Cell Stress and Chaperones (2013) 18:535–558
DOI 10.1007/s12192-013-0426-y

MINI REVIEW

# Dynamic *O*-GlcNAcylation and its roles in the cellular stress response and homeostasis

**Jennifer A. Groves · Albert Lee · Gokben Yildirir · Natasha E. Zachara**

Received: 17 September 2012 / Revised: 29 March 2013 / Accepted: 1 April 2013 / Published online: 26 April 2013
© Cell Stress Society International 2013

**Abstract** *O*-linked *N*-acetyl-β-D-glucosamine (*O*-GlcNAc) is a ubiquitous and dynamic post-translational modification known to modify over 3,000 nuclear, cytoplasmic, and mitochondrial eukaryotic proteins. Addition of *O*-GlcNAc to proteins is catalyzed by the *O*-GlcNAc transferase and is removed by a neutral-*N*-acetyl-β-glucosaminidase (*O*-GlcNAcase). *O*-GlcNAc is thought to regulate proteins in a manner analogous to protein phosphorylation, and the cycling of this carbohydrate modification regulates many cellular functions such as the cellular stress response. Diverse forms of cellular stress and tissue injury result in enhanced *O*-GlcNAc modification, or *O*-GlcNAcylation, of numerous intracellular proteins. Stress-induced *O*-GlcNAcylation appears to promote cell/tissue survival by regulating a multitude of biological processes including: the phosphoinositide 3-kinase/Akt pathway, heat shock protein expression, calcium homeostasis, levels of reactive oxygen species, ER stress, protein stability, mitochondrial dynamics, and inflammation. Here, we will discuss the regulation of these processes by *O*-GlcNAc and the impact of such regulation on survival in models of ischemia reperfusion injury and trauma hemorrhage. We will also discuss the misregulation of *O*-GlcNAc in diseases commonly associated with the stress response, namely Alzheimer's and Parkinson's diseases. Finally, we will highlight recent advancements in the tools and technologies used to study the *O*-GlcNAc modification.

Jennifer A. Groves and Albert Lee contributed equally to this paper.

J. A. Groves · A. Lee · G. Yildirir · N. E. Zachara (✉)
The Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 725 N. Wolfe St, Baltimore, MD 21205-2185, USA
e-mail: nzachara@jhmi.edu

**Keywords** *O*-GlcNAc · Stress · Signal transduction · *O*-GlcNAc transferase · *O*-GlcNAcase · Post-translational modification

**Abbreviations**

| | |
|---|---|
| 4-OHT | 4-Hydroxytamoxifen |
| AD | Alzheimer's disease |
| APP | Amyloid β precursor protein |
| Aβ | Amyloid β |
| Bcl-2 | B-cell lymphoma 2 |
| BEMAD | Beta elimination and Michael addition |
| CaMK | $Ca^{2+}$/calmodulin-dependent kinase |
| CCE | Capacitative $Ca^{2+}$ entry |
| CID | Collision-induced dissociation |
| DON | 6-Diazo-5-oxo-L-norleucine |
| Emeg32 | Glucosamine-6-phosphate acetyltransferase |
| eNOS | Endothelial nitric oxide synthase |
| ESI | Electrospray ionization |
| ETD | Electron transfer dissociation |
| FDGlcNAc | Fluorescein di-*N*-acetyl-β-D-glucosaminide |
| Foxo | Forkhead box |
| FRET | Förster resonance energy transfer |
| GalNAz | *N*-azidoacetylgalactosamine |
| GalT1 | UDP-Gal/GlcNAcβ-1,4-galactosyltransferase |
| GE | Gel electrophoresis |
| GFAT | Glutamine/fructose-6-phosphate amidotransferase |
| GlcNAz | *N*-azidoacetylglucosamine |
| GSK3β | Glycogen synthase kinase 3β |
| HAT | Histone acetyltransferase |
| HBP | Hexosamine biosynthetic pathway |

| | |
|---|---|
| Hex | Hexosaminidase |
| HK | Hexokinase |
| HSC | Heat shock cognate |
| HSF | Heat shock factor |
| HSP | Heat shock protein |
| I/R | Ischemia–reperfusion |
| IKKβ | Inhibitor of NFκB kinase |
| IL | Interleukin |
| $IP_3$ | Inositol (3,4,5)-trisphosphate |
| IRS-1 | Insulin receptor substrate 1 |
| K18 | Cytokeratin 18 |
| LC | Liquid chromatography |
| LWAC | Lectin weak affinity chromatography |
| MALDI | Matrix-assisted laser desorption ionization |
| MAP3K7 | Mitogen-activated protein kinase kinase kinase 7 |
| MAPK | Mitogen-activated protein kinase |
| Mgea5 | Meningioma-expressed antigen 5 |
| mOGT | Mitochondrial OGT |
| mPTP | Mitochondrial permeability transition pore |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| nCl | Negative chemical ionization |
| ncOGT | Nuclear/cytoplasmic OGT |
| NFκB | Nuclear factor κB |
| NO | Nitric oxide |
| *O*-GlcNAc | *O*-linked *N*-acetyl-β-D-glucosamine |
| *O*-GlcNAcase (OGA) | *N*-acetyl-β-glucosaminidase |
| *O*-GlcNAcylated | *O*-GlcNAc-modified |
| OGT (*O*-GlcNAc transferase) | UDP-*N*-acetylglucosamine/peptide *N*-acetylglucosaminyl-transferase |
| PD | Parkinson's disease |
| PGC1α | Peroxisome proliferator-activated receptor gamma coactivator 1-α |
| Pgm3 | *N*-acetylglucosamine-phosphate mutase |
| PI3K | Phosphoinositide 3-kinase |
| $PIP_3$ | Phosphatidylinositol (3,4,5)-trisphosphate |
| PNGase | F Peptide *N*-glycosidase F |
| *p*NP-β-GlcNAc | *p*-Nitrophenol-*N*-acetyl-β-D-glucosamine |
| PRMT4/Carm1 | Protein arginine methyltransferase 4 |
| PTM | Post-translational modification |
| PUGNAc | *O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-*N*-phenylcarbamate |
| QTOF | Quadrupole time-of-flight |
| Rb | Retinoblastoma protein |
| ROS | Reactive oxygen species |
| RT-PCR | Real-time polymerase chain reaction |
| SILAC | Stable isotope labeling with amino acids in cell culture |
| STZ | Streptozotocin |
| sWGA | Succinylated WGA |
| TAB1 | TAK1-binding protein |
| TAD | Transactivation domain |
| TAK1 | TGF-β-activated kinase I |
| TGF | Transforming growth factor |
| TMG | (thiamet-G) 2-Ethylamino-3aR, 6S, 7R, 7aR-tetrahydro-5R-hydroxymethyl-5H-pyrano[3, 2-d]thiazole-6,7-diol |
| TNF-α | Tumor necrosis factor-α |
| TRP | Tetratricopeptide |
| Uap1 | UDP-GlcNAc pyrophosphorylase |
| UDP | Uridine diphosphate |
| UL32 | Human cytomegalovirus tegument basic phosphoprotein |
| VDAC | Voltage-dependent anion channel |
| WGA | Wheat germ agglutinin |

## Introduction

*O*-linked *N*-acetyl-β-D-glucosamine (*O*-GlcNAc) is a dynamic post-translational modification (PTM) of more than 3,000 nuclear, cytoplasmic, and mitochondrial proteins (Carapito et al. 2009; Gurcel et al. 2008; Rexach et al. 2008; Teo et al. 2010; Vosseller et al. 2006; Wang et al. 2010a, b; Wells et al. 2002b; Torres and Hart 1984). While *O*-GlcNAc appears more common in metazoans, there is evidence for this PTM in simple eukaryotes such as *Aspergillus niger* (Machida and Jigami 1994) and in some prokaryotes (Shen et al. 2006). *O*-GlcNAc is cycled on and off serine (Ser) and/or threonine (Thr) residues by just two enzymes: the *O*-GlcNAc transferase (OGT) that catalyzes the addition of *O*-GlcNAc and the *O*-GlcNAcase that removes *O*-GlcNAc (Fig. 1). Highlighting the importance of *O*-GlcNAc in cellular homeostasis, deletion of OGT, *O*-GlcNAcase, and other key enzymes in the hexosamine biosynthetic pathway (HBP) is lethal in mammals (Mio et al. 1999; Greig et al. 2007; Boehmelt et al. 2000a, b; Shafi et al. 2000; Forsythe et al. 2006; Yang et al. 2012).

Intracellular proteins that are *O*-GlcNAc-modified (*O*-GlcNAcylated) fall into diverse functional groups. Examples of *O*-GlcNAcylated proteins include chromatin-associated proteins and histones, transcription factors, ribosomal proteins, proteasomal proteins, cytoskeletal proteins, and many different types of signaling proteins such as kinases and metabolic enzymes (Carapito et al. 2009; Gurcel et al. 2008; Rexach et al. 2008; Teo et al. 2010; Vosseller et al. 2006; Wang et al. 2010a, b; Wells et al. 2002b). *O*-

GlcNAc is thought to regulate these proteins in a manner analogous to protein phosphorylation. *O*-GlcNAc has been demonstrated to alter numerous protein functions that include: DNA binding and transactivation (Jackson and Tjian 1989; Ozcan et al. 2010; Comer and Hart 1999; Gao et al. 2003; Sayat et al. 2008), protein–protein interactions (Lim and Chang 2010; Ise et al. 2010; Guinez et al. 2010; Guinez et al. 2007; Guinez et al. 2006), protein degradation (Cheng and Hart 2001; Han and Kudlow 1997; Zhang et al. 2003), protein and enzyme activity (Kim et al. 2006b; Rengifo et al. 2007; Zhang et al. 2003; Dias et al. 2009; Bimboese et al. 2011), and protein localization (Sayat et al. 2008; Dudognon et al. 2004) among many others (Hart et al. 2011).

Like phosphorylation, the levels of *O*-GlcNAc respond to both intracellular and extracellular stimuli including insulin, nutrient levels, and cellular stress (Whelan et al. 2008, 2010; Walgren et al. 2003; Kearse and Hart 1991; Zachara et al. 2004b; Song et al. 2008; Hart et al. 2011), with the latter being the main focus of this review. In response to diverse forms of cellular stress, *O*-GlcNAc levels are elevated on numerous proteins (Zachara et al. 2004b). Augmenting *O*-GlcNAc levels promotes survival while suppressing *O*-GlcNAc levels sensitizes cells to death, suggesting that *O*-GlcNAc is a key regulator of the cellular stress response (Zachara et al. 2004b). While the exact mechanism(s) by which *O*-GlcNAc alters protein function leading to cell survival has not been defined, *O*-GlcNAc is known to regulate many signaling events and pathways, and this review will specifically focus on (1) the phosphoinositide 3-kinase (PI3K)/Akt pathway, (2) heat shock protein (HSP) expression, (3) calcium homeostasis, (4) reactive oxygen species (ROS) generation, (5) mitochondrial dynamics, (6) inflammation, and (7) the interplay with other PTMs. *O*-GlcNAc modulates these aspects of the cellular stress response to protect against ischemia reperfusion injury, trauma hemorrhage, and neurodegeneration. We will also discuss the interplay between *O*-GlcNAc and other post-translational modifications, future directions regarding novel technologies for detecting *O*-GlcNAc, and potential roles for *O*-GlcNAc in other disease mechanisms.

## Biosynthesis of *O*-GlcNAc

Two enzymes modulate protein *O*-GlcNAcylation: UDP-*N*-acetyl-β-D-glucosamine/peptide *N*-acetylglucosaminyltransferase (OGT), which catalyzes the addition of *O*-GlcNAc, and a neutral *N*-acetyl-β-glucosaminidase (*O*-GlcNAcase), which is responsible for the removal of *O*-GlcNAc (Fig. 1). The levels of *O*-GlcNAc appear to be regulated by five main events: (1) expression and activity of OGT, (2) expression and activity of *O*-GlcNAcase, (3) the concentration of UDP-GlcNAc, the sugar nucleotide donor of OGT, (4) the availability of protein substrates, and (5) targeting of the enzymes to their substrates.

