without precursor accumulation
    Type 1: Decrease or deletion of G_{M3},[a] G_{D3}: increase of LacCer, GlcCer
    Type 2: Decrease or deletion of G_{M1}, G_T, $G_{D1a/or\ b}$; increase of G_{M3}, G_{M2}
    Type 3: Decrease or deletion of Gb_4, Gb_5 or other longer neutral glycosphingolipids
    Type 4: Accumulation of asialo core (Gg_4, Gg_3, nLc_4) which is normally absent
    Type 5: Accumulation of G_{D3} or G_{D2}
  b. Neosynthesis (activation of a new addition of a glycosyl residue)
    Type 6: Neosynthesis of Gb_5 (Forssman) and other Forsmann hapten
    Type 7: Neosynthesis of incompatible blood group antigen foreign to the host
      A-like antigen in gastrointestinal tumors with Blood Group O or B host
      Pk antigen in Burkitt's lymphoma (irrespective of host blood group P status)
      P-like and P_1 antigen in the gastric cancer of the host with *pp* genotype
    Type 8: A linear chain elongation of type 2 chain coupled with $\alpha 1 \rightarrow 3$ fucosylation at every GlcNAc residue
    Type 9: Enhanced synthesis of X-hapten and its sialosylation
    Type 10: Enhanced synthesis of Le[a] hapten and its sialosylation
B. In glycoproteins
  a. Increased branch: GlcNAc-mannosyl core structure of asparagine-linked oligosaccharide is increased
  b. Increased density: *O*-glycoside mucin-type oligosaccharide chain
  c. Changes in peripheral region: The same as incomplete synthesis or neosynthesis in glycolipids (types 1 to 10)

[a] For glycolipid structures and abbreviations, see Refs. 38 and 39.

change in glycoprotein carbohydrates in tumors is the appearance of larger-molecular-weight glycopeptides than those in corresponding fractions derived from nontransformed progenitor cells (Warren-Blick-Buck phenomenon; for a review, see Ref. 110); these glycopeptides have been partially characterized as having a larger antennary structure due to increased branching at the mannosyl core of *N*-asparagine-linked oligosaccharide, *i.e.*, an increase in Galβ1→4GlcNAcβ1-Man[2] branches and bisecting GlcNAcβ1→4Man structure (85, 96, 103, 116). In addition, a second category of changes has recently been detected in *O*-glycosylated mucin-type structures by a specific reactivity defined by a few tumor-associated monoclonal antibodies (4, 7, 11, 69, 79). The antibodies seem to be directed to the sialosyl residue of a densely glycosylated region of sialosyl oligosaccharides linked to proteins (8, 33), although exact structural information is yet unknown.

The exact aberrant glycosylation pattern characteristic of a given type of tumor can be recognized more easily by the study of glycolipid rather than glycoprotein structure, since a specific glycolipid of a tumor cell can be isolated as a single component the structure of which can be exactly defined. This approach is difficult to apply to glycoproteins because glycoprotein carbohydrates are always heterogeneous. Thus, our current knowledge of the structure of tumor-associated carbohydrate antigens is essentially based on glycolipid studies. Application of well-defined anti-carbohydrate monoclonal antibodies (see, for a review, Ref. 37) makes it easier to identify carbohydrate structures in glycoproteins.

Tumor-associated Carbohydrate Markers Defined by Monoclonal Antibodies. Either precursor accumulation due to blocked synthesis or the appearance of a neoglycolipid due to neosynthesis results in the formation of a tumor-associated carbohydrate marker characteristic of a specific type of tumor cell. These glycolipid antigens have been detected by classical chemical-immunological analysis (Refs. 47, 95, and 118; see, for review, Refs. 40 and 43) as well as by the recently evolved monoclonal antibody approach which defines tumor-associated antigens (13, 16, 24, 29, 30, 31, 44, 45, 46, 54, 63, 64, 75, 83, 84, 91, 120). Gg_3 in murine lymphoma L5178 (120) and Kirsten murine sarcoma virus tumor (95) and Gb_3 in rat fibrosarcoma (KMT-17) (54) are typical examples of precursor accumulation in experimental cancers; G_{D_3} in human melanoma (83, 91) and human acute nonlymphocytic leukemia (100), G_{D_2} in human neuroectodermal tumors (16), Gg_3 in Hodgkin's lymphoma (62), and Gb_3 in Burkitt's lymphoma (84) are typical examples of tumor antigens representing precursor accumulation in human cancer. In these tumors, glycolipids with longer carbohydrate chains are deleted, and their precursors are preponderant, dominantly immunogenic, and recognized as tumor antigens by their respective monoclonal antibodies.

Accumulation of a large variety of fucosylated glycolipids (fucolipids) (13, 44, 118) and their sialosylated derivatives (fucogangliosides) (24, 31, 46, 64, 75) (see Table 3) has been found in a wide variety of human cancers, particularly those originating from such endodermal epithelia as gastrointestinal, lung, and mammary gland, which are the sites of the most common human cancers. These structures are defined by specific monoclonal antibodies with highly restricted specificities as listed in Table 3. However, none of them is the specific product of a given tumor. Some are present in small quantity in stem cells or in a specific type of cells in normal tissue from which the tumor was derived, and others are present in unrelated cells in normal tissues. The antigens in normal cells are often cryptic and immunologically undetectable. The appearance of these antigens could be due to activation of aberrant fucosyl- or sialosyltransferases and can be considered to be the result of neosynthesis (see Chart 1).

Expression of glycolipid antigens at the cell surface is not only controlled by the balance of synthesis and degradation but is also greatly influenced by their organization at the cell surface (58, 107), such as: (*a*) state of cluster, which depends on the antigen concentration in membranes; (*b*) state of coexisting glycolipids and proteins, which will mask the antigen; and (*c*) ceramide composition, which may determine the steric stability and orientation of the antigen. The factors controlling glycolipid crypticity were reviewed recently (40, 43).

[2] The abbreviations used are: Gal, galactose; Lac, lactose; Cer, ceramide; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; Man, mannose; G_{D_3}, G_{D_2}, disialogangliosides; G_{M_1}, G_{M_2}, G_{M_3}, monosialogangliosides; SSEA-1, stage-specific embryonic antigen 1; BHK, baby hamster kidney; HMG high-mobility globin; Gg_3, gangliotriaosylceramide; Gb_3, globotriaosylceramide.

Table 3

Novel fucolipids and fucogangliosides as human tumor-associated markers defined by specific monoclonal antibodies

	Association	Structure	Antibody	Ref.
A. Lacto-series type 1 chain				
Sialosyl Le^a	Gastrointestinal/pancreas cancer	Galβ1→3GlcNAcβ1→3Galβ1→R 3 4 ↑ ↑ NeuAcα2 Fucα1	N-19-9	75
B. Lacto-series type 2 chain				
Difucosyl Y_2 IIIV^{3}Fuc$_2$nLc6	Gastrointestinal/lung/breast cancer	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 3 ↑ ↑ Fucα1 Fucα1	FH4	29, 44
Sialosyldifucosyl Y_2 II^{3}V^{3}FucVI^{3}NeuAc	As above	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 3 3 ↑ ↑ ↑ NeuAcα2 Fucα1 Fucα1	FH6	31
Sialosyl Le^x	As above	Galβ1→4GlcNAcβ1→3Galβ1→R 3 3 ↑ ↑ NeuAcα2 Fucα1	CSLEXI	32
C. Globo series	Breast cancer	Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer 2 ↑ Fucα1	MBrl	13
D. Ganglio series[a]	Small cell lung carcinoma	Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer 2 3 ↑ ↑ Fucα1 NeuAcα2		82

[a] Monoclonal antibody defining this structure has been established (L. Svennerholm, personal communication).

Tumor-associated Carbohydrate Determinants Shared by Glycolipids and Glycoproteins. Many tumor-associated determinants that belong to the lacto- series (see Table 3) can be shared by glycolipids and glycoproteins such as X hapten, Y hapten, sialosyl Le^a, and sialosyl X. These determinants were found to be accumulated in various tumors and are defined by their respective antibodies. More recently, the same determinants have been found in the peripheral region of glycoprotein side chains (17, 18, 74, 97). X hapten structure was chemically identified in glycoproteins of human neuroblastoma tumors (97) and in carcinoembryonic antigens (17). Sialosyl X structure was identified in α_1-acid glycoprotein of liver metastases from various tumors (18), and sialosyl Le^a was found in serum glycoproteins of patients with various cancers (74). However, the exact proportion of glycolipid *versus* glycoprotein antigens has not been determined for any given tumor due to technical difficulty. On the other hand, the structures belonging to the globo- and ganglio- series shown in Table 3 are mainly expressed in glycolipids. G_{D_3} antigen of human melanoma (83, 91), Gb_3 antigen of Burkitt lymphoma (84), and Gg_3 antigen of Hodgkin's lymphoma (62), defined by their specific monoclonal antibodies, are not found in glycoproteins.

Interestingly, some antigens, particularly fucosyl-sialosyl determinants (sialosyl Le^a and sialosyl X), not only are present in cancer tissue but are also released into circulating blood; thus, the level of the antigen in body fluids can offer a useful criterion for diagnosis of human cancer (52, 64). The major sialosyl Le^a antigen in plasma of patients has been identified as a mucin-type glycoprotein rather than a glycolipid (74).

