Possible Roles of Tumor-associated Carbohydrate Antigens¹

Minoru Fukuda²

The Glycobiology Program, La Jolla Cancer Research Center, The Burnham Institute, La Jolla, California 92037

Abstract

Recent progress in the studies on the roles of carbohydrates has brought about critical discoveries, which allow us to have working hypotheses for understanding the roles of tumor-associated carbohydrate antigens. In this report, I focus my description on three different aspects of this progress. I discuss: (a) the immunological response to oligosaccharides aberrantly expressed under pathological conditions; (b) possible roles of carbohydrate-dependent cell adhesion during tumor metastasis; and (c) the roles of carbohydrates in modulating the functions of proteins that attach those carbohydrates. These three areas in the roles of carbohydrates will likely be the target for continuous research efforts to reveal the roles of tumor-associated carbohydrates.

Introduction

Cell surface glycoproteins play essential roles in maintaining the function as well as the structure of cells. The importance of cell surface carbohydrates is particularly evident during the course of development and differentiation, as shown by sequential expression and disappearance of cell surface carbohydrates specific to each cell lineage or maturation stage in a given cell lineage (1–4). At the end, mature cells acquire a vast array of different functions and cell surface structures unique to each cell type.

In the past decade, a large amount of knowledge has been accumulated on the carbohydrate structures present in glycoproteins and glycolipids. These studies revealed that those carbohydrates can be classified into several groups, for instance those attached to proteins through asparagylglycosylamine linkage, N-glycans, and those through O-glycosidic linkage, O-glycans. There appears to be a tremendous heterogeneity among oligosaccharides belonging to the same group, but yet those of apparent complexity can be deduced into the common core structure and the variation in the side chains attached to the core structure (5).

One of the most important findings in the past decade is that these variations of oligosaccharides are well controlled during development and differentiation. Thus, oligosaccharides are very often characteristic of different cell types. Under pathological conditions such as tumor cells, changes in carbohydrate structures can be almost always observed (6).

In this report, I would like to present three examples that exemplify the roles of those oligosaccharides present under pathological conditions. I will thus first describe the changes in O-linked oligosaccharides during inflammation and then extend the analysis into the roles of carbohydrate-dependent adhesion during metastasis. In the third part, I will discuss the roles of carbohydrates as a modulator of protein function. In particular, I will focus on the roles of polysialic acid in the N-CAM3 during development and tumor metastasis. Rather than collect a vast amount of knowledge on changes of cell surface carbohydrates in tumor cells, I have selected these three topics. By doing this, I hope that I will present clear examples that can be a model for working on other systems. Readers are encouraged to read other review articles to see other carbohydrate changes reported in tumor cells (6–9).

Expression of Branched Hexasaccharides Attached to Leukosialin (CD43) during Development and Malignancy

Analysis of cell surface glycoproteins of human leukocytes and leukemic cell lines revealed that the structures of O-glycans are cell type-specific. Erythrocytes express a tetrasaccharide, NeuNAc α2–3Galβ1–3(NeuNAc α2–6)GalNAc, whereas granulocytes express a hexasaccharide, NeuNAc α2–3Galβ1–3(NeuNAc α2–6)Galβ1–4GlcNAc β1–6GalNAc (10). The large majority of T-lymphocytes in peripheral blood express the same tetrasaccharide as erythrocytes express. T-lymphocytes are activated when engaging in immune response. Those activated T-lymphocytes now acquire the hexasaccharide (11). This conversion is caused by an increase in core 2 GnT, which forms a critical branch. In resting T-lymphocytes, the same enzyme is negligibly present, thus forming instead the tetrasaccharide (Fig. 1). The expression of core 2 GnT suppresses the expression of the tetrasaccharide, because this enzyme competes with α-2,6-sialyltransferase at the same C-6 position of N-acetylgalactosamine.

