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Review

O-GlcNAcylation and neurodegeneration

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ABSTRACT

O-GlcNAcylation is a dynamic form of protein glycosylation which involves the addition of β-D-N-acetylglucosamine (GlcNAc) via an O-linkage to serine or threonine residues of nuclear, cytoplasmic, mitochondrial and transmembrane proteins. The two enzymes responsible for O-GlcNAc cycling are O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA); their expression and activities in brain are age dependent. More than 1000 O-GlcNAc protein targets have been identified which play critical roles in many cellular processes. In mammalian brain, O-GlcNAc modification of Tau decreases its phosphorylation and toxicity, suggesting a neuroprotective role of pharmacological elevation of brain O-GlcNAc for Alzheimer's disease treatment. Other observations suggest that elevating O-GlcNAc levels may decrease protein clearance or induce apoptosis. This review highlights some of the key findings regarding O-GlcNAcylation in models of neurodegenerative diseases.

Contents

1. Introduction

The discovery of protein O-GlcNAcylation dates back to the 1980s, in which Torres and Hart et al. described a novel single carbohydrate N-acetylglucosamine (GlcNAc)-peptide linkage on monocyte cell surface (Torres and Hart, 1984). Later studies defined it as the addition of GlcNAc via an O-linkage on serine and threonine residues of target proteins (Torres and Hart, 1984; Haltiwangeret al., 1990). In contrast to classical O- and N-linked glycosylation, which mainly occurs on endoplasmic reticulum and Golgi resident proteins, O-GlcNAcylation has been detected on proteins in all the major compartments of the cell including the membranes, cytoplasm, mitochondria and nucleus (Varki et al., 2009).

O-GlcNAcylation is often referred to as a nutrient sensitive pathway and this nutrient sensitivity is attributed to the levels of

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UDP-GlcNAc, which is dependent on flux through the hexosamine biosynthetic pathway (HBP). Approximately 2–5% of all glucose entering into the cell is channeled into the HBP to generate UDP-GlcNAc (Marshall et al., 1991). However, this estimate is based on cultured adipocytes; consequently, the relative flux of glucose into the HBP in highly metabolic tissues such as the brain is not known. Glutamine–fructose-6-phosphate amido transferase (GFAT), which converts fructose-6-phosphate to glucosamine-6-phosphate, is the rate limiting enzyme of the (HBP). GFAT is feedback inhibited by UDP-GlcNAc, the major end product of the HBP. O-GlcNAcylation of many proteins is consequently affected by the changes in UDP-GlcNAc levels (Marshall et al., 1991).

Mammalian cells subjected to various cellular stresses, including oxidative, osmotic and chemical stress, exhibit increased global O-GlcNAcylation (Zachara et al., 2004). Thus, O-GlcNAcylation is a unique metabolic signaling mechanism allowing cells to detect and respond to stress and thereby influence cell survival. However, whether changes in this post-translational modification promote cell death or cell survival depends highly on the cellular context. In this review we will summarize mechanisms and regulation of O-GlcNAc cycling, as well as what is known about its involvement in neurodegenerative diseases.

2. O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA)

Despite being an abundant post-translational modification, O-GlcNAcylation is regulated by the concerted action of only two enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) (Kreppel and Hart, 1999) (Fig. 1). OGT catalyzes the addition of N-acetylglucosamine to target proteins (Haltiwanger et al., 1990) and is highly conserved from *C. elegans* to humans, including plants (Kreppel et al., 1997; Lubas et al., 1997). Although OGT mRNA is ubiquitously expressed in all tissues, the highest levels of OGT mRNA are found in pancreas followed by brain compared to other organs (Kreppel et al., 1997; Lubas et al., 1997; Shafi et al., 2000). Cytosolic OGT activity is 10 times higher in brain compared to muscle, adipose tissue, heart, and liver (Okuyama and Marshall, 2003). The *Ogt* gene is localized at Xq13 and its deletion in mice causes embryonic lethality (Shafi et al., 2000). Parkinsonian-dystonia (DYT3) has been mapped to the X chromosomal region that includes the *Ogt* locus (Nemeth et al., 1999). A rare type of glycosylation has been recently reported, which involves the O-GlcNAc modification of extracellular proteins containing folded EGF-like domains such as Notch receptors (Matsuura et al., 2008). This O-GlcNAc modification is catalyzed by a distinct OGT isoform, endoplasmic reticulum-resident O-GlcNAc transferase, EOGT (Sakaidani et al., 2012).