### The hexosamine biosynthetic pathway

The synthesis of UDP-GlcNAc occurs via the hexosamine biosynthesis pathway (HBP; Fig. 1). Upon entering cells, glucose is rapidly converted to glucose-6-phosphate by hexokinase (HK), and subsequently to fructose-6-phosphate by glucose-6-phosphate isomerase. The first step of the HBP is rate limiting and comprises of the conversion of fructose-6-phosphate to glucosamine-6-phosphate by glutamine/fructose-6-phosphate amidotransferase (GFAT). Notably, glutamine is required for this step and can be used to alter cellular *O*-GlcNAc levels as well as the levels of other sugar metabolites (Marshall et al. 1991). Several subsequent reactions result in the production of UDP-GlcNAc, the sugar nucleotide donor substrate used by OGT and other glycosyltransferases. The HBP accounts for ~2–5 % of glucose flux in 3T3-L1 adipocytes (Marshall et al. 1991). These and other observations have led some to suggest that *O*-GlcNAc regulates cellular function in a glucose-dependent manner. Although little is known about the regulation of GFAT, it appears to be inhibited by high concentrations of UDP-GlcNAc (Traxinger and Marshall 1991). Interestingly, free radicals promote GFAT activity suggesting one mechanism by which cellular stress and high glucose can lead to upregulation of UDP-GlcNAc pools (Du et al. 2000). Mutations in genes encoding enzymes that significantly lower *O*-GlcNAc levels, such as *emeg32* (glucosamine-6-phosphate acetyltransferase) and *pgm3* (*N*-acetylglucosamine-phosphate mutase), are embryonic lethal highlighting the importance of *O*-GlcNAc and other forms of protein glycosylation that rely on UDP-GlcNAc (Boehmelt et al. 2000a, b; Greig et al. 2007).

### *O*-GlcNAc transferase

Unlike many other glycosyltransferases, OGT is a soluble protein that is predominantly localized to the nucleus, mitochondria, and cytoplasm of all tissues studied thus far (Kreppel et al. 1997; Haltiwanger et al. 1992; Lubas et al. 1997; Love et al. 2003; Hanover et al. 2003). Two functional domains characterize OGT: an N-terminal tetratricopeptide repeat (TPR domain) and a C-terminal catalytic domain belonging to the glycogen phosphorylase superfamily (Kreppel et al. 1997; Lubas et al. 1997; Wrabl and Grishin 2001). Recently, the structure of OGT and the TPR domain have been solved (Lazarus et al. 2011; Martinez-Fleites et al. 2008; Jinek et al. 2004). The TPR domain forms an extended alpha-helix similar to importin-α (Jinek et al. 2004), and is important for mediating protein–protein interactions and enzyme activity (Kreppel and Hart 1999; Lubas and Hanover

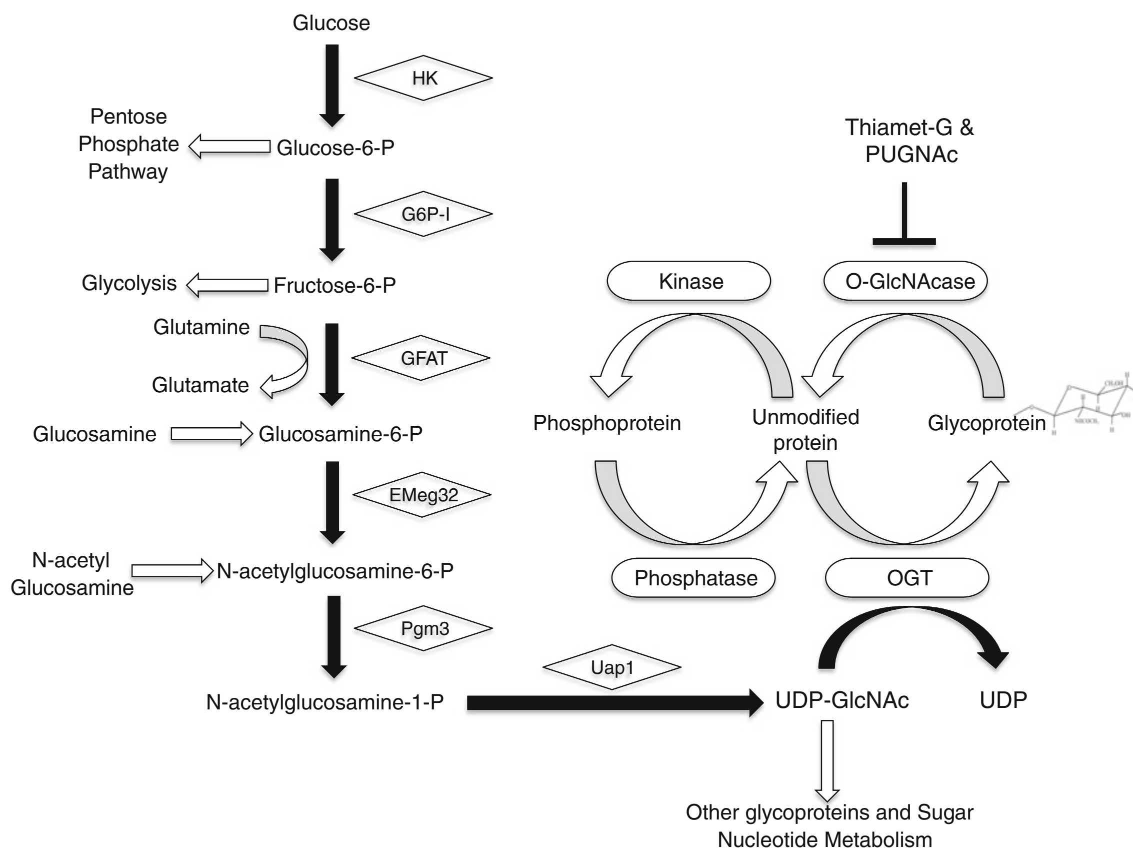


**Fig. 1** The hexosamine biosynthetic pathway provides the donor substrate for *O*-GlcNAcylation and other types of protein glycosylation. Upon entry into the cell, a small percentage of Glucose (2–5 % in adipocytes) is directed into the hexosamine biosynthetic pathway and converted into UDP-GlcNAc via glutamine:fructose-6-phosphate amidotransferase (GFAT), glucosamine-6-phosphate acetyltransferase (*EMeg32*), *N*-acetylglucosamine-phosphate mutase (*pgm3*), and UDP-GlcNAc pyrophosphorylase (*uap1*). OGT catalyzes the addition of the glycoside to yield *O*-GlcNAcylated nuclear, cytoplasmic, and mitochondrial proteins, whereas the enzyme *O*-GlcNAcase catalyzes the removal *O*-GlcNAc from proteins. *O*-GlcNAc-modified proteins are involved in many cellular processes including the cellular stress response. *HK* hexokinase, *G6P-I* glucose 6-phosphate isomerase, *Glc* glucose, *NAc N*-acetyl

2000). The catalytic domain contains two Rossman folds, separated by an intervening sequence. Notably, this linker is missing in the bacterial homologs of OGT that do not appear to modify protein substrates (Lazarus et al. 2011).

The gene for OGT maps to Xq13 on the mammalian X chromosome and encodes three well-characterized variants: short OGT, mitochondrial OGT (mOGT), and nuclear/cytoplasmic OGT (Kreppel et al. 1997; Lubas et al. 1997; Love et al. 2003; Hanover et al. 2003). mOGT has an alternative mitochondrial targeting sequence on its *N*-terminus. Interestingly, overexpression of the mOGT appears to induce apoptosis (Shin et al. 2011). OGT is essential for embryonic stem cell viability and somatic cell function (O'Donnell et al. 2004; Shafi et al. 2000). Deletion of OGT in *Arabidopsis* (Jacobsen et al. 1996), *Drosophila* (Sinclair et al. 2009; Gambetta et al. 2009), and mice is lethal (Shafi et al. 2000), and leads to dauer phenotypes in *Caenorhabditis elegans* (Hanover et al. 2005), suggesting that *O*-GlcNAc is essential for cellular homeostasis.

The mechanism of OGT substrate specificity is not fully understood; however, deletions in the TPR domain can affect the ability of OGT to *O*-GlcNAcylate protein and peptide substrates. It is generally thought that protein–protein interactions of OGT form a series of OGT complexes with different substrate specificities (Kreppel and Hart 1999). In support of this hypothesis, OGT has been found to associate with numerous proteins (Cheung et al. 2008) and is targeted to its substrates by p38 mitogen-activated protein kinase (MAPK) under conditions of nutritional deprivation (Cheung and Hart 2008). Moreover, OGT can bind phosphatidylinositol (3,4,5)-trisphosphate, which targets OGT to the plasma membrane during insulin signaling (Yang et al. 2008). These observations may also explain why there is no exact consensus sequence or motif for the addition of *O*-GlcNAc, although a recent paper has

developed a neural network for predicting *O*-GlcNAc modification sites (Wang et al. 2011).

The activity of OGT appears to be regulated by substrate targeting (discussed above), the levels of UDP and UDP-GlcNAc, and potentially by other post-translational modifications. OGT is both tyrosine phosphorylated (Kreppel et al. 1997; Kreppel and Hart 1999) and *O*-GlcNAc-modified (Kreppel and Hart 1999; Lubas and Hanover 2000), although the consequence of these modifications has not been defined. Interestingly, OGT is phosphorylated and activated by active $Ca^{2+}$/calmodulin-dependent kinase (CaMK) IV (Song et al. 2008). Recently, it has been demonstrated that OGT is *S*-nitrosylated in resting cells and its denitrosylation following induction of the innate immune response results in increased catalytic activity (Ryu and Do 2011). While UDP-GlcNAc levels may alter OGT activity by providing more substrate, there is also evidence that UDP-GlcNAc alters the substrate specificity of OGT. Thus, OGT may glycosylate one group of proteins at low concentrations of UDP-GlcNAc, but at higher concentrations may target a completely different panel of proteins (Kreppel and Hart 1999).

Little is known about how stress affects the activity of OGT, but stress alters glucose uptake and the activity of the HBP (discussed above) presumably augmenting UDP-GlcNAc pools. During heat stress, the activity of OGT is upregulated threefold by currently unknown mechanisms (Zachara et al. 2004b) and OGT translocates to the nucleus (Kazemi et al. 2010). Suggesting yet another mechanism by which stress alters *O*-GlcNAc levels, stressors such as sodium chloride, ethanol, and arsenite appear to alter the expression of OGT (Zachara et al. 2004b). During glucose deprivation, the expression of OGT is partially dependent on AMP-activated protein kinase, and OGT is targeted to its substrates by p38 MAPK (Cheung and Hart 2008). This results in a dramatic increase in *O*-GlcNAcylation of the neuronal protein neurofilament H, suggesting a possible mechanism by which defective glucose metabolism in the brain may directly contribute to the loss of axonal structure and stability (Cheung and Hart 2008).

Several assays have been used to detect OGT activity. The most common involves the addition of a tritiated UDP-GlcNAc ($^3$H-UDP-GlcNAc) to a peptide substrate (Haltiwanger et al. 1997). Recently, an effective fluorescence-based substrate analogue displacement assay was developed to assess the activity of OGT (Gross et al. 2005). The spatio-temporal dynamics of *O*-GlcNAc have also been characterized using a series of genetically based *O*-GlcNAc FRET (Förster resonance energy transfer) sensors targeted to specific subcellular compartments (Carrillo et al. 2011; Carrillo et al. 2006). For example, a study utilizing the *O*-GlcNAc FRET sensor found that during serum-stimulated signal transduction rapid increases in *O*-GlcNAcylation were observed at the plasma membrane and in the nucleus, with a concomitant decrease in *O*-GlcNAcylation in the cytoplasm (Carrillo et al. 2011).

### *O*-GlcNAcase

*O*-GlcNAcase is approximately 103 kDa in size and catalyzes the removal of *O*-GlcNAc from proteins. *O*-GlcNAcase appears to be predominantly cytoplasmic and like OGT is ubiquitously expressed (Dong and Hart 1994). The gene-encoding *O*-GlcNAcase was identified as meningioma-expressed antigen 5 (*mgea5*) and characterized as a hyaluronidase (Heckel et al. 1998). However, further analysis indicated that the product of the *mgea5* gene was active at neutral pH, localized to the cytoplasm, and would remove *O*-GlcNAc from peptides suggesting that it was the *O*-GlcNAcase biochemically characterized by Dong and Hart (Comtesse et al. 2001; Gao et al. 2001). *O*-GlcNAcase exists as two isoforms: the full-length isoform with a histone acetyltransferase (HAT) domain in its C-terminus and a short isoform lacking the C-terminal HAT domain.

*O*-GlcNAcase is serine phosphorylated and *O*-GlcNAcylated, although the functional significance of these modifications has yet to be elucidated (Gao et al. 2001; Wells et al. 2002a). Like OGT, *O*-GlcNAcase from bovine brain appears to exist in complexes with other proteins including HSP110, HSC70, amphiphysin, and dihydropyriminidase-related protein-2. Finally, *O*-GlcNAcase is cleaved by caspase-3 during apoptosis (Wells et al. 2002a). Notably, the cleavage products appear to remain associated with each other and the activity of *O*-GlcNAcase is unaffected (Butkinaree et al. 2008). Thus far, little is known about the regulation of *O*-GlcNAcase during the cellular stress response.

