*Biochemical Society Transactions* (2017) **45** 237–249
DOI: 10.1042/BST20160153

Review Article

# Stress-induced O-GlcNAcylation: an adaptive process of injured cells

**Marissa R. Martinez[1], Thiago Braido Dias[1,2,*], Peter S. Natov[1,†,*] and Natasha E. Zachara[1]**

[1]Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205-2185, U.S.A. and [2]Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Av Bandeirantes 3900, Ribeirao Preto, 14049-900 SP, Brazil

Correspondence: Natasha E. Zachara (nzachara@jhmi.edu)

In the 30 years, since the discovery of nucleocytoplasmic glycosylation, *O*-GlcNAc has been implicated in regulating cellular processes as diverse as protein folding, localization, degradation, activity, post-translational modifications, and interactions. The cell co-ordinates these molecular events, on thousands of cellular proteins, in concert with environmental and physiological cues to fine-tune epigenetics, transcription, translation, signal transduction, cell cycle, and metabolism. The cellular stress response is no exception: diverse forms of injury result in dynamic changes to the *O*-GlcNAc subproteome that promote survival. In this review, we discuss the biosynthesis of *O*-GlcNAc, the mechanisms by which *O*-GlcNAc promotes cytoprotection, and the clinical significance of these data.

## Introduction

Cells and tissues respond to environmental and physiological injury by reprogramming transcription, translation, metabolism, and signal transduction to affect repair and survival, and if necessary to promote programmed cell death. Collectively, this cell-wide reprogramming is known as the cellular stress response and is characterized by the induction of chaperones known as heat shock proteins (HSPs) [1–3]. In 2004, we reported that global *O*-GlcNAc (modification of intracellular proteins by monosaccharides of O-linked β-*N*-acetylglucosamine) levels were induced in a dose-dependent manner in response to a wide range of cellular stressors in several mammalian cell lines and that augmentation of *O*-GlcNAc levels promoted the induction of HSPs and cell survival in a model of heat stress (Table 1) [4]. Combined, these data suggested that stress-induced O-GlcNAcylation was one target of the mammalian stress response. Since then, numerous reports have demonstrated that this response, termed the *O*-GlcNAc-mediated stress response, is conserved in both transformed and primary cells as well as several forms of physiological injury. Furthermore, augmentation of O-GlcNAcylation promotes cell and tissue survival in models ranging from heat stress to myocardial ischemia–reperfusion (I/R) injury (Table 1) [5].

Several reports suggest that stress-induced changes to the *O*-GlcNAc subproteome are more complicated than originally proposed. Studies in models of oxidative stress, in particular *in vivo* I/R injury and trauma hemorrhage, demonstrate that O-GlcNAcylation can decline [6–13]. In some models, this decline precedes an increase in O-GlcNAcylation [6], and in others, it is associated with the onset of apoptosis and tissue death [7–10,13]. The identification of proteins dynamically O-GlcNAcylated in response to injury (oxidative, heat stress, and trauma hemorrhage) supports these studies [6,7,12]. Notably, in a model of oxidative stress, a subset of proteins are targeted for deglycosylation even when global O-GlcNAcylation is increased [6]. Collectively, these data lead us to amend the original model proposed [4]: preventing deglycosylation of key proteins acts in concert with enhanced glycosylation on other proteins to prevent apoptosis/necrosis and to promote survival (Figure 1).

*These authors contributed equally.
†Present address: Tufts University School of Medicine, Boston, MA 02111, U.S.A.

Received: 25 October 2016
Revised: 30 November 2016
Accepted: 14 December 2016

Version of Record published: 15 February 2017

Biochemical Society Transactions (2017) **45** 237–249
DOI: 10.1042/BST20160153

**Table 1 Summary of stressors that induce the *O*-GlcNAc modification and models in which enhanced O-GlcNAcylation is protective.**

