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Review

Modulation of transcription factor function by O-GlcNAc modification

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ABSTRACT

O-linked beta-N-acetylglucosamine (O-GlcNAc) modification of nuclear and cytoplasmic proteins is important for many cellular processes, and the number of proteins that contain this modification is steadily increasing. This modification is dynamic and reversible, and in some cases competes for phosphorylation of the same residues. O-GlcNAc modification of proteins is regulated by cell cycle, nutrient metabolism, and other extracellular signals. Compared to protein phosphorylation, which is mediated by a large number of kinases, O-GlcNAc modification is catalyzed only by one enzyme called O-linked N-acetylglucosaminyl transferase or OGT. Removal of O-GlcNAc from proteins is catalyzed by the enzyme beta-N-acetylglucosaminidase (O-GlcNAcase or OGA). Altered O-linked GlcNAc modification levels contribute to the establishment of many diseases, such as cancer, diabetes, cardiovascular disease, and neurodegeneration. Many transcription factors have been shown to be modified by O-linked GlcNAc modification, which can influence their transcriptional activity, DNA binding, localization, stability, and interaction with other co-factors. This review focuses on modulation of transcription factor function by O-linked GlcNAc modification.

1. Introduction

O-linked GlcNAc modification is an important posttranslational modification that modulates the function of many nuclear and cytoplasmic proteins. Proteins are modified at serine or threonine residues by attachment of a single N-acetylglucosamine (GlcNAc) molecule catalyzed by the enzyme O-linked N-acetylglucosaminyl transferase OGT [1–4]. OGT is encoded by a single gene on the X-chromosome and its function is critical for mouse development, since OGT knockout mice are embryonically lethal [5,6]. In general, OGT is ubiquitously expressed with high transcript levels in macrophages, pancreas, and the nervous system [7]. Although, there is only one OGT enzyme, the OGT gene encodes for several splice variants, which differ in the length of the N-terminal tetratricopeptide (TPR) repeats and are targeted to the cytosol, nucleus, and mitochondria [8,9]. The specificity of OGT may be regulated by posttranslational modifications and by its association with different targeting subunits.

The substrate UDP-GlcNAc for OGT is synthesized by the hexosamine biosynthetic pathway (HBP), which uses the glycolytic metabolite fructose-6-phosphate and glutamine (Fig. 1). Only a small fraction of glucose (2–5%) enters the HBP as fructose 6-phosphate [10,11]. The HBP together with O-linked GlcNAc modification of proteins has been suggested to function as a nutrient sensor for the cell [12,13]. Consistent with this idea, exposure to high glucose leads to increased flux via the HBP and results in elevated levels of O-GlcNAc modified proteins. However, recent data suggest that O-GlcNAc modification of a number of proteins, including glycogen synthase is stimulated during nutrient deprivation via upregulation of OGT expression [14–16]. This suggests that different classes of proteins are modified during glucose excess versus glucose deprivation.

O-linked GlcNAc modification of nuclear and cytoplasmic proteins is dynamic and reversible. It has been suggested that O-linked GlcNAcylation and phosphorylation play a reciprocal role in regulation of protein function by competing for modification of the same serine or threonine residues. This reciprocal relationship has been demonstrated for several proteins, including RNA Pol II, estrogen receptor beta, and c-myc [17,18]. However, there are many examples where O-GlcNAc modification has been shown to be promixal or distant from important phosphorylation sites within the same protein [19]. Nevertheless, there is an extensive crosstalk between O-GlcNAc modification and phosphorylation in regulation of protein function.

Altered O-linked GlcNAc modification has been linked to various human diseases, including cardiovascular disease [20,21], neurodegenerative disorders [22–24], diabetes mellitus [18,25–27], and cancer [28]. A single nucleotide polymorphism in O-GlcNAcase (OGA; MGEA5) has been associated with increased susceptibility to type 2 diabetes in Mexican Americans [29]. Interestingly, the human OGT gene is localized on the chromosome X q13.1 region that has been linked to X-linked Dystonia Parkinsonism [30,31].

Many nuclear proteins that are modified by O-linked GlcNAc include transcription factors, such as Pdx-1 [32,33], Sp1 [34–36], c-myc [28,37], NF-κB [38–40], NFAT [39], p53 [41,42] STAT5A [43], FoxO-1 [44,45],

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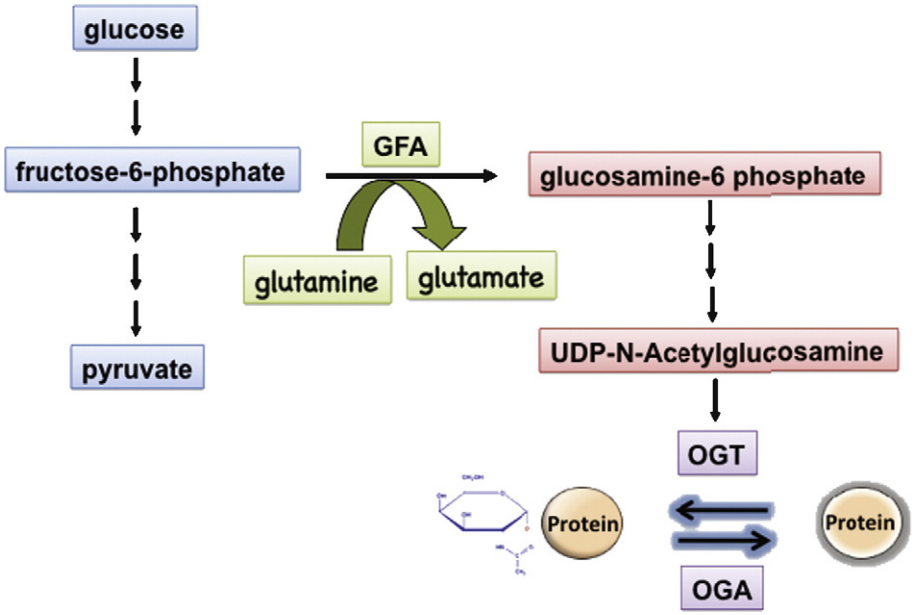