While little is known about the regulation of *O*-GlcNAcase, the activity of *O*-GlcNAcase can be conveniently assayed in vitro with a synthetic substrate *p*-nitrophenol-β-GlcNAc (*p*NP-β-GlcNAc) (Dong and Hart 1994). However, care must be taken to exclude the activities of the lysosomal hexosaminidases (HexA and HexB) in total cell/tissue lysate. Recent studies have shown that fluorogenic substrates exhibit higher sensitivity and can be used to study both *O*-GlcNAcase isoforms. Both fluorescein di-β-GlcNAc (FDGlcNAc; Kim et al. 2006a) and 4-methylumbelliferyl-2-*N*-acetyl-2-deoxy-β-D-glucopyranoside have been used successfully (Macauley et al. 2005); however, the latter is tenfold less sensitive than FDGlcNAc (Kim et al. 2006a).

## *O*-GlcNAc and the cellular stress response

In response to injury, cells have the ability to modify their metabolic, transcriptional, translational, and signaling pathways to promote survival, collectively this is known as the "cellular stress response" (Lindquist 1986; Nollen and

Morimoto 2002). Recent data suggests that *O*-GlcNAc is one component of the cellular stress response that is relevant to a variety of models of injury in several cell and tissue types. *O*-GlcNAc levels become elevated in response to numerous forms of cell stress and tissue injury including: heat stress (Sohn et al. 2004; Zachara et al. 2004b), oxidative stress (Jones et al. 2008; Zachara et al. 2004b), ethanolic stress (Zachara et al. 2004b; Ngoh et al. 2009b), genotoxic stress (doxorubicin, belocin, and UVB irradiation; Zachara et al. 2004b, 2011a; Love et al. 2010), reductive stress (iodoacetamide; Zachara et al. 2004b), ER stress (dithiothreitol and tunicamycin; Zachara et al. 2004b; Ngoh et al. 2009b), hypoxia reoxygenation (Ngoh et al. 2009a; Ngoh et al. 2008; Ngoh et al. 2011), osmotic stress (NaCl, sorbitol, and sucrose; Zachara et al. 2004b; Zou et al. 2007), ATP depletion (sodium arsenite; Zachara et al. 2004b), ischemia reperfusion injury (Champattanachai et al. 2007, 2008; Fulop et al. 2007a, b; Hwang et al. 2010; Jones et al. 2008; Laczy et al. 2010; Liu et al. 2006, 2007; Nagy et al. 2006; Ngoh et al. 2008, 2009a, b, 2011; Pang et al. 2002; Zou et al. 2009), and trauma hemorrhage (Not et al. 2007, 2010; Yang et al. 2006a; Zou et al. 2007, 2009). This response occurs in primary and transformed cells, as well as in tissues in vivo and ex vivo. Suggesting that the addition of *O*-GlcNAc to proteins is part of a prosurvival signaling program, elevating *O*-GlcNAc levels prior (Zachara et al. 2004b; Chatham et al. 2008; Ngoh et al. 2008) or immediately following (Liu et al. 2007) cellular injury dramatically improves survival. Conversely, lowering *O*-GlcNAc levels sensitizes cells and tissues to injury (Zachara et al. 2004b; Ngoh et al. 2009a; Ngoh et al. 2008; Ngoh et al. 2011).

Modulating the levels of *O*-GlcNAc in a manner consistent with improved survival regulates a number of biological pathways. Some examples include: (1) increased protein solubility during heat stress (Cheung and Hart 2008; Lim and Chang 2006) or Alzheimer's disease (Yuzwa et al. 2012); (2) reduced calcium overload (Liu et al. 2006, 2007); (3) decreased calpain activation (Liu et al. 2007); (4) altered p38 MAPK activation in response to ischemia reperfusion injury (Fulop et al. 2007b; Cheung and Hart 2008); (5) modulation of proinflammatory cytokines (Huang et al. 2007; Zou et al. 2009); (6) reduced mitochondrial permeability transition pore (mPTP) formation (Jones et al. 2008); (7) increased B-cell lymphoma 2 (Bcl-2) expression and translocation (Champattanachai et al. 2008); and (8) regulation of the expression of a subset of molecular chaperones (Zachara et al. 2004b; Kazemi et al. 2010; Sohn et al. 2004).

Numerous reports have suggested that *O*-GlcNAc regulates many processes in the heart and vasculature (Champattanachai et al. 2008; Chatham and Marchase 2010; Fulop et al. 2007a; Liu et al. 2007; Ngoh et al. 2011) in models of ischemia–reperfusion (I/R) injury. For example, *O*-GlcNAc appears to regulate many of the hallmarks of I/R injury such as calcium overload, oxidative damage, ER stress, and mitochondrial aberrations (Chatham and Marchase 2010). Elevating *O*-GlcNAc levels with glucosamine, PUGNAc (*O*-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-*N*-phenylcarbamate), or thiamet G (TMG, 2-ethylamino-3aR, 6S, 7R, 7aR-tetrahydro-5R-hydroxymethyl-5H-pyrano[3, 2-d]thiazole-6, 7-diol) leads to improved function and reduced tissue death in both ex vivo and in vivo models of I/R injury in the heart (Liu et al. 2007; Liu et al. 2006; Fulop et al. 2007b; Jones et al. 2008). Collectively, these data suggest that the stress-induced elevation of the *O*-GlcNAc modification is a pro-survival response of cells and tissues (Champattanachai et al. 2007, 2008; Hwang et al. 2010).

## The PI3K/Akt pathway

The role of *O*-GlcNAc in the PI3K/Akt pathway has been extensively studied in the context of nutrient sensing, insulin resistance, and type II diabetes, and readers are directed to pertinent reviews on this topic for more extensive details (Akimoto et al. 2005; Slawson et al. 2010; Buse 2006; Hart et al. 2011). In contrast to these data, the interplay between *O*-GlcNAc and Akt signaling has been observed to inhibit apoptosis in a number of models including murine pancreatic β-cells (Kang et al. 2008), mouse embryonic fibroblasts (Kazemi et al. 2010), and murine liver (Ku et al. 2010), as discussed below.

Recently, it was demonstrated that *O*-GlcNAcylation of cytokeratin 18 (K18) is a regulator of Akt1 phosphorylation, which promotes downstream anti-apoptotic and cytoprotective signaling (Ku et al. 2010). Mouse mutants expressing human K18 S30/31/49A mutations, which cannot be glycosylated (K18–Gly$^{-}$), were more susceptible to liver and pancreatic injury induced by treatment with either streptozotocin (STZ) or combined *O*-GlcNAcase inhibition and Fas administration (Ku et al. 2010). It is thought that the prosurvival mechanism is *O*-GlcNAcylation of K18, which leads to its interaction with Akt1 and keratin 8 (K8) ultimately promoting the phosphorylation of Akt1$^{Thr308}$. Akt is activated by phosphorylation at Thr308 and then phosphorylates substrates such as forkhead box protein (Foxo) and glycogen synthase kinase 3β (GSK3β), thereby enhancing cell survival (Ku et al. 2010; Fig. 2). It is also important to note that Akt itself is dynamically modified by *O*-GlcNAc at Ser473 (Gandy et al. 2006; Kang et al. 2008). While hyper-*O*-GlcNAcylation of Akt has deleterious effects in hepatic cell and tissue models of euglycemia (Soesanto et al. 2008) and in pancreatic β-cells under hyperglycemic conditions (Kang et al. 2008), it is unclear if Akt is glycosylated in models of tissue injury.

## Heat shock protein expression

One mechanism by which *O*-GlcNAc is thought to regulate cell survival is by modulating the expression of HSPs, the

sentinels of the cellular stress response. Initial reports demonstrated that elevating O-GlcNAc levels augments the heat-induced expression of HSP72 and HSP40 (Zachara et al. 2004b; Sohn et al. 2004). Conversely, in cells in which O-GlcNAc levels had been lowered, by inhibition of the HBP or deletion of OGT, the expression of HSPs was suppressed (Zachara et al. 2004b; Kazemi et al. 2010). Using a real-time polymerase chain reaction (RT-PCR) array of 84 chaperones, Kazemi et al. demonstrated that the expression of 18 molecular chaperones was inhibited when O-GlcNAc levels were lowered by deletion of OGT (Kazemi et al. 2010). These chaperones included HSP72, HSP40 J-domain containing proteins (Dnaja1, Dnaja2, Dnaja3, Dnaja4, Dnajb1, and Dnajb7), the chaperone regulators Bag2 and Bag3, α-crystallin (*Cryab*, *Hspb8*), HSP25 (*Hspb1*), HSP110, HSP90α, and HSP60 (Kazemi et al. 2010). Interestingly, the expression of three proteins was upregulated (Dnajb8, Dnajc5b, and Dnajc5g; Kazemi et al. 2010).

The expression of HSPs is regulated by two key transcription factors: heat shock factor 1 (HSF-1) and Sp1. Several studies have demonstrated that O-GlcNAc regulates both of these transcription factors, directly and indirectly, leading to the regulation of chaperone expression. One example by which O-GlcNAc is thought to regulate HSP expression is by promoting the phosphorylation and inactivation of the kinase GSK3β at Ser9 (Kazemi et al. 2010). In cells with reduced O-GlcNAcylation, increased inhibitory phosphorylation of HSF-1 at Ser303 is observed (Kazemi et al. 2010). Conversely, elevating O-GlcNAc levels suppresses the phosphorylation of HSF-1 at Ser303, promoting both its activation and downstream HSP expression (Kazemi et al. 2010). Recent reports suggest that GSK3β may suppress HSF-1 activity by phosphorylating Ser/Thr residues independent of Ser303 (Batista-Nascimento et al. 2011). Nonetheless, consistent with a model in which increased GSK3β activity suppresses HSF-1 activation in the OGT null, inhibition of GSK3β rescues HSP72 expression in these cells (Kazemi et al. 2010). Further supporting this model, a reduction of Ser9 inhibitory phosphorylation of nuclear GSK3β was observed in cells with lower O-GlcNAc levels (Kazemi et al. 2010). Notably, unlike the study by Gandy et al. (2006), the stress-induced

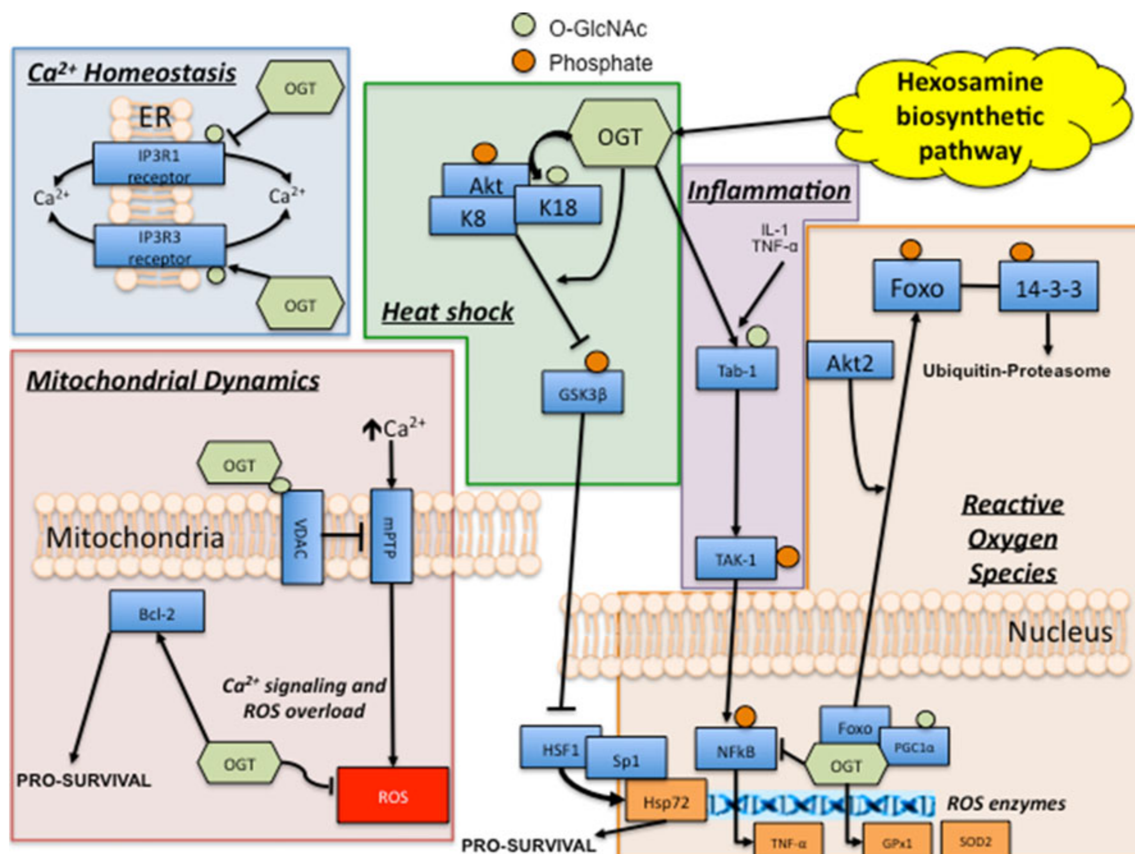


**Fig. 2** O-GlcNAc is involved in the cellular stress response. Glucose and glucosamine can enter cells via glucose transporters and are directed towards the HBP for conversion to UDP-GlcNAc. O-GlcNAcylation of key proteins is essential for maintaining cellular homeostasis and promoting survival during stress and injury. Key survival pathways modulated by O-GlcNAc include: (1) the PI3K/Akt pathway, (2) heat shock protein expression, (3) calcium homeostasis, (4) reactive oxygen species regulation, (5) mitochondrial dynamics, (6) inflammation, and (7) PTM interplay. Note that proteins are shown as *light blue squares*, O-GlcNAc as *green circles*, and phosphates as *orange circles*. *Arrows* denote activation, whereas *blunt ends* denote inhibition. *OGA* O-GlcNAcase, *OGT* O-GlcNAc transferase

phosphorylation of Akt was unaffected in this model, demonstrating that *O*-GlcNAc can regulate numerous points upstream of GSK3β.

