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Review

Review

Functional crosstalk among oxidative stress and O-GlcNAc signaling pathways

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Abstract

In metazoans, thousands of intracellular proteins are modified with O-linked β-*N*-acetylglucosamine (O-GlcNAc) in response to a wide range of stimuli and stresses. In particular, a complex and evolutionarily conserved interplay between O-GlcNAcylation and oxidative stress has emerged in recent years. Here, we review the current literature on the connections between O-GlcNAc and oxidative stress, with a particular emphasis on major signaling pathways, such as KEAP1/NRF2, FOXO, NFκB, p53 and cell metabolism. Taken together, this work sheds important light on the signaling functions of protein glycosylation and the mechanisms of stress responses alike and illuminates how the two are integrated in animal cell physiology.

Key words: KEAP1, NRF2, O-GlcNAc, oxidative stress

Introduction

O-GlcNAcylation is a reversible form of glycosylation on serine and threonine side-chains of intracellular proteins in a wide variety of animal and plant species. In mammals, O-GlcNAc is added to substrates from a uridine diphosphate (UDP)-GlcNAc donor by a single O-GlcNAc transferase (OGT) and is removed by a single glycoside hydrolase, O-GlcNAcase (OGA). In many organisms, including mammals, O-GlcNAcylation is essential for cell viability and embryonic development. Consistent with this critical role, O-GlcNAc is thought to participate in a long list of cellular functions. For example, global O-GlcNAc levels fluctuate in response to many noxious stimuli, including heat shock, nutrient depletion, endoplasmic reticulum dysfunction and redox imbalance (Zachara et al. 2004; Zachara and Hart 2004; Groves et al. 2013; Reeves et al. 2014). Moreover, (de)glycosylation of particular substrates governs stress responses themselves, pointing to a complex, reciprocal interplay between stress pathways and O-GlcNAc signaling. However, in most cases, the regulatory mechanisms and most important O-GlcNAc substrates remain enigmatic. Here, we review the connections between O-GlcNAc and oxidative stress in metazoans as a prototypical example of stress signaling. Understanding this crosstalk is essential for building an integrated model of the oxidative stress response and also serves as a useful case study for elucidating how O-GlcNAcylation participates in a critical aspect of cellular homeostasis.

Oxidative stresses affect O-GlcNAcylation

In aerobic environments, cells frequently encounter stress caused by excessive oxidants, most prominently reactive oxygen species (ROS), including the superoxide anion, hydroxyl radicals and hydrogen peroxide (Schieber and Chandel 2014). Indeed, various species of ROS are generated by mitochondrial respiration, central metabolism and environmental stimuli (Holmstrom and Finkel 2014). ROS can act not only as signal transduction molecules, but can also damage proteins, nucleic acids and lipids, resulting in stress (Circu and Aw 2010; Panieri and Santoro 2016). The accumulation of oxidative damage and the resulting dysregulation of cellular processes increase the danger of genome instability, cell death and tumorigenesis (Cui 2012; Costa et al. 2014; Sies et al. 2017).

Animal cells have evolved several well-known mechanisms for defending against oxidative damage, including redox buffering by glutathione and NADP/NADPH, the regeneration of thioredoxin,

the action of ROS-detoxifying enzymes, and the sequestration of iron, an essential redox-active metal (Gorrini et al. 2013; Panieri and Santoro 2016). Interestingly, the level of O-GlcNAcylation also responds to oxidative stress. For example, Hart and colleagues reported that treatment of COS7 cells or mouse embryonic fibroblasts (MEFs) with hydrogen peroxide or arsenite enhanced global O-GlcNAcylation (Zachara et al. 2004; Zachara and Hart 2004). Extending these observations to intact tissues, Peternelj et al. (2015) subsequently found that global O-GlcNAcylation is induced in rat white gastrocnemius muscle after exercise and recovery in the presence of diethyl maleate, which raises ROS levels by depleting glutathione. Moreover, the authors observed that exercise and recovery triggered fluctuations in the mRNA levels of OGT, OGA and isoforms of glutamine:fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme in the hexosamine biosynthetic pathway (HBP), though the mechanistic underpinnings of this relationship remain unclear (Peternelj et al. 2015). Conversely, manipulating O-GlcNAcylation can also alter intracellular ROS levels. For instance, Goldberg et al. (2011) showed that knockdown of OGT lowers hyperglycemia-triggered ROS in murine glomerular mesangial cells, perhaps in part through the modulation of the stress-induced MAP kinase p38. As these examples illustrate, the crosstalk between O-GlcNAcylation and oxidative stress signaling is complex and reciprocal. In the following sections, we discuss how O-GlcNAc responds to different oxidative stress stimuli and how O-GlcNAcylation impacts on phenotypic outcomes under oxidative stress conditions.