OGA, the enzyme that removes O-GlcNAc from proteins, was first isolated from crude cellular extracts and was initially named hexosaminidase C to distinguish it from the lysosomal β-hexosaminidase (Braidman et al., 1974; Overdijk et al., 1981). Whereas lysosomal hexosaminidases have an acidic pH optimum and can use both O-linked N-acetylgalactosamine (O-GalNAc) and O-GlcNAc as their substrates, OGA has a neutral pH optimum and selectively uses O-GlcNAc as its substrate but not O-GalNAc (Gao et al., 2001). OGA is expressed from a single gene, which is annotated as meningioma expressed antigen 5 (MGEA5) and is expressed at the highest levels in pancreas, brain, and thymus, with lesser amounts in other tissues (Gao et al., 2001), the highest levels of OGA transcripts are also found in brain (Gao et al., 2001). OGA is localized to the long arm of chromosome 10(10q24), a region highly associated with late-onset Alzheimer's disease (AD) (Bertram et al., 2000).

O-GlcNAcylated proteins, OGT and OGA are abundant throughout the brain, including the synapses and post-synaptic density preparations (Rexach et al., 2012; Khidekel et al., 2004; Vosseller et al., 2006; Taylor et al., 2014; Liu, 2012; Cole and Hart, 2001). In hippocampus, O-GlcNAcylated proteins are present in pyramidal cells, GABAergic interneurons, and astrocytes (Taylor et al., 2014). In cerebellar cortex, OGT mRNA and protein, as well as O-GlcNAcylated proteins are expressed in neurons, especially in Purkinje cells. Using immuno-gold labeling, it has been found that within Purkinje cells, OGT and O-GlcNAcylated proteins are present in nucleus, cytoplasmic matrix, and around microtubules in dendrites. Presynaptic terminals, especially around synaptic vesicles, have more OGT and O-GlcNAcylated proteins than postsynaptic terminals (Akimoto et al., 2003). In adult rat brains, OGT and OGA immunostaining show similar patterns with higher staining in the dorsal parietal cortex and hippocampus than other brain regions (Liu, 2012). Regarding age dependent O-GlcNAcylation, in the Wistar rats, various isoforms of OGT and OGA proteins are differentially expressed with age. ncOGT (116 kDa) decreases from Embryonic Day 15 (E15)-Postnatal 15 (P15) to postnatal 3 months and continues to decrease to postnatal 2 years, while sOGT (70 kDa) increases and peaks at postnatal 3–6 months. OGT activity as assayed using nucleoporin p62 as a substrate is increased from E19 to P30. OGA full-length has 2 immunoreactive species with an upper band (~150 kDa) exhibiting increased levels with age and a lower band (~120 kDa) exhibiting decreased levels with age and the sum peaked at P5 and decrease with age and OGA nuclear variant (~80 kDa) only expressed at embryonic stages. Overall OGA activity is lower at P15-postnatal 2 year compared to E15-P5 using *p*-nitrophenyl-*N*-acetyl-b-D-glucosaminide as a substrate. O-GlcNAc levels are decreased significantly from E15 to postnatal 5 days to 6 months, and slightly increased from 6 months to 2 years (Liu, 2012). Conversely studies in Brown-Norway rats found that brain O-GlcNAc levels increased ~30% in 24-month compared to 5-month old rats, without any changes in the OGT or OGA mRNA expression (Fülöp et al., 2008). Collectively, these studies suggest an involvement of O-GlcNAc modification in diverse brain functions throughout the lifespan.

3. Regulation of OGT and OGA

OGT protein consists of two major domains. The N-terminal domain is comprised of several tetratricopeptide (TPR) repeats and the C-terminal domain exhibits glycosyltransferase activity and binds UDP-GlcNAc. The TPR repeats serve as protein:protein docking sites for substrate targeting proteins (Kreppel et al., 1997; Iyer and Hart, 2003). Surprisingly, OGT itself undergoes O-GlcNAc modification, as well as tyrosine phosphorylation; the impact of these modifications on OGT activity is currently unknown (Kreppel et al., 1997). However, S-nitrosation of OGT strongly inhibits its catalytic activity whereas de-nitrosation activates it, leading to protein hyper-O-GlcNAcylation (Ryu and Do, 2011). OGT mRNA undergoes alternative splicing giving rise to transcripts encoding distinct OGT isoforms, which includes nucleocytoplasmic isoform (ncOGT), the mitochondrial isoform (mOGT), and the short isoform (sOGT) (Hanover et al., 2003; Love et al., 2003). These different OGT isoforms differ in their N-terminal sequence: the ncOGT isoform (116 kDa) contains 12 tetratricopeptide repeats (TPRs), the mOGT (103 kDa) contains 9 TPRs, and a mitochondrial targeting sequence, while the sOGT (75 kDa) contains 2 TPRs (Hanover et al., 2003). This difference in number of TPRs in each isoform may confer differential substrate specificity (Lubas and Hanover, 2000).

OGA is also a dual function enzyme with both an N-terminal catalytic domain and a C-terminal histone acetyltransferase (HAT) domain. Although it has been shown that O-GlcNAcase has histone acetyltransferase activity *in vitro* (Toleman et al., 2004), this remains an area of controversy (He et al., 2014). OGA is a caspase-3

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