Glutamine has been demonstrated to protect cells and tissues from diverse forms of cellular stress and tissue injury (Wischmeyer et al. 2001). As glutamine is utilized by the HBP in the generation of UDP-GlcNAc (Fig. 1), it has been postulated that glutamine may work in an *O*-GlcNAc-dependent manner. In support of this hypothesis, in vivo treatment with glutamine has been shown to elevate *O*-GlcNAc levels (Singleton and Wischmeyer 2008; Hamiel et al. 2009). Moreover, the ability of glutamine to induce HSP70 is attenuated in cells in which *O*-GlcNAc levels have been lowered (Hamiel et al. 2009). In heat-stressed cells and a septic mouse model, glutamine treatment appears to promote the nuclear accumulation of HSF-1 and Sp1 in an *O*-GlcNAc-dependent manner (Singleton and Wischmeyer 2008; Hamiel et al. 2009). Although the mechanisms underlying the above observations remain unclear, it may be explained in part by the observations of Lim and Chang who demonstrated that increased levels of *O*-GlcNAc are associated with a more soluble pool of Sp1 during thermal stress (Lim and Chang 2006). This may represent altered localization of Sp1, or that Sp1 exhibits increased thermal stability when it is *O*-GlcNAc-modified (Lim and Chang 2006) similar to Tau (Yuzwa et al. 2012). Together, the data discussed above suggest that *O*-GlcNAc can regulate the expression of HSPs by regulating both HSF-1 and Sp1.

## Calcium homeostasis

Another mechanism by which *O*-GlcNAc may promote cell survival is by regulating calcium handling. Angiotensin II, an $IP_3$-generating agonist, is a potent stimulator of cardiomyocyte hypertrophy that can elevate cytoplasmic $Ca^{2+}$ during vasoconstriction, increased blood pressure, and hemodynamic stress. A number of studies have demonstrated that angiotensin II treatment leads to an increase in basal $Ca^{2+}$ levels in adult and neonatal cardiomyocytes (Kem et al. 1991; Shao et al. 1998; Hunton et al. 2004), with sustained exposure affecting numerous cellular pathways (Meldrum et al. 1996; Liu et al. 2004b; Zhang et al. 2004) and eventually resulting in cell death (Goldenberg et al. 2001; Kajstura et al. 1997). Elevating UDP-GlcNAc and *O*-GlcNAc levels through treatment with glucosamine or the *O*-GlcNAcase inhibitor PUGNAc attenuates angiotensin II-induced CCE during I/R injury (Nagy et al. 2006).

Further suggesting that regulation of calcium homeostasis in the heart is modulated by HBP flux and altered *O*-GlcNAc levels, *O*-GlcNAc appears to regulate a number of pathways downstream of calcium in models of ischemic injury. Consistent with decreased calcium influx, CaMKII phosphorylation is reduced, as are downstream events such as calpain proteolysis of structural proteins such as α-fodrin (Liu et al. 2007). Moreover, augmented *O*-GlcNAc signaling, via inhibition of *O*-GlcNAcase or overexpression of OGT, has been shown to attenuate oxidative stress and calcium overload in cardiomyocytes (Ngoh et al. 2011). Although the mechanism by which this occurs is unknown, it is accompanied by a reduction in ROS release and reduced formation of the mPTP (Ngoh et al. 2011).

While the mechanism by which *O*-GlcNAc regulates calcium handling in the ischemic heart has not been parsed out, recent studies have demonstrated that several $IP_3$ receptors are *O*-GlcNAc-modified suggesting a potential molecular mechanism by which *O*-GlcNAc can modulate $Ca^{2+}$ flux. The $IP_3$ type I receptor is the principle intracellular calcium release channel in many cell types including neurons and it is regulated by calcium, ATP, and *O*-GlcNAc. *O*-GlcNAcylation of the $IP_3$ type I receptor decreased its activity, while the removal of *O*-GlcNAc restored its function (Rengifo et al. 2007). Following this study, it was demonstrated that the $IP_3$ type III receptor is functionally regulated by *O*-GlcNAc, except that *O*-GlcNAcylation enhanced its activity while *O*-GlcNAc removal negated its function (Bimboese et al. 2011). Thus, *O*-GlcNAcylation of $IP_3$ type I and III receptors regulates their function(s) in controlling $Ca^{2+}$ flux and ultimately attenuating $Ca^{2+}$-dependent apoptosis (Rengifo et al. 2007).

## Reactive oxygen species

ROS participate in many intracellular signaling pathways that can ultimately lead to alterations in gene transcription, protein synthesis, and overall cellular function. Stressors such as I/R injury, which induces mitochondrial $Ca^{2+}$ overload (discussed previously) and ROS generation, favor the formation of the mPTP (discussed in the next section) and ultimately lead to cell death. Augmenting *O*-GlcNAc levels by adenoviral OGT overexpression or PUGNAc treatment has been shown to attenuate hypoxia and oxidative stress-induced ROS production. Conversely, suppression of *O*-GlcNAcylation by adenoviral *O*-GlcNAcase overexpression exacerbated ROS levels (Ngoh et al. 2011). One mechanism by which *O*-GlcNAc is thought to suppress ROS levels is by promoting the expression of antioxidant enzymes by stimulating the activity of Foxo1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α; Housley et al. 2009). For example, it was demonstrated that induction of PGC1α in response to ROS leads to its *O*-GlcNAcylation and activation by OGT. Subsequently, the OGT-PGC1α complex is targeted to and hyper-*O*-GlcNAcylates Foxo1 (Fig. 2) to promote increased transcription of ROS responsive enzymes, such as glutathione peroxidase 1 and superoxide dismutase 2, which protect against oxidative damage (St-Pierre et al. 2006; Housley et al. 2008, 2009). While the above studies were performed in

hepatocytes, subsequent studies in a model of myocardial damage demonstrated that catalase mRNA levels were augmented above baseline following inhibition of *O*-GlcNAcase (PUGNAc), while overexpression of *O*-GlcNAcase reduced baseline catalase mRNA levels (Ngoh et al. 2011). Notably, in contrast to the studies discussed here, *O*-GlcNAc is thought to promote ROS generation in models of hyperglycemia and glucose toxicity (Goldberg et al. 2011), although the molecular basis for this paradox remains to be defined (Lima et al. 2012).

Mitochondrial dynamics

The mitochondrion is the powerhouse of the cell and is responsible for ATP production, maintenance of cellular byproducts, and overall regulation of cellular homeostasis. Altered mitochondrial dynamics leads to activation of the mitochondrial death pathway, which ultimately results in formation of the mPTP. Pore formation is activated by calcium surplus and ROS generation (previously discussed, Fig. 2), and is responsible for nonspecifically allowing small molecules (<1.5 kDa) to enter and exit the mitochondrial matrix (Crow et al. 2004). One postulated mechanism for the cytoprotective nature of *O*-GlcNAc is through the regulation of mitochondrial function (Ngoh et al. 2008; 2010, 2011; Jones et al. 2008) by reducing calcium overload, dampening ROS production, and preventing mPTP formation (Ngoh et al. 2008, 2011; Jones et al. 2008). It has been suggested that *O*-GlcNAc may regulate the mPTP directly by modifying and modulating the voltage-dependent anion channel (VDAC; Ngoh et al. 2008; Jones et al. 2008; Fig. 2). For example, deletion of OGT led to reduced *O*-GlcNAcylation of VDAC, sensitizing cells to mitochondrial membrane potential collapse and mPTP formation (Ngoh et al. 2008; Jones et al. 2008). Notably, formation of the mPTP is also promoted by active GSK3β (Juhaszova et al. 2004; Miura and Miki 2009), which is inhibited in some models by stress-induced *O*-GlcNAcylation (Kazemi et al. 2010; Ku et al. 2010).

In addition to modulating VDAC, *O*-GlcNAc has been demonstrated to modulate Bcl-2 and the electron transport chain. Mitochondrial localization of Bcl-2 has been postulated to inhibit mPTP opening (Tsujimoto et al. 2006; Tsujimoto 2003), protect against a loss in mitochondrial membrane potential (Tsujimoto et al. 2006; Tsujimoto 2003; Murphy et al. 2005), prevent cytochrome c release (Tsujimoto et al. 2006; Tsujimoto 2003; Murphy et al. 2005), and attenuate mitochondria-mediated apoptosis (Tsujimoto et al. 2006; Tsujimoto 2003; Murphy et al. 2005). One study demonstrated that elevation of *O*-GlcNAc levels in hydrogen peroxide-treated cells, an ex vivo model of ischemia–reperfusion injury, led to increased mitochondrial Bcl-2 translocation (Champattanachai et al. 2008). There is some debate surrounding this finding, as PUGNAc did not appear to alter I/R-induced increases in mitochondrial Bcl-2 levels (Champattanachai et al. 2008). Finally, elevating levels of glucose in neonatal cardiomyocytes resulted in augmented *O*-GlcNAc levels and subsequently reduced function of mitochondrial electron transport complexes I, III, and IV (Hu et al. 2005), however, its role in stress has not been studied yet. Interestingly, overexpressing *O*-GlcNAcase to reduce *O*-GlcNAc levels can reverse the effects of reduced mitochondrial function when glucose levels are elevated (Hu et al. 2005).

Inflammation

Trauma hemorrhage injury, like infection, induces an acute inflammatory response necessary for tissue repair. However, a hyperinflammatory response can compromise healthy tissue, leading to cell and tissue death (Nathan 2002; Jarrar et al. 1999). The inflammatory response is driven by secreted tissue-derived cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6), that are responsible for inflammation in the damaged area. Recent studies have shown that augmenting *O*-GlcNAc levels prior to and following trauma hemorrhage injury improves survival, and this protective phenotype is associated with reduced expression of TNF-α and IL-6 and improved recovery of multiple tissues and blood pressure (Chatham et al. 2008; Not et al. 2007, 2010; Yang et al. 2006a).

*O*-GlcNAc has also been demonstrated to suppress acute inflammation in other models of injury presumably promoting cell survival (Xing et al. 2008; Pathak et al. 2012; Not et al. 2007). For example, in a rat model of arterial injury, elevating *O*-GlcNAc levels by administration of glucosamine or PUGNAc-attenuated inflammatory mediator expression, leukocyte infiltration, and neointima formation (Xing et al. 2008). Reduced inflammation was attributed to decreased TNF-α-induced phosphorylation of the nuclear factor κB (NFκB) p65 subunit and subsequent inhibition of NFκB transcriptional activation and signaling (Xing et al. 2008). Additionally, augmenting *O*-GlcNAc levels reduced the expression of a number of chemokines (cytokine-induced neutrophil chemoattractant-2β and monocyte chemotactic protein-1) and adhesion molecules (vascular cell adhesion molecule-1 and P-selectin; Xing et al. 2008).