In T-lymphocytes, these O-glycans are attached to leukosialin (CD43, sialophorin). It has been shown that leukosialin is involved in cell-cell interaction: (a) the adhesion of T-lymphocytes to HeLa cells through LFA-1 binding to ICAM-1 was inhibited by expression of CD43 in HeLa cells. This inhibitory activity was abolished, however, when HeLa cells expressing leukosialin was treated with sialidase (12); (b) similarly, leukosialin was found to be concentrated in the cleavage furrow during cell division (13). Such highly restrictive distribution of leukosialin likely provides repulsion between dividing cells; (c) leukosialin is shed from neutrophils inhibiting the spreading and adhesion of activated neutrophils (14). Furthermore, activated T lymphocytes also shed leukosialin (15). Secretion of leukosialin appears to be required for cell adhesion, since leukosialin works as an anti-adhesive molecule. In fact, absence of leukosialin is present in the plasma, and its carbohydrate composition is consistent in that the leukosialin was derived from monocytes and lymphocytes (16).

More recent studies suggest that mucin-type O-glycans and leukosialin play a critical role in thymocyte development and cells of T lymphocytes, which originated from the bone marrow.
ROLES OF TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS

Fig. 1. The biosynthetic pathways of O-glycans (A) and poly-N-acetyllactosaminyl O-glycans (B). A. It has been shown that the tetrasaccharide (bottom left) is formed by sequential action of α2→3 sialyltransferase, followed by α2→6 sialyltransferase. When β1→6 N-acetylgalactosaminyltransferase, core 2 GnT, is present, the branched hexasaccharide (bottom right) is formed (based on Refs. 10, 11, and 22). B, poly-N-acetyllactosaminyl chain can be extended from the GlcNAcβ1→6 linkage formed in core 2. Sialylated poly-N-acetyllactosaminyl extension or N-acetyllactosamine can be further modified by α1→3 fucosyltransferase, forming sialyl Leα termini. The core 2 branch is emphasized by a box (based on Fukuda et al. (10), Piller et al. (11), and Maemura and Fukuda (47)).

It was discovered that the cortical thymocytes are strongly stained by a monoclonal antibody, T305, which preferentially reacts with the branched hexasaccharide, NeuNac α2→3 Galβ1→3 (NeuNac α2→3 Galβ1→4 GlcNAcβ1→6) GalNAc attached to leukosialin (17). In contrast, medullary thymocytes are negligibly stained by the same antibody. Consistent with this finding, the mRNA of core 2 GnT, which forms the core 2 branches, is abundantly present in cortical thymocytes whereas it is not detectable in medullary thymocytes (17). These results clearly indicate that the O-glycans attached to leukosialin undergo a profound change from the hexasaccharides and larger ones to the tetrasaccharides once thymocytes mature from the cortical thymus to the medullary thymus.

It is known that the majority of active thymocytes die before they enter into the medullary thymus. These results thus suggest a very provocative possibility that the selection for further maturation may be dependent on the turning off of core 2 GnT. Ectopic expression of core 2 GnT in the medullary thymus and its knock-out in the thymus should yield critical information to test this hypothesis.

It was also shown that adhesion of thymocytes to thymus epithelium can be inhibited by T305 antibody (17). It has been demonstrated that interaction between thymocyte-thymus epithelium plays an essential role in thymocyte maturation (19). These results combined together indicate that the interaction between the hexasaccharides on thymocytes and thymus epithelia plays a critical role(s) during thymocyte maturation in the cortical thymus. Once thymocytes are relatively matured, they no longer require the hexasaccharides for attachment to thymus epithelia.

Production of Autoantibodies against the Branched Oligosaccharides under Pathological Conditions

In the previous section, I have described the presence of the hexasaccharides in immature cortical thymocytes and its absence in undesirable thymocytes apparently takes place in the medullary thymus. These results thus suggest a very provocative possibility that the selection for further maturation may be dependent on the turning off of core 2 GnT. Ectopic expression of core 2 GnT in the medullary thymus and its knock-out in the thymus should yield critical information to test this hypothesis.