Part 1 of 2

| Model | Cell/tissue type | Stress-induced elevation in *O*-GlcNAc[†] | *O*-GlcNAc protective | Method of modulation[‡] | Citation |
|---|---|---|---|---|---|
| Arsenite | Cos7, HepG2, MEFs, U2OS, | ✓ | N/A | N/A | [4,77,106,107] |
| Bleocin (DNA damage) | Cos-7 | ✓ | N/A | N/A | [12] |
| Brefeldin A | NRVM | ✓ | ✓ | Genetic, pharmacological | [72] |
| Cobalt chloride | Cos-7 | ✓ | N/A | N/A | [4] |
| Doxorubicin (DNA damage) | Cos-7 | ✓ | N/A | N/A | [12] |
| DTT (ER stress) | Hek293, HepG2, MEFs | ✓ | ✓ | eIF2α mutation | [74,106] |
| Ethanol | Cos-7 | ✓ | N/A | N/A | [4,106] |
| Glucose deprivation | Cardiac stem cells, C2C12, HEK293, HeLa, Neuro2-a | ✓ | N/A | N/A | [22,32,108] |
| Glyoxal | HRMEC | ✓ | ✓ | Genetic, pharmacological | [109] |
| Heat shock | Cos-7, Chang, Cho, HCAEC, Hep3B, HepG2, HEK293, HeLa, L929, MEFs, Neuro2A, NVRM | ✓ (one report decreased) | ✓ | Genetic, metabolic, pharmacological | [4,27,57,58,60,106,107,110] |
| Hydrogen peroxide | Cos-7, HeLa, HepG2, HREC, MEFs, NRVM, RGC5 | Complex | ✓ | Genetic, metabolic, pharmacological | [4,6,13,106] |
| Hypoxia and hypoxia reoxygenation | A549, cardiac stem cells, H1299, HEK293T, mESC, NVRMs, renal proximal tubule cells | ✓ | ✓ | Genetic, metabolic, pharmacological; PFK1 Ser 529; G6PD Ser84 | [9,70,72,73,89,90,108,111] |
| I/R, IPC, rIPC, simulated I/R | Brain tissue (murine and rat), heart tissue (rat, murine, and human), NRVM | Complex | ✓ | Genetic, metabolic, pharmacological | [8,10,13,28,29,37,60,95,98,100] |
| Iodoacetamide | Cos-7 | ✓ | N/A | N/A | [4] |
| Lipopolysaccharide | Raw164.7, human macrophage | ✓ | N/A | N/A | [34] |
| Lysosomal inhibition | Cos-7 | ✓ | N/A | N/A | [4] |

Continued

**Table 1 Summary of stressors that induce the *O*-GlcNAc modification and models in which enhanced O-GlcNAcylation is protective.**

Part 2 of 2

| Model | Cell/tissue type | Stress-induced elevation in *O*-GlcNAc[†] | *O*-GlcNAc protective | Method of modulation[‡] | Citation |
|---|---|---|---|---|---|
| Osmotic stress (hyper) | Cho, Cos-7, HCAEC, HeLa, HEK293T, HepG2, MEFs, Neuro2A | Complex | N/A | N/A | [4,12,80,106,107] |
| Osmotic stress (hypo) | HEK293T | Reduced | ✓ | Genetic, metabolic | [112] |
| Peritonial dialysis fluid — osmotic stress, low pH, glucose degradation products | HPMC, MeT-5A | ✓ | ✓ | Metabolic, pharmacological | [113] |
| Proteasomal inhibition | Cos-7, U2OS | ✓ | N/A | N/A | [4,77] |
| Ribosomal inhibition | Cos-7, MEFs | ✓ | N/A | N/A | [4,106] |
| Streptozotocin and Fas ligand/PUGNAc | Liver, pancreas, kidney (mouse) | ✓ | ✓ | K18 S30/31/49 | [59] |
| Thapsigargin (ER stress) | Hek293, HepG2 | ✓ | ✓ | eIF2α mutation | [74] |
| Trauma hemorrhage | Rats | Complex | ✓ | Metabolic, pharmacological | [7,36,84,85,96] |
| Tunicamycin (ER stress) | Cos-7, MEFs, NVVMs | ✓ | ✓ | Genetic, pharmacological | [12,72,106] |
| UV | Cos-7 | ✓ | N/A | N/A | [4] |
| γ-Irradiation | HeLa | ✓ | N/A | N/A | [114] |

Abbreviations: A549, human lung carcinoma (epithelial); C2C12, mouse muscle cell (myoblast); Chang, HeLa cell derivative (epithelial); Cho, Chinese hamster ovary cells (epithelial); Cos-7, African green monkey kidney fibroblast-like (fibroblast); DAOY, human medulloblastoma cells (polygonal); H1299, human lung carcinoma (non-small-cell lung cancer, epithelial); H9C2, rat heart/myocardium myoblast; HCAECs, human coronary artery endothelial cells, primary; HeLa, human cervical adenocarcinoma (epithelial); HEK293, human embryonic kidney (epithelial); HEK293T, human embryonic kidney (epithelial); Hep3B, human liver hepatocellular carcinoma (epithelial); HepG2, human liver hepatocellular carcinoma (epithelial); HPMCs, human peritoneal mesothelial cells, primary; HRECs, human retinal microvascular endothelial cells, primary; L929, mouse fibroblasts; mESCs, mouse stem cells; MEFs, mouse embryonic fibroblasts; MeT-5A, human mesothelium (epithelial); NRVMs, neonatal rat ventricular myocytes, primary; Neuro2A, murine neuroblastoma (neuroblast); RGC5, retinal ganglion cells; U2OS, human bone osteosarcoma (epithelial); N/A, not addressed in this model.