Proinflammatory cytokines, such as TNF-α and interleukin 1 (IL-1), activate downstream signals in a manner mediated by TGF-β-activated kinase I (TAK1), also known as mitogen-activated protein kinase kinase kinase 7 (Sakurai et al. 2000; Wang et al. 2001). A recent study showed that *O*-GlcNAcylation of TAK1-binding protein (TAB1) modulates TAK1-mediated cytokine release when induced by IL-1 and osmotic stress (Pathak et al. 2012). Utilizing wild-type and *O*-GlcNAc-deficient

mutant TAB1 (Ser395) in TAB1$^{-/-}$ mouse embryonic fibroblasts, this study demonstrated that the *O*-GlcNAc modification of TAB1 at Ser395 is essential for full TAK1 autophosphorylation at Thr187 and activation upon stress, as well as the subsequent downstream activation of NFκB and production of IL-6 and TNF-α (Pathak et al. 2012).

Interplay between *O*-GlcNAc and other PTMs

One mechanism by which *O*-GlcNAc is thought to regulate proteins is by directly or indirectly regulating other post-translational modifications including phosphorylation, nitrosylation, and ubiquitination.

*Phosphorylation*

Phosphate ($PO_4^{3-}$) and GlcNAc ($C_8H_{15}NO_6$) moieties are both covalently and reversibly added in an *O*-linkage to Ser/Thr residues in a manner known as phosphorylation and *O*-GlcNAcylation respectively. There is extensive crosstalk between these two modifications and this dynamic interplay is implicated in regulating numerous signaling networks (Wang et al. 2007, 2008, 2010b; Hart et al. 2011). For a subset of proteins (Cheng and Hart 2001; Kelly et al. 1993; Comer and Hart 2001; Du et al. 2001), *O*-GlcNAc and phosphorylation appear to compete for the same Ser/Thr residue, such as on the proto-oncogene c-myc (Chou et al. 1995). Alternatively, for numerous proteins *O*-GlcNAc can sterically hinder nearby phosphorylation sites (Dias et al. 2009; Yang et al. 2006b; Housley et al. 2008; Wang et al. 2010b; Tarrant et al. 2012), for example in the CK2 protein where *O*-GlcNAcylation of Ser347 prevents its phosphorylation at nearby amino acid Thr344 (Tarrant et al. 2012). The idea that these modifications are reciprocal comes from data demonstrating that phosphorylation of subsets of proteins is suppressed when global *O*-GlcNAc levels are elevated (Griffith and Schmitz 1999; Lefebvre et al. 2001; Wang et al. 2008). One elegant study demonstrated that in vitro *O*-GlcNAcylation of the C-terminal domain of RNA polymerase II blocked its phosphorylation and vice versa (Comer and Hart 2001). Suggesting that this interplay also alters protein function in vivo, additional studies have demonstrated that tau is hyperphosphorylated in a brain OGT knockout (O'Donnell et al. 2004). However, readers should be cautioned as the reciprocal relationship between *O*-GlcNAc and phosphorylation exists only for a subset of proteins; while for others, there is no direct association and proteins can be both phosphorylated and *O*-GlcNAcylated simultaneously, such as insulin receptor substrate-1 (Ball et al. 2006).

In addition to cross-talk associated with site occupancy, there is cross talk between the enzymes responsible for *O*-GlcNAcylation and phosphorylation. OGT and *O*-GlcNAcase are known to associate with and modify kinase and phosphatase enzymes (Khidekel et al. 2004; Wang et al. 2010b; Wells et al. 2004; Slawson et al. 2008; Dias et al. 2009, 2012; Cheung et al. 2008). For example, CaMKIV is inhibited by *O*-GlcNAc modification within its ATP-binding region, and de-*O*-GlcNAcylation and subsequent phosphorylation is required for CaMKIV activation (Dias et al. 2009). Several proteomic analyses have been performed to further delineate the interplay between *O*-GlcNAc and phosphorylation in cell cycle progression. One of these studies demonstrated that the kinase Aurora B, which regulates mitotic progression, forms a transient complex with OGT and *O*-GlcNAcase in M phase of the cell cycle and colocalizes with OGT to midbodies during telophase and cytokinesis (Slawson et al. 2008). Inhibition of Aurora B leads to diminished midbody localization of OGT and an increase in global *O*-GlcNAc levels (Slawson et al. 2008). This study also demonstrated that vimentin, a substrate for Aurora B and OGT during mitosis, exhibited alterations in its *O*-GlcNAcylation and phosphorylation patterns upon modulation of OGT or *O*-GlcNAcase activity (Slawson et al. 2008). A second study identified 141 previously unreported *O*-GlcNAc sites on proteins that are involved in spindle assembly and cytokinesis, many of which are the same site as or are in close proximity to sites of phosphorylation (Wang et al. 2010b). Finally, modulation of *O*-GlcNAc levels was found to alter the phosphorylation status of proteins localized to the mitotic spindle and midbody, for example upon OGT overexpression, cyclin-dependent kinase 1 becomes hyperphosphorylated thereby reducing its activity and subsequently decreasing the phosphorylation status of its target proteins (Wang et al. 2010b).

Endothelial nitric oxide synthase (eNOS) generates nitric oxide and stimulates vasodilation by inhibiting smooth muscle contraction and platelet aggregation, with these functions reported to be impaired in diabetes-associated erectile dysfunction. One study demonstrated that in rats treated with alloxan to induce diabetes, eNOS was hyper-*O*-GlcNAcylated and hypo-phosphorylated at Ser1177 (Musicki et al. 2005). Phosphorylation of eNOS at this site is mediated by Akt phosphorylation and this study demonstrated that the phosphorylation of both Akt and eNOS was decreased in the diabetic model, implicating that *O*-GlcNAcylation at Ser1177 is one potential mechanism for eNOS inactivation and thereby diminished vasodilation (Musicki et al. 2005).

*Other PTM roles*

There is emerging evidence for the interplay of *O*-GlcNAc with other post-translational modifications such as protein ubiquitination and nitrosylation, although it is important to note that the exact mechanisms by which these modifications interact are still being elucidated.

A growing body of evidence suggests that *O*-GlcNAc may be a regulator of protein degradation. This includes data demonstrating that enhanced *O*-GlcNAcylation is associated with an extended half-life of proteins, such as with Sp1 (Han and Kudlow 1997) and the murine β-estrogen receptor (Jiang and Hart 1997; Cheng and Hart 2001). At least three mechanisms have been postulated to underlie these observations. Firstly, *O*-GlcNAc can block the phosphorylation of Pro-Glu-Ser-Thr domains in the murine β-estrogen receptor thereby preventing its degradation (Cheng et al. 2000; Cheng and Hart 2001). Secondly, as discussed below, data suggests that *O*-GlcNAc may regulate ubiquitination. Thirdly, *O*-GlcNAc is implicated in regulating the proteasome directly, as highlighted by studies showing that that numerous proteins in the proteasome are *O*-GlcNAc-modified and that enhanced *O*-GlcNAcylation may inhibit proteasome function (Zhang et al. 2003; Sumegi et al. 2003).

One study demonstrated that both ubiquitination and *O*-GlcNAcylation increase upon heat-shock treatment and that these PTMs can occur on some proteins concomitantly (Guinez et al. 2008). *O*-GlcNAc-modified proteins were not observed to be stabilized following proteasome inhibition, and ubiquitination could be enhanced or reduced by increasing or decreasing *O*-GlcNAc levels respectively (Guinez et al. 2008). It was also speculated that an E1 ubiquitin-activating enzyme interacts with Hsp70 only in its *O*-GlcNAcylated form (Guinez et al. 2008). An agonistic relationship between these two modifications is suggested such that *O*-GlcNAc may alter the activity of E1 enzymes to modulate stress-induced ubiquitination (Shrikhande et al. 2010; Shimura et al. 2001; Fujiki et al. 2011). It has also been shown that *O*-GlcNAcylation of a protein may facilitate its subsequent ubiquitination. For example, *O*-GlcNAcylation of histone H2B at Ser112 facilitates monoubiquitination at Lys120 to regulate transcription (Fujiki et al. 2011).

*S*-nitrosylation is a cysteine modification that is found and maintained on OGT in resting cells and *S*-nitrosylated OGT has greatly reduced catalytic activity compared to the native form of the protein (Ryu and Do 2011). OGT becomes denitrosylated in macrophages treated with lipopolysacharides to trigger an innate immune response, which leads to increased catalytic activity and hyper-*O*-GlcNAcylation of proteins (Haberhausen et al. 1995). While other PTMs such as acetylation, methylation, sumoylation, and many others have pivotal roles in regulating protein function and signal transduction, the interplay of these modifications with *O*-GlcNAcylation has yet to be defined.

## Molecular mechanisms of disease

There are many facets of the cellular stress response whose misregulation can contribute to neurodegenerative disease pathophysiology, and *O*-GlcNAc is implicated in the etiology of some of these diseases. Notably, the OGT gene is on the X chromosome at position Xq13.1, the same locus associated with dystonia–parkinsonism syndrome (Haberhausen et al. 1995), and *O*-GlcNAcase maps to chromosome 10 near the locus associated with late-onset Alzheimer's disease (10q24.1–q24.3; Bertram et al. 2000).

### Alzheimer's disease

Alzheimer's disease (AD) is a form of dementia that is characterized by reduced brain function and cognitive deficits, where both genetic and environmental factors play a role in pathogenesis (Hardy and Allsop 1991). AD phenotypes are often the result of unfolded or aggregated proteins, aberrant signaling, and oxidative stress. While there are many mechanisms by which these events are regulated, the most well-studied proteins involved in AD are amyloid β precursor protein (APP), amyloid β (Aβ), and tau (Hardy and Allsop 1991). Misregulation of APP, Aβ plaque formation, tau phosphorylation, and tangle formation dominate the cascade of events leading to AD and ultimately to neuronal death (Dong et al. 2004).

AD transgenic mouse models are characterized by Aβ plaque formation and this leads to decreased cell proliferation and defective contextual memory (Kang et al. 2007). APP, the precursor protein to Aβ, is *O*-GlcNAc-modified although the importance of this modification remained elusive for many years (Jacobsen and Iverfeldt 2011). It was later demonstrated that inhibiting *O*-GlcNAcase with PUGNAc or reducing the expression of *O*-GlcNAcase (siRNA) increased the amount of APP *O*-GlcNAcylation and non-amyloidogenic α-secretase processing (Jacobsen and Iverfeldt 2011). This has further downstream effects such as increasing levels of the neuroprotective sAPPα fragment, which reduces Aβ secretion and suggests a protective role for *O*-GlcNAc (Kim et al. 2012). Finally, treatment with the *O*-GlcNAcase inhibitor NButGT in a mouse model of AD resulted in reduced γ-secretase activity and subsequent attenuation of Aβ plaque production and inflammation in vivo, further highlighting the important protective role of *O*-GlcNAc under conditions of stress and neurodegeneration (Rissman et al. 2007, 2012).

Tau was demonstrated to be directly *O*-GlcNAc-modified (Liu et al. 2004a; Arnold et al. 1996) and it was later shown that *O*-GlcNAcylation negatively regulates site-specific phosphorylation of tau in the human brain (Liu et al. 2004a). Notably, increased phosphorylation of Tau has been implicated in the formation of the toxic tangles associated with neurogeneration. Recent data have demonstrated that inhibition of *O*-GlcNAcase, which leads to an increase in *O*-GlcNAc levels, in a murine model of AD led to increased *O*-GlcNAcylation of tau, decreased tau aggregation, and diminished neuronal cell death. Together, these data highlight

the protective role of *O*-GlcNAc and suggest that *O*-GlcNAcase could be a therapeutic target for slowing AD progression (Yuzwa et al. 2012). Interestingly, low glucose uptake and metabolism during aging has been postulated to lead to decreased *O*-GlcNAcylation of key proteins such as Tau, potentially exacerbating the AD phenotype during aging (Yuzwa et al. 2012).

### Parkinson's disease

Parkinson's disease (PD) is a brain disorder characterized by the degeneration of midbrain dopaminergic neurons, which is often accompanied by the formation of Lewy bodies (abnormal protein aggregates; Henchcliffe and Beal 2008). Together, this leads to improper signaling and loss of muscle function. Genetic alterations in the nucleus have been linked to disrupted mitochondrial morphology and function that can then modulate protein activity, trigger apoptosis, or lead to accumulation of misfolded or damaged proteins in PD pathogenesis (Henchcliffe and Beal 2008).