Plasma Gangliosides Shed from Tumors: Are They Immunosuppressive? In plasma, glycolipids are usually associated with lipoproteins (108). The increased level of gangliosides in plasma of tumor-bearing animals (61, 70, 101) and in patients with some human cancers (22, 61, 90) has been well documented. There is increasing experimental evidence that gangliosides may modulate the physiological response of lymphocytes as initially postulated by Esselman and Miller (23). Some investigators such as Krishnaraj *et al.* (66, 67, 71) and Ladish *et al.* (68) have extended the idea to imply that a higher level of gangliosides, particularly polysialosyl species, may contribute to the immunosuppressive status of tumor-bearing animals as well as patients with human cancer. Portoukalian *et al.* (89) observed that natural killer cell activity can be suppressed by G_{D_3} ganglioside *in vitro*. Although a general idea that gangliosides are the suppressor factor in tumor-bearing plasma is highly attractive, the data are still all based on *in vitro* assay. Thus, it is not clear whether the phenomenon can be applied for the role of gangliosides shed from tumors in immunosuppression *in vivo*. Recently, an excellent critical review on this topic was written by Marcus (77).

Enzymatic Basis of Aberrant Glycosylation: Is the Mechanism under the Control of Oncogenes? Although classical enzymology has focused on correlating the activities of glycosyltransferases and hydrolases with the patterns of aberrant glycosylation, the exact mechanism involved is far from understood. That synthesis of various glycolipids is blocked by suppression of glycosyltransferase activity has been demonstrated in a number of transformants induced by various tumor viruses (see, for review, Refs. 10, 40, and 43). In contrast, only a few examples of enhanced glycosyltransferases have been demonstrated, which include enhanced GalNAc transferase for synthesis of Gg_3 in 3T3 cells transformed by Kirsten strain murine

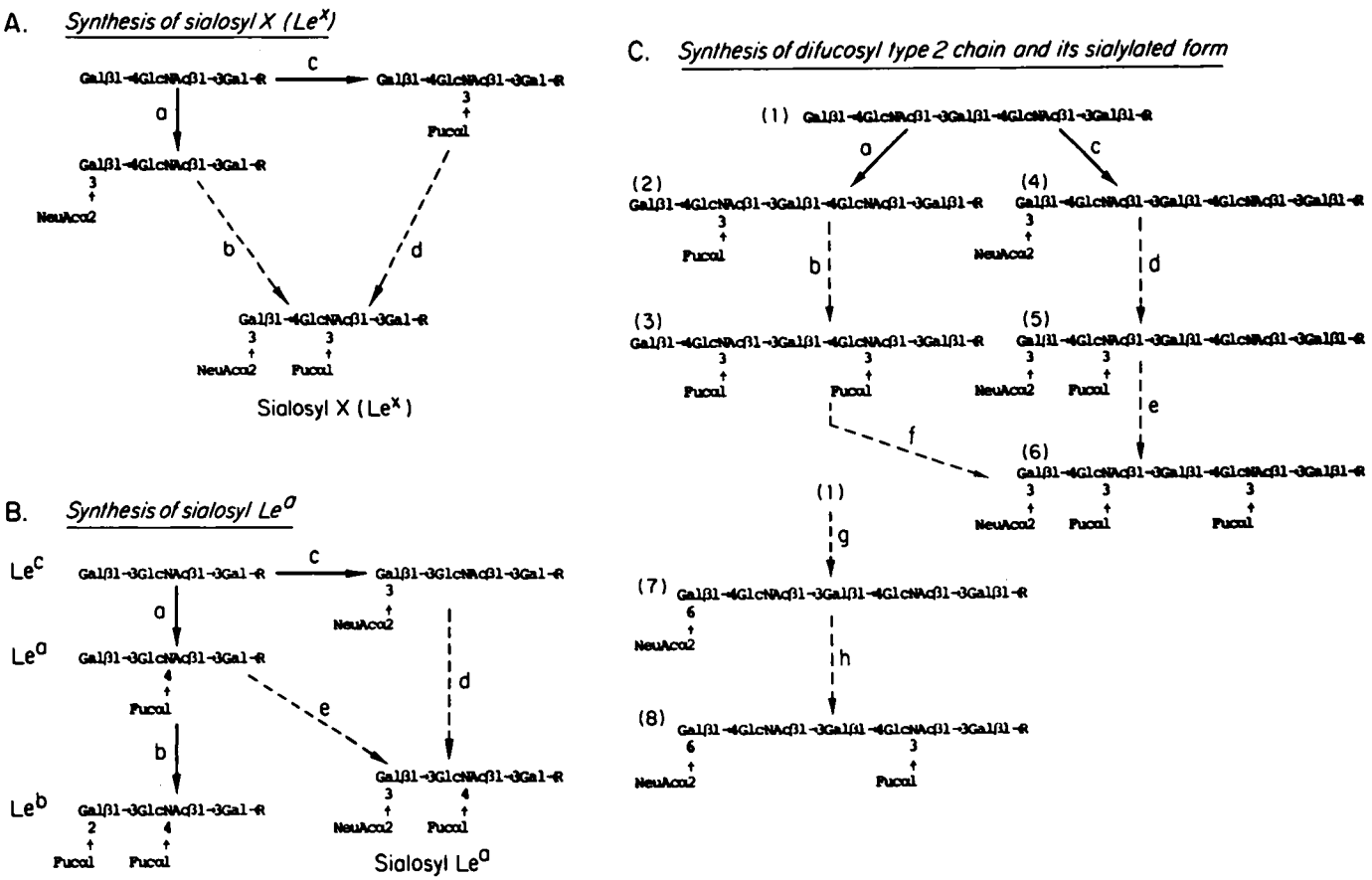

Chart 1. Possible synthesis of tumor-associated carbohydrate antigens based on the presence of glycosyltransferases with less restricted specificity than that of enzymes in normal cells. Tumor cells could be characterized by the presence of glycosyltransferases with less restricted substrate specificity than enzymes in normal cells. Examples are shown for synthesis of 3 structures: sialosyl X (Le^x) (*A*); sialosyl Le^a (*B*); and difucosyl type 2 chain and its sialosyl derivative (*C*). Synthesis of sialosyl X (Le^x) may precede sialylation of type 2 chain (*Route a* in *A*) followed by $\alpha 1 \rightarrow 3$ fucosylation (*Route b* in *A*). Alternatively, $\alpha 1 \rightarrow 3$ fucosylation (*Route c* and *A*) may be followed by sialylation (*Route d* in *A*). The former pathway (*Route a*→*b*) is more plausible than the latter (*Route c*→*d*) (51).[3] In many normal tissues (with the exception of kidney tubules and possibly some unknown tissues), fucosylation adjacent to sialosyl residues or sialylation adjacent to fucosyl residues is restricted, while in tumor cells either a sialosyltransferase or a fucosyltransferase may be less restricted, resulting in synthesis of sialosyl X through *Route c* or *d* in *A*. Similarly, synthesis of sialosyl Le^a requires sialylation of type 1 chain (*Route a* in *B*) followed by $\alpha 1 \rightarrow 4$ fucosylation (*Route d* in *b*).[4] This fucosylation may occur in both Le^a and Le^b individuals, but not in Le^{a-b-} individuals. An alternative route is sialylation of Le^a, which may also require a sialosyltransferase with less restricted substrate specificity. For synthesis of dimeric X (Le^x) structure, the unbranched type 2 chain (*Structure 1* in *C*) can be $\alpha 1 \rightarrow 3$ fucosylated at the penultimate GlcNAc residue (*Route a* in *C*) in normal tissue in advance of the $\alpha 1 \rightarrow 3$ fucosylation at the internal GlcNAc residue (*Route b* in *C*), which is catalyzed by an identical or similar α-fucosyltransferase, but the reaction is highly restricted in normal cells. Both routes (*a* and *b*) are characteristically enhanced in many human adenocarcinomas (51). The type 2 chain can be $\alpha 2 \rightarrow$ 3 sialylated (*Route c* in *C*) followed by $\alpha 1 \rightarrow 3$ fucosylation at the penultimate GlcNAc (*Route d* in *C*) as well as at the internal GlcNAc (*Route e* in *C*). Thus, a cancer-associated difucosylganglioside (*Structure 6* in *C*) (31) is accumulated. Alternatively, the difucosyl type 2 chain can be $\alpha 2 \rightarrow 3$ sialylated (*Route f* in *C*). *Routes d, e,* and *f* should be highly restricted in normal tissues and less restricted/or enhanced in human cancer. The $\alpha 2 \rightarrow 6$ sialylation of type 2 chain (*Route g* in *C*) is greatly enhanced in human adenocarcinoma. The sialosyl 2→6 type 2 chain can be fucosylated only at the internal GlcNAc (IIIGlcNAc) (*Route h* in *C*), and fucosylation of VGlcNAc is restricted (42). Normally restricted routes, which are less restricted or activated in cancer, are shown by dotted lines.

sarcoma virus (72), an induced fucosyltransferase for synthesis of fucosyl G_{M_1} in precancerous livers of rats fed with the chemical carcinogen 2-fluoroenylacetamide and in rat hepatoma (50), and an enhanced fucosyltransferase for synthesis of X determinant and polyfucosylated type 2 chain (51). The enzymatic basis of aberrant glycosylation in the core structure of glycoproteins, as described under "Chemistry of Aberrant Glycosylation" and in Table 2B, is of crucial importance. An aberrant β-GlcNAc transferase that makes the GlcNAcβ1→4Man structure must be a key enzyme. An enhanced β-GlcNAc transferase activity to make a bisecting GlcNAc in human lymphoma cells has been reported in a preliminary note (81). In general, enzymes involved in neosynthesis of aberrant carbohydrate chains may have a slightly less restricted substrate specificity than do normal enzymes, as shown in Chart 1. Subtle changes in substrate specificity may be caused by posttranslational modification of the enzymes.