Hydrogen peroxide

In 2016, the Zachara group performed quantitative, proteome-wide profiling of hydrogen peroxide-induced changes in O-GlcNAc in MEFs (Lee et al. 2016). Importantly, O-GlcNAcylated proteins were affinity-enriched by a 'G5 Lectibody' approach, using a combination of O-GlcNAc-binding lectins and monoclonal antibodies to purify proteins of interest. In this work, hydrogen peroxide treatment increased the enrichment of ~26% of identified proteins, whereas 11% or 17% of identified proteins were depleted at 1- and 2-h time-points, respectively. In parallel, the authors showed that 2-h hydrogen peroxide increased the O-GlcNAcylation of high molecular weight proteins (>72 kDa). The differentially O-GlcNAcylated proteins identified by the authors participate in a range of cellular processes, including chromatin remodeling (SWItch/sucrose non fermenting complex), transcriptional control (mediator complex), RNA and stress granule biogenesis and post-translational signaling (14-3-3 family).

In recent complementary work, the same group used quantitative proteomics and a proximity biotinylation strategy to analyze the oxidative stress-dependent OGA interactome (Groves et al. 2017). Consistent with their prior study, the authors found that hydrogen peroxide treatment increased global O-GlcNAcylation in human U2OS osteosarcoma cells. Surprisingly, however, total OGA protein levels and activity increased under the same conditions. A possible explanation for this apparent paradox was provided by the discovery that hydrogen peroxide treatment promoted the interaction between OGA and the metabolic enzyme fatty acid synthase (FAS), reducing OGA activity by ~85%. These results suggest that hydrogen peroxide may downregulate global OGA activity through its sequestration by FAS. Indeed, the authors found that FAS overexpression further augments global hydrogen peroxide-induced O-GlcNAc levels. However, the biochemical details of OGA/FAS interaction, as well as the functional significance of most other hydrogen peroxide-induced changes to the OGA interactome, remain to be characterized.

Other groups have made analogous observations in complementary experimental systems. For example, Nagy and colleagues reported that a 2-h treatment of hydrogen peroxide modestly induced global O-GlcNAc levels and GFAT mRNA in SH-SY5Y neuroblastoma cells, suggesting that this response is conserved across multiple cell types (Katai et al. 2016). On the other hand, in retinal ganglion cells, a 1-h hydrogen peroxide treatment reduced global O-GlcNAcylation, but supplementation with glucosamine (which promotes UDP-GlcNAc biosynthesis and O-GlcNAcylation) reduced hydrogen peroxide-induced cell death, suggesting that the protective effects of O-GlcNAc may be more universal than the ability to regulate O-GlcNAc in response to this particular stimulus (Chen, Huang et al. 2015).

Given the pleiotropic effects of hydrogen peroxide, more work will be required to dissect the kinetics, dose-response relationship, cell type-specific events and key glycoprotein substrates that trigger protective, stress-induced increases in O-GlcNAc. However, one recent study provided a potentially important clue to this mode of signaling. Han et al. (2017) demonstrated that hydrogen peroxide treatment (as well as genotoxic or nutrient stress) induced the O-GlcNAcylation of the SIRT1 sirtuin deacetylase on Ser549. Interestingly, SIRT1 glycosylation potentiated its deacetylase activity and enhanced cellular stress resistance, at least in part by promoting the deacetylation of the tumor suppressor protein p53. While SIRT1 Ser549 glycosylation was essential for these effects, the authors found evidence of additional, as-yet unidentified O-GlcNAcylation sites on SIRT1 as well, suggesting that other stimuli or stresses may influence SIRT1 activity through glycosylation at distinct sites. Clearly, we have more to learn about the role of O-GlcNAcylation in hydrogen peroxide stress, with respect to both SIRT1 in particular and other, uncharacterized glycosylation changes in additional signaling pathways.

Hypoxia and ischemia

In any pathological condition of vascular insufficiency, including most solid tumors (Dewhirst and Chi 2013), hypoxia and ischemia are prominent features. After a hypoxic or ischemic event, tissue reperfusion and re-oxygenation increase oxidative stress, a disease-relevant example of redox imbalance (Giordano 2005). Changes in O-GlcNAc signaling have been implicated in this context as well. For example, the Jones group has addressed the pathophysiological connection between ischemia-induced ROS and O-GlcNAcylation in neonatal rat cardiac myocytes (NRCMs). In one study, the authors demonstrated that adenovirus-mediated overexpression of OGA (AdOGA) in NRCMs increased cytotoxicity after hypoxia/re-oxygenation, whereas inhibition of OGA by siRNA or the small molecule PUGNAc produced the opposite effect. These effects correlated with lower mitochondrial membrane potential and higher calcium overload in AdOGA-infected NRCMs, suggesting a possible mechanism for the cytoprotective effects of O-GlcNAcylation in this tissue (Ngoh et al. 2009). The same lab subsequently showed that global O-GlcNAcylation was reduced after 40 min of myocardial ischemia, but temporarily increased in the ischemic zone at 1 h after reperfusion. Similarly, O-GlcNAcylation in NRCMs was decreased soon after hypoxia but increased at 6 h after re-oxygenation. Interestingly, adenovirus-mediated OGT overexpression or PUGNAc treatment decreased ROS production after hypoxia/re-oxygenation or hydrogen peroxide treatment, whereas OGA overexpression produced the opposite result (Ngoh et al. 2011). This work confirms the protective effects of increased O-GlcNAcylation in a well-established model of cardiovascular disease.