Aggregation of the α-synuclein protein or mutations in the α-synuclein gene (A53T) which lead to an impairment of mitochondrial dynamics, morphology, and movement have been linked to rare inherited forms of PD (Polymeropoulos et al. 1997; Spillantini et al. 1997; Xie and Chung 2012). One study identified a 22 kDa *O*-glycosylated form of α-synuclein in a protein complex with parkin (an E3 ubiquitin ligase) and UbcH7 (an E2 ubiquitin-conjugating enzyme). These studies suggested that the glycosylated form of α-synuclein was bound and ubiquitinated by parkin (Shimura et al. 2001). On the contrary, a subsequent study postulated that α-synuclein is modified by *O*-GlcNAc and that its nonglycosylated form interacts with parkin in embryonic hippocampal cells, although the specific type of glycosylation was never confirmed (Kim et al. 2003). Recently, the *O*-GlcNAc modification status of α-synuclein was mapped to Thr72 (Wang et al. 2010a), which has been demonstrated to prevent its aggregation (Marotta et al. 2012). Further data (discussed previously) highlights the interplay between *O*-GlcNAcylation and ubiquitination to regulate protein stability and degradation, although a direct link between these phenomena and PD has yet to be elucidated.

## Methods to study *O*-GlcNAcomics

In this section, we will discuss the current methodologies used to modulate *O*-GlcNAc levels, enrich *O*-GlcNAc-modified proteins, and identify sites of *O*-GlcNAcylation.

### In vitro and in vivo modulation of *O*-GlcNAc levels

*O*-GlcNAc levels can be directly or indirectly altered in vitro and in vivo by modulating the activity or expression of OGT (Gross et al. 2005, 2011; Dorfmueller et al. 2011), *O*-GlcNAcase (Dong and Hart 1994; Haltiwanger et al. 1998; Liu et al. 2002; Dorfmueller et al. 2006; Whitworth et al. 2007; Macauley et al. 2008; Knapp et al. 2007; Beer et al. 1990; Yuzwa et al. 2008; Konrad et al. 2002; Laczy et al. 2010), or other key enzymes in the HBP (Buse et al. 2002; McClain 2002; Patti et al. 1999; Ross et al. 2000; Marshall et al. 1991; Traxinger and Marshall 1991). Techniques such as over expression of OGT (Zachara et al. 2004b), *O*-GlcNAcase (Slawson et al. 2005), and GFAT (Chen et al. 1997; Marshall et al. 2004; James et al. 2000) by both transient transfection and viral transduction have been successful, as has reducing the expression of these enzymes using RNA interference (Zachara et al. 2004b; Ngoh et al. 2009a; Hsieh et al. 2012). OGT has also been overexpressed using a tetracycline-inducible OGT stably expressed in HeLa cells, although for effective overexpression a histone deacetylase inhibitor was included with the tetracycline for appropriate regulation of OGT (Marshall et al. 2003).

#### *Murine models and the $OGT^{F/Y,mER\text{-}Cre\text{-}2A\text{-}GFP}$ cell line*

Several mouse models and cell lines exist in which the levels of *O*-GlcNAc can be modulated. Notably, in murine models deletion of OGT (Shafi et al. 2000; O'Donnell et al. 2004), EMeg32 (Boehmelt et al. 2000b), or *pgm3* (Greig et al. 2007) leads to embryonic lethality, whereas deletion of *O*-GlcNAcase leads to perinatal lethality (Yang et al. 2012). A number of hypomorphic alleles of *pgm3* have been characterized, leading to cells and mice with different concentrations of UDP-GlcNAc (Greig et al. 2007). Unlike deletion of EMeg32, deletion of OGT is lethal in isolated embryonic fibroblasts (O'Donnell et al. 2004). To overcome this challenge, researchers have immortalized cells in which the first two exons of OGT are flanked by loxP recombination sites (O'Donnell et al. 2004; Kazemi et al. 2010). These cells ($OGT^{F/Y}$) have been stably transfected with a Cre recombinase estrogen receptor chimera (Cre-ERT2-GFP) that is induced by 4-hydroxytamoxifen (4-OHT; ($OGT^{F/Y,mER\text{-}Cre\text{-}2A\text{-}GFP}$); Kazemi et al. 2010). Addition of 4-OHT activates Cre recombinase leading to the deletion of OGT.

#### *Modulating O-GlcNAc levels using inhibitors of OGT, O-GlcNAcase, and the HBP*

A large number of inhibitors have been developed for blocking the activity of *O*-GlcNAcase in both cell culture and animal models (Dong and Hart 1994; Haltiwanger et al. 1998; Liu et al. 2002; Dorfmueller et al. 2006; Whitworth et al. 2007; Macauley et al. 2008; Knapp et al. 2007; Beer et al. 1990; Yuzwa et al. 2008; Konrad et al. 2002), and here we will focus on those that are commercially available.

PUGNAc was the first *O*-GlcNAcase inhibitor isolated and inhibits *O*-GlcNAcase at a $K_i$ of 52 nM in vitro (Haltiwanger et al. 1998; Dong and Hart 1994). PUGNAc is suitable for use in cells (10–100 μM, 4–18 h) and in animals (50 mg/kg, 4–12 h) (Jones et al. 2008); however, prolonged use (>36 h) can lead to cell cycle defects (Slawson et al. 2005). While widely used, recent evidence demonstrates that PUGNAc can also inhibit other lysosomal glycosidases including HexA and HexB (Macauley et al. 2005; Ficko-Blean et al. 2008), suggesting that PUGNAc may have effects on other cellular pathways aside from *O*-GlcNAcylation. STZ has also been widely used, but only inhibits *O*-GlcNAcase at very high concentrations (Toleman et al. 2006; Gao et al. 2000; Liu et al. 2002; Okuyama and Yachi 2001; Roos et al. 1998). It is important to note that STZ is also a DNA-alkylating agent and can release nitric oxide, making it difficult to determine whether the observed effects are due to *O*-GlcNAcase inhibition or STZ toxicity (Kwon et al. 1994). Alloxan has been suggested to inhibit *O*-GlcNAcase (Lee et al. 2006), but has been reported to also inhibit OGT and as such should be used with extreme caution (Konrad et al. 2002; Macauley et al. 2005). More recently, TMG has been introduced, and unlike PUGNAc, TMG is effective in crossing the blood–brain barrier and does not appear to inhibit HexA or HexB (Yuzwa et al. 2008).

Several OGT inhibitors have also been isolated that work well in vitro (Gross et al. 2005), and thus far these have been useful in isolated neonatal cardiomyocytes (5 μM; Ngoh et al. 2008) and breast cancer cells (200 μM; Caldwell et al. 2010). A more recent study has focused on synthesizing novel OGT inhibitors with structural analogues to UDP-GlcNAc/UDP (Dorfmueller et al. 2011). While the synthesized compounds inhibit OGT in the micromolar range, it should be noted that they are inactive when used on living cells (Dorfmueller et al. 2011). Another recent study developed a synthetic carbohydrate precursor, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-5-thio-α-d-glucopyranose, which can be successfully converted by the cell to produce UDP-5SGlcNAc in cell culture and in vitro models (Gloster et al. 2011). UDP-5SGlcNAc is not an efficient substrate for OGT, but excludes binding of UDP-GlcNAc therefore inhibiting OGT function (Gloster et al. 2011). It is important to note that these inhibitors are not yet commercially available.

There are numerous commercially available chemicals for supressing flux through the HBP (Buse et al. 2002; McClain 2002; Patti et al. 1999; Ngoh et al. 2010; Ross et al. 2000; Marshall et al. 1991; Traxinger and Marshall 1991), which reduced UDP-GlcNAc levels and thus the *O*-GlcNAc modification. Treatment with exogenous glucose, glutamine, or glucosamine leads to increased *O*-GlcNAc levels by increasing flux through the HBP (Traxinger and Marshall 1991), although it is important to note that these compounds enter numerous pathways in the cell and observed effects may not be due to changes in *O*-GlcNAcylation. Azaserine and DON (6-diazo-5-oxo-L-norleucine) are GFAT inhibitors, and treatment with these compounds decreases flux through the HBP thereby reducing *O*-GlcNAc levels (Marshall et al. 1991). Despite their common usage, both of these GFAT inhibitors do not act specifically on the HBP and may have effects on other enzymes and cellular pathways, especially those involved in glutamine metabolism.

## Isolation, purification, and detection of *O*-GlcNAc and *O*-GlcNAc-modified proteins

Our understanding of the role of *O*-GlcNAc has been hampered due to the lack of specific and sensitive tools. Many challenges arise as the most commonly used analytical protein techniques, including gel electrophoresis (GE), liquid chromatography (LC), and mass spectrometry (MS), often fail to detect *O*-GlcNAc. This is due to a number of factors such as (1) the addition of a monosaccharide of *O*-GlcNAc does not generally affect the migration of the glycoprotein during either 1D- or 2D-GE; (2) the *O*-GlcNAc modification is rapidly hydrolyzed by hexosaminidases during protein isolation unless appropriate inhibitors are included; (3) the β-*O*-glycosidic linkage is chemically labile and is rapidly released under conditions of mild acid or base; (4) due to its lability, *O*-GlcNAc is usually lost at the ion source of electrospray ionization (ESI) or during ionization by matrix-assisted laser desorption ionization (MALDI) mass spectrometry; and (5) ionization of peptides modified by *O*-GlcNAc is suppressed during mass spectrometry and as such need to be enriched. Here, we will review the most robust techniques for the detection and purification of *O*-GlcNAc-modified proteins, as well as techniques for defining sites of *O*-GlcNAcylation.

### *Detecting O-GlcNAc-modified proteins by [$^3$H]-galactose labeling*

The gold standard for the detection of *O*-GlcNAc-modified proteins has been UDP-Gal:GlcNAc β-1,4-galactosyltransferase (GalT1) labeling, which transfers $^3$H-galactose from UDP-[$^3$H]-Gal to any terminal GlcNAc residue (Torres and Hart 1984; Roquemore et al. 1994). One advantage of this technique is the generation of a radiolabeled sugar ($^3$H-Gal-R) that can be followed in subsequent analyses. As GalT1 will transfer galactose to any terminal GlcNAc reside, it is important to treat samples with peptide *N*-glycosidase F (PNGase F) to enzymatically remove *N*-glycans prior to $^3$H-galactose labeling. Recently, this technique has also been adapted to incorporate an unnatural *N*-azidoacetylgalactosamine (GalNAz) onto proteins (see section Click-iT).

*Immunopurification with antibodies and lectins*

In contrast to protein phosphorylation where a range of pan- and site-specific phospho-antibodies are commercially available, only a few such tools exist for the *O*-GlcNAc modification. These antibodies fall into two classes: those that can be considered pan-specific and others that are site specific (Zachara et al. 2011c). The two most commonly used pan-specific antibodies for detecting *O*-GlcNAc-modified proteins are CTD110.6 (Comer and Hart 2001), a mouse IgM antibody raised against the C-terminus of RNA Pol II, and RL2 (Snow et al. 1987), a mouse IgG antibody raised against *O*-GlcNAc-modified components of the nuclear pore complex. Recently, a number of monoclonal IgG antibodies have been introduced for detecting *O*-GlcNAc-modified proteins including 1F5.D6(14), 18B10.C7(3), and 9D1.E3(10) (Teo et al. 2010). These antibodies were raised against a three-component immunogen-containing epitope regions from the casein kinase α-subunit 15, a mouse major histocompatibility complex class II-restricted helper T-cell epitope, and a Toll-like receptor 2 agonist (Teo et al. 2010). Like RL2, these antibodies are mouse IgGs and appear to only recognize *O*-GlcNAc in certain three-dimensional environments. In addition to the antibodies discussed previously, antistreptococcal monoclonal mouse IgG antibodies have been commonly used for detecting proteins containing GlcNAc residues. These antibodies were raised against streptococcal group A carbohydrates, which is composed of a polyrhamnose backbone with GlcNAc side chains (Turner et al. 1990).

