Transcriptional Regulation of Glycan Expression

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Abstract

Glycan structures on the cell surface are altered in various human diseases and disorders. These changes are, in most cases, associated with aberrant changes in glycosyltransferase expression. The activities of glycosyltransferases are controlled by several mechanisms, including the regulation of the expression of their transcripts. Many studies have revealed the important role of transcription factors in the transcriptional regulation of glycosyltransferases that are involved in the regulation of glycans on several types of glycoconjugates. Addressing the regulatory role of transcription factors not only sheds considerable light on the molecular mechanisms underlying the glycosyltransferase gene regulation but also provides a solid foundation for understanding the potential contribution of transcription factor-mediated regulation to particular diseases and identifying potential, novel targets for therapeutic intervention. In this chapter, the regulation of glycans, in particular N- and O-linked glycoprotein glycans, by transcription factors is summarized and a perspective on this regulation is presented.

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Introduction

Protein glycosylation is the most prevalent and complex posttranslational modification (Varki 1993). Glycan structures associated with proteins and lipids have been shown to regulate numerous biological and physiological events, such as protein folding and quality control, protein function and signaling, protein half-life and endocytosis, cell-cell interaction, autoimmunity, hormone bioactivity, gene expression, tumor metastasis, and many others (Moremen et al. 2012; Varki 1993). Most glycans on membrane and secreted proteins can be classified into two major types based on their linkage to polypeptide structures: N-linked glycans and O-linked glycans. Glycans attached to Asn side chains of polypeptides form *N*-linked glycans, while *O*-glycosylation is initiated by the addition of O-linked *N*-acetylglucosamine (GalNAc) to serine or threonine, catalyzed by a family of polypeptide *N*-acetylgalactosaminyltransferases (ppGalNAcT) enzymes. In addition to O-linked GalNAc, commonly referred to as mucin-type *O*-glycosylation, additional, distinct glycan types linked to Ser or Thr with defined functions have been identified, including *O*-mannose,*O*-Fuc, and *O*-GlcNAc (Moremen et al. 2012). Glycan structures on glycoproteins are complex with a great diversity for branching and anomeric linkages. Although the biosynthesis of the polypeptides of glycoproteins is controlled by the transcription and translation of their corresponding genes, the posttranslational glycosylation of these glycoproteins is not template driven. Instead, hundreds of proteins are involved in the biosynthesis of the various kinds of glycan structures. Among these, about 250 open reading frames in the mouse and human genomes are identified as glycosyltransferases, the enzymes that catalyze the addition of glycans to polypeptides using nucleotide or lipid-linked sugars as activated donor substrate. Therefore, the expression levels of glycans on glycoconjugates are determined primarily by the activities of corresponding glycosyltransferases. The transcripts and expression of glycosyltransferases are regulated during embryonic development, cellular differentiation, and proliferation. Aberrant expression of these enzymes results in aberrant glycans, which have been associated with a wide range of human diseases, including tumor, diabetes, cardiovascular, immunological, infectious, and congenital disorders (Marth and Grewal 2008).

The expression of glycosyltransferases is controlled by many biological effectors, including the Golgi complex itself, which can regulate the function of glycosyltransferases, and various transcriptional regulators that control glycosyltransferase gene expression, such as the binding of transcription factors to promoter regions, as well as epigenetic regulation. Transcription factors are a group of proteins that recognize and bind to a segment of DNA in the promoter and/or enhancer region, recruiting cofactors and RNA polymerase II and regulating the expression of specific genes (Lee and Young 2013; Spitz and Furlong 2012).

There are two different types of transcription factors based on their regulatory responsibilities, those that control initiation and those that control elongation. Most transcription factors are believed to control transcription initiation by recruiting co-activators, such as the Mediator complex, P300, and general transcription factors (Juven-Gershon and Kadonaga 2010; Lee and Young 2013; Taatjes 2010). Gene regulation by transcription factors plays a pivotal role in many cell events, and several human diseases and syndromes have been linked to the mutations in transcription factors, including diabetes, cardiovascular diseases, autoimmunity, and certain cancers (Lee and Young 2013).

Regulation of Glycans by Transcription Factors

Many studies have investigated the transcriptional regulation of glycosyltransferase gene expression by transcription factors using various strategies, such as rapid amplification of 5′ cDNA ends (5′ RACE), luciferase reporter assays, series deletion of promoter regions, site-directed mutagenesis, electrophoretic mobility shift assays (EMSA), and chromatin immunoprecipitation (ChIP) assays. Results show that the expression of glycosyltransferases associated with the initiation, extension, branching, and modification of both N- and O-linked glycans are regulated by transcription factors in a tissue- or cell-specific manner. The types of transcription factors involved in this regulation vary from the common families of transcription factors, such as Ets and AP-2, to some differentiation inducers and cytokines that directly bind to cis-regulatory elements in flanking sequences of glycosyltransferase genes, such as ATRA and IL13. The binding of transcription factors to promoter regions influences either positively or negatively the transcription of specific genes. Table 1 summarizes the involvement of transcription factors in the regulation of the expression of particular glycosyltransferases.

