Current Topics

Frontier in Tumor Glycobiology

Recent Progress in Carbohydrate Biosynthesis and Function in Relation to Tumor Biology

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Recent development in carbohydrate markers and functions are described. Identification of carbohydrate epitope for cancer-specific antibody is introduced. This novel approach involves the key glycosyltransferases that synthesize tumor-associated carbohydrate-antigens and elucidate the biosynthetic pathways. This is the true determination of carbohydrate ligands and glycan array is secondary to determine the epitope. Tumor suppressor activity of carbohydrate is described. Cell surface carbohydrate, which expressed in normal cells is diminished in cancer cells, function as a tumor suppressor. Glycans attached to α-dystroglycan function as laminin-binding glycans. In cancer cells, oncogene downregulates laminin-binding glycans and they do not bind to laminin in extracellular matrix, making cells to mobile. Thus, laminin-binding glycans function to suppress the cell mobility, thereby suppressing tumor formation in normal cells. This article summarizes the recent progress in the regulation of carbohydrate function in cancer cells. Since the review is short and not comprehensive, other several important topics may be missing.

Key words  cancer specific carbohydrate antigen; antibody epitope; glycosyltransferase

1. CARBOHYDRATES LIGANDS NEED TO BE SYNTHESIZED

In the past three decades, the changes in cell surface carbohydrates in malignant transformation showed that the changes can be classified as follows: 1) Carbohydrates that are substantially increased in cancer cells compared to parent untransformed cells, 2) carbohydrates that decreased in cancer cells compared to parent untransformed cells, and 3) carbohydrates that are essentially unchanged after oncogenic transformation. In these three different groups, studies on the first group have been extensively carried out.1–3) In particular, the development of monoclonal antibodies specific to cancer cells has been very powerful in identifying carbohydrates that are enriched in cancer cells. By using these monoclonal antibodies, the expression of carbohydrates can be regarded as tumor-associated carbohydrate antigens.

In early studies however, these carbohydrates were not necessarily discovered as carbohydrate antigens were highly expressed in cancer cells, but these carbohydrates are in subset of cells such as embryonic cells. In other cases, a subset of normal cells express those carbohydrate antigens and cancer cells express the same carbohydrates in increased amounts and the difference in the expression in normal and cancer cell is its quantity. One of such example is sialyl Lewis X that is present in neutrophils (granulocytes) in blood. Sialyl Lewis X present on neutrophils function as a selectin ligand in neutrophils recruitment to inflammatory sites. In sulfated form, such as 6-sulfosialyl Lewis X, sialic acid α2→3Galβ1→4(Fucα1→3(sulfo→6))GlcNAc, this carbohydrate is present in unique vascular cells, high-endothelial (HEV) cells and is recognized by λ-selectin in lymphocytes when lymphocytes are recruited to lymph nodes and endothelial cells expressed at chronic inflammatory sites.4) Although an isomer of 6-sulfosialyl Lewis X, 6′-sulfo sialyl Lewis X sulfo→6Galβ1→4 (Fucα1→3)GlcNAc was also proposed as a selectin ligand,5) this was later excluded. This is because sulfation takes place before α1→3 fucosylation and α1,3-fucosyltransferase cannot add 6′-sulfated N-acetyllactosamine. In the same reason, sialic acid α2→6Galβ1→4GlcNAc is not utilized as a substrate for α1,3-fucosyltransferases (Fig. 1).

It is assumed that 6′-linked sialic acid or sulfated group can make steric hindrance for α1,3-fucosyltransferase act on 3-positive of N-acetylglucosamine. This exemplifies the importance for biosynthetic studies of carbohydrates antigens. Those carbohydrates that cannot be synthesized by glycosyltransferases cannot be an epitope for a monoclonal antibody or selectin ligands since such structures do not exist in nature.