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Production of Autoantibodies against the Branched Oligosaccharides under Pathological Conditions

In the previous section, I have described the presence of the hexasaccharides in immature cortical thymocytes and its absence in
relatively matured medullary thymocytes as well as resting T lymphocytes in the peripheral blood. Once activated, T lymphocytes acquire again the hexasaccharides. This is a typical example in which proliferating matured cells show a phenotype similar to their immature precursor cells. This similarity can be extended further to the property displayed by T lymphocytes under pathological conditions:

(a) It was shown that leukemic cells derived from T lymphocytes in the peripheral blood are characterized by having a significant amount of the branched hexasaccharides (20, 21). This increase in the hexasaccharides is more prominent in those patients with acute lymphocytic leukemia than those with chronic lymphocytic leukemia (20).

(b) The increase of the hexasaccharides in the peripheral blood is observed in patients with the Wiskott-Aldrich syndrome (22, 23). The Wiskott-Aldrich syndrome is inherited as an X-linked recessive trait, most likely due to a defect(s) in a single locus of the X chromosome (24). The most recent study identified a gene, which is defective in the syndrome (25). The putative protein product of this gene has been sequenced with prolines, which is characteristic for SH3 binding, and a nuclear localization signal. In fact, a mouse homologue of the Wiskott-Aldrich gene, named N-3AP1, was cloned by virtue of its binding to the SH3 domains of the Nck oncogene.4 Nck protein is phosphorylated in response to activation of various cytoplasmic and receptor tyrosine kinases, and Nck has been shown to bind, via its SH2 domain, to activated platelet growth factor and epidermal growth factor receptors (for an example, see Ref. 26). These results indicate that the signal pathways by Nck may be regulated by its binding to the Wiskott-Aldrich gene product. It will be exciting to determine the roles of the Wiskott-Aldrich gene product in the signal pathways.

This syndrome is characterized by severe eczema, thrombocytopenia, and susceptibility to opportunistic infection. Patients with this syndrome fail to respond to polysaccharide antigens, rendering them susceptible to bacterial infection. The second critical problem is that T-cell number and function decline quickly with age. T-cell-dependent areas of lymph nodes and spleen becomes progressively depleted (24). This raised the possibility that one of the defects in this syndrome may be the defect in the thymus. Before the advent of better therapy such as bone marrow transplantation, the median life span of these patients was found to be 5.7 years. The cause of death is infection (53% of the total deaths), hemorrhage (27%), and malignant tumors (5%), which are mostly leukemia and Hodgkin's disease (24).

From the early stages of the studies, it was discovered that leukosialin behaves abnormally in leukocytes of patients having the Wiskott-Aldrich syndrome. Initially, the studies appeared to suggest that leukosialin is missing in leukocytes from the patients with this syndrome (27). However, it turned out that leukosialin is present but with a high molecular weight form that is the same as that found in activated T lymphocytes (22, 23). It was demonstrated that a substantially increased amount of the branched hexasaccharides are present in leukosialin derived from patients with the Wiskott-Aldrich syndrome. As described above, the hexasaccharides are present in the immature cortical thymocytes but absent in the mature resting T lymphocytes. The expression of the hexasaccharides thus appear to reflect that lymphocytes from the patients with the Wiskott-Aldrich syndrome are functionally immature as well. These results strongly suggest that the conversion of the hexasaccharide to the tetrasaccharide is critical for the maturation of T lymphocytes and its failure might induce premature release of immature T lymphocytes from the thymus.

Continuous expression of the hexasaccharide is due to the increased amount of core 2 GnT. It is thus tempting to speculate that the transcription of core 2 GnT is suppressed by the product of the wild-type gene, which is mutated in the Wiskott-Aldrich syndrome. Because of the mutation in the Wiskott-Aldrich syndrome, such gene product may not suppress the core 2 GnT gene expression any longer.