Perspective

A study that cataloged changes of glycosyltransferase transcript expression, as well as changes in glycan structures, during mouse embryonic stem cell differentiation also compared these two sets of data to determine the degree to which the changes were associated (Nairn et al. 2012). The conclusion was that in by far the majority of cases, there was a positive association between changes in a particular glycosyltransferase transcript and the changes in glycan expression whose levels these enzymes should impact. This result suggests that the most common mechanism for regulation of glycan expression is due to changes in glycosyltransferase transcript expression. There were, however, a significant number of cases where there were changes in a transcript but no corresponding change in the glycan products synthesized by the enzyme encoded by the transcript, or vice versa, there were no changes in transcripts but there were observed changes in corresponding glycan products attributed to the enzyme encoded by these transcripts. These intriguing

Table 1 Regulation of glycans by transcription factors

Gene name	Enzyme	Transcription factors	References
***N*-linked**			
DPAGT1	Dolichyl-phosphate GlcNAc-1-P transferase	STAT5a, TCF/LEF	Zhang et al. 2003; Sengupta et al. 2010
MGAT2	*N*-Acetylglucosaminyltransferase II	Ets-1, Ets-2	Zhang et al. 2000
MGAT3	*N*-Acetylglucosaminyltransferase III	TCF/LEF-1	Xu et al. 2011
MGAT4a	*N*-Acetylglucosaminyltransferase IVa	FOXA2, HNF A	Ohtsubo et al. 2011
MGAT5	*N*-Acetylglucosaminyltransferase V	Ets-1	Kang et al. 1996; Buckhaults et al. 1997
***O*-linked**			
GALNT3	Polypeptide GalNAc transferase III	AP-2 and NRF-1	Nomoto et al. 1999; Izumi et al. 2003
GCNT1	Core 2 β1,6 *N*-acetylglucosaminyltransferase 1	Sp-1	Falkenberg et al. 2007
GCNT2	Core 2 β1,6 *N*-acetylglucosaminyltransferase 2	ATRA, IL-13	Tan et al. 2007
POMGNT1	POM β1,2-*N*-acetylglucosaminyltransferase 1[a]	ZNF202	Raducu et al. 2012
Lewis antigen-related			
B4GALT1	β1,4-Galactosyltransferase I	E1AF, E2F1	Zhu et al. 2005; Wei et al. 2010
B4GALT5	β1,4-Galactosyltransferase V	Est-1, Sp-1, E1AF	Sato et al. 2004, 2007; Jiang et al. 2007
B3GALT5	β1,3-Galactosyltransferase V	Cdx1, Cdx2, HNF1α, HNF1β	Isshiki et al. 2003
Sialylation			
ST3GAL1	α2,3-Sialyltransferase I	Sp1, USF, NF-kB	Taniguchi et al. 2001; Higai et al. 2006
ST3GAL3	α2,3-Sialyltransferase III	Sp-1	Taniguchi et al. 2003a
ST3GAL4	α2,3-Sialyltransferase IV	AP-2, c-Ets	Taniguchi et al. 2003b
ST6GAL1	α2,6-Sialyltransferase I	HNF-1, DBP, Sp-1, Oct-1	Svensson et al. 1990; Taniguchi 2000
ST6GAL2	α2,6-Sialyltransferase II	NF-kB, NRSF, Sox5, Pura, Olf1	Lehoux et al. 2010

(*continued*)

Table 1 (continued)

Gene name	Enzyme	Transcription factors	References
ST6GALNAC3	ST6GalNAc transferase III	Sp-1	Takashima et al. 2000
ST6GALNAC4	ST6GalNAc transferase IV	Sp1 and MZF1	Takashima et al. 2000; Kang et al. 2004
St8sia2	α2,8-Sialyltransferase II/STX	Sp-1	Yoshida et al. 1996
Poly-lactosamine branching			
GCNT2	β6-GlcNAc-transferase (I branching)	C/EBPα	Twu et al. 2007, 2010
Fucosylation			
FUT1	Fucosyltransferase I	Elk-1	Taniuchi et al. 2013
FUT3	Fucosyltransferase III	AP-1, HNF-1α	Dabrowska et al. 2005; Lauc et al. 2010
FUT6	Fucosyltransferase VI	HNF-4α, Oct-1, HNF-1α	Higai et al. 2008; Lauc et al. 2010
FUT10	Fucosyltransferase X	HNF-1α, HNF-4α	Lauc et al. 2010

[a]*POM* protein *O*-mannose

examples demonstrate not only that transcript expression is only one of many regulatory mechanisms for glycan expression but also that our understanding of these mechanisms is very incomplete. A study of some of these examples may, therefore, allow us to identify additional regulatory mechanisms for glycan expression. It is also clear that the expression of at least one glycosyltransferase is regulated epigenetically (Kizuka et al. 2011). Presently, the precise mechanisms that regulate glycosyltransferase transcripts have only been studied in detail for about two dozen enzymes (Table I). As more and more functions for specific glycan structures are discovered and the mechanisms by which their expression become relevant, this list of enzymes and the relevant transcription factors in play will undoubtedly grow. Some glycosyltransferase transcripts have extensive 3′-untranslated regions; therefore, it is quite likely that there is control of glycosyltransferase expression by posttranscriptional mechanisms, as well.

Further studies are needed to elucidate precisely how expression of glycosyltransferases is controlled by transcription factors in specific tissues and cell types. Since many diseases and syndromes are associated with mutations in both transcription factors and regulatory regions of promoters (Lee and Young 2013), analyses of genetic variations in transcription factors and regulatory regions of glycosyltransferase genes will likely define a research direction for the study

of the transcriptional regulation of glycans expression. Indeed, mutations in the *cis*-elements of promoter regions of glycosyltransferases, leading to the generation of additional binding sites for certain transcription factors, have been reported recently (Raducu et al. 2012).

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