Aberrant glycosylation in glycolipids or glycoproteins must be under the control, if not the direct influence, of transforming genes. Reversible shifts in glycolipids and in the core structure of glycopeptides were observed in cell lines transformed by temperature-sensitive mutants of tumor viruses at permissive and nonpermissive temperatures (see, for review, Refs. 40 and 43) and in cells transfected-transformed by human cancer DNAs (34, 105). Neosynthesis of G_{D_3} and enhanced synthesis of G_{M_2} and G_{M_1} associated with a decrease of G_{M_3} were found in rat cells transfected-transformed with a DNA segment derived from human adenovirus type 12 transforming gene (80). Thus, it is increasingly possible that aberrant glycosylation could result from a series of cascade mechanisms triggered by oncogene activation.

[3] E. Holmes and S. Hakomori, unpublished data.

[4] G. C. Hansson and D. Zopf, personal communication and Biosynthesis of the cancer-associated sialyl-Le^a antigen, J. Biol. Chem., in press, 1985.

Cell Biological Significance of Aberrant Glycosylation

Differentiation-dependent and Oncofetal Expression of Glycosylation. Essentially all carbohydrate chains in glycoproteins or in glycolipids undergo rapid and dramatic changes during development and differentiation. Well-defined examples are (*a*) alteration of branching structure in lacto-series carbohydrate chains identified as I/i interconversion in preimplantation embryo (59) and myelogenous leukemia or erythroleukemic cell differentiation (57, 104); (*b*) modification of type 2 chains responsible for the appearance of X-hapten (SSEA-1) (36, 45); (*c*) transition of globo-series antigen expression through P^k, P, and Forssman (113); and (*d*) switching of core structure from one series to another series of glycolipid in early embryogenesis (56) and in murine myelogenous leukemia cell differentiation (57). A multimeric lacto-*N*-fucopentaose III-lysyllysine conjugate has been shown to strongly inhibit compaction of morulae (25), indicating that X-hapten structure is essential in cell-cell recognition in morula-stage embryo for subsequent differentiation. An analogous role for each carbohydrate in every step of differentiation and development is predicted (41).

Aberrant glycosylation in cancer cells, therefore, may represent retrogenetic expression of carbohydrate synthesis to a certain stage of embryogenesis and fetal development, although evidence for this idea has been rather limited because an analytical technique for highly limited quantities of fetal tissue has not been available. However, monoclonal antibodies that define specific carbohydrate structures have provided opportunities for studying specific glycosylation in tissue at various stages of development. Recent immunohistological study of the distribution of mono- and difucosylated antigens defined by monoclonal antibodies FH3 and FH4 (30) in fetal tissue confirmed this idea.

Cell Growth Regulation and Ganglioside Changes. Several lines of evidence, shown in Table 4, indicate that glycolipids may regulate cell growth (for a review, see Ref. 38). Retinoid-induced G_{M_3} synthesis as related to cell growth inhibition or contact inhibition (88) has been specifically correlated with enhanced activity of CMP-sialic acid:lactosylceramide sialyltransferase (15). A decrease or deletion of G_{M_3} or G_{M_1} in many fibroblasts associated with oncogenic transformation may be related to a loss of growth control in those transformed cells, since exogenous addition of G_{M_3} or G_{M_1} restored normal cell growth and reduced saturation density (references in Ref. 38). Recent studies of cell growth in chemically defined media indicated that exogenous addition of G_{M_3} or G_{M_1} altered the binding affinity of cells to platelet-derived growth factor, epidermal growth factor, or fibroblast growth factor. The phenomenon is coupled to the inhibition of growth factor-dependent tyrosine phosphorylation of the platelet-derived growth factor receptor (12) as well as epidermal growth factor[5] receptor. Thus, a loss or decrease of cell type-nonspecific gangliosides such as G_{M_1} or G_{M_3} in certain transformed fibroblasts can be related to a loss of the regulatory mechanism of growth factor-dependent receptor function (Chart 2, *b–b′*).

Cell Adhesion and Ganglioside Changes. G_{M_3} or G_{M_1} of BHK cells (86) and G_{D_2} and G_{D_3} of melanoma cells (19) are present in the cell attachment matrix and may function to regulate adhesive proteins and their receptors. Metabolic turnover of G_{M_3} in transformed cell matrices was shown to be much higher than in normal cell matrices, although the chemical quantity of G_{M_3} in whole cells was much reduced in transformed BHK cells (86). G_{M_3} inhibited BHK cell attachment and spreading on plastic surfaces with or without fibronectin coating (86). G_{D_2} and G_{D_3} were detected by indirect immunofluorescence in adhesion plaques of human melanoma cells (19). Polysialosyl gangliosides inhibited fibronectin-mediated cell attachment (60) as well as lectin-mediated cell attachment (92), and a mutant cell line deficient in polysialoganglioside showed defective distribution of pericellular fibronectin and did not show fibronectin-dependent cell attachment (114). Thus, many gangliosides, particularly cell type-nonspecific gangliosides, may regulate cell adhesiveness; the decrease of cell adhesiveness and anchorage-independent cell growth may well be related to a loss of some ganglioside and accumulation of a tumor cell-associated ganglioside (Chart 2, *c–c′*).

Table 4

Evidence that glycolipids may regulate cell proliferation (items abstracted from Ref. 38)

1. Contact inhibition of cell growth accompanies change of glycolipid synthesis.
2. Various glycolipids are more highly exposed at G_1 phase, and some are more highly exposed at G_2 phase.
3. Butyrate induces cell growth inhibition and enhances G_{M3} synthesis.
4. Retinoids induce contact inhibition, enhance G_{M3} synthesis and glycolipid response.
5. Antibodies to G_{M3}, but not to globoside, inhibit 3T3 and NIL cell growth and enhance G_{M3} synthesis.
6. Exogenous addition of glycolipids incorporated into cell membranes inhibits cell growth through extension of G_1 phase.

Trends in Future Studies and Problems to Be Confronted

Mechanism of Aberrant Glycosylation and a Possible Genetic and Epigenetic Control. Aberrant glycosylation in tumor cells and its correlation with oncogene expression is undoubtedly one of the major problems to be solved. In many transformed cells, a specific enzyme defect or specific enhancement of enzyme activity can be demonstrated as a basis of aberrant glycosylation (see, for reviews, Refs. 10, 40, and 43). However, some transformed cells show a clear chemical change in carbohydrates, although the enzyme activity responsible for such carbohydrate changes cannot be demonstrated. For example, no positive correlation was found between CMP-sialic acid:LacCer sialyltransferase activity and the chemical level of G_{M_3} in chick embryonic fibroblasts infected with temperature-sensitive mutants of Rous sarcoma virus.[6] Burkitt's lymphoma contains a large quantity of Gb_3, which is defined by a monoclonal antibody directed to Burkitt's lymphoma antigen, but the enzyme for synthesis of this glycolipid (UDP-Gal:LacCer α-galactosyltransferase) cannot be exactly correlated with the level of Gb_3 accumulated in Burkitt's lymphoma (111). It is therefore possible that carbohydrate structures not only are controlled by the quantity and quality of glycosyltransferases but also can be regulated by the assembly and organization of multiglycosyltransferase complexes in membranes (*i.e.*, at the level of membrane organization). Such an organization and assembly of glycosyltransferases may be posttranslationally controlled by phosphorylation (15, 78) or glycosylation (55) of enzymes. The G_{M_3}:GalNAc transferase for synthesis of G_{M_2} was greatly en-

[5] E. Bremer, S. Hakomori, and J. Schlessinger, unpublished data.

[6] K. Itaya and S. Hakomori, unpublished observation.