During the analysis of lymphocytes from various patients, it was discovered that the peripheral lymphocytes from AIDS patients also contained an increased amount of the hexasaccharide (20). When two different specimens were analyzed, the patient with more full-blown AIDS symptoms showed an increased amount of the hexasaccharide. The patients who were HIV-infected but asymptomatic in AIDS did not show an increase of the hexasaccharide. Interestingly, the sera from HIV-infected individuals were found to contain antibodies reactive with leukosialin, and the sera reacted with leukosialin in thymocytes but not with peripheral blood lymphocytes (28). When peripheral T lymphocytes are partially digested by neuraminidase, the reactivity with the AIDS patients' sera was increased. Leukosialin in thymocytes carry not only the hexasaccharides but also the pentasaccharides, Galβ1→3(NeuNAc α2→3 Galβ1→4 GlcNAcβ1→6) GalNAc or NeuNAc α2→3 Gal (Galβ1→4 GlcNAcβ1→6) GalNAc, which are partially sialylated oligosaccharides (20). In fact, the amount of Galβ1→3 GalNAc α2,3-sialyltransferase transcript is more abundant in medullary thymocytes than cortex thymocytes (29).

Taken together, these results strongly suggest that AIDS patients produce antibodies against leukosialin, responding to the increased amount of the partially sialylated oligosaccharides present on leukosialin under such pathological conditions.

It has been shown that antibodies to leukosialin can induce biochemical and functional changes in T lymphocytes in vitro (for an example, see Ref. 30). It is thus tempting to speculate that human anti-leukosialin antibodies in vivo may have similar effects on thymocytes, resulting in inappropriate activation of thymocytes during the process of maturation. It is presumed that the thymus is required for normal replenishment of CD4+ lymphocytes. Thymic dysfunction thus would be expected to prevent the replenishment of mature CD4+ cells killed by HIV-1.

Complementary to the above discoveries, it was found that the HIV-1-infected leukemic T-cell line CEM produced leukosialin that was undersialylated. Such hyposialylation was found to be associated with an impairment of leukosialin-mediated homotypic aggregation (31).

Although there has been no report on autoantibodies in the Wiskott-Aldrich syndrome, patients with the Wiskott-Aldrich syndrome may produce similar autoantibodies. If that is the case, those antibodies will likely induce the depletion of thymocytes, which is one of the major symptoms in this syndrome. The presence of autoantibodies in both syndromes could heighten the possibility that these autoantibodies are involved in the pathogenesis of these diseases.

Roles of CD43 (Leukosialin) Revealed by Its Overexpression or Gene Knock-out in Mice

The above described studies mostly address the roles of glycans attached to leukosialin. To directly determine the roles of leukosialin expression, overexpression of leukosialin in B-cell lineage and knock-out of the leukosialin gene were carried out in mice.

As described above, leukosialin is present in early B-cell progenitors but absent during maturation. Once B lymphocytes differentiate into plasma cells, leukosialin is expressed again. By fusing with the immunoglobulin heavy chain enhancer, leukosialin was continuously expressed in B lymphocytes (32). Those B lymphocytes obtained in the transgenic mice survive longer in vitro and show less sensitivity to apoptosis. These results suggest that leukosialin may deliver a signal

4 W. Li, personal communication (see also Refs. 91 and 92).
that reduces the number of B lymphocytes that would have normally undergone programmed cell death.

When the leukosialin gene was knocked out in mice, the knockout mice developed normal hematopoiesis. However, T lymphocytes from leukosialin-deficient mice showed an accelerated kinetics of T-cell activation than T lymphocytes from wild-type mice (33). This increased T-cell activation was found to be due to an increase in the proliferation ability of the leukosialin-deficient cells rather than in the number of lymphocytes in the allo-specific precursor pool. To determine how these proliferative abilities were achieved, the adhesion of thymocytes and splenic T lymphocytes were investigated. Thymocytes from leukosialin-deficient mice showed substantially increased homotypic adhesion compared with thymocytes from wild-type litter mates. Binding of leukosialin-deficient splenic T lymphocytes was nearly 100% greater than binding of leukosialin-containing cells to fibronectin and 50% greater to ICAM-1.

When mice were infected with vaccinia virus, leukosialin-deficient mice showed considerably greater CTL activity. Moreover, infectious virus was recovered from lungs and gonads, which was not observed from wild-type mice. These results suggest that defects involving tissue localization of effector cells or post-lysing viral clearance by phagocytes might occur in the absence of leukosialin.