Fig. 1. O-GlcNAc modification is linked to glycolysis via the hexosamine biosynthetic pathway (HBP). Only a small fraction of the glucose (2–5%) enters the HBP, which starts with the conversion of the glycolytic metabolite fructose-6-phosphate and glutamine to glucosamine-6-phosphate and glutamate by the rate-limiting enzyme GFA. The end product of HBP is UDP-N-acetylglucosamine, which is used by OGT as substrate to modify proteins by O-GlcNAc linkages. This modification is reversible, and proteins are deglycosylated by the O-GlcNAcase (OGA).

and co-activators CRTC2 [46] and PGC-1α [47]. In fact, over 25% of the O-GlcNAc modified proteins are involved in transcriptional regulation. O-GlcNAc modification of transcription factors is important in regulation of gene expression in various tissues [48]. Many transcription factors are modified by O-linked GlcNAcylation in response to physiological stimuli, cell cycle stage, and developmental stage, and this modification can modulate their function in different ways [49–51]. O-linked GlcNAc moieties on transcription factors may be recognized by various components of the transcriptional machinery, serve as a nuclear localization signal, antagonize the action of protein kinases by masking the potential serine and threonine sites for phosphorylation, modulate the DNA binding activity or the half-life, and increase the stability of transcription factors in the cell.

There are already several excellent reviews focusing on detection of O-GlcNAc modification on proteins [52–54] and on the regulation of signal transduction pathways by O-GlcNAc cycling [7,55–58]. Thus, this review focuses on O-linked GlcNAc modification of transcriptional regulators and the role of O-linked GlcNAcylation in modulation of transcription factor function. We will discuss the function of O-linked GlcNAc modification in regulating the stability, localization, protein–protein interaction, and DNA binding ability of transcription factors. Although a large number of transcription factors have been demonstrated to be O-GlcNAc modified, this review will focus only on a selected number of transcriptional regulators, where the role of O-GlcNAc modification has been studied in detail.

2. Regulation of protein–protein interaction by O-GlcNAc modification

2.1. NF-κB

The transcription factor NF-κB (nuclear factor-kappaB) serves as a critical regulator of cytokine production, lymphocyte activation, and proliferation [59,60]. NF-κB is present as a dimer consisting of p65 (RelA) and p50 subunits in most cell types. This dimer is localized to the cytoplasm and binds the inhibitor IκB (Fig. 2). Treatment with TNFα or other activating agents stimulate IκB kinase (IKK), which phosphorylates IκB and thereby induces its degradation. This leads to dissociation and translocation of NF-κB into the nucleus and activation of target genes [59,61].

Activation of NF-κB requires posttranslational modifications, including phosphorylation and acetylation. O-linked GlcNAc modification of NF-κB regulates its nuclear localization by disrupting its interaction with the inhibitor IκB [39,40]. In T lymphocytes, NF-κB p65 subunit has been shown to be O-GlcNAc modified, which causes its translocation into the nucleus [38,39]. Furthermore, a recent report demonstrates that modification of NF-κB by O-GlcNAc decreases its binding to IκBα in vascular smooth muscle cells (VSMCs) and increases its transcriptional activity in response to hyperglycemia [40]. The modification sites within NF-κB have been identified as Thr-322 and Thr-352. O-GlcNAc modification of NF-κB at Thr-352 in response to high glucose has been shown to inhibit the interaction of NF-κB with IκB, causing the nuclear translocation of NF-κB and activation of its target genes [40].

NF-κB has also been shown to be O-GlcNAc modified in mesangial cells and accumulates in the nucleus by treatment with high glucose or glucosamine [38]. This leads to activation of NF-κB-dependent genes, such as VCAM-1, TNF-α, and IL-6 (Fig. 2). Overexpression of GFA (glutamine:fructose-6-phosphate amidotransferase) also leads to NF-κB dependent gene expression, indicating a positive role for O-GlcNAc in regulation of NF-κB function in mesangial cells [38]. It is likely that O-GlcNAc modification of NF-κB in mesangial cells disrupts the interaction of NF-κB with IκB in the cytoplasm and causes its nuclear accumulation as observed in VSMCs. Thus, the primary function of O-GlcNAc modification of NF-κB is to disrupt its interaction with IκB, which results in increased nuclear accumulation of NF-κB and stimulation of NF-κB-dependent gene expression (Fig. 2). However, it is possible that O-GlcNAc modification of NF-κB not only disrupts the interaction with IκB, but may also be directly involved in nuclear translocation of NF-κB.

2.2. Stat5a

Signal transducer and activator of transcription (STAT) proteins mediate cellular responses to cytokines, hormones, and growth factors [62]. Mammalian cells contain seven isoforms of Stat proteins (Stat1, Stat2, Stat3, Stat4 Stat5a, Stat5b and Stat6), which are involved