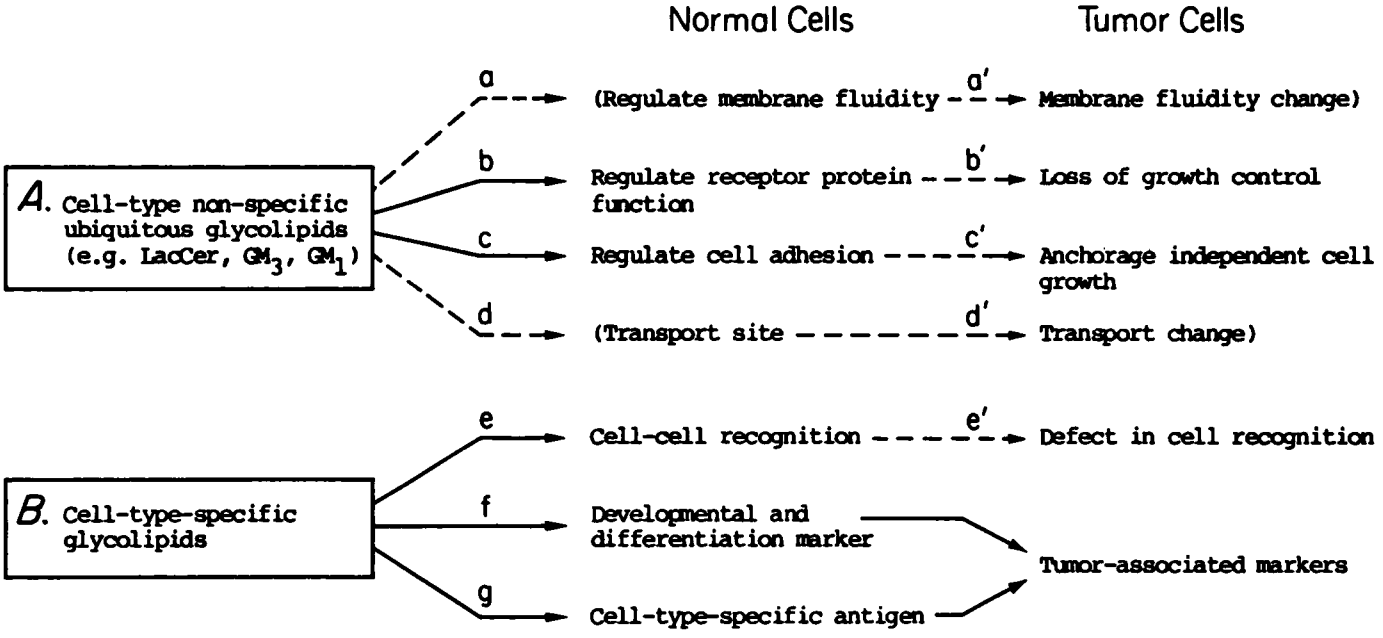

Chart 2. Glycosphingolipids as multifunctional membrane modulators. There are 2 classes of membrane glycolipids. One consists of cell type-nonspecific glycolipids (such as LacCer, G_{M_3}, G_{M_1}) distributed ubiquitously in various types of cells (Class A). The other class is made up of cell type-specific glycolipids which have complex structures (Class B). The former may function in regulating the intrinsic properties of membranes, while the latter may orient cell-cell interaction and define cell-social activity. Class A glycolipids may regulate fluidity and rigidity of membranes (*a*) and may affect the function of membrane receptors (*b*) and adhesive proteins (*c*). Although no strong evidence has been provided, this class may also affect the function of membrane transporters (*d*). Class B glycolipids are mainly involved in cell-cell or cell-environment interactions (*e*), and they are recognized as differentiation stage-specific, cell type-specific, or allogeneic antigens (*f*, *g*). Various phenotypes characteristic of tumor cells, such as membrane fluidity changes (*a'*), loss of growth control (*b'*), anchorage-independent cell growth (*c'*), and transport changes (*d'*), are closely associated with or indirectly caused by modification of Class A glycolipids. A defective cell recognition (*e'*) in tumor cells can be closely associated with the expression of tumor-associated markers which include a large variety of Class B glycolipids.

hanced by various factors that stimulate cyclic AMP-dependent protein kinases (78). The LacCer:sialyltransferase activity for synthesis of G_{M_3} was reduced by dephosphorylation and enhanced by ATP and cyclic AMP-dependent protein kinase (15). Although these data are very preliminary, phosphorylation and dephosphorylation may regulate glycosyltransferase activity. In addition, conformation of oligosaccharides may greatly affect the susceptibility of a substrate to glycosyltransferases and hydrolases (14, 98). Thus, the incorporation of a key glycosyl residue into an oligosaccharide may convert a nonsubstrate to a substrate for transferases and hydrolases. Such a mechanism might be important to create a large diversity of branched structures in *N*-linked oligosaccharides (14, 98).

If aberrant glycosylation is related to an enhanced or blocked synthesis of glycosyltransferases, the mechanism of activation or inactivation of transcription could be the major focus of future studies. According to the current concept of transcription control (48), changes in the promotor sequences (transcriptional control sequence), particularly the structural or organizational change of the initiator for the enhancer sequence for RNA polymerase II-encoding glycosyltransferase, would be of major interest. Deletion mutations and multiple- or single-point mutations should also be considered. On the other hand, various elements that define inactive or active transcription in chromatin, such as hypersensitivity to nucleases, presence of HMG, modification of both histone and non-histone proteins, and DNA methylation, etc. (20), should be considered. Reeves *et al.* (93, 94) demonstrated that HMGs which are preferentially, if not exclusively, associated with regions of active chromatin are highly glycosylated, particularly HMG 14 and HMG 17. For either possibility, cloning of genes for the transformation-sensitive glycosyltransferases and for their specific probes should be of immediate future concern. Such probes will be useful for clarifying chromosomal localization and organization and the mechanism of the activation of glycosyltransferase genes. The activation mechanism of the promotor, enhancement of glycosyltransferase genes, and the organization of a "hypersensitive" domain affecting glycosyltransferase genes should be eventually elucidated.

If aberrant glycosylation is caused by an organizational change of the enzymes, the mechanism involved must be posttranslational modification of the enzyme, *i.e.*, either phosphorylation or glycosylation. A reversible change of glycolipid synthesis triggered by oncogene activation, such as "incomplete synthesis," may also be caused by transcriptional or posttranslational change of glycosyltransferase.

How Aberrant Glycosylation Correlates with the Expression of Tumor Cell Phenotypes. Despite our increasing structural knowledge of aberrant glycosylation in tumor cells, very little is known about its cell biological significance. Do the glycosylation patterns correlate with the degree of malignancy? Or with the metastatic potentials? Or do they cause aberrant cell recognition and cell adhesion? Various cell-social abnormalities displayed in cancer cells should be studied as related to specific glycosylation patterns (see, for a review, Ref. 87). Meaningful efforts along this line have been made, *e.g.*, studies using lectin-selected variants of melanoma cells as related to metastatic potential (27, 28). A number of variants of sarcomas, melanomas, and lymphomas selected by different metastatic potentials have been correlated with the degree of sialylation (26, 119) and exposure of GalNAc→Gal residue (2, 99) and with the presence of a tumor-associated lectin that induces homotypic aggregation of tumor cells (73). However, knowledge of the distribution pattern of

carbohydrates and the change in various species of membrane components is important in order to correlate the glycosylation pattern with its cell biological function. The use of immunoblot labeling with radioactive lectins and monoclonal anticarbohydrate antibodies as applied on gels (53) and thin-layer chromatography plates (76) deepens our knowledge of the structural basis of aberrant glycosylation in tumors, and the knowledge will provide clues for subsequent biological studies.

Despite progress on a possible role of G_{M_1} or G_{M_3} in cell growth through tyrosine phosphorylation of growth factor receptors (12), little is known of a functional role of tumor-associated glycolipid antigens accumulated in tumor cells. Correlation between Gg_3 expression in mouse lymphoma and transferrin-dependent cell growth has been observed. Transferrin is the sole growth factor required for L5178 lymphoma in chemically defined media. A cross-linking of Gg_3 through biotinylated anti-Gg_3 IgM (2D4 antibody) completely inhibited the cell growth and transferrin internalization of the lymphoma; *i.e.*, a cross-linking of Gg_3 caused dysfunction of transferrin receptor function.[7] A similar mechanism can be applied to the growth inhibition of melanoma induced by anti-G_{D_3} antibody (R24 antibody) (21). Extension of studies along this line, exploring the effect of gangliosides and their antibodies on other receptors and transporters is certain to be fruitful.

Since a possible role of gangliosides in cell adhesion at the cell adhesion matrix has been postulated (19, 86), a specific role of gangliosides in cell adhesion and its modification in transformed cells may be important topics in the immediate future. The presence of a high concentration of G_{D_2} and G_{D_3} (19) in the melanoma cell matrix may correlate with the infiltrative property of melanoma cells.

Glycolipids present in the outer leaflet of the plasma membrane may greatly contribute to the rigidity and fluidity of the plasma membrane, an idea which has been supported by a few physical determinations (see, for review, Refs. 38 and 115). Dramatic changes in glycolipid composition in transformed cells may affect the change of membrane fluidity that has been widely observed in many transformed cells (Chart 2, *a–a'*). A direct correlation between glycolipid changes and membrane fluidity changes in tumor cells has not been examined and is an important item to be studied in the future. Classical studies in neurochemistry and plant physiology indicate that glycolipids may affect ion transport through membranes, although much work remains to be done to substantiate this hypothesis. The possibility exists, therefore, that transport changes in many transformed cells may also be correlated with glycolipid changes (Chart 2, *d–d'*). Since cell-cell recognition takes place by specific interaction through cell surface glycoconjugates and their recognition proteins, the altered composition of cell surface glycoconjugates in tumor cells may result in a defect in cell recognition. This idea has been widely considered; nevertheless, solid data to support this mechanism have not been furnished. Extensive study is needed in the future.