These results, taken together, strongly suggest that leukosialin acts as an antiadhesive property that retards T-lymphocyte interactions. Its carbohydrate moiety, composed of a significant amount of sialic acid, clearly contributes to this function. In this context, the presence of partially sialylated oligosaccharides in leukosialin from the immunodeficient patients may have more significance than they act as immunogens to produce autoantibodies. Moreover, it is possible that the complexity of these oligosaccharides influences the antiadhesive property of leukosialin and possibly its susceptibility to proteases.

In summary, I have described the roles of mucin-type O-glycans attached to leukosialin and leukosialin itself in immunodeficient syndromes. This field may be one of the few fields where studies have been extensively carried out on both a protein and the glycosylation in a particular glycoprotein. Because of recent isolation of genes that might determine the expression of core 2 GnT, further studies will be expected to provide more insightful and exciting results that would reveal the roles of this interesting molecule. Such studies are expected to shed light on possible roles of autoantibodies formed against tumor-associated carbohydrate antigens.

Oligosaccharides in Inflammation and Tumor Metastasis

In the past several years, significant progress was made in understanding the roles of oligosaccharides in inflammation. This series of developments was initiated by structural studies on the cell surface carbohydrates of neutrophils. These early studies demonstrated that neutrophils contain \( \text{Le}^a, \text{Galβ1→4(Fucα1→3)GlcNAc→R} \) and sialyl \( \text{Le}^a, \text{NeuNαcα2→3 Galβ1→4(Fucα1→3)GlcNAc→R} \) (34, 35), but not blood group ABO antigens, which are the major oligosaccharides present in erythrocytes.

In 1989, the cDNAs for three different adhesion molecules, now collectively called "selectin," that bind to neutrophils and monocytes or endothelial cells were cloned. Among them, E- and P-selectin appear in the onset of inflammation, and binding of these selectins to neutrophils and monocytes results in the recruitment of these leukocytes into inflammatory sites (reviewed in Ref. 36).

Since all of these selectins were found to contain a carbohydrate-binding motif observed in animal lectins, it was natural to test if selectins bind to carbohydrates. These studies revealed that E- and P-selectin binds to sialyl \( \text{Le}^a \), present as a minor component in neutrophils (37–39). This binding of E-and P-selectins slows down the movement of neutrophils along the endothelial wall, which then allows leukocytes to have enough access to chemokines. Once chemokines are bound to their receptors on leukocytes, a signal is presumably transmitted through G-proteins, which activates integrins on the surfaces of leukocytes, establishing a firm attachment to endothelia. This firm attachment then leads into extravasation, possibly through homophilic interaction of platelet/endothelial cell adhesion molecule 1 (PECAM-1) (Fig. 2). It is possible that sialyl \( \text{Le}^a \) and E-selectin interaction also plays a role in cell-cell interaction during development. In fact, it has been shown that the interaction of E-selectin and sialyl \( \text{Le}^a \) is involved in capillary tube formation from bovine endothelial cells (40).

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two selectins differ in the requirement for how these oligosaccharide ligands are presented. It has been observed repeatedly that E-selectin binds to cells as long as they express sialyl Le\(^a\) structures. However, P-selectin binds to sialyl Le\(^a\) structures only when they are attached to particular glycoproteins. One of these carrier glycoproteins, PSGL-1, was cloned by the virtue of its high affinity binding to P-selectin (45). The amino acid sequence of PSGL-1 was found to contain a domain composed of tandem repeats that should attach a large number of mucin-type O-glycans. Similarly, the high affinity ligand for L-selectin is presented by two mucin-type glycoproteins, CD34 and GlyCAM-1 (46). These results strongly suggest that P- and L-selectins bind mucin-type O-glycans that are present as a cluster. It is likely that the multiple presentation of ligands leads into high affinity binding of P- and L-selectin. In contrast, E-selectin apparently binds to the carbohydrate ligands, regardless of how they are presented (37).

For the synthesis of the sialyl Le\(^a\) structure in mucin-type oligosaccharides, the presence of core 2 GnT is essential (Fig. 1; Ref. 47). Only those cells expressing core 2 GnT can present efficient ligands for P- and L-selectin. In some instances, it may even be important to have sialyl Le\(^a\) in mucin-type oligosaccharides for E-selectin. For example, activated T-lymphocytes adhere to E-selectin whereas resting T lymphocytes do not (48). As described above, activated T lymphocytes express core 2 GnT, thus allowing the expression of sialyl Le\(^a\) in mucin-type oligosaccharides present in activated T lymphocytes. These results strongly suggest that the expression of the branched hexasaccharide and its undersialylated forms widely play roles in cell-cell interaction.