The mechanisms linking ischemic insults to O-GlcNAc changes remain incompletely understood. However, one potentially important player is the hypoxia-inducible factor 1α (HIF-1 α), a transcription

factor stabilized under low oxygen conditions. A report by the Reginato group (Ferrer et al. 2014) demonstrated that OGT facilitates HIF-1α stabilization and the expression of its target gene, GLUT1, during hypoxia in both in vitro and in vivo breast cancer models. Conversely, the authors found that OGT depletion raised the levels of several metabolites, including α-ketoglutarate (α-KG), which in turn destabilizes HIF-1α protein by proline hydroxylation, recruitment of the von Hippel–Lindau protein (VHL), and subsequent VHL-mediated degradation. Because of the importance of hypoxia and HIF-1α in a wide variety of human diseases, these results suggest that an OGT/HIF-1α regulatory connection may be important in diverse pathological contexts. Testing this possibility will be a priority for future studies.

Oxidative stress signaling pathways regulated by O-GlcNAc

Here, we focus on individual signaling pathways directly regulated by O-GlcNAcylation during redox stress. To provide a broad perspective on how O-GlcNAc cycling affects redox homeostasis, we review studies across different aspects of the antioxidant response in diverse model systems (Figure 1).

Central carbon metabolism

Because O-GlcNAc is a nutrient-sensitive modification, it is perhaps no surprise that O-GlcNAcylation and redox signaling are intimately connected through core cellular metabolism, itself a major source of endogenous ROS. In one key example, Hsieh-Wilson and colleagues reported that the glycolytic enzyme phosphofructokinase 1 (PFK1) is an OGT substrate (Yi et al. 2012). PFK1 phosphorylates fructose-6-phosphate to generate fructose 1,6-biphosphate, which is then cleaved by aldolase to produce glyceraldehyde 3-phosphate for glycolysis. Increased PFK1 activity is associated with higher glycolytic flux (Yalcin et al. 2009; Mor et al. 2011) and several studies have shown that PFK1 activity is governed in part by various post-translational modifications (PTMs; Smerc et al. 2011, Yi et al. 2012, Li et al. 2016). Extending these observations, the Hsieh-Wilson Lab found that OGT