Future Trends of Study Utilizing Tumor-associated Carbohydrate Markers

Diagnosis of Cancer and Precancerous States. The presence of chemically well-characterized tumor-associated carbohydrate antigens defined by monoclonal antibodies is undoubtedly one of the major thrusts in current tumor immunology; its diagnostic application has at least partially proven to be rewarding. A few, if not many, antigens are detectable in circulating blood and thus are useful aids for early diagnosis (52, 64). A large variety of tumor-associated carbohydrate markers as discussed above and listed in Table 3 may be useful in histological diagnosis of *in situ* cancer. Some of them may be useful in premalignant diagnosis, since a premalignant change of a specific ganglioside marker has been well established (50). A labeled antibody could be used for tumor localization by scintillographic photoscanning (35). An effective accumulation of radiolabeled anti-SSEA-1 antibody to MH15 mouse teratocarcinoma and heterotransplanted choriocarcinoma grown *in vivo* was reported (6). Although SSEA-1 antigen is highly expressed in normal kidney tubules, radiolabeled antibody was minimally found in kidney (6). Data indicate that a successful immunolocalization of antibody may not require absolute tumor specificity but rather accessibility of the antigen to the antibody.

Therapeutic Applications. Antibodies to tumor-associated carbohydrates can be useful under certain circumstances to suppress tumor growth, if the right isotopes of antibodies and susceptible antigens are selected (5, 49, 120) and a single high dose of antibody is given (5). Effective antibody isotopes in mice are IgG3 (120) or IgG2a (5, 49), but not IgM. The mechanism of tumor suppression by these antibodies in mice tumors could be largely due to macrophage activation or to "antibody-dependent cytotoxicity" (1). On the other hand, a great deal of effort has been focused on a possible application of "immunotoxins" to tumor cells. A successful application of immunotoxins to an established mouse lymphoma has been reported (*e.g.*, Ref. 109). Glycolipid antigens appear to be more susceptible to immunotoxins than are other surface markers (112). A successful tumor cell killing *in vitro* dependent on biotinyl-anti-Gg_3 antibody, biotinylneocarzinostatin, and avidin was reported (106). Further studies on application of this or some other system of targeting immunotoxins *in vivo* is of great importance in practical application.

Tumor-associated glycolipid antigens are able to induce specific immune response in tumor-bearing hosts (102), and glycolipid-liposomes can inhibit tumor growth *in vivo* (3).[8] Thus, immunotherapy by active immunization with tumor-associated glycolipids is warranted in the immediate future.

The major drawback to targeting immunotoxins as well as immunotherapy in general is the heterogeneity of antigens expressed in tumor cells and the shedding of antigens from cell surfaces. Each tumor may have a different degree of cell heterogeneity and shedding of antigens therefrom. Exact knowledge of the antigen profiles and the degree of antigen shedding is essential, since these 2 factors are the major source of tumor cell growth escape from cytotoxic immune attack and antibody-mediated killing of tumor cells. It is important to establish the molecular basis of the heterogeneity of tumor cells. Mouse lymphoma that survived passive immunotherapy by IgG3 monoclonal antibody, directed to the glycolipid antigen Gg_3, showed the same karyotype as those that were killed but did not contain Gg_3 (118, 121). This heterogeneity of Gg_3 expression was induced by IgG3 antibody although its mechanism is unknown. Thus, an approach that combines multiple tumor markers is

[7] Y. Okada, H. Matsuura, and S. Hakomori, unpublished observation.

[8] T. Kaizu, J. S. Sundsmo, and S. Hakomori, unpublished observation.

important to suppress tumor growth *in vivo*. Further knowledge on the chemical properties of tumor markers will be essential to determine suitable therapy.

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Note Added in Proof

Since submission of this manuscript, some important information on fucoganglioside antigens has been published. Sialyl Le^a antigen defined by N-19-9 antibody was found in large quantity (1,300–15,000 units/ml) in human seminal fluid. This quantity is three times higher than that in tumor cells (Uhlenbruck, G., van Meensel-Maene, U., Hanisch, F.-G., and Dienst, C. Hoppe-Seyler's Z. Physiol. Chem., *365:* 613–617, 1984). The seminal plasma antigen was identified as being *O*-glycosidically-linked to serine or threonine (Hanisch, F.-G., Uhlenbruck, G., and Dienst, C. Eur. J. Biochem., *144:* 467–474, 1984). Nevertheless, expression of the marker in other normal adult tissue is restricted to gall bladder epithelia, ductal epithelia of pancreas, and salivary glands. The antigen was not found in other normal adult tissues as previously observed (63, 74). The antigen sialyl Le^x is highly expressed in esophagus mucosa, proximal tubuli, and Henle's loop and is moderately or weakly expressed in deep crypt of colon, alveolar macrophage, acinar cells of pancreas, hepatic cells, Kupfer cells, ureter, and granulocytes. No other adult normal tissues express this antigen (Chia, D., Terasaki, P. I., Suyama, N., Galton, J., Hirota, M., and Katz, D. Cancer Res., *45:* 435–437, 1985). In striking contrast, sialyl dimeric Le^x (31) is expressed only in proximal tubuli, Henle's loop, and granulocytes. No other adult tissues, including crypt area of gastrointestinal mucosa, express this antigen (Fukushi, Y., Kannagi, R., Hakormori, S., Shepard, T., Kulander, B. G., and Singer, J. W. Cancer Res., *45:* in press, 1985. These fucoganglioside antigens are expressed with characteristic incidence and intensity in gastrointestinal, colorectal, pancreatic liver, breast, and/or lung cancers. The serum level of all these antigens in patients with various cancers increases significantly as compared with the serum level in patients with nonmalignant diseases and normal subjects, although a clear elevation has only been observed in stage III and IV cancers (Chia *et al.* and Fukushi *et al.*, cited above). Further improvement of methods for the determination of antigen levels in serum will be required for practical use of these antigens for early diagnosis of human cancer.

Recently, a successful immunotherapy by large dose infusions of anti-G_{D_3} IgG3 antibody to melanoma patients has been reported (Houghton, A. N., Mintzer, D., Cordon-Cardo, C., Welt, S., Fliegel, B., Vadhan, S., Carswell, E., Melamed, M. R., Oettgen, H. F., and Old, L. J. Proc. Natl. Acad. Sci. USA, *82:* 1242–1246, 1985). Interestingly, G_{D_3} ganglioside is not only present in melanoma in high quantity, but is also found in high quantity in retina and in moderate quantity in kidney and intestinal tissue. Nevertheless, patients who received anti-G_{D_3} treatment did not develop neurological or kidney disorders. Only melanomas regressed. This finding greatly encourages the possibility that some of the IgG3 anti-glycolipid antibodies described in this review could be useful in the treatment of various types of human cancer. Glycolipid antigens present in normal tissue may well be organized in such a way that antibodies may not damage the tissue, as discussed in the test (6). Although no carbohydrate antigens have been shown to be "tumor-specific" (as reviewed in Ref. 40), many "tumor-associated" glycolipids found in appropriate density and organization at the cell surface could be useful targets for immunotherapy, if specific antibodies with suitable affinity and appropriate isotype were provided. This hope is contradictory to a prevailing pessimism (e.g., Feizi, T. Nature 314: 53–57, 1985), but further critical evaluation is essential.