[†]In some models, *O*-GlcNAc levels decline, before becoming elevated. This phenotype is annotated as 'complex'. In other models of injury, *O*-GlcNAc levels decrease. This phenotype is annotated as 'reduced'. Models in which there are conflicting data, which may result from experimental differences, are also labeled 'complex'.

[‡]Pharmacological modulation of *O*-GlcNAc refers to the inhibition of OGA with Thiamet-G, NAG-thiazalone, or PUGNAc or the *O*-GlcNAc transferase with TT04, 4Ac-SGlcNAc, or OSIM-1. Genetic modulation refers to the manipulation of OGT or OGA expression by deletion, RNA interference, or overexpression. Metabolic manipulation refers either to inhibition of GFAT with DON or Azaserine, or to feeding cells with metabolites of the HBP such as glucosamine, *N*-acetylglucosamine, and glutamine.

Dynamic O-GlcNAcylation is well positioned to reprogram cellular pathways in response to cellular stress. To date, over a thousand proteins have been described as O-GlcNAcylated, many of which play important roles in mediating cellular homeostasis with well-described roles in epigenetics, transcription, mRNA biogenesis, protein degradation, signal transduction, and metabolism [14,15]. Consistent with these data, *O*-GlcNAc has been implicated in mediating cell survival decisions via numerous pathways that include transcription, stress granule formation, HSP synthesis, altered metabolic flux, reduced endoplasmic reticulum (ER) stress, and improved mitochondrial function. These data, and the clinical implications of the *O*-GlcNAc-mediated stress response, are discussed below.