In this regard, 6′-sulfosialyl Lewis X was originally reported as a ligand to SigLec8.6) This was determined by glycan array using chemical synthesized oligosaccharides. However, this oligosaccharide is not present in nature, and this conclusion was not correct. Later 6′-sulfo N-acetyllactosamine was also identified as a ligand. This ligand, sulfated→6Galβ1→4GlcNAc can be naturally synthesized and we have finally reached the correct conclusion. This exemplifies the conclusion that synthetic pathway of a given ligand oligosaccharide has to be determined before the oligosaccharide structure ligand was identified.

Recent studies on oligosaccharide ligands determination combined biosynthetic studies and glycan array using synthetic oligosaccharides have advantage. Cells defective with a desired epitope are transfected with a mixture of glycosidase and a mixture of sulfotransferase. Once such a transfection...
results in positivity of an antigen, first a mixture of glycosyltransferases and a mixture of sulfotransferases are separately transfected. The result will show if both glycosyltransferases and sulfotransferases are necessary. Then a sulfotransferases is deleted from a mixture of glycosyltransferase and a mixture of sulfotransferases. If the sulfotransferases is necessary for epitope oligosaccharide, its deletion results in no expression of epitope structure. By deleting one by one, the glycosyltransferases and sulfotransferases that are necessary for epitope expression is identified. This procedure will yield a synthetic pathway based on the epitope biosynthesis. Experiments using glycan array confirmed the biosynthetic studies. This combined approach would thus elucidate the biosynthetic pathways for forming the epitope.7) If a given cell line has an epitope oligosaccharide, short interfering RNA (siRNA)-mediated downregulation confirms that identified glycosyltransferases or sulfotransferases are critical in forming the epitope of oligosaccharides.

2. CELL SURFACE CARBOHYDRATES FUNCTION AS A TUMOR SUPPRESSOR

It has been extensively reported that cancer cells acquire carbohydrates, which are almost absent in normal cells. This type of carbohydrate can be used as tumor marker, and thus antibody specific to tumor-associated antigen recognize tumor cells. Conjugating toxic chemicals allow tumor-specific treatment using tumor-specific carbohydrates. With tumor associated carbohydrates specific or enriched in tumor cells, conjugate of tumor homing peptide anti-cancer can be used as tumor-targeting treatment described in a separate article.8,9)

Carbohydrates present in normal cells that are diminished in cancer cells are also present. This type of carbohydrates is studied only recently. In this article, we describe a few examples of this type of carbohydrates. α-Dystroglycan (α-DG) is attached to epithelial cells and in particular neuron cells, muscle cells, prostate and breast cells of epithelial cell origin. In wild-type cells, α-DG contained unique carbohydrate and it plays in interaction of epithelial cells and basement membrane.

The interaction is entirely dependent on carbohydrates in epithelial cells and protein (lamina) present in extracellular matrix and carbohydrates attached to α-DG.10) To synthesize a laminin-binding activity, the synthesis require unique enzyme LARGE.12) Enzymatic activity of LARGE is identified only very recently as α1,4-xyllosyltransferase and β1,3-glucuronyltransferases activity.13) These two enzymatic activities uniquely acts alternatively with xylα1→4GlcAβ1→3, thus forming a polymer of xylα1→4GlcAβ1→3.14) The polymer of laminin-binding activity is apparently at the reducing end linked to GalNAcβ1→3GlcNAcβ1→2(6-phosphate mannose).

In muscular dystrophy syndrome, glycosyltransferases and other enzymes participated in laminin glycan and synthesis is mutated, causing pathology of muscular dystrophy due to decreased α-DG–laminin interaction. In cancer cells, apart from this mutation, the enzymatic activity is apparently downregulated.11,15) This is particularly true for β3-N-acetylgalactosaminyltransferases-1 or β3GnT-1, which collaborates with LARGE to form laminin-binding activity.

In human prostate cancer, laminin-binding glycan is progressively decreased as tumor progresses.16) By lower binding of α-dystroglycan to laminin in the extracellular matrix, cells are mobile and more freely move due to integrin mediated signaling.
On the other hand, normal cells that have enough amount of laminin-binding glycans is co-expressed so that interaction of laminin and laminin-binding glycan suppress integrin-mediated cell migration. Since this synthesis require multiple enzymatic activities, it is reasonable to presume that one or more proteins regulate simultaneously multiple glycosyltransferases that synthesize laminin-binding glycan.