References

1. Adams, D. O., Hall, T., Steplewski, Z., and Koprowski, H. Tumors undergoing rejection induced by monoclonal antibodies of the IgG2a isotype contain increased numbers of macrophages activated for a distinctive form of antibody-dependent cytosis. Proc. Natl. Acad. Sci. USA, *81:* 3506–3510, 1984.
2. Altevogt, P., Fogel, M., Cheingsong-Popov, R., Dennis, J., Robinson, P., and Schirrmacher, V. Different patterns of lectin binding and cell surface sialylation detected on related high- and low-metastatic tumor lines. Cancer Res., *43:* 5138–5144, 1983.
3. Ansel, S., and Blangy, D. *In vivo* induction of tumor-specific immunity by glycolipid extracts of SV40-transformed cells. Int. J. Cancer, *34:* 555–559, 1984.
4. Ashall, F., Bramwell, M. E., and Harris, H. A new marker for human cancer cells. 1. The Ca antigen and the Ca1 antibody. Lancet, *2:* 1–6, 1982.
5. Badger, C., and Bernstein, I. Therapy of murine leukemia with monoclonal antibody against a normal differentiation antigen. J. Exp. Med., *157:* 828–842, 1983.
6. Ballou, B., Jaffe, R., Taylor, J., Solter, D., and Hakala, T. R. Tumor radioimmunolocation: differential antibody retention by antigenic normal tissue and tumor. J. Immunol., *132:* 2111–2116, 1984.
7. Bast, R., Jr., Klug, T., St. John, E., Jenison, E., Niloff, J., Lazarus, H., Berkowitz, R., Leavitt, T., Griffiths, T., Parker, L., Zurawski, V., Jr., and Knapp, R. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N. Engl. J. Med., *309:* 883–887, 1983.
8. Bhavanandan, V. P., Kemper, J. G., and Bystyn, J. C. Purification and partial characterization of a murine melanoma-associated antigen. J. Biol. Chem., *255:* 5145–5153, 1980.
9. Bishop, J. M. Cellular oncogenes and retroviruses. Annu. Rev. Biochem., *52:* 301–354, 1983.
10. Brady, R. O., and Fishman, P. Biosynthesis of glycolipids in virus-transformed cells. Biochim. Biophys. Acta., *335:* 121–148, 1974.
11. Bramwell, M. E., Bhavanandan, V. P., Wiseman, G., and Harris, H. Structure and function of the Ca antigen. Br. J. Cancer, *48:* 177–183, 1983.
12. Bremer, E. G., Hakomori, S., Bowen-Pope, D. F., Raines, E., and Ross, R. Ganglioside-mediated modulation of cell growth, growth factor binding and receptor phosphorylation. J. Biol. Chem., *259:* 6818–6825, 1984.
13. Bremer, E. G., Levery, S. B., Sonnino, S., Ghidoni, R., Canevari, S., Kannagi, R., and Hakomori, S. Characterization of a glycosphingolipid antigen defined by the monoclonal antibody MBrl expressed in normal and neoplastic epithelial cells of human mammary gland. J. Biol. Chem., *259:* 14773–14777, 1984.
14. Brisson, J.-R., and Carver, J. P. The relation of three-dimensional structure to biosynthesis in the *N*-linked oligosaccharides. Can. J. Biochem. Cell Biol., *61;* 1067–1078, 1983.
15. Burczak, J. D., Soltysiak, R. M., and Sweeley, C. C. Regulation of membrane-bound enzymes of glycolipid biosynthesis. J. Lipid Res., in press, 1985.
16. Cahan, L. D., Irie, R. I., Singh, R., and Cassidenti, A. Identification of human neuroectodermal tumor antigen (OFA-1-1) as ganglioside G_{D_2}. Proc. Natl. Acad. Sci. USA, *80:* 5392–5396, 1982.
17. Chandrasekaran, E. V., Davila, M., Nixon, D. W., Goldfarb, M., and Medicino, J. F. Isolation and structures of the oligosaccharide units of carcinoembryonic antigen. J. Biol. Chem., *258:* 7213–7222, 1983.
18. Chandrasekaran, E. V., Davila, M., Nixon, D., and Mendicino, J. Structures of the oligosaccahride chains of two forms of α_1-acid glycoprotein purified from liver metastases of lung, colon, and breast tumors. Cancer Res., *44:* 1557–1567, 1984.
19. Cheresh, D. A., Harper, J. R., Schultz, G., and Reisfeld, R. A. Localization of the gangliosides GD_2 and GD_3 in adhesion plaques and on the surface of human melanoma cells., Proc. Natl. Acad. Sci. USA, *81:* 5767–5771, 1984.
20. Conklin, K. F., and Groudine, M. Chromatin structure and gene expression. *In:* A. Razin, H. Cedar, and A. Riggs (eds.), DNA Methylation: Biochemistry and Biological Significance, pp. 293–351. Berlin: Springer-Verlag, 1984.
21. Dippold, W. G., Knuth, A., and Meyer zum Buschenfelde, K.-H. Inhibition of human melanoma cell growth *in vitro* by monoclonal anti-G_{D_3}-ganglioside antibody. Cancer Res., *44:* 806–801, 1984.
22. Dnistrian, A. M., Schwartz, M. K., Katopodis, N., Frachia, A. A., and Stock, C. C. Serum lipid-bound sialic acid as a marker in breast cancer. Cancer (Phila.) *50:* 1815–1819, 1982.
23. Esselman, W. J., and Miller, H. Modulation of B cell responses by glycolipid released from antigen-stimulated T cells. J. Immunol., *119:* 1994–2000, 1977.
24. Falk, K.-E., Karlsson, K.-A., Larson, G., Thurin, J., Blaszcyk, M., Steplewski, Z., and Koprowski, H. Mass spectrometry of a human tumor glycolipid antigen being defined by mouse monoclonal antibody NS-19-9. Biochem. Biophys. Res. Commun., *100:* 383–391, 1983.
25. Fenderson, B. A., Zehavi, U., and Hakomori, S. A multivalent lacto-*N*-fucopentaose III-lysyllysine conjugate decompacts preimplantation-stage mouse embryos, while the free oligosaccharide is ineffective. J. Exp. Med., *160:* 1591–1596, 1984.
26. Fogel, M., Altevogt, P., and Schirrmacher, V. Metastatic potential severely altered by changes in tumor cell adhesiveness and cell-surface sialylation. J. Exp. Med., *157:* 371–376, 1983.
27. Finne, J., Burger, M., and Prieels, J.-P. Enzymatic basis for a lectin-resistant phenotype: increase in fucosyltransferase in mouse melanoma cells. J. Cell Biol., *92:* 277–282, 1982.
28. Finne, J., Tao, T.-W., and Burger, M. Carbohydrate changes in glycoproteins of a poorly metastasizing wheat germ agglutin-resistant melanoma clone. Cancer Res., *40:* 2580–2587, 1980.
29. Fukushi, Y., Hakomori, S., Nudelman, E., and Cochran, N. Novel fucolipids accumulating in human adenocarcinoma. II. Selective isolation of hybridoma antibodies that differentially recognize mono-, di-, and trifucosylated type 2 chain. J. Biol. Chem., *259:* 4681–4685, 1984.
30. Fukushi, Y., Hakomori, S., and Shepard, T. Localization and alteration of mono-, di-, and trifucosyl α1→3 type 2 chain structures during human embryogenesis and in human cancer. J. Exp. Med., *159:* 506–520, 1984.
31. Fukushi, Y., Nudelman, E., Levery, S. B., Rauvala, H., and Hakomori, S. A hybridoma antibody (FH6) defining a human cancer-associated difucoganglioside ($VI^3NeuAcV^3III^3Fuc_2nLc_6$); novel fucolipids accumulating in human cancer. III. J. Biol. Chem., *259:* 10511–10517, 1984.
32. Fukushima, J., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, H., Chia, D., Suyama, N., Fukushi, Y., Nudelman, E. and Hakomori, S. Characterization of a new tumor-associated antigen: sialosylated $Lewis^x$. Cancer Res., *44:* 5279–5285, 1984.