Since both tumor cells and neutrophils contain sialyl Le\(^a\) on the cell surface, it is an attractive hypothesis that tumor cells use the same selectin-carbohydrate interaction during metastasis. When tumor cells disseminate, blood-borne tumor cells must adhere to endothelial cells at metastatic sites (49). Once tumor cells adhere to endothelial cells, they then penetrate through endothelial cells, moving into subendothelial tissues. Once tumor cells grow beneath the endothelia, tumor metastasis is established (see also Fig. 2). In fact, it was shown in early studies that tumor cells bind to endothelial cells by E-selectin/carbohydrate interaction (50).

It has been demonstrated that highly metastatic colon carcinoma cells bind more strongly to E-selectin expressed on activated, human endothelial cells than low-metastatic counterparts (51). A similar difference was found in the binding of the same colon carcinoma cells to mouse endothelial cells that were activated (51). The latter findings indicate that metastasis in nude mice mostly reflects tumor metastasis in humans, at least at the level of E-selectin-mediated adhesion. Poorly metastatic tumor cells expressing low levels of sialyl Le\(^a\) structure were genetically engineered to increase the amount of sialyl Le\(^a\). Resultant cells were found to adhere more strongly to E-selectins (52). More importantly, it was discovered that the amount of sialyl Le\(^a\) on colon carcinoma cells is directly correlated to the prognosis of the patients with these carcinomas (53). The patients with a higher expression of cell surface sialyl Le\(^a\) structures display much poorer prognosis than those with lower expression of sialyl Le\(^a\), although other histological criteria were apparently similar.

These combined studies strongly suggest that one of the key factors in metastatic spread is the abundance of sialyl Le\(^a\) structure and possibly sialyl Le\(^a\), the ligand for E- and P-selectin expressed in tumor cells. There are several points that need to be understood:

(a) It has not been determined whether tumor cells bind to P-selectin as well. This is a critical point since tumor cells aggregate around platelets (49) and there is a possibility that this aggregation is due to the interaction of P-selectin on platelets and carbohydrates on tumor cells. In this regard, it is important to point out that PSGL-1 contains not only sialyl Le\(^a\) but also sulfated tyrosine, which appears to play critical roles for the binding of PSGL-1 to P-selectin (54–56). Consistent with this result, it was reported that different kinds of tumor cells bind more efficiently to P-selectin after the cells were transfected to express PSGL-1 (57). These tumor cells apparently contain sulfated glycans that inhibit sialyl Le\(^a\) and sialyl Le\(^a\)-dependent adhesion to P-selectin (58). However, a recent report indicates that colonic carcinoma cells, the same cells used in the above studies (51, 52), can adhere to P-selectin under flow conditions (59), which should be closer to the actual situation when tumor cells adhere to platelets. These colonic tumor cells were shown to lack PSGL-1 (59). It is thus possible that tumor cells express a P-selectin ligand presenter(s) that is different from PSGL-1.

(b) In tumor cell binding to selectins, which N-glycans or mucin-type glycans primarily carry high-affinity ligands for selectins? In this regard, it is interesting to note that colo 205 colonic carcinoma cells express sialyl Le\(^a\) in mucin-type glycoprotein leukosialin, which may be involved in E- and P-selectin-mediated adhesion (60).