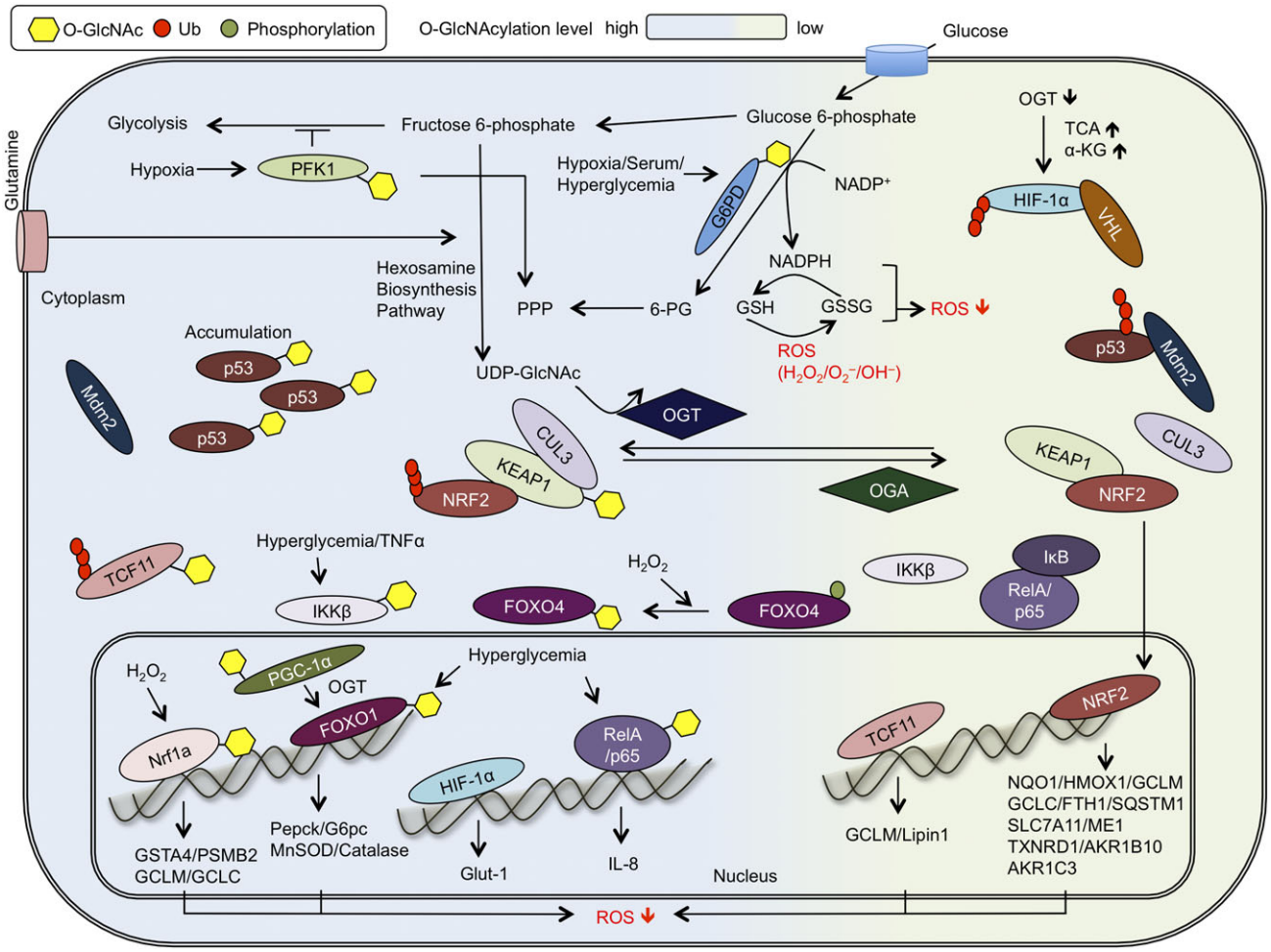

Fig. 1. O-GlcNAcylation regulates oxidative stress signaling pathways. Summary of selected connections among O-GlcNAcylation and major redox stress signaling pathways described in the text. OGT glycosylates PFK1 and G6PD, leading to the inhibition of glycolysis while promoting flux through the pentose phosphate pathway (PPP). O-GlcNAcylation of IKKβ and RelA/p65 enhances RelA/p65 transactivation activity. O-GlcNAcylation of p53 reduces its polyubiquitination via decreased interaction with MDM2. KEAP1 is likely constitutively O-GlcNAcylated to facilitate its interaction with CUL3 and subsequent NRF2 polyubiquitination. Upon KEAP1 deglycosylation, an NRF2-mediated antioxidant response is activated. OGT inhibition increases TCF11 protein level and target gene expression, and Nrf1a O-GlcNAcylation, stimulated by hydrogen peroxide exposure, also promotes its transcriptional activation. OGT inhibition increases HIF-1α polyubiquitination by VHL, suppressing HIF-1α signaling, whereas OGT overexpression increases the expression of the HIF-1α target gene GLUT1. FoxO4 O-GlcNAcylation is enhanced upon hydrogen peroxide treatment and FoxO1 O-GlcNAcylation promotes expression of stress response genes. Please see text for details and references.

overexpression or PUGNAc treatment decreased glycolytic rates, lactate production and PFK1 activity alike (Yi et al. 2012). In dissecting the mechanism of these observations, the authors found that O-GlcNAcylation of PFK1 at Ser529 affects its oligomerization and represses PFK1 activity under hypoxic conditions. Interestingly, O-GlcNAcylation of PFK1 on Ser529 redirects glucose flux from glycolysis to the pentose phosphate pathway (PPP), leading to higher levels of NADPH and reduced glutathione (GSH). Moreover, the authors also showed that OGT-overexpressing H1299 cells exhibited both lower ROS upon treatment with the oxidizing agent diamide and resistance to hydrogen peroxide-induced cell death. This mode of metabolic regulation may contribute to cancer cell growth and proliferation because the authors showed that ablation of PFK1 Ser529 glycosylation inhibited cancer cell division in vitro and tumor formation in vivo. Taken together, these results demonstrate that redox stress resistance can be regulated by the glycosylation of a key glycolytic enzyme, with important implications for understanding both normal cell physiology and cancer metabolism.

In another example of crosstalk, Rao et al. (2015) reported that glucose-6-phosphate dehydrogenase (G6PD) activity and oligomerization are also regulated by O-GlcNAc cycling. G6PD is the rate-limiting enzyme of the PPP, catalyzing the conversion of glucose-6-phosphate to 6-phosphogluconate. Therefore, G6PD contributes to the regeneration of NADPH, a major ROS scavenger. The authors found that OGT glycosylates G6PD at Ser84 and showed that A549 cells expressing a Ser84Val G6PD mutant (compared with WT control) exhibited lower PPP metabolite levels (e.g., 6-phosphogluconate), without affecting TCA or glycolytic metabolites. Overexpression of OGT in cells expressing WT G6PD increased the relative amounts of NADPH and GSH but failed to do so in S84V G6PD-expressing cells. Consistently, A549 cells expressing a Ser84Val G6PD mutant were sensitized to hydrogen peroxide, hypoxia and diamide, relative to WT-expressing controls. Importantly, the authors provided in vivo evidence that O-GlcNAcylation of G6PD is increased in human lung malignancies, suggesting that these observations, like those with PFK1 glycosylation, may have direct relevance to oncogenesis or cancer treatment.

The KEAP1/NRF2 axis

NRF2 is a transcription factor and a master regulator of the cellular response to oxidative stress and xenobiotics (Jaramillo and Zhang 2013; Menegon et al. 2016). In unstressed cells, NRF2 associates with a complex comprising the cytoplasmic KEAP1 adaptor protein and CUL3 E3 ubiquitin ligase, which ubiquitinates NRF2, targeting it for proteasome-mediated destruction (Jaramillo and Zhang 2013). During stress, various toxicants (e.g., electrophiles or oxidizing agents) covalently modify redox-active cysteines in KEAP1, impairing the ability of KEAP1/CUL3 to ubiquitinate NRF2 (Jaramillo and Zhang 2013; Menegon et al. 2016). Free NRF2 then migrates to the nucleus, where it binds to promoters containing antioxidant response elements (AREs) and upregulates antioxidant defense genes, such as the glutathione biosynthetic pathway, xenobiotic efflux pumps and drug-metabolizing enzymes (Hayes et al. 2010; Jaramillo and Zhang 2013; Menegon et al. 2016).