33. Funakoshi, I., and Yamashina, I. Structure of *O*-glycosidically linked sugar units from plasma membranes of an ascites hepatoma, AH-66. J. Biol. Chem., *257:* 3782–3787, 1982.
34. Glick, M. C., Santer, U. V., and Gilbert, F. Glycopeptide change after transfection of NIH 3T3 with human tumor DNA. Fed. Proc., *42:* 2189, 1983.
35. Goldenberg, D. M., DeLand, F., Kim, E., Bennett, S., Primus, F. J., van Nagell, J. R., Estes, N., DeSimone, P., and Rayburn, P. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N. Engl. J. Med., *298:* 1384–1388, 1978.
36. Gooi, H. C., Feizi, T., Kapadia, A., Knowles, B. B., Solter, D., and Evans, J. M. Stage-specific embryonic antigen involves α1→3 fucosylated type 2 blood group chains. Nature (Lond.), *292:* 156–158, 1981.
37. Hakomori, S. Monoclonal antibodies directed to cell-surface carbohydrates. *In:* R. H. Kennett, K. B. Bechtol, and T. J. McKearn, (eds.), Monoclonal Antibodies and Functional Cell Lines, pp. 67–100. New York: Plenum Publishing Corp., 1984.
38. Hakomori, S. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. Annu. Rev. Biochem., *50:* 733–764, 1981.
39. Hakomori, S. Chemistry of glycosphingolipids. *In:* J. N. Kanfer and S. Hakomori (eds.), Handbook of Lipid Research, Vol. 3, Chap. 1, pp. 1–165. New York: Plenum Publishing Corp., 1983.
40. Hakomori, S. Tumor-associated carbohydrate antigens. Annu. Rev. Immunol., *2:* 103–126, 1984.
41. Hakomori, S, Fukuda, M., and Nudelman, E. Role of cell surface carbohydrates in differentiation: Behavior of lactosaminoglycan in glycolipids and glycoproteins. *In:* T. Muramatsu, G. Gachelin, A. A. Moscona, and Y. Ikawa (eds.), Teratocarcinoma and Embryonic Cell Interactions, pp. 179–200. Tokyo: Japan Scientific Societies Press, 1982.
42. Hakomori, S., Fukuda, M., Sekiguchi, K., and Carter, W. G. Chemistry and function of pericellular and intercellular glycoproteins: fibronectin, laminin, and other matrix components. *In:* K. Piez and A. H. Reddi (eds.), Extracellular Matrix Biochemistry, Chap. 7, pp. 229–275. Amsterdam: Elsevier/North-Holland Biomedical Press, Inc., 1984.
43. Hakomori, S., and Kannagi, R. Glycosphingolipids as tumor-associated and differentiation markers. J. Natl. Cancer Inst., *71:* 231–251, 1983.
44. Hakomori, S., Nudelman, E., Levery, S. B., and Kannagi, R. Novel fucolipids accumulating in human adenocarcinoma. I. Glycolipids with di- or trifucosylated type 2 chain. J. Biol. Chem., *259:* 4672–4680, 1984.
45. Hakomori, S., Nudelman, E., Levery, S., Solter, D., and Knowles, B. B. The hapten structure of a developmentally regulated glycolipid antigen (SSEA-1) isolated from human erythrocytes and adenocarcinoma: a preliminary note. Biochem. Biophys. Res. Commun., *100:* 1578–1586, 1981.
46. Hakomori, S., Nudelman, E., Levery, S. B., and Patterson, C. M. Human cancer-associated gangliosides defined by a monoclonal antibody (IB9) directed to sialosyl α2→6galactosyl residue: a preliminary note. Biochem. Biophys. Res. Commun., *113:* 791–798, 1983.
47. Hakomori, S., Wang, S.-M., and Young, W. W., Jr. Isoantigenic expression of Forssman glycolipid in human gastric and colonic mucosa: its possible identity with "A-like antigen" in human cancer. Proc. Natl. Acad. Sci. USA, *74:* 3023–3027, 1977.
48. Hansen, U., and Sharp, P. A. Transcription by RNA polymerase II. Compr. Virol., *19:* 65–97, 1984.
49. Herlyn, D. M., Steplewski, Z., Herlyn, H. F., and Koprowski, H. Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. Cancer Res., *40:* 717–721, 1980.
50. Holmes, E. H., and Hakomori, S. Enzymatic basis for changes in fucoganglioside during chemical carcinogenesis: induction of a specific α-fucosyltransferase and status of an α-galactosyltransferase in precancerous rat liver and hepatoma. J. Biol. Chem., *258:* 3706–3713, 1983.
51. Holmes, E. H., Ostrander, G., and Hakomori, S. Enzymatic basis for the accumulation of glycolipids with X and dimeric X determinants in human lung cancer cells (NCI-H69). J. Biol. Chem., in press, 1985.
52. Iguro, T., Wakisaka, A., Terasaki, P. I., Hirota, M., Suyama, N., Fukushima, K., and Chia, D. Sialylated Lewis^x antigen detected in the sera of cancer patients. Lancet, in press, 1985.
53. Irimura, T., and Nicholson, G. Carbohydrate-chain analysis by lectin binding to mixtures of glycoproteins, separated by polyacrylamide slab-gel electrophoresis, with *in situ* modifications. Carbohydr. Res., *115:* 209–220, 1983.
54. Ito, M., Suzuki, E., Naiki, M., Sendo, F., and Arai, S. Carbohydrates as tumor-associated antigens. Int. J. Cancer, *34:* 689–697, 1984.
55. Ivatt, R. J. Regulation of glycoprotein synthesis by formation of specific glycosyltransferase complexes. Proc. Natl. Acad. Sci. USA, *78:* 4021–4025, 1981.
56. Kannagi, R., Cochran, N. A., Ishigami, F., Hakomori, S., Andrews, P. W., Knowles, B. B., and 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., *2:* 2355–2361, 1983.
57. Kannagi, R., Levery, S. B., and Hakomori, S. Sequential change of carbohydrate antigen associated with differentiation of murine leukemia cells: li-antigenic conversion and shifting of glycolipid synthesis. Proc. Natl. Acad. Sci. USA, *80:* 2844–2848, 1983.
58. Kannagi, R., Stroup, R., Cochran, N. A., Urdal, D. L., Young, W. W., Jr., and Hakomori, S. Factors affecting expression of glycolipid tumor antigens: influence of ceramide composition and coexisting glycolipid on the antigenicity of gangliotriaosylceramide in murine lymphoma cells. Cancer Res., *43:* 4997–5005, 1983.
59. Kapadia, A., Feizi, T., and Evans, M. J. Changes in the expression and polarization of blood group I and i antigens in post-implantation embryos and teratocarcinomas of mouse associated with cell differentiation. Exp. Cell Res., *131:* 185–195, 1981.
60. Kleinman, H. D., Martin, G. R., and Fishman, P. H. Ganglioside inhibition of fibronectin-mediated cell adhesion to collagen. Proc. Natl. Acad. Sci. USA, *76:* 3367–3371, 1979.
61. Kloppel, T. M., Keenan, T. W., Freeman, M. J., and Morre, D. J. Glycolipid-bound sialic acid in serum: increased levels in mice and human bearing mammary carcinomas. Proc. Natl. Acad. Sci. USA, *74:* 3011–3013, 1977.
62. Kniep, B., Monner, D. A., Burrichter, H., Diehl, V., and Muhlradt, P. F. Gangliotriaosylceramide (asialo G_{M_2}), a glycosphingolipid marker for cell lines from patients with Hodgkin's disease. J. Immunol., *131:* 1591–1594, 1983.
63. Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D., and Fuhrer, P. Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet., *5:* 957–972, 1979.
64. Koprowski, H., Herlyn, M., Steplewski, Z., and Sears, H. F. Specific antigen in serum of patients with colon carcinoma. Science (Wash. DC), *212:* 53–55, 1981.
65. Kornfeld, R., and Kornfeld, S. Structure of glycoproteins and their oligosaccharide units. *In:* W. J. Lennarz (ed.), The Biochemistry of Glycoproteins and Proteoglycans, pp. 1–34. New York: Plenum Publishing Corp., 1981.
66. Krishnaraj, R., Lengle, E. E., and Kemp, R. G. Murine leukemia, proposed role for gangliosides in immune suppression. Eur. J. Cancer Clin. Oncol., *18:* 89–98, 1982.
67. Krishnaraj, R., Lin, J., and Kemp, R. G. Lectin- and ionophore-stimulated Ca^{++} influx in murine lymphocytes: inhibition by disialoganglioside. Mol. Immunol., *78:* 152–160, 1983.
68. Ladisch, S., Gillard, B., Wong, C., and Ulsh, L. Shedding and immunoregulatory activity of YAC-1 lymphoma cell ganglioside. Cancer Res., *43:* 3808–3813, 1983.
69. Lan, M. S., Finn, O. J., Fernsten, P. D., and Metzgar, R. Isolation and properties of a human pancreatic adenocarcinoma-associated antigen, DU-PAN-2. Cancer Res., *45:* 305–310, 1985.
70. Lengle, E. E. Increased levels of lipid-bound sialic acid in thymic lymphocytes and plasma from leukemia AKR/J mice. J. Natl. Cancer Inst., *62:* 1565–1567, 1979.
71. Lengle, E. E., Krishnaraj, R., and Kemps, R. G. Inhibition of the lectin-induced mitogenic response of thrombocytes by glycolipids. Cancer Res., *39:* 817–822, 1979.
72. Lockney, M. W., Soltysiak, R. M., and Sweeley, C. C. Glycolipid *N*-acetylgalactosaminyltransferase activity in normal and Kirsten murine sarcoma virus transformed Balb/c 3T3 cells. Adv. Exp. Med. Biol. Monogr., *152:* 139–147, 1982.
73. Lotan, R., and Raz, A. Low colony formation *in vivo* and in culture as exhibited by metastatic melanoma cells selected for reduced homotypic aggregation. Cancer Res., *43:* 2088–2093, 1983.
74. Magnani, J., Steplewski, Z., Koprowski, H., and Ginsburg, V. Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody 19-9 in the sera of patients as mucin. Cancer Res., *43:* 5489–5492, 1983.
75. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopr, D., Steplewski, Z., Koprowski, H., and Ginsburg, V. A monoclonal antibody-defined associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-*N*-fucopentaose II. J. Biol. Chem., *257:* 14365–14369, 1982.
76. Magnani, J. L., Smith, D. F., and Ginsburg, V. Detection of gangliosides that bind cholera toxin: direct binding of ^{125}I-labeled toxin to thin-layer chromatograms. Anal. Biochem., *109:* 399–402, 1980.
77. Marcus, D. M. A review of the immunogenic and immunodulatory properties of glycosphingolipids. Mol. Immunol., *21:* 1083–1091, 1985.
78. McLawhon, R. W., Schuon, G. S., and Dawson, G. Glycolipids and opiate action. Eur. J. Cell Biol., *25:* 353–358, 1981.
79. Metzger, R. S., Gaillard, M. T., Levine, S. J., Tuck, F. L., Bossen, F. H., and Borowitz, M. J. Antigens of human pancreatic adenocarcinoma cells defined by murine monoclonal antibodies. Cancer Res., *42:* 601–608, 1982.
80. Nakamura, H., Sanai, Y., Shiroki, K., and Nagai, Y. Appearance of GD_3 ganglioside in rat cells on transfection with transforming gene E1 of human adenovirus type 12 DNA and its transcriptional subunits. J. Biochem. (Tokyo), *96:* 1471–1480, 1984.
81. Narasimhan, S., Shirley, M., Hewitt, J. M., Freedman, M. H., Gelfand, E. W., and Schachter, H. UDP-GlcNAc:GnGn (GlcNAc to Manβ1→4) β4-GlcNAc-transferase III (Gn-T III) in human tissues. Fed. Proc., *42:* 2199, 1983.
82. Nillson, O., Mansoon, J.-E., Brezicka, T., Holmgren, J., Lindholm, L., Sorenson, S., Yngvason, F., and Svennerholm, L. Fucosyl-GM_1—a ganglioside associated with small cell lung carcinomas. Glycoconjugate J. *1:* 43–49, 1984.
83. Nudelman, E., Hakomori, S., Kannagi, R., Levery, S., Yeh, M.-Y., Hellström, K. E., and Hellström, I. Characterization of a human melanoma-associated