(c) How can the amount of sialyl Le\(^a\) in tumor cells be correlated to the presence of other carbohydrates present in tumor cells? For example, the presence of sialyl Tn, NeuNAca2→6GalNAca1→Ser/Thr or Tn antigen GalNAca1→Ser/Thr in mucin-type oligosaccharides likely indicates that the mucin-type O-glycans present in those tumor cells are shorter than those that do not express sialyl Tn or Tn antigen (61). If this is the case, those tumor cells likely contain less amounts of sialyl Le\(^a\) and sialyl Le\(^a\) structures. It is then possible that colonic carcinoma cells expressing sialyl Tn or Tn antigen is less metastatic than those that express less of the antigen. Contrary to this, it was reported that compared to patients with colonic tumors containing less amounts of sialyl Tn and Tn, those containing increased amounts of these antigens showed a poorer prognosis (62). Further studies are thus necessary to clarify this point. Those studies addressing the above three points should provide a comprehensive understanding on the roles of tumor-associated carbohydrate antigens during metastasis.

It is also necessary to mention here that carbohydrate-protein interaction may play a role beyond the scope of selectin-carbohydrate interaction. In fact, it has been shown that galectin-1 on endothelial cells, which belongs to a family of S-type lectin, is involved in apoptosis during T-cell development (63). It is noteworthy that the binding of galectin-1 to N-glycans on thymocytes is critical in this process. Mucin-type O-glycans are inhibiting this process (63). Considering that the conversion of O-glycans from the hexasaccharide to the tetrasaccharide in thymocyte development (17), it is likely that bulkier O-glycans are more efficient in inhibiting the interaction between galectin-1 and N-glycans. These results suggest that carbohydrate-protein interaction in tumor metastasis may also use mechanisms other than selectin-carbohydrate interaction. Further studies will be of great significance to reveal such interactions.

**Modulation of the N-CAM by Polysialic Acid**

Polysialic acid is a developmentally regulated carbohydrate composed of a linear homopolymer of α-2,8-linked sialic acid residues. Polysialic acid is mainly attached to the N-CAM. Although polysialylated N-CAM is abundant in embryonic tissues, the majority of N-CAM in adult tissues lacks this unique glycan (64–66). The presence of this large negatively charged carbohydrate modulates the adhesive property of N-CAM, and removal of polysialic acid increases binding between N-CAM-bearing liposomes and neuroblastoma cells expressing N-CAM (67, 68). During embryonic development, the polysialylated, embryonic N-CAM is restricted to specific cell types where cells are migrating (69, 70), and the removal of...
polysialic acid from the N-CAM was reported to influence motor neuron projections in the embryonic tissues (69). Other studies suggest that the polysialylated form of N-CAM not only exhibits antiadhesive properties on homophilic N-CAM interaction but also attenuates cell-cell interactions carried by other cell adhesion molecules (65, 71, 72). The recent studies of N-CAM knock-out mice demonstrated a defect in spatial learning and memory, due to an anomaly of the olfactory bulb and hippocampus (73 74), where polysialic acid is continuously expressed also in adult brain (75, 76). These results strongly suggest that polysialylation regulates the function of N-CAM. Due to the presence of polysialic acid in these tissues undergoing synaptic rearrangement and cell migration, polysialic acid is implicated in reducing N-CAM adhesion and thus perhaps allowing increased neurite outgrowth and cellular mobility.

Although the expression of polysialic acid is diminished in the majority of tissues in the adult, some tumors are known to re-express polysialic acid; thus, polysialic acid represents an oncodevelopmental antigen. The first example of this expression was discovered in Wilms’ tumor (77). Wilms’ tumor is a primary renal tumor of childhood and is characterized by the presence of structural elements, which are found during embryonic development of the human kidney. In particular, undifferentiated blastoma regions, which are composed of small closely packed cells, express the highest amount of polysialic acid. Regions showing epithelial differentiation, such as glomeruloid structures and tubules, exhibited only a weak staining. The majority of these polysialic acid glycans were found to be attached to N-CAM. These results clearly establish that Wilms’ tumors express polysialic acid, which is suppressed in normal adult kidney.

Very recently, a cDNA encoding a PST was cloned (78, 79). The predicted amino acid sequences of both human and hamster PST show that the enzyme consists of 359 amino acid residues and has homology with other sialyltransferases belonging to the sialyltransferase superfamily. It was also demonstrated that PST can form all α-2,8-sialic acid residues necessary for polysialic acid formation (80). Consistent with the above results on tissue distribution, mRNA of PST is substantially present in fetal kidney but absent in adult kidney (78). It will be thus of interest to test if the amount of the PST transcript is elevated in Wilms’ tumor. It will be also significant if Wr-I, the tumor suppressor gene identified in Wilms’ tumor (81), regulates the gene expression of PST.