Several biochemical and functional connections between O-GlcNAcylation and NRF2 have been reported. For example, genetic knockdown of OGT induced NRF2 target gene expression in cultured human cell lines (Chu et al. 2014), human embryonic stem cells (Andres et al. 2017) and the murine forebrain (Wang et al. 2016). Consistent with these results, two other studies observed correlations between global O-GlcNAcylation, NRF2 levels and the cellular antioxidant response. In particular, α-lipoic acid (LA) treatment decreased global O-GlcNAcylation in the streptozotocin (STZ)-induced diabetic rat kidney (Arambasic et al. 2013) and rat liver (Dinic et al. 2013). Moreover, these authors observed an increase in NRF2 nuclear translocation and elevated mRNA/protein levels of antioxidant enzymes, such as manganese superoxide dismutase (SOD), copper/zinc SOD and catalase. Together, these reports hint at an inverse correlation between global O-GlcNAcylation and NRF2 signaling.

Consistent with this notion, a recent report by the Slawson group found that very long (3-week) treatment of SH-SY5Y neuroblastoma cells with the OGA inhibitor Thiamet-G and the UDP-GlcNAc precursor glucosamine (GlcN) reduced NRF2 protein and NRF2 target gene expression (Tan et al. 2017). Conversely, genetic ablation of OGT in the murine liver caused the opposite effect, with increased NRF2 levels and activity. In this case, the authors reported that increased global O-GlcNAcylation suppressed ROS levels while slightly increasing the NAD+/NADH ratio. These observations pointed to a likely functional connection between O-GlcNAc signaling and the NRF2 pathway but the relevant OGT substrates and biochemical mechanisms remained unclear (Tan et al. 2014, 2017).

In recent work, we unexpectedly discovered that the pharmacological inhibition or genetic knockdown of OGT in numerous human cell types elicits an antioxidant response by activating the NRF2 pathway (Chen, Chi et al. 2017; Chen, Smith et al. 2017). Interestingly, we did not observe increased ROS production or a reduced:oxidized glutathione imbalance upon OGT inhibition, suggesting that NRF2 activation is due to a specific O-GlcNAc-mediated signal, and not to nonspecific stress. Indeed, we found that KEAP1 physically interacts with OGT and is thereby O-GlcNAcylated under unstressed conditions at eleven candidate sites that we mapped by mass spectrometry. A panel of site-specific glycosylation-null mutants identified KEAP1 Ser104 as the most functionally relevant site. Abrogation of KEAP1 glycosylation, by inhibiting OGT or by mutating KEAP1 Ser104, reduced the interaction of KEAP1 with CUL3, resulting in a loss of NRF2 ubiquitination. Thus, our data indicate that, under homeostatic conditions, site-specific O-GlcNAcylation of KEAP1 at Ser104 is required for its optimal activity, mediating NRF2 ubiquitination and restraining the NRF2 pathway. Consistent with this idea, we also found that KEAP1 O-GlcNAcylation correlates with glucose availability, as hypoglycemic conditions triggered KEAP1 deglycosylation and subsequent NRF2 signaling. Inhibition of OGA blocked hypoglycemia-induced KEAP1 deglycosylation and prevented NRF2 induction, indicating that glucose availability is sensed in part through site-specific KEAP1 O-GlcNAcylation. Overall, these results revealed a new connection between nutrient sensing and redox metabolism through the conduit of KEAP1 glycosylation.

NRF2 belongs to the cap'n'collar (Cnc) protein family, which includes the related mammalian transcription factors NFE2, NRF1 and NRF3, as well as orthologs in other animals (Sykiotis and Bohmann 2010). Interestingly, the functional connections among O-GlcNAc and Cnc family transcription factors extend beyond NRF2 itself, and even beyond mammalian systems. For example, Hanover and colleagues first reported that SKN-1, the sole Cnc family member in *Caenorhabditis elegans*, translocated to nucleus in *ogt-1(ok430)*-null worms with kinetics similar to that of sodium azide-induced SKN-1 nuclear translocation in a WT strain (Hanover et al. 2005). Like mammalian NRF2, nematode SKN-1 responds to oxidative stress to upregulate the expression of detoxifying enzymes (Walker et al. 2000, An and Blackwell 2003, An et al. 2005; Inoue et al. 2005). However, no KEAP1 ortholog has been identified in *C. elegans*, suggesting that the regulation of SKN-1 and NRF2 by O-GlcNAc are likely distinct. It was