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DOI: 10.1042/BST20160153

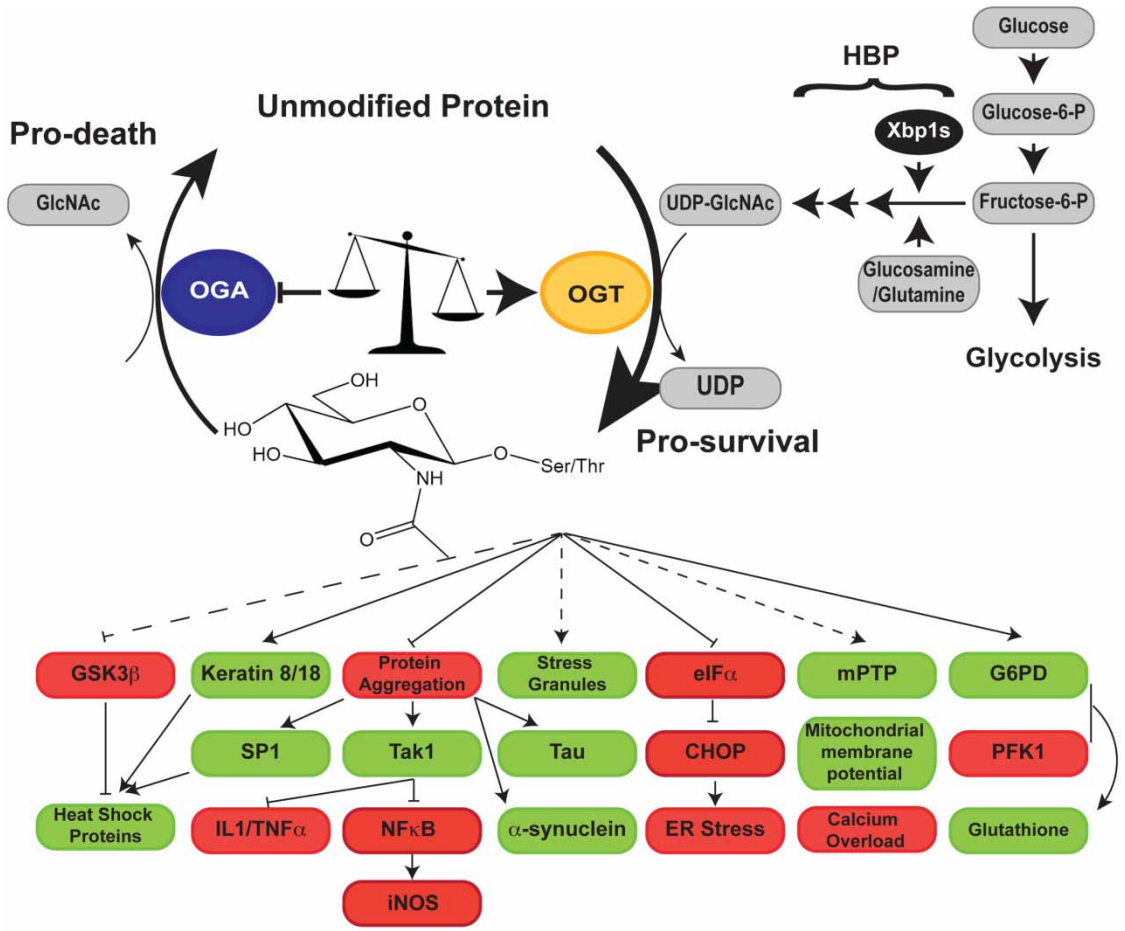


**Figure 1. In response to injury, there are significant and dynamic changes to the *O*-GlcNAc subproteome.**
As increasing *O*-GlcNAc levels promotes survival, and as decreasing *O*-GlcNAc levels promotes apoptosis and necrosis, stress-induced changes in O-GlcNAcylation are thought to reprogram cellular pathways promoting survival. Stress-induced changes in the *O*-GlcNAc modification correlate with increased expression, activity, and substrate targeting of OGT (yellow) and decreased expression and activity of OGA (blue), as well as increased flux through the hexosamine biosynthetic pathway. One mechanism supporting metabolic remodeling is increased expression of GFAT, the rate-limiting enzyme in the hexosamine biosynthetic pathway, which is mediated by the transcription factor Xbp1s. *O*-GlcNAc has been demonstrated to activate proteins/pathways (green) leading to cell survival (e.g. heat shock protein expression) and to inhibit proteins (red) that promote cell death (e.g. CHOP activation), collectively increasing cellular protection. For some pathways, the molecular mechanism by which *O*-GlcNAc mediates survival/inhibition is unknown (dashed lines), whereas for others the O-GlcNAcylated proteins and sites have been identified (solid lines). Nonetheless, additional work is required to fully delineate how *O*-GlcNAc promotes a pro-survival phenotype. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; GFAT, glutamine fructose-6-phosphate aminotransferase; GSK3β, glycogen synthase kinase 3β; mPTP, mitochondrial permeability transition pore; OGA, *O*-GlcNAcase; OGT, *O*-GlcNAc transferase; IL-1, interleukin 1; PFK1, phosphofructokinase 1; TNF-α, tumor necrosis factor α; Xbp1s, spliced X-box binding protein 1.

## Regulation of O-GlcNAcylation during injury

The *O*-GlcNAc modification is cycled by two enzymes, the *O*-GlcNAc transferase (OGT) and the *O*-GlcNAcase (OGA). OGT, which catalyzes the addition of the *O*-GlcNAc residue, exists as three well-characterized isoforms: nucleocytoplasmic, mitochondrial, and short [16,17]. Each isoform contains a C-terminal glycosyltransferase domain and a variable number of N-terminal tandem tetratricopeptide repeats (TPRs) [18–21]. The substrate specificity of OGT is postulated to be regulated by the TPR domain and protein interactors of OGT [22,23]. Deletions in the TPR domain that do not affect the catalytic activity of OGT alter

OGT's ability to glycosylate protein substrates [24,25]. This model is further supported by findings that the three isoforms have different peptide and protein substrates [25,26].

In cell culture models, stress-induced O-GlcNAcylation is coincident with increased enzymatic activity, protein expression, and nuclear localization of OGT [4,27]. Similarly, in isolated heart tissue subjected to *ex vivo* or remote ischemic preconditioning (rIPC), increased *O*-GlcNAc levels were associated with elevated OGT expression and activity [28,29]. In neuroblastoma cells exposed to glucose deprivation, p38 mitogen-activated protein kinase is critical for targeting OGT to specific substrates such as neurofilament protein H [22]. In the aforementioned model, glucose deprivation induced OGT expression in an AMP-activated protein kinase (AMPK)-dependent manner [22]. Regulation of OGT expression at the post-transcriptional level has also been demonstrated. In patients with congestive heart failure, microRNA-423-5p was found to be elevated. In cultured cardiomyocytes, microRNA-423-5p was demonstrated to target the 3′-untranslated region (3′-UTR) of OGT mRNA, suppressing OGT expression [30].

Post-translational modifications (PTMs) also regulate OGT. In fact, OGT is itself O-GlcNAcylated at two sites within the catalytic domain [18,19,31]. OGT is phosphorylated at Tyr979, Thr444, and Ser3 or Ser4 [18,32,33]. While the implications of these PTMs are unknown in models of injury, phosphorylation at Thr444 is catalyzed by AMPK *in vitro* and augments OGT nuclear localization in glucose-deprived muscle cells [32]. In the fine-tuning of the circadian clock, glycogen synthase kinase 3β (GSK3β) phosphorylates OGT at Ser3 or Ser4, resulting in activation of OGT [33]. Finally, it has been shown *in vitro* and *in vivo* that OGT is basally S-nitrosylated in macrophages, inhibiting catalytic activity, and is activated by de-nitrosylation when macrophages are stimulated by lipopolysaccharide [34].