To identify the regulator, siRNA-library was screened for the inhibitor of laminin-binding glycans. As the product is determined by specific antibodies, activator or inhibitor can be identified. Indeed, high-throughput screening of siRNA library for kinase library showed that several genes are found to be inhibitor or activator. Among them Fer kinase is identified and noted as inhibitor for laminin-binding glycan in breast and prostate carcinoma. More importantly, Fer kinase lowers the transcription of β3GnT-1 and LARGE. Fer kinase was reported to play a role in prostate cancer formation. This may be at least partly due to downregulation of laminin-binding synthesis.

Most likely β3GnT-1 and LARGE are regulated indirectly by Fer through regulation of proteins. In order to identify a novel pathway, Fer kinase substrates are being isolated. If completed, this is the first clear demonstration of kinase-mediated gene regulation of glycosyltransferase.

Similar studies were carried out on sialyl Lewis X expression. Colonic cells are induced for epithelial-mesenchymal transition (EMT) by EGF-bFGF treatment. Mesenchymal-like cells expressed sialyl Lewis X and this was due to induced expression of sialyltransferases (ST3Gal, 3, 4) and fucosyltransferases (FUT3 and FUT6). This was most likely due to activation of Myc and expression of CDX2. This mechanism works for colonic cell surface carbohydrate during change in E-M transition.

Another glycan known to be a tumor suppressor is a core 3 oligosaccharide. Mucin-type O-glycans are classified according to their core structures. First, N-acetylgalactosamine is added by α-N-acetylgalactosaminyltransferase, forming α-GalNAc-Thr/Ser. α-GalNAc then serves as acceptor for β1,3-galactosyltransferase (core 1 forming enzyme, core 1 synthase). Core 1 forming enzyme requires a chaperone, Cosmc for core 1 synthase to be transferred to cis-Golgi. Missing in Cosmc but core 1 synthesis results in not active core 1 synthase activity. Core 1 oligosaccharide serves as a substrate for core 2 β1,6-N-acetylgallosaminyltransferase (C2GnT).

Resultant oligosaccharide is core 2 oligosaccharide. In a less frequent occasion, core 1 is not synthesized and instead core 3 β-N-acetylgalcosaminyl works (core 3 oligosaccharides). Core 3 oligossaccharide often remains, but further added core 4- forming enzyme. The core forming 4 enzyme activity is usually carried out by mucin core 2 β1,6-N-acetylgallosaminyltransferase (β1,6GlcNAc-2). This enzyme acts on both core 1 and core 3 that form both core 2 and core 4 oligosaccharides. Core 3 structure is expressed in normal tissue but disappear in cancer cells of gastrointestinal origin. Core 3 gene is knocked out in mouse and mutant mouse is much more susceptible to carcinogen. In colon carcinoma core 4 structures and its enzyme core2GnT-2 are found to be tumor suppressor genes. Core 3 synthesis lead to core 3 oligosaccharide in integrin thus, interfering in the formation of integrin 2β complex. Moreover, the absence of core 2 oligosaccharide leads to anomaly of intestine differentiation, probably due to incomplete maturation of surface mucosa. Terminal sugars can function as tumor suppressor. For
α1,4-linked GlcNAc to core 2 oligosaccharide prevent gastric mucosa from carcinoma in situ. α1,4-N-Acetlyglucosaminyltransferase was inactivated in mouse and mutant mice developed gastric adenocarcinomas in situ. This carcinogen takes place without inflammation, providing a novel model for carcinogenesis.\textsuperscript{23} α1,4-GlcNAc plays an antibiotic activity toward \textit{Helicobacter pylori},\textsuperscript{26} and comprehensive studies of this unique carbohydrate is worth pursuing.

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REFERENCES