reported previously that DAF-2 (insulin/insulin-like growth factor receptor) activation facilitates AKT-mediated phosphorylation of SKN-1 and inhibits SKN-1 from translocating to the nucleus (Cohen and Dillin 2008; Tullet et al. 2008), keeping the pathway off. Interestingly, Li et al. (2017) subsequently found that SKN-1 is O-GlcNAcylated. In this work, the authors discovered that O-GlcNAc cycling also regulates SKN-1 nuclear localization, demonstrating that SKN-1 mislocalizes in *ogt-1(ok1474)* or *oga-1(ok1207)* null worms upon treatment with the ROS inducer *tert*-butyl hydroperoxide. The translocation of SKN-1 and expression of its target genes (e.g., *gcs-1*, *gst-4* and *gst-7*) are induced in *oga*-null worms. Importantly, SKN-1 interacts with OGT and is O-GlcNAcylated at Ser470 and Thr493, and reconstitution of *skn-1*-null worms with an S470A/T493A SKN-1 mutant reduced lifespan under oxidative stress conditions. These effects may be due to a lost regulatory interplay between glycosylation and phosphorylation because the authors demonstrated that phosphorylation of SKN-1 at Ser483 by GSK-3 is repressed by its oxidative stress-induced O-GlcNAcylation.

Two recent reports showed that mammalian NRF1 is glycosylated as well (Chen, Liu et al. 2015; Han et al. 2017). NRF1 (NFE2L1) is expressed as several isoforms, including NRF1a, NRF1b, LCRF1 and TCF11. Like NRF2, NRF1 interacts with ARE-containing promoters, but the PTMs of NRF1 and NRF2 are distinct. Chen, Liu et al. (2015) reported that OGT interacts with TCF11 and promotes its ubiquitination in 293T cells. Genetic depletion of OGT increased both TCF11 protein level and the mRNA and protein of its downstream targets GCLM and lipin1, whereas OGT overexpression decreased TCF11 protein. These results suggest that the NRF1 isoform TCF11 is O-GlcNAcylated, but specific glycosylation sites have not yet been mapped. In separate work, Han et al. (2017) found that NRF1a interacts with OGT and the heavily glycosylated transcriptional coactivator host cell factor 1 (HCF1). NRF1a is O-GlcNAcylated, especially under oxidative stress conditions (i.e., arsenic or *tert*-butylhydroquinone treatment), although, once again, the glycosylated residues have not been pinpointed. Nevertheless, these observations likely have functional significance, because NRF1a polyubiquitination was reduced and its half-life increased by transient overexpression of OGT or PUGNAc treatment, both of which boost global O-GlcNAc levels. Interestingly, several recent reports have also suggested a role for ER lumenal glycosylation of NRF1 in regulating its function (Radhakrishnan et al. 2014; Tomlin et al. 2017; Widenmaier et al. 2017). It remains unknown whether and how the distinct O-GlcNAcylation and secretory pathway glycosylation of NRF1 are coordinated.

Taken together, these studies indicate an evolutionarily conserved connection between O-GlcNAc cycling and redox stress signaling through the Cnc transcription factor family. It is tempting to speculate that this mode of regulation has been preserved from worms to mammals in the case of SKN-1/NRF1 glycosylation, whereas the analogous regulation of the NRF2 pathway has shifted to the O-GlcNAcylation of KEAP1, an upstream regulator in mammals that is absent from *C. elegans*. Testing this model would be an interesting object of future study.

The FOXO family

The mammalian forkhead box (FOX) class O transcription factors, including FoxO1, FoxO3, FoxO4 and FoxO6, are important regulators of oxidative stress signaling and glucose metabolism (Nakae et al. 2008). As with NRF2, crosstalk among FOXO family members and O-GlcNAcylation is an evolutionarily conserved feature of redox stress signaling. In *C. elegans*, DAF-16 (an ortholog of FoxO) was found to be inhibited by the insulin/IGF-1-like signaling pathway. In the presence of insulin-like peptide, DAF-2 is activated and triggers a phosphorylation cascade involving AGE-1, PDK1, AKT1/2, and DAF-16. Phosphorylation of DAF-16 prevents its nuclear accumulation and thereby blocks the upregulation of its target genes. Interestingly, several studies reported genetic interactions between OGT and the insulin/IGF-1-like signaling pathway in *C. elegans* (Hanover et al. 2005; Forsythe et al. 2006; Lee et al. 2010; Love et al. 2010; Rahman et al. 2010). For instance, Hanover and colleagues found that dauer formation in *daf-2* worms is affected by O-GlcNAc cycling in a temperature-sensitive manner (Hanover et al. 2005; Forsythe et al. 2006). In addition, multiple groups reported a connection between O-GlcNAc cycling and lifespan, a phenotype heavily influenced by DAF-2/DAF-16 signaling. Rahman et al. (2010) reported that *ogt-1* mutant animals have a shorter lifespan and Love et al. (2010) discovered that *oga-1* mutation extended lifespan. Importantly, both effects were suppressed in a *daf-16* mutant background. Moreover, *daf-16;oga-1* double mutant animals exhibited reduced survival after treatment with the ROS inducer paraquat, as compared with single *oga-1* mutants. Similarly, *ogt-1;daf-2* double mutant animals lost the survival advantage of *daf-2* single mutants under oxidative stress conditions. These results indicate that genetic interactions between OGT/OGA and the DAF-2/DAF-16 pathway influence lifespan and oxidative stress resistance. However, DAF-16 nuclear localization is slightly increased in both *ogt-1* and *oga-1* mutants, and whether DAF-16 is directly O-GlcNAcylated in worms remains unclear. Complementary biochemical and genetic experiments will be required to elucidate the precise mechanism by which O-GlcNAcylation regulates DAF-16 activity in *C. elegans*.