Another regulator of OGT activity is the availability of its nucleotide sugar substrate, UDP-GlcNAc [24]. Elevated UDP-GlcNAc levels increase OGT's affinity for its peptide substrates [18,24]. Immediate elevations in UDP-GlcNAc levels are observed in cancer cell lines in response to treatment with the chemotherapeutic agent cisplatin [35]. Moreover, modest elevations in UDP-GlcNAc levels have been reported in myocardial I/R injury [10,36,37]. Increased flux through the hexosamine biosynthetic pathway (HBP), which generates UDP-GlcNAc, may be a result of increased glucose flux, which is a common response of cells to injury [38]. Recent data suggest that cellular stress directly up-regulates the HBP [37]. Spliced X-box binding protein 1 (Xbp1s) is a transcription factor that promotes the expression of chaperones and other mediators of the unfolded protein response (UPR) [37]. One target of Xbp1s is the gene encoding glutamine fructose-6-phosphate aminotransferase 1 (GFAT1), the enzyme catalyzing the rate-limiting step of the HBP. Xbp1s was demonstrated to increase GFAT1 expression *in vivo* in a murine model of I/R injury, elevating flux through the HBP and global *O*-GlcNAc levels [37]. Similarly, another study demonstrated that prostate cancer cells overexpressing the enzyme UDP-*N*-acetylglucosamine pyrophosphorylase 1, which catalyzes the final step of the HBP, have significantly up-regulated HBP flux and are protected from ER stress [39].

The removal of *O*-GlcNAc is catalyzed by OGA, a soluble *N*-acetylglucosaminidase [40–42]. There are two well-characterized isoforms of OGA: full-length OGA and short OGA [41–43]. Short OGA preferentially localizes to the nucleus and lipid droplets [43], whereas the full-length isoform is found in the nucleus, cytoplasm, and mitochondria [41,42,44]. Less is known about the regulation of OGA, although it can be cleaved by caspase 3 [45] and is O-GlcNAcylated [46], phosphorylated, ubiquitinated, and acetylated [47–50]. The impact of these PTMs on the localization, substrate specificity, or activity of OGA has not been reported. Little is known about the regulation of OGA levels in stressed cells. In models of rIPC, increased *O*-GlcNAc levels are associated with decreased OGA activity [28]. In a cell culture model of I/R injury and murine infarct-induced heart failure, miRNA-539 levels were up-regulated, resulting in decreased OGA expression [51].

Several regulatory mechanisms of *O*-GlcNAc levels in cells and tissues have been discussed. However, it remains unclear if cells and tissues co-ordinate all of these mechanisms to affect stress-induced changes in O-GlcNAcylation, or if different cells and tissues induce specific pathways depending on the type of stress.

## Mechanisms by which *O*-GlcNAc promotes survival

Elevating O-GlcNAcylation augments survival in a broad range of cells and tissues challenged with diverse stressors, suggesting that stress-induced changes in O-GlcNAcylation are a conserved defense against injury that enable cellular remodeling to promote survival (Table 1). Several studies have begun to delineate the mechanisms by which *O*-GlcNAc confers cytoprotection.

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DOI: 10.1042/BST20160153

## Regulation of HSP expression

HSPs and heat shock factors (HSFs), the transcription factors that mediate HSP synthesis, are central mediators of proteostasis during the cellular stress response [52] and are emerging as a key pathway regulated by *O*-GlcNAc. Specifically, pharmacologically elevating O-GlcNAcylation prior to heat stress increases HSP72 and HSP40 expression, whereas blocking the HBP reduces the expression of HSP72 [4]. To determine if *O*-GlcNAc modulates the expression of other chaperones, Kazemi et al. developed a cell line in which OGT deletion could be induced with 4-hydroxytamoxifen. Deletion of OGT alters the basal and stress-induced expression of 18 of the 84 chaperones tested [27]. Under basal conditions, GSK3β inhibits HSF1 via phosphorylation of Ser303 [53,54]. Elevating O-GlcNAcylation suppresses HSF1 Ser303 phosphorylation, which is in part mediated by inhibition of GSK3β by phosphorylation at Ser9 [27]. The regulatory effect of *O*-GlcNAc on chaperone expression has been recapitulated in many cell and physiological models of injury [13,55–58].

Regulation of HSP expression via the GSK3/AKT axis has also been demonstrated with another stress-induced regulator, Keratin 18 (K18). Glycosylation of K18 at Ser30, Ser31, and Ser49 [59] is protective following acute liver injury, with K18 triple glycosylation mutant (K18Gly-) mice exhibiting higher susceptibility to streptozotocin or PUGNAc/Fas ligand-induced death [59]. Phosphorylation of AKT at Thr308 was decreased following PUGNAc/Fas ligand-induced injury with a concomitant decrease in phosphorylation of GSK3α at Ser21 in K18Gly-mice, as well as a decrease in HSP72 expression [59]. Taken together, these studies indicate that glycosylation is required for AKT activation of a pro-survival phenotype. In addition to direct regulation of HSP pathways, O-GlcNAcylation may prevent protein aggregation as in the case of αB crystalline [60] and the transcription factor Sp1 [57], allowing them to bind to and initiate the transcription of HSP, respectively. Furthermore, Sp1 is stabilized through the co-translational addition of *O*-GlcNAc to nascent polypeptide chains, preventing its proteasomal degradation [61]. Taken together, these data suggest that *O*-GlcNAc positively regulates HSP pathways through a variety of mechanisms.