Small cell lung carcinoma is a highly malignant tumor showing some neuroendocrine characteristics. It was found that human small cell lung carcinomas, irrespective of their histological type, express polysialic acid (82). Metastatic tumor cells, derived from small cell lung carcinomas, also express polysialic acid. In contrast, polysialic acid was not detected in the bronchial as well as gastrointestinal carcinoids. In one of the cell lines derived from a human small cell lung carcinoma (NCI-H69), a subline containing a negligible amount of polysialic acid (E2) and that containing a significant amount of polysialic acid (F3), respectively, were established (83). More importantly, compared to the polysialic acid-negative clonal subline, (E2), polysialic acid-positive clonal subline (F3) forms much more colonies in soft-agar or methyl cellulose. Most significantly, i.e. metastasis took place when F3 subline was s.c. injected into nude mice. In contrast, the polysialic acid-negative subline produced only a negligible amount of metastasis, despite the fact that both E2 and F3 express comparable amounts of N-CAM (83).

These results strongly suggest that masking or weakening of N-CAM homophilic or heterophilic interaction may allow those tumor cells to detach more easily from primary tumor. They also may be able to survive in the blood stream by preventing the attachment to endothelial cells.

Consistent with these discoveries, the amount of N-CAM itself is reduced in some tumor metastases (85, 86). In these tumors, either decreasing of N-CAM itself or weakening the adhesive capability of N-CAM by polysialic acid results in increased metastasis.

When tumor cells migrate and adhere to certain tissues, polysialic acid-containing N-CAMS appear to play roles. Natural killer cell-derived lymphomas express polysialylated N-CAM, and their metastatic patterns are characterized by unusual tissue involvement, such as the central nervous system, nasopharyx, gastrointestinal tract, skeletal muscle, and kidney (87, 88). The recent studies showed that mRNA of PST can be detected in all of these tissues (78). Moreover, those tissues express polysialic acid detected by anti-polysialic acid antibody. These results strongly suggest that lymphoma cells expressing polysialic acid and N-CAM migrate to the tissues where N-CAM and polysialic acid are expressed, achieving the binding of those lymphoma cells at the metastatic sites. For successful attachment of lymphoma cells, however, the amount of polysialic acid should be moderate in both lymphomas and tissues where the attachment takes place. If the polysialic acid content is too high, the detachment of lymphoma cells can take place, but the attachment of those cells to particular tissues may not be possible. Although there is no quantitative data of the polysialic acid content on lymphoma cells, it is noteworthy that the heart, which expresses a very high amount of polysialic acid (78), is not a preferential site for the attachment of lymphoma cells.

It has been shown clearly that neurite outgrowth is facilitated much better on substrate cells that express both N-CAM and polysialic acid than those expressing N-CAM alone (Fig. 3; Ref. 78). The presence of polysialic acid thus allows cells to migrate more efficiently, preventing static adhesion (89). Such modulated cell movement may be also critical when tumor cells eventually adhere to the sites where they
grow, after invading into subendothelial tissues and further migrating into the metastatic sites. Further studies will be important to determine the roles of polysialic acid by testing the metastatic capability of tumor cells that express a different amount of polysialic acid by gene manipulation. In this regard, it is also now possible to determine the roles of polysialylated gangliosides by using a cDNA encoding the newly identified ganglioside-specific polysialyltransferase (90).

In summary, I have described three different roles of tumor-associated carbohydrate antigens in the pathogenesis of tumor formation. The first section concerned immunogenicity toward gangliosides that are aberrantly synthesized. The second section concerned the ligand function of oligosaccharides in protein-carbohydrate interaction. The third section described the modulatory function of gangliosides attached to functional proteins. I believe that these three different functions of oligosaccharides will be continuously revealed in further studies on tumor-associated carbohydrate antigens.

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References


Possible Roles of Tumor-associated Carbohydrate Antigens

Minoru Fukuda