Hyperglycemia is a prominent feature of uncontrolled diabetes and glucose control is one of the best strategies to prevent long-term diabetic complications. One prevailing hypothesis is that dysregulated O-GlcNAcylation may contribute directly to hyperglycemia-induced diabetic symptoms. Indeed, hyperglycemia is known to induce mitochondrial superoxide and activate the hexosamine biosynthesis pathway (Du et al. 2000; Nishikawa et al. 2000). Interestingly, insulin resistance can be recapitulated by elevating O-GlcNAcylation level as well (Marshall et al. 1991; McClain et al. 2002; Vosseller et al. 2002). Furthermore, the O-GlcNAc/FoxO axis may underlie these observations. For example, Housley et al. (2008) found that the DAF-16 ortholog FoxO1 is O-GlcNAcylated at on several residues in Fao rat hepatoma cells. In this context, OGT enhances FoxO1 transactivation via O-GlcNAcylation at Thr317. FoxO1 target genes involved in gluconeogenesis (e.g., phosphoenolpyruvate carboxykinase (Pepck) and the Glucose-6-Phosphatase catalytic subunit (G6pc)) and the oxidative stress response (e.g., MnSOD and catalase) are also induced upon exposure to high glucose (25 mM). Notably, while high glucose enhances O-GlcNAcylation of FoxO1, insulin treatment reduces its O-GlcNAcylation. The authors also discovered that the transcriptional coactivator PGC1α, which binds directly to FoxO1, is O-GlcNAcylated at Ser333. The PGC1α/OGT complex promotes O-GlcNAcylation of FoxO1 and FoxO3 (Housley et al. 2009), but the functional relevance of these observations is not yet certain. In parallel, another study suggested that FoxO4 is O-GlcNAcylated in 293 cells, though the specific O-GlcNAc sites were not identified (Ho et al. 2010). In this system, the authors found that hydrogen peroxide treatment induces an OGT/FoxO4 interaction, resulting in increased FoxO4 glycosylation and decreased FoxO4 phosphorylation at Ser193, a known AKT target site (Brownawell et al. 2001). In addition, OGT

expression promotes FoxO4 transactivation activity while OGA expression decreases it, suggesting a direct functional connection between FoxO4 and O-GlcNAc cycling. Additional biochemical and cellular studies will be necessary to understand the molecular details and complex interplay between O-GlcNAcylation and phosphorylation in regulating FoxO activity during oxidative stress.

NFκB

The canonical NFκB transcriptional pathway is induced by pro-inflammatory molecules, such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1). Activation of the TNF or IL-1 receptor induces phosphorylation of the IKK (IκB kinase, an inhibitor of κB kinase) complex, which leads to the subsequent phosphorylation and degradation of IκB. The RelA (also called p65)/p50 complex (or RelB/p52, in an alternative pathway) then dissociates from IκB and translocates into nucleus to induce the expression of cytokines, chemokines, adhesion molecules and antioxidant enzymes (Lawrence 2009).

In addition to pro-inflammatory signals, the NFκB pathway is also regulated by ROS through PTMs on NFκB subunits. For example, oxidation or glutathionylation of p50 at Cys62 reduces its DNA binding activity (Pineda-Molina et al. 2001; Morgan and Liu 2011). More recently, O-GlcNAc has been shown to regulate the NFκB pathway as well. For instance, Yang et al. (2008) reported that RelA/p65 is O-GlcNAcylated in rat vascular smooth muscle cells and MEFs. OGA overexpression reduced NFκB p65 transactivation activity, whereas OGT overexpression increased it. The authors further found that RelA/p65 O-GlcNAcylation at Thr352 regulates its interaction with IκBα and transcriptional activity. In subsequent work, Allison et al. (2012) also reported that RelA/p65 is O-GlcNAcylated in 293T cells, with specific O-GlcNAc sites regulating its transactivation activity by affecting the acetylation of RelA by p300. The authors showed that glycosylation of RelA/p65 Thr305 affects its acetylation on Lys310. NFκB transactivation activity was repressed by silencing OGT in the presence of TNFα stimulation, and cells expressing Thr205Ala or Lys310Arg mutant RelA/p65 were sensitized to TNFα or etoposide, as compared with cells expressing WT RelA/p65. Consistent with these results, another study found that hyper-O-GlcNAcylation of RelA/p65 confers resistance to apoptosis in pancreatic cells (Ma et al. 2013). Together, these studies demonstrate a clear functional connection between NFκB protein O-GlcNAcylation and downstream stress resistance.