## *O*-GlcNAc disrupts protein aggregation in neurodegenerative diseases

Previous studies have begun to delineate the protective effects of *O*-GlcNAc in diseases of protein aggregation, which play an important role in neurodegenerative diseases like Parkinson's disease where hallmark proteinaceous inclusions contribute to pathophysiology [62]. α-Synuclein is the major structural component of Lewy bodies in Parkinson's disease, first aggregating to form oligomeric species that ultimately polymerize into fibers [62]. α-Synuclein is O-GlcNAcylated *in vivo* at residues Thr64, Thr72, and Ser87 [63–65]. α-Synuclein glycosylated at Thr72, generated semisynthetically by expressed protein ligation, inhibited α-synuclein aggregation and fiber formation in a dose-dependent manner, with fully glycosylated species exhibiting little to no aggregation [66]. In addition, cellular proliferation was increased, and α-synuclein-induced toxicity decreased in cells treated with α-synuclein glycosylated at Thr72 compared with unmodified protein [66]. A similar phenotype has been demonstrated for Tau, the oligomeric species in Alzheimer's disease. O-GlcNAcylation of Tau at Ser400 inhibited oligomerization *in vitro*, which was not seen with Tau S400A [67]. In the JNPL3 model of Alzheimer's disease, which harbors the FTDP-17 tau$^{P301L}$ transgene, mice treated with Thiamet-G (TMG) to elevate *O*-GlcNAc levels displayed 1.4 times as many motor neurons and had decreased pathologic Tau phosphorylation (Ser202) compared with controls. TMG treatment increased glycosylation of Tau at Ser400 in these mice, suggesting that protective effects of TMG are mediated through a disruption in Tau aggregation [68].

## *O*-GlcNAc inhibits the hallmarks of I/R injury

Much of the work delineating how *O*-GlcNAc regulates cellular injury has been done in models of I/R injury. In neonatal rat ventricular myocytes (NRVMs) subjected to hypoxia–reoxygenation, elevating O-GlcNAcylation by overexpressing OGT or inhibiting OGA reduced reactive oxygen species and calcium overload [9]. Some of these protective effects originate in the mitochondria where *O*-GlcNAc is necessary for maintenance of mitochondrial membrane potential and prevents the formation of the mitochondrial membrane transition pore [13,69,70]. One mechanism by which these effects may occur is through direct O-GlcNAcylation and regulation of the voltage-dependent anion channel, which regulates flux of $Ca^{2+}$ through the mitochondria [13,69,70].

## *O*-GlcNAc reduces ER stress

The protective effects of *O*-GlcNAc are not limited to mitochondrial function, but also rescue injury caused by ER stress. The accumulation of misfolded proteins in the ER signals through IRE1 PERK1 (PKR-like ER

kinase) and ATF6 to recruit chaperones, such as BiP, in order to increase folding capacity and eliminate misfolded proteins [71]. In the event that protein misfolding cannot be contained, the cell signals through eukaryotic initiation factor 2α (eIF2α) and the transcription factor C/EBP homologous protein (CHOP) to induce apoptosis [71]. In NRVMs treated with the ER stressors brefeldin A and tunicamycin, *O*-GlcNAc attenuates the activation of the maladaptive arm of the UPR and reduces cardiomyocyte death. Reduced cell death has been correlated with decreased activation of CHOP [72]. Similar results have been demonstrated in a rabbit model of renal I/R injury where CHOP levels were suppressed upon administration of glucosamine [73]. eIF2α phosphorylation by PERK is required for CHOP activation. O-GlcNAcylation of eIF2α (Ser219, Thr239, and Thr241) may hinder its phosphorylation, thus suppressing CHOP activation [74]. In addition, OGT stabilizes hypoxia-inducible factor 1α (HIF1α), a transcription factor, by reducing its hydroxylation and subsequent degradation via the ubiquitin E3 ligase von Hippel–Lindau [75]. Depletion of OGT alone induced the maladaptive arm of the ER stress response, and this phenotype was reversed by overexpression of hydroxylation/degradation-resistant HIF1α mutants [75]. Further function has been demonstrated for *O*-GlcNAc in mitigating ER stress through the formation of stress granules (SGs) and processing bodies (PBs), which primarily regulate mRNA translation and degradation, respectively [76]. OGT and the HBP are required for stress-induced stress granule (SG) assembly and constitutive PB assembly [77]. Upon stress, O-GlcNAcylated proteins, such as receptor for activated C kinase 1 (RACK1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and ribosomal subunit proteins, are found in SG, whereas most of OGT is localized elsewhere. These observations indicate that OGT modifies proteins that are subsequently recruited to SG. Combined, these data suggest that OGT and *O*-GlcNAc play central roles to mitigate ER stress and prevent apoptosis.