There has only been a small cohort of studies that have incorporated a top-down shotgun proteomics approach for identifying *O*-GlcNAc-modified proteins using the antibodies previously discussed. These studies have been hampered because none of the discussed antibodies appear to bind *O*-GlcNAc-modified peptides. As such, proteins that interact with *O*-GlcNAc-modified proteins are also identified, and in most cases *O*-GlcNAc-modified peptides are suppressed in the MS making *O*-GlcNAc modification sites difficult to map. Nonetheless, a number of studies have been performed highlighting proteins that are potentially *O*-GlcNAc-modified in response to cellular stress and tissue injury (Jones et al. 2008; Teo et al. 2010; Zachara et al. 2011c), and the *O*-GlcNAcylation status of a number of proteins has been confirmed by independent techniques (Table 1). One recent study used 1F5.D6(14), 18B10.C7(3), and 9D1.E3(10) to immunoprecipitate *O*-GlcNAc-modified proteins followed by large-scale shotgun proteomics to identify more than 200 differentially expressed glycoproteins from HEK293 cells and rat livers responding to trauma hemorrhage and resuscitation (Teo et al. 2010). Another recent study relied on Stable Isotope Labeling with Amino acids in Cell culture (SILAC; Ong et al. 2002; 2003) in combination with CTD110.6 immunoprecipitation (Zachara et al. 2011a). Here, Cos-7 cells were isotopically labeled in SILAC media ($^{13}C_6$ L-arginine and $^{13}C_6{}^{15}N_4$ L-arginine) and treated with heat shock or an inhibitor of *O*-GlcNAcase, PUGNAc (Zachara et al. 2011a). Labeled proteins were combined in equal ratios and *O*-GlcNAc-modified proteins were immunoprecipitated using CTD110.6 immobilized to agarose and subsequently identified by LC-MS/MS (Zachara et al. 2011a). Numerous proteins with diverse functions were identified, including nuclear factor 90, RuvB-like 1, RuvB-like 2, and several COPII vesicle transport proteins. Many of these proteins bind double-stranded DNA-dependent protein kinase (DNA PK) or double-stranded DNA breaks, suggesting a role for *O*-GlcNAc in regulating DNA damage signaling or repair (Zachara et al. 2011a).

*Lectin affinity chromatography*

Wheat germ agglutinin (WGA) is a commonly used plant lectin for enriching and detecting *O*-GlcNAc-modified proteins (Zachara et al. 2004a, 2011b; Vosseller et al. 2006). WGA has weak affinity for single GlcNAc residues, but its affinity is dramatically increased for GlcNAcs that are clustered (Finlay et al. 1987; Lundquist and Toone 2002; Lee and Lee 2000). The drawback of WGA as a tool for *O*-GlcNAc enrichment and detection is its additional recognition of sialic acid (NeuAcα(2–3)) residues (Monsigny et al. 1980). Treating samples initially with PNGase F to remove *N*-glycans, and by performing nuclear and cytoplasmic protein extractions (Zachara et al. 2011b) can resolve these shortcomings. Succinylation of WGA (sWGA) increases its specificity for GlcNAc as it ablates its reactivity to sialic acid (Monsigny et al. 1980). However, succinylation reduces the affinity of sWGA for *O*-GlcNAc and thereby decreases its utility for immunoprecipitation.

Lectin weak affinity chromatography (LWAC) utilizes the affinity of WGA in an extended column format (>3 m in length) with a small diameter (~1 cm) to enrich *O*-GlcNAc-modified peptides (Vosseller et al. 2006). This chromatography technique employs low flow rates and an isocratic elution buffer containing low concentrations of GlcNAc (Vosseller et al. 2006). LWAC has been adapted successfully for shotgun characterization of *O*-GlcNAc-modified proteomes from postsynaptic density preparations (Vosseller et al. 2006), *Arabidposis thaliana* (Xu et al. 2012), and mouse embryonic stem cells (Myers et al. 2011).

*Click-iT and other chemically based strategies to detect O-GlcNAc-modified proteins*

Click chemistry is a chemoenzymatic strategy for quickly and reliably synthesizing compounds of interest. By this method, target substrates are labeled with a probe of interest

**Table 1** A summary of *O*-GlcNAc-modified proteins, their biological significance, and the methods by which they were detected. This table highlights the wide range of techniques and tools for the enrichment and detection of *O*-GlcNAc-modified proteins, including but not limited to *O*-GlcNAc antibodies, click-iT methods, immunoprecipitation, and proteomics tools

| *O*-GlcNAc modified protein | Biological function, implication, significance | Cell type/model | Detection | Secondary confirmation | Reference |
|---|---|---|---|---|---|
| Casein kinase II Casein kinase II α subunit | *O*-GlcNAc antagonizes Thr344 phosphorylation, promotes proteasomal degradation, and alters substrate specificity | HEK293T cells | Western blotting (CTD110.6, 1F5.D6(14), 18B10.C7(3) and 9D1.E3(10)) | Ion trap ETD MS/MS, GalT1 LC-MS/MS | Tarrant et al. (2012) |
| Protein arginine methyltransferase 4 (PRMT4/ Carm1) | *O*-GlcNAcylated Carm1 does not exhibit mitotic phosphorylation or proper mitotic cellular localization | Neuro2a cells | Western blotting (CTD110.6) | Immunoprecipitation, yeast-2-hybrid | Cheung and Hart (2008), Sakabe et al. (2010), |
| Forkhead Box O1 (Foxo1) | *O*-GlcNAc regulates activation in response to glucose, resulting in increased expression of gluconeogenic genes and genes encoding enzymes that detoxify ROS | Fao rat hepatoma cells | Western blotting (CTD110.6) | OGT assay, Immunoprecipitation, Ion trap ETD MS/MS | Housley et al. (2008), Housley et al. (2009), Kuo et al. (2008) |
| Inhibitor of NFκB kinase (IKKβ) | Catalytic activity of IKKβ is enhanced through *O*-GlcNAcylation and loss of p53 | $p53^{-/-}$, $p65^{-/-}$, and $p53^{-/-}p65^{-/-}$MEFs | Western blotting (CTD110.6) | Immunoprecipitation | Kawauchi et al. (2009) |
| NFκB | Increased *O*-GlcNAc levels is associated with reduced activation of NFκB and reduced expression of proinflammatory cytokines | HeLa cells | Azide-tag, nano-HPLC/tandem MS | Immunoprecipitation and Western blot | Golks et al. (2007), Nandi et al. (2006) |
| p53 | Modification of p53 with *O*-GlcNAc regulates p53 stability by blocking ubiquitin-dependent proteolysis | MCF-7 and H1299 cells | Western blotting (CTD110.6) | 2D-GE, BEMAD and QTOF MS | Yang et al. (2006b) |
| $IP_3$ receptor type I | Regulation (up or down/ in or out) of calcium flux through the ER and $Ca^{2+}$ signaling | SH-SY5Y and DT40 B cells, Mouse and Rat Cerebella | Western blotting (CTD110.6 and RL2) | Immunoprecipitation | Rengifo et al. (2007) |
| $IP_3$ receptor type III | Regulation (up or down/ in or out) of calcium flux through the ER and $Ca^{2+}$ signaling | AR4-2J cells | Western blotting (RL2) | Immunoprecipitation | Bimboese et al. (2011) |
| TAB1 | *O*-GlcNAcylation of TAB1 is required for full TAK1 activation upon stimulation by IL-1/osmotic stress, and downstream activation of NFκB, and production of IL-6 and TNFα | TAB1($^{-/-}$) Mouse embryonic fibroblasts | Western blotting (CTD110.6) and LC-ETD MS/MS | Immunoprecipitation (*O*-GlcNAc specific TAB1 S395 antibody), In vitro *O*-GlcNAc assay, GalT1 label | Pathak et al. (2012) |
| Insulin receptor substrate 1 (IRS-1) | *O*-GlcNAc contributes to insulin resistance by inhibiting phosphorylation at the PI3K p85 binding motif in IRS-1 | HEK293T cells | BEMAD and ion trap MS/MS/ MS | Western blotting (CTD110.6) | Whelan et al. (2010), Ball et al. (2006) |
| HSP70 and HSP90 | No function currently associated with glycosylation of these proteins | HeLa cells | Azide-tag, nano-HPLC/tandem MS | Immunoprecipitation and Western blot | Walgren et al. (2003), Wells et al. (2002b), Nandi et al. (2006) |
| K18 | Glycosylation of K18 increases upon heat stress. In models of liver damage, *O*-GlcNAcylated K18 promotes the phosphorylation and activation of Akt | Transgenic K18–Gly mice & BHK21 cells | Western blotting (CTD110.6) | Immunostaining, Immunofluorescence and GalT1 activity assays | Ku et al. (2010) |
| HSF-1 | *O*-GlcNAcylation of HSF-1 promotes its nuclear ranslocation and activation leading to HSP expression | C57Bl/6 mice | Western blotting (CTD110.6) | Immunoprecipitation | Singleton and Wischmeyer (2008) |
| Calcium/calmodulin-dependent kinase IV (CaMKIV) | *O*-GlcNAcylation of the CaMKIV active site blocks its phosphorylation at Thr200 and ATP binding, inhibiting its kinase activity | HEK293A cells | Western blotting (CTD110.6) | Immunoprecipitation, GalT1 label, BEMAD and Ion trap MS/MS | Dias et al. (2009) |
| Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1α) | Co-localizes with OGT and targets the enzyme to FoxOs, leading to increased transcriptional of metabolic enzymes | HEK293T cells | Ion trap ETD MS/MS | Western blot (CTD110.6), sWGA, immunoprecipitation, GalT1 label, OGT activity assay | Housley et al. (2009) |

**Table 1** (continued)

| *O*-GlcNAc modified protein | Biological function, implication, significance | Cell type/model | Detection | Secondary confirmation | Reference |
|---|---|---|---|---|---|
| Voltage-dependent anion channel (VDAC) | *O*-GlcNAc–modified VDAC is associated with reduced calcium-induced swelling of the mitochondria | Mouse hearts and isolated cardiac myocytes | Immunoprecipitation (CTD110.6) | | Jones et al. (2008), Ngoh et al. (2008) |

*ETD MS/MS* electron transfer dissociation mass spectrometry, *GalT1 LC-MS/MS* β1-4 galactosyltransferase labeling and liquid chromatography mass spectrometry, *BEMAD* beta elimination and Michael addition, *QTOF MS* quadrupole time-of-flight mass spectrometry, *2D-GE* two-dimensional gel electrophoresis

that can be derivatized to detect or enrich proteins and peptides. This technique has been adapted for the detection and enrichment of *O*-GlcNAc-modified proteins and peptides. Sugars containing either ketones or azido groups are incorporated into *O*-GlcNAc-modified proteins using one of two techniques: (1) a mutant GalT1 (Y289L) with an enlarged active site is used to add UDP-GalNAz to terminal GlcNAc residues similar to the GalT1 labeling discussed above (Khidekel et al. 2003); and (2) in cells by metabolic labeling with a peracetylated azido-GlcNAc substrate (Vocadlo et al. 2003; Sprung et al. 2005), as many of the enzymes in the HBP will tolerate unnatural sugars. However, the kinetics of *O*-GlcNAz removal from proteins by *O*-GlcNAcase is significantly reduced. These unnatural sugars can be derivatized further to incorporate a biotin tag, which facilitates the detection and enrichment of *O*-GlcNAc-modified proteins and peptides (Wang et al. 2010a). Alternatively, this method has been used to incorporated polyethylene glycol moieties (Rexach et al. 2010), which results in a molecular weight shift (~2 kDa) for each GlcNAc residue found on a protein and thus makes it possible to estimate the stoichiometry of modification. Additionally, this method has been used to incorporate fluorescent tags, allowing researchers to quantitatively measure and image intracellular *O*-GlcNAc-modified proteins in vivo (Clark et al. 2008). Although these established methods provided great advancements for the detection of *O*-GlcNAc-modified proteins, the weak nature of metabolic labeling with peracetylated azido-GlcNAc has led to the development of better technologies. Subsequent studies have shown that GalNAz can be converted to UDP-GalNAz and epimerized to UDP-GlcNAz by mammalian biosynthetic enzymes, which is then be added to cells by OGT (Boyce et al. 2011). Proof-of-principle experiments highlighting this metabolic labeling demonstrated that numerous proteins are *O*-GlcNAc-modified, laying a solid framework for future studies to visualize and characterize dynamic *O*-GlcNAc-mediated signaling events (Boyce et al. 2011).

*Mass spectrometry*

Mass spectrometry-based methods have become widely and successfully employed for studying proteins and proteomes as well as post-translational modifications such as phosphorylation and methylation using collision-induced dissociation (CID) in tandem mass spectrometry (MS/MS). However, detection and site mapping of *O*-GlcNAc-modified peptides by mass spectrometric techniques is challenging. This is largely due to the labile nature of the β-linkage in the gas phase, which is lost prior to fragmentation of the peptide backbone and often prevents correct peptide identification, localization of the *O*-GlcNAc site, and consequently relative quantification. More importantly, distinguishing *O*-GlcNAc

peptides from a complex mixture is problematic because ion intensities of *O*-GlcNAc peptides are often suppressed when compared to unmodified peptides. Despite these pitfalls, the generation of diagnostic fragment ions of *O*-GlcNAc-modified peptides, for example $[M+H]^{1+}$ of *m*/*z* 204.1 for GlcNAc oxonium ions (Carr et al. 1993; Huddleston et al. 1993), can be used to distinguish *O*-GlcNAc-modified peptides from complex mixtures. By optimizing fragmentation energy to release *O*-GlcNAc (*m*/*z* 204.1), *O*-GlcNAc-modified peptides can be detected by ESI-MS, which has been employed in mapping *O*-GlcNAcylation sites in human cytomegalovirus tegument basic phosphoprotein (UL32) to serine 921 and serine 952 (Greis et al. 1994).