Other components of the NFκB pathway are regulated by O-GlcNAc signaling as well. For example, Kawauchi et al. (2009) reported that IKKβ is O-GlcNAcylated at Ser733. Similarly, Ramakrishnan et al. (2013) showed that c-Rel is O-GlcNAcylated at Ser350 in lymphocytes, and this modification influences the binding of c-Rel to DNA containing the CD28 response element. We have also observed the concerted transcriptional downregulation of several NFκB target genes (e.g., IL-8 and COX-2) in response to OGT inhibition (Chen, Smith et al. 2017), though the relevant OGT substrate(s) in this case remain unidentified.

In sum, these studies demonstrate that multiple components of the NFκB pathway are directly regulated by O-GlcNAcylation, with functional relevance for gene expression and phenotypic responses. Although NFκB signaling is well-known to influence cell survival in response to redox stress and other noxious stimuli, more work will be required to determine whether the influence of O-GlcNAc cycling is critical for NFκB-mediated redox stress resistance in particular.

p53

ROS are a major cause of DNA damage, leading to the activation of the tumor suppressor protein p53 (Sablina et al. 2005; Liu and Xu 2011). p53 induces a variety of transcription-dependent and -independent cellular responses, including DNA repair, cell cycle arrest, apoptosis, and ferroptosis (Kastenhuber and Lowe 2017). p53 activity is tightly regulated, and PTMs provide one important layer of control (Dai and Gu 2010). Yang et al. (2006) first reported that p53 is O-GlcNAcylated at Ser149 in human breast cancer cells. The nonspecific OGA inhibitor streptozotocin (STZ) enhanced p53 O-GlcNAcylation and reduced its polyubiquitination by interrupting its binding to MDM2, an E3 ubiquitin ligase. Cells expressing a Ser149Ala mutant of p53 exhibited moderate resistance to STZ treatment combined with the genotoxic drug doxorubicin, as compared with doxorubicin alone. In more recent, complementary work, Shtraizent et al. (2017) discovered a novel form of crosstalk between p53 O-GlcNAcylation and the metabolic enzyme mannose phosphate isomerase (MPI) in zebrafish. MPI interconverts mannose-6-phosphate and fructose-6-phosphate, regulating the levels of a key upstream substrate for the HBP. The authors found that p53 accumulated when MPI was depleted in zebrafish, MEFs, and human liver cancer cells, and this effect was caused by increased flux through the HBP and subsequent p53 O-GlcNAcylation.

Although these studies point to a functional connection between O-GlcNAcylation and p53, the role of this mode of regulation in the redox stress response remains to be investigated. Interestingly, however, O-GlcNAcylation is reported to regulate several DNA damage-sensing and repair pathways beyond p53, suggesting that O-GlcNAc may be relevant in the p53 response to diverse stimuli (Yang et al. 2006; Miura et al. 2012; Chen and Yu 2016). This possibility will be an important focus for future studies.

Concluding remarks

Many studies have demonstrated that critical regulators of redox homeostasis are governed by dynamic O-GlcNAcylation in a wide range of tissues, organisms and pathophysiological contexts. Moreover, changes in O-GlcNAcylation can decisively affect phenotypic outcomes under redox stress conditions. Together, these observations point to a complex and reciprocal interplay between a nutrient-sensitive form of glycosylation and a stress caused largely by byproducts of aerobic metabolism.

However, it remains incompletely understood how O-GlcNAc signaling integrates metabolic and oxidative stress cues to respond to noxious stimuli. Though some key O-GlcNAcylated substrates have been discovered, more work is needed to determine the complete 'parts list' of glycoproteins that transduce these signals. In addition, a better understanding is needed of the kinetics and stoichiometry of dynamic O-GlcNAc cycling during oxidative stress, both on specific substrates of interest, and globally within cells and tissues. We expect that recent advances in chemical and mass spectrometry-based technologies will greatly accelerate these efforts (Tarrant et al. 2012; Myers et al. 2013; Raj et al. 2016). Indeed, future studies may provide a more comprehensive understanding of the role of O-GlcNAc in oxidative stress signaling and suggest new opportunities to manipulate these responses for therapeutic benefit in diseases as diverse as cancer, diabetes and neurodegeneration.

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Conflict of interest statement

None declared.

Abbreviations

O-GlcNAc, O-linked β-N-acetylglucosamine; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; ROS, reactive oxygen species; MEF, mouse embryonic fibroblast; GFAT, glutamine:fructose-6-phosphate amidotransferase; HBP, hexosamine biosynthetic pathway; FAS, fatty acid synthase; NRCMs, neonatal rat cardiac myocytes; AdOGA, adenovirus-mediated overexpression of OGA; HIF-1 α, hypoxia-inducible factor 1α; α-KG, α-ketoglutarate; VHL, von Hippel–Lindau protein; PPP, pentose phosphate pathway; PFK1, phosphofructokinase 1; G6PD, glucose-6-phosphate dehydrogenase; AREs, antioxidant response elements; SOD, superoxide dismutase; HCF1, host cell factor 1; FOX, forkhead box; Pepck, phosphoenolpyruvate carboxykinase; TNFα, tumor necrosis factor α; STZ, streptozotocin; MPI, mannose phosphate isomerase.

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