Branched N-glycans and their implications for cell adhesion, signaling and clinical applications for cancer biomarkers and in therapeutics

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Branched N-glycans are produced by a series of glycosyltransferases including N-acetylglucosaminyltransferases and fucosyltransferases and their corresponding genes. Glycans on specific glycoproteins, which are attached via the action of glycosyltransferases, play key roles in cell adhesion and signaling. Examples of this are adhesion molecules or signaling molecules such as integrin and E-cadherin, as well as membrane receptors such as the EGF and TGF-β receptors. These molecules also play pivotal roles in the underlying mechanism of a variety of disease such as cancer metastasis, diabetes, and chronic obstructive pulmonary disease (COPD). Alterations in the structures of branched N-glycans are also hallmark and are useful for cancer biomarkers and therapeutics against cancer. This mini-review describes some of our recent studies on a functional glycomics approach to the study of branched N-glycans produced by N-acetylglucosaminyltransferases III, IV, V and IX (Vb) (GnT-III, GnT-IV, V and IX (Vb)) and fucosyltransferase 8 (Fut8) and their pathophysiological significance, with emphasis on the importance of a systems glycobiology approach as a future perspective for glycobiology. [BMB reports 2011; 44(12): 772-781]

INTRODUCTION

As compared to the genome, transcriptome and proteome, the glycome is much more complex and heterogeneous and glycosylation is one of the most abundant protein modification reactions. In fact, over 50% of proteins have undergone glycosylation (1). Moreover structural studies of glycans are much more difficult because there are no methods for sequencing or synthesizing them, like DNA and proteins and no cloning techniques such as PCR are currently available. Even though capillary electrophoresis, Mass-spectrometry and NMR techniques have been extensively developed for the analysis of glycans, problems remain in terms of sensitivity and the quantitative analysis of glycans.

For the last 20 year or so, our group has been interested in the functions of N-linked glycans, especially glycosyltransferases that are involved in the N-linked glycan branching (2-8). Over time, we purified a series of glycosyltransferases to homogeneity using classical but, in fact, unique and sophisticated methodology such as affinity chromatography using donor or acceptor substrates. We were able to obtain partial amino acid sequences for these glycosyltransferases and to then successfully clone their cDNAs and genes. These studies made it possible to analyze the structure and function of N-linked glycans (8)

This mini-review will introduce our investigations and studies by other group as well in terms of the onset of disease, cancer biomarkers and therapeutics especially antibody therapy.

Biosynthesis of N-glycan branching structures

N-glycan branching structures are biosynthesized by various glycosyltransferases such as GnTs (N-acetylglucosaminyltransferases), Futs (fucosyltransferases) and GalTs (galactosyltransferases) and STs (sialyltransferases) in the Golgi apparatus.

N-acetylglucosaminyltransferases (GnTs) I to -VI act on a common core structure of Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn (9, 10) but in this mini-review we will focus on GnT-III, IV, V and IX (Vb) and Fut8 (Fig. 1).

Bisecting GlcNAc and GnT-III

GnT-III catalyzes the formation of a unique structure, namely the "bisecting GlcNAc", and is involved in the biosynthesis of complex and hybrid types of N-glycans. The enzyme was first reported by Narasimhan (11), Gleson and Schachter (12). On the other hand our group found that the enzyme which is involved in the turnover of glutathione, γ-glutamyltranspeptidase, is highly activated in the process of azo-dye induced hepatoma and that the same enzyme from various sources had identical properties, except for the sugar chains (13, 14). Subsequently, in collaboration with Kobata’s group, we identified the bisecting GlcNAc
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Fig. 1. Branched N-glycans and their relevant glycosyltransferases. Each GlcNAc branch may be elongated with galactose, poly-N-acetyllactosamine, sialic acid and fucose. GnT-VI is not found in humans.

structure in a γ-glutamyltranspeptidase purified from ascites hepatoma cells but this structure was not present in the enzyme from a normal liver (15). Our group developed an assay method for GnT-III that involved the use of pyridylaminated sugars as a fluorescent probe which was developed by Hase’s group (16-18). GnT-III activity was found to be very high in those tissues, including hepatoma tissues, kidney and brain of rats, whereas in normal liver tissue, the activity is almost negligible (19). We then purified this enzyme to homogeneity from rat kidney using an affinity column to which a biantennary sugar chain was immobilized. The enzyme was purified to homogeneity and a partial amino acid sequence was obtained and the cDNA and its gene was then cloned (20). Using a functional glycomics technique (21) we found various target proteins toward GnT-III on which bisecting GlcNAc was attached in vitro and in vivo. The GnT-III gene suppressed cancer metastasis in vivo in a model of lung cancer metastasis (22). We then found that one of the target proteins toward GnT-III was E-cadherin (23) which plays a key role in the suppression of cancer metastasis in an experimental rat model. The addition of bisecting GlcNAc to E-cadherin alters its distribution in the cell and most of the glycosylated E-cadherin is recruited to the cell surface of tumor cells and enhances cell-cell contacts of tumor cells (6, 23). Very recently Pinho et al. also reported the similar data (24) and also found that GnT-III induced a stabilizing effect on E-cadherin at the cell membrane by inducing a delay in the turnover rate of the protein that promotes the formation of a stable and functional adherens-junction, and further prevents clathrin-dependent E-cadherin endocytosis (unpublished). The other major glycoprotein on the cell surface is integrin. In fact, the overexpression of GnT-III resulted in a reduction in β1, 6 GlcNAc branching structures, along with an increase in the bisected N-glycans on integrins (6, 25, 26). This resulted in the inhibition of integrin-mediated cell spreading and migration, and the level of cellular phosphorylation. Conversely, the knockdown of endogenous GnT-III expression resulted in an increased cell migration, concomitant with an increase in β1-6GlcNAc-branched N-glycans on integrins. Thus, N-glycans can be considered to be either a positive or negative regulator of the biological functions of integrin. Zhao et al. reported a similar type of regulation in α3 integrin (27), α5 integrin (28) and laminin 332 (29). Therefore GnT-III and GnT-V have adverse effects on cancer invasion and metastasis by adding bisecting GlcNAc or β1-6GlcNAc branching (Fig. 2) to a glycan structure. Detailed information on GnT-V will be described below.

GnT-III also regulates various signaling molecules or signaling pathways by adding the bisecting GlcNAc to these mole-