In contrast to CID, electron capture dissociation and electron transfer dissociation (ETD) are alternative fragmentation methods used to preserve more labile modifications such as phosphorylation, methylation, acetylation, glycosylation, nitrosylation, and sulfation, and allow for direct mapping of peptide/protein modifications (Mikesh et al. 2006; Syka et al. 2004; Udeshi et al. 2007, 2008; Wu et al. 2007; Wang et al. 2010a; Sobott et al. 2009). ETD fragmentation cleaves along the peptide backbone between the Cα-N producing c and z ions while still maintaining peptide side chains and modifications (Syka et al. 2004; Sobott et al. 2009). ETD-MS has been utilized to identify dynamically *O*-GlcNAcylated proteins and their modification sites in excitatory neurons in the brain (Khidekel et al. 2007), the cell adhesion protein paxillin (Kwak et al. 2010), transcription activation of Foxo1 in response to glucose (Housley et al. 2008), examination of the extensive crosstalk between phosphorylation and *O*-GlcNAcylation during cytokinesis (Wang et al. 2010b), and many other processes (Chou et al. 1995). Although the emergence of ETD-MS has proven to be a successful technique, again enrichment of *O*-GlcNAcylated peptides is ideal for effective sequencing and identification of these peptides by MS.

## Future directions

Phosphorylation and other PTMs have historically been the exclusive focus of studies on cellular signal transduction events. As discussed in this review, *O*-GlcNAc is emerging as a PTM that should also be considered as an equally important contributor and regulator of signaling pathways, especially those involved in regulating the cells response to stress and injury. Previously, it has proven difficult to detect *O*-GlcNAc and *O*-GlcNAc-modified proteins. The work of numerous groups has resulted in the development of alternative approaches that solve many of the caveats associated with studying this labile protein modification. The application of these techniques will answer many of the remaining questions: Which proteins are *O*-GlcNAc modified in response to stress?; Are the proteins modified in a stress- and/or tissue-specific manner?; How does the *O*-GlcNAc modification alter the function of these proteins at a molecular level?; How are OGT and *O*-GlcNAcase regulated during stress and injury?; and importantly, how is the *O*-GlcNAc-mediated stress response misregulated in disease?

**Acknowledgments** We apologize to our colleagues whose work was not cited in this review due to theme or space restrictions. Natasha E. Zachara, Ph.D., is funded by grants from the American Heart Association (SD0930162N) and the National Heart, Lung, and Blood Institute (R21-HL-108003 and 1P01-HL-107153). Albert Lee, Ph.D., receives funding as a GCF fellow from the National Heart, Lung and Blood Institute PEG Program (P01-HL-107153).

**Disclosures** There are no conflicts of interest, financial or otherwise, declared by the authors of this paper.

## References

Akimoto Y, Hart GW, Hirano H, Kawakami H (2005) *O*-GlcNAc modification of nucleocytoplasmic proteins and diabetes. Med Mol Morphol 38(2):84–91

Arnold CS, Johnson GV, Cole RN, Dong DL, Lee M, Hart GW (1996) The microtubule-associated protein tau is extensively modified with *O*-linked *N*-acetylglucosamine. J Biol Chem 271(46):28741–28744

Ball LE, Berkaw MN, Buse MG (2006) Identification of the major site of *O*-linked beta-*N*-acetylglucosamine modification in the C terminus of insulin receptor substrate-1. Mol Cell Proteomics 5(2):313–323

Batista-Nascimento L, Neef DW, Liu PC, Rodrigues-Pousada C, Thiele DJ (2011) Deciphering human heat shock transcription factor 1 regulation via post-translational modification in yeast. PLoS One 6(1):e15976

Beer D, Maloisel J-L, Rast DM, Vasella A (1990) Synthesis of 2-acetamido-2-deoxy-D-gluconhydroximolactone and chitobionhydroximolactone-derived *N*-phenylcarbamates, potential inhibitors of beta-*N*-acetylglucosaminidase. Helv Chim Acta 73(7):1918–1922

Bertram L, Blacker D, Mullin K, Keeney D, Jones J, Basu S, Yhu S, McInnis MG, Go RC, Vekrellis K, Selkoe DJ, Saunders AJ, Tanzi RE (2000) Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. Science 290(5500):2302–2303

Bimboese P, Gibson CJ, Schmidt S, Xiang W, Ehrlich BE (2011) Isoform-specific regulation of the inositol 1,4,5-trisphosphate receptor by *O*-linked glycosylation. J Biol Chem 286(18):15688–15697

Boehmelt G, Fialka I, Brothers G, McGinley MD, Patterson SD, Mo R, Hui CC, Chung S, Huber LA, Mak TW, Iscove NN (2000a) Cloning and characterization of the murine glucosamine-6-phosphate acetyltransferase EMeg32. Differential expression and intracellular membrane association. J Biol Chem 275(17):12821–12832

Boehmelt G, Wakeham A, Elia A, Sasaki T, Plyte S, Potter J, Yang Y, Tsang E, Ruland J, Iscove NN, Dennis JW, Mak TW (2000b) Decreased UDP-GlcNAc levels abrogate proliferation control in EMeg32-deficient cells. EMBO J 19(19):5092–5104

Boyce M, Carrico IS, Ganguli AS, Yu SH, Hangauer MJ, Hubbard SC, Kohler JJ, Bertozzi CR (2011) Metabolic cross-talk allows labeling of *O*-linked beta-*N*-acetylglucosamine-modified proteins via the *N*-acetylgalactosamine salvage pathway. Proc Natl Acad Sci U S A 108(8):3141–3146

Buse MG (2006) Hexosamines, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab 290(1):E1–E8

Buse MG, Robinson KA, Marshall BA, Hresko RC, Mueckler MM (2002) Enhanced *O*-GlcNAc protein modification is associated with insulin resistance in GLUT1-overexpressing muscles. Am J Physiol Endocrinol Metab 283(2):E241–E250

Butkinaree C, Cheung WD, Park S, Park K, Barber M, Hart GW (2008) Characterization of beta-*N*-acetylglucosaminidase cleavage by caspase-3 during apoptosis. J Biol Chem 283(35):23557–23566

Caldwell SA, Jackson SR, Shahriari KS, Lynch TP, Sethi G, Walker S, Vosseller K, Reginato MJ (2010) Nutrient sensor *O*-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. Oncogene 29(19):2831–2842

Carapito C, Klemm C, Aebersold R, Domon B (2009) Systematic LC-MS analysis of labile post-translational modifications in complex mixtures. J Proteome Res 8(5):2608–2614

Carr SA, Huddleston MJ, Bean MF (1993) Selective identification and differentiation of *N*- and *O*-linked oligosaccharides in glycoproteins by liquid chromatography-mass spectrometry. Protein Sci 2(2):183–196

Carrillo LD, Froemming JA, Mahal LK (2011) Targeted in vivo *O*-GlcNAc sensors reveal discrete compartment-specific dynamics during signal transduction. J Biol Chem 286(8):6650–6658

Carrillo LD, Krishnamoorthy L, Mahal LK (2006) A cellular FRET-based sensor for beta-*O*-GlcNAc, a dynamic carbohydrate modification involved in signaling. J Am Chem Soc 128(46):14768–14769

Champattanachai V, Marchase RB, Chatham JC (2007) Glucosamine protects neonatal cardiomyocytes from ischemia–reperfusion injury via increased protein-associated *O*-GlcNAc. Am J Physiol Cell Physiol 292(1):C178–C187

Champattanachai V, Marchase RB, Chatham JC (2008) Glucosamine protects neonatal cardiomyocytes from ischemia–reperfusion injury via increased protein *O*-GlcNAc and increased mitochondrial Bcl-2. Am J Physiol Cell Physiol 294(6):C1509–C1520

Chatham JC, Marchase RB (2010) The role of protein *O*-linked beta-*N*-acetylglucosamine in mediating cardiac stress responses. Biochim Biophys Acta 1800(2):57–66

Chatham JC, Not LG, Fulop N, Marchase RB (2008) Hexosamine biosynthesis and protein *O*-glycosylation: the first line of defense against stress, ischemia, and trauma. Shock 29(4):431–440

Chen H, Ing BL, Robinson KA, Feagin AC, Buse MG, Quon MJ (1997) Effects of overexpression of glutamine: fructose-6-phosphate amidotransferase (GFAT) and glucosamine treatment on translocation of GLUT4 in rat adipose cells. Mol Cell Endocrinol 135(1):67–77

Cheng X, Cole RN, Zaia J, Hart GW (2000) Alternative *O*-glycosylation/*O*-phosphorylation of the murine estrogen receptor beta. Biochemistry 39(38):11609–11620

Cheng X, Hart GW (2001) Alternative *O*-glycosylation/*O*-phosphorylation of serine-16 in murine estrogen receptor beta: post-translational regulation of turnover and transactivation activity. J Biol Chem 276(13):10570–10575

Cheung WD, Hart GW (2008) AMP-activated protein kinase and p38 MAPK activate *O*-GlcNAcylation of neuronal proteins during glucose deprivation. J Biol Chem 283(19):13009–13020

Cheung WD, Sakabe K, Housley MP, Dias WB, Hart GW (2008) *O*-linked beta-*N*-acetylglucosaminyltransferase substrate specificity is regulated by myosin phosphatase targeting and other interacting proteins. J Biol Chem 283(49):33935–33941

Chou T-Y, Hart GW, Dang CV (1995) c-Myc is glycosylated at threonine 58, a known phosphorylation site and a mutational hot spot in lymphomas. J Biol Chem 270(32):18961–18965

Clark PM, Dweck JF, Mason DE, Hart CR, Buck SB, Peters EC, Agnew BJ, Hsieh-Wilson LC (2008) Direct in-gel fluorescence detection and cellular imaging of *O*-GlcNAc-modified proteins. J Am Chem Soc 130(35):11576–11577

Comer FI, Hart GW (1999) *O*-GlcNAc and the control of gene expression. Biochim Biophys Acta 1473(1):161–171

Comer FI, Hart GW (2001) Reciprocity between O-GlcNAc and *O*-phosphate on the carboxyl terminal domain of RNA polymerase II. Biochemistry 40(26):7845–7852

Comtesse N, Maldener E, Meese E (2001) Identification of a nuclear variant of MGEA5, a cytoplasmic hyaluronidase and a beta-*N*-acetylglucosaminidase. Biochem Biophys Res Commun 283(3):634–640

Crow MT, Mani K, Nam YJ, Kitsis RN (2004) The mitochondrial death pathway and cardiac myocyte apoptosis. Circ Res 95(10):957–970

Dias WB, Cheung WD, Hart GW (2012) *O*-GlcNAcylation of kinases. Biochem Biophys Res Commun 422(2):224–228

Dias WB, Cheung WD, Wang Z, Hart GW (2009) Regulation of calcium/calmodulin-dependent kinase IV by *O*-GlcNAc modification. J Biol Chem 284(32):21327–21337

Dong DL-Y, Hart GW (1994) Purification and characterization of an *O*-GlcNAc selective *N*-acetyl-beta-D-glucosaminidase from rat spleen cytosol. J Biol Chem 269(30):19321–19330

Dong H, Goico B, Martin M, Csernansky CA, Bertchume A, Csernansky JG (2004) Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. Neuroscience 127(3):601–609

Dorfmueller HC, Borodkin VS, Blair DE, Pathak S, Navratilova I, van Aalten DM (2011) Substrate and product analogues as human *O*-GlcNAc transferase inhibitors. Amino Acids 40(3):781–792

Dorfmueller HC, Borodkin VS, Schimpl M, Shepherd SM, Shpiro NA, van Aalten DM (2006) GlcNAcstatin: a picomolar, selective *O*-GlcNAcase inhibitor that modulates intracellular *O*-glcNAcylation levels. J Am Chem Soc 128(51):16484–16485

Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M (2001) Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest 108(9):1341–1348

Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M (2000) Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proc Natl Acad Sci U S A 97(22):12222–12226

Dudognon P, Maeder-Garavaglia C, Carpentier JL, Paccaud JP (2004) Regulation of a COPII component by cytosolic *O*-glycosylation during mitosis. FEBS