ganglioside antigen defined by a monoclonal antibody. J. Biol. Chem., *257:* 12752–12756, 1982.
84. Nudelman, E., Kannagi, R., Hakomori, S., Parsons, M., Lipinski, M., Wiels, J., Fellous, M., and Tursz, T. A glycolipid antigen associated with Burkitt lymphoma defined by a monoclonal antibody. Science (Wash. DC), *220:* 509–511, 1983.
85. Ogata, S., Muramatsu, T., and Kobata, A. New structural characteristic of the large glycopeptides from transformed cells. Nature (Lond.), *259:* 580–582, 1976.
86. Okada, Y., Mugnai, G., Bremer, E. G., and Hakomori, S. Glycosphingolipids in detergent-insoluble substrate attachment matrix (DISAM) prepared from substrate attachment material (SAM): their role in regulating cell adhesion. Exp. Cell Res., *155:* 448–456, 1984.
87. Olden, K., Parnet, J. B., and White, S. L. Carbohydrate moieties of glycoproteins, a reevaluation of their function. Biochim. Biophys. Acta, *650:* 209–232, 1982.
88. Patt, L., Itaya, K., and Hakomori, S. Retinol induces density-dependent growth and changes in glycolipids and LETS. Nature (Lond.), *273:* 379–381, 1978.
89. Portoukalian, J., Geneve, J., Gerard, J.-P., and Dore, J.-F. Influence of melanoma tumor gangliosides on lymphocyte stimulation and NK activity in normal donors and melanoma patients. *In:* Proceedings of the 13th International Cancer Congress, Abstract 2545, p. 446. Seattle: 13th International Cancer Congress, Inc., 1982.
90. Portoukalian, J., Zwingelstein, G., Abdul-Malak, N., and Dore, J. F. Alterations of gangliosides in plasm and red cells of humans bearing melanoma tumors. Biochem. Bipohys. Res. Commun., *85:* 916–920, 1978.
91. Pukel, C. S., Lloyd, K. O., Trabassos, L. R., Dippold, W. G., Oettgen, J. F., and Old, L. J. GD_3 A prominent ganglioside of human melanoma: detection and characterization by mouse monoclonal antibody. J. Exp. Med., *155:* 1333–1147, 1982.
92. Rauvala, H., Carter, W. G., and Hakomori, S. Studies on cell adhesion and recognition. I. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (glycosidases and lectins) and fibronectin. J. Cell Biol., *88:* 127–137, 1981.
93. Reeves, R., and Chang, D. Investigations of the possible functions for glycosylation in the high mobility group proteins: evidence for a role in nuclear matrix association. J. Biol.Chem., *258:* 679–687, 1983.
94. Reeves, R., Chang, D., and Chung, S.-C. Carbohydrate modifications of the high mobility group proteins. Proc. Natl. Acad. Sci. USA, *78:* 6704–6708, 1981.
95. Rosenfelder, G., Young, W. W., Jr., and Hakomori, S. Association of the glycolipid pattern with antigenic alterations in mouse fibroblasts transformed by murine sarcoma viruses. Cancer Res., *37:* 1333–1339, 1977.
96. Santer, U. V., and Glick, M. C. Partial structure of a membrane glycopeptide from virus-transformed hamster cells. Biochemistry, *18:* 2533–2540, 1979.
97. Santer, U. V., Glick, M. C., van Halbeek, H., and Vliegenthart, J. F. G. Characterization of the neutral glycopeptides containing the structure α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucose from human neuroblastoma cells. Carbohydr. Res., *120:* 197–213, 1983.
98. Scachter, H., Narasimhan, S., Gleeson, P., and Vella, G. Control of branching during the biosynthesis of asparagine-linked oligosaccharides. Can. J. Biochem. Cell Biol., *61:* 1049–1066, 1983.
99. Schirrmacher, V., Altevogt, P., Fogel, M., Dennis, J., Waller, C. A., Barz, D., Schwartz, R., Cheingsong-Popov, R., Springer, G., Robinson, P. J., Nege, T., Brossmer, W., Vlodavsky, I., Paweletz, N., Zimmerman, P.-H., and Uhlenbruck, G. Importance of cell surface carbohydrates in cancer cell adhesion, invasion and metastasis. Invasion Metastatis, *2:* 313–360, 1982.
100. Siddiqui, B., Buehler, J., De Gregorio, M. W., and Macher, B. Differential expression of ganglioside GD_3 by human leukocytes and leukemia cells. Cancer Res., *44:* 5262–5265, 1984.
101. Skipski, V. P., Katopodis, N., Prendergast, J. S., and Stock, C. C. Gangliosides in blood serum of normal rats and Morris hepatoma 5123tc-bearing rats. Biochem. Biophys. Res. Commun., *67:* 1122–1127, 1975.
102. Sundsmo, J. S., and Hakomori, S. Lacto-*N*-neotetraosylceramide ("paragloboside") to a possible tumor-associated surface antigen of hamster NILpy tumor. Biochem. Biophys. Res. Commun., *68:* 799–806, 1976.
103. Takasaki, S., Ikehira, H., and Kobata, A. Increases of asparagine-linked oligosaccharides with branched outer chains caused by cell transformation. Biochem. Biophys. Res. Commun., *92:* 735–742, 1980.
104. Testa, U., Henri, A., Bettaieb, A., Titeux, M., Vainchenker, W., Tonthat, H., Docklear, M. C., and Rochant, H. Regulation of i- and I-antigen expression in the K562 cell line. Cancer Res., *42:* 4694–4700, 1982.
105. Tsuchiya, S., and Hakomori, S. Cell surface glycolipids of transformed NIH 3T3 cells transfected with DNAs of human bladder and lung carcinomas. EMBO J., *2:* 2323–2326, 1983.
106. Urdal, D. L., and Hakomori, S. Tumor-associated ganglio-*N*-triosylceramides target for antibody dependent, avidin mediated drug killing of tumor cells. J. Biol. Chem., *255:* 10509–10516, 1980.
107. Urdal, D. L., and Hakomori, S. Characterization of tumor-associated ganglio-*N*-triaosylceramide in mouse lymphoma and the dependency of its exposure and antigenicity on the sialosyl residues of a second glycoconjugate. J. Biol. Chem., *258:* 6869–6874, 1983.
108. van den Bergh, F. A. J. T. M., and Tager, J. M. Localization of neutral glycosphingolipids in human plasma. Biochim. Biophys. Acta, *441:* 391–402, 1976.
109. Vitetta, E. S., Krolick, K. A., Miyama-Inaba, M., Cushley, W., and Uhr, J. W. Immunotoxins: a new approach to cancer therapy. Science (Wash. DC), *219:* 644–650, 1983.
110. Warren, L., Buck, C. A., and Tuszynski, G. P. Glycopeptide changes and malignant transformation. A possible role for carbohydrate in malignant behavior. Biochim. Biophys. Acta, *516:* 97–123, 1978.
111. Wiels, J., Holmes, E. H., Cochran, N., Tursz, T., and Hakomori, S. Enzymatic and organizational difference in expression of a Burkitt lymphoma-associated antigen (globotriasoylceramide) in Burkitt lymphoma and lymphoblastoid cell lines. J. Biol. Chem., *259:* 14783–14787, 1984.
112. Wiels, J., Junqua, S., Dujardin, P., LePecq, J.-B., and Tursz, T. Properties of immunotoxins against a glycolipid antigen associated with Burkitt's lymphoma. Cancer Res., *44:* 129–133, 1984.
113. Willison, K. R., Karol, R. A., Suzuki, A., Kundu, S. K., and Marcus, D. M. Neutral glycolipid antigens as developmental markers of mouse teratocarcinoma and early embryos: an immunologic and chemical analysis. J. Immunol., *129:* 603–609, 1982.
114. Yamada, K. M., Critchley, D. R., Fishman, P. H., and Moss, J. Exogenous gangliosides enhance the interaction of fibronectin with ganglioside-deficient cells. Exp. Cell Res., *143:* 295–309, 1983.
115. Yamakawa, T., and Nagai, Y. Glycolipids at the cell surface and their biological significance. Trends Biochem. Sci., *3:* 128–131, 1978.
116. Yamashita, K., Hitoi, A., Taniguchi, N., Yokosawa, N., Tsukada, Y., and Kobata, A. Comparative study of the sugar chains of α-glutamyltranferases purified from rat liver and rat AH-66 hepatoma cells. Cancer Res., *43:* 5059–5063, 1983.
117. Yamashita, K., Tachibana, Y., Takeuchi, T., and Kobata, A. Structural study of the sugar chains α-amylases produced ectopically in tumors. J. Biochem. (Tokyo), *90:* 1281–1289, 1981.
118. Yang, H.-J., and Hakomori, S. A sphingolipid having a novel type of ceramide and lacto-*N*-fucopentaose III. J. Biol. Chem., *246:* 1192–1200, 1971.
119. Yogeeswaran, G., and Salk, P. L. Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines. Science (Wash. DC), *212:* 1514–1516, 1981.
120. Young, W. W., Jr., and Hakomori, S. Therapy of mouse lymphoma with monoclonal antibody to glycolipid: selection of low antigenic variants *in vivo*. Science (Wash. DC), *211:* 487–489, 1981.
121. Young, W. W., Jr., Tamura, Y., Johnson, H. S., and Miller, D. A. Decreased glycolipid antigen expression in lymphoma cell variants escaping from anti-glycolipid serotherapy. J. Exp. Med., *157:* 24–33, 1983.