## *O*-GlcNAc-mediated regulation of the inflammatory-driven stress response

Activation of inflammatory signaling pathways is characteristic of the stress response [78,79]. Transforming growth factor-β-activated kinase 1 (TAK1) is a serine/threonine kinase composed of a catalytic subunit in complex with regulatory subunits [TAK-binding proteins (TAB) 1/2/3] [80]. TAK1 activates various kinases, such as c-Jun N-terminal kinase 1 ( JNK1), ERK1, and nuclear factor κB (NF-κB), that result in the production of pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) [81–83]. TAB1 is O-GlcNAcylated at Ser295 both basally and in response to IL-1 and osmotic stress [80]. O-GlcNAcylation results in elevated TAK1 activity, including phosphorylation of the downstream targets JNK1/2 and Ikappaβα (Iκβα). Activation of TAK1 is blocked with mutation of TAB1 S295A, also diminishing NF-κB activation and subsequent production of TNF-α and IL-6 [80].

Elevating O-GlcNAcylation has also been demonstrated to reduce pro-inflammatory signaling through decreased NF-κB activation in models of trauma hemorrhage [36,84,85] and arterial injury [86–88]. Here, NF-κB phosphorylation at Ser536 decreases concomitant with increased glycosylation on the regulatory subunit p65, promoting Iκβα binding [86]. Decreased NF-κB signaling attenuates chemokine production and subsequent infiltration of neutrophils and macrophages [86] as well as a reduction in inducible nitric oxide synthase (iNOS) expression and nitrotyrosine formation [87].

## Retuning metabolism with *O*-GlcNAc enables cancer cells to resist stressful tumor microenvironments

Inhibiting OGA or overexpressing OGT to elevate O-GlcNAcylation decreases the rate of glucose metabolism under normoxia and hypoxia, with a concomitant decrease in lactate and ATP levels [89]. This change in metabolism is regulated by glycosylation of phosphofructokinase 1 (PFK1) at Ser529, an intermediate enzyme in glycolysis that converts fructose-6-phosphate into fructose-1,6-bisphosphate, and results in decreased catalytic activity [89]. Suppressing glycolysis activates the pentose phosphate pathway (PPP) to provide pentose sugars for nucleotide and nucleic acid biosynthesis as well as NADPH for the synthesis of the cellular antioxidant glutathione. Furthermore, PFK1 was glycosylated in various solid tumors and malignant tissues, suggesting that cancer cells may inhibit PFK1 activity through glycosylation to enhance flux through the PPP and to promote cellular proliferation [89]. Further studies have demonstrated a direct role for *O*-GlcNAc within the PPP. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the PPP, is *O*-GlcNAc modified in response to hypoxia at Ser84 [90]. Increasing O-GlcNAcylation enhanced G6PD oligomerization and activity in cells expressing wild-type, but not G6PD S84V, protein. G6PD O-GlcNAcylation was accompanied by increased flux through the PPP and an enhanced proliferative phenotype. Similar to the aforementioned study,

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DOI: 10.1042/BST20160153

glycosylation of G6PD enhanced tumor formation and was more abundant in human lung cancer tissue [90]. Collectively, these and other data suggest that cancer cells have hijacked the *O*-GlcNAc-mediated stress response to promote growth in adverse environments [91].

## Clinical implications of *O*-GlcNAc-mediated cytoprotection

The conservation of the *O*-GlcNAc-mediated stress response highlights the potential for modulation of this pathway in a wide range of clinical models (Table 1). Critically, in models of *ex vivo* myocardial I/R injury [92] and *in vivo* trauma hemorrhage [36,84], elevating O-GlcNAcylation post-injury is protective. Moreover, OGT is necessary for postinfarct remodeling of the heart [93]. Pharmacological inhibitors of OGA, such as TMG, have been used to suppress protein aggregates in models of tauopathies, are orally bioavailable, and appear nontoxic [68,94]. One alternative to pharmacological modulation are metabolites that augment flux through the HBP (Figure 1). Both glucosamine and glutamine have been demonstrated to improve survival and organ function [10,11,36,56,73,95–98], although the effectiveness of oral glucosamine supplementation is controversial [99]. Alternatively, glucose–insulin–potassium administration both augments *O*-GlcNAc levels and promotes cardioprotection [100].

While elevating O-GlcNAcylation transiently in the models discussed above is protective, chronic elevation of O-GlcNAcylation has been associated with the development of hypertension [101], heart failure [51,102,103], glucose toxicity, and type II diabetes [14]. The molecular mechanisms underlying the transition from protection to pathology are unknown. One simple model is that elevating O-GlcNAcylation acutely is protective, whereas chronic elevation is toxic. Such timing is not unique among stress response pathways: chronic up-regulation of HSPs is toxic in cells challenged with a chronic proteostasis stress [104]. In all likelihood, the model is more complex because of an altered molecular landscape, including changes in the proteome and PTMs therein. Ultimately, maladaptation of cellular pathways is predicted to lead to decreased *O*-GlcNAc cycling at specific sites and altered substrate specificity and activity of the enzymes that cycle *O*-GlcNAc. Indeed, Ma and coworkers recently demonstrated that the protein interactors of OGT were changed in a model of streptozotocin-induced diabetes [44]. Collectively, these data lead to a critical yet unanswered question: how do chronic diseases, such as diabetes and hypertension, alter the *O*-GlcNAc-mediated stress response? Two recent studies provide some insights into this question. Jensen and coworkers showed that rIPC was ineffective on diabetic atrial trabeculae, and unlike control samples, had no effect on O-GlcNAcylation [28]. In the second study, *O*-GlcNAc levels did not respond to brain I/R injury in old animals, whereas *O*-GlcNAc levels were elevated in the brains of young mice [105]. These data suggest that simple modulation of *O*-GlcNAc levels may not be specific enough in clinical models.

## Concluding remarks

Collectively, the data discussed suggest that targeting O-GlcNAcylation of key proteins may lead to the development of novel therapeutics for ameliorating cell and tissue death in a wide range of injury models. To achieve an understanding of how O-GlcNAcylation is relevant to translational medicine, there are several barriers that need to be overcome and include: (1) identifying proteins and quantifying sites of O-GlcNAcylation targeted during injury and defining the role of *O*-GlcNAc on these proteins/sites; (2) characterizing the mechanisms that lead to protein-specific changes in the *O*-GlcNAc modification during injury, especially the targeting of OGT and OGA to substrates; and (3) determining how conditions, such as aging, which alter the ability of tissues to respond to injury, affect the *O*-GlcNAc-mediated stress response. Answering such challenges will build a map of the *O*-GlcNAc signaling network from which the role of *O*-GlcNAc in diverse models can be probed, providing critical insights into the role of this modification in cellular homeostasis and disease pathology.

### Abbreviations

3′-UTR, 3′-untranslated region; AMPK, AMP-activated protein kinase; CHOP, transcription factor C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; G6PD, glucose-6-phosphate dehydrogenase; GFAT1, glutamine fructose-6-phosphate aminotransferase 1; GSK3β, glycogen synthase kinase 3β; HBP, hexosamine biosynthetic pathway; HIF1α, hypoxia-inducible factor α; HSF, heat shock factors; HSP, heat shock protein; Iκβα, Ikappaβα; IL-1, interleukin-1; I/R, ischemia–reperfusion; iNOS, inducible nitric oxide synthase; JNK1, c-Jun N-terminal kinase 1; K18, Keratin 18; K18Gly, K18 triple glycosylation mutant; NF-κB, nuclear factor κB; NRVM, neonatal rat ventricular myocyte; *O*-GlcNAc, modification

of intracellular proteins by monosaccharides of O-linked β-*N*-acetylglucosamine; OGA, *O*-GlcNAcase; OGT, *O*-GlcNAc transferase; PB, processing bodies; PERK, PKR-like ER kinase; PFK1, phosphofructokinase 1; PPP, pentose phosphate pathway; PTM, post-translational modifications; rIPC, remote ischemic preconditioning; SG, stress granules; TAB, TAK-binding protein; TAK1, transforming growth factor-β-activated kinase; TMG, Thiamet-G; TNF-α, tumor necrosis factor α; TPR, tetratricopeptide repeat; UPR, unfolded protein response; Xbp1s, spliced X-box binding protein 1.

### Author Contribution

P.S.N. composed the section 'regulation of OGT and OGA during injury'. M.R.M. wrote the section 'mechanisms by which *O*-GlcNAc promotes survival'. T.B.D. composed the section 'clinical implications of *O*-GlcNAc-mediated cytoprotection'. N.E.Z. wrote all other sections and edited the manuscript.

### Funding

This work is supported by grants to N.E.Z. from the National Institutes of Health [P01HL107153] (National Heart Lung and Blood Institute) and [R21DK108782] (National Institute of Diabetes and Digestive and Kidney Diseases).

### Acknowledgements

The authors thank Kamau Fahie PhD and Jennifer Groves for their constructive criticism of the manuscript. We acknowledge the work of researchers whose data were not discussed due to space limitations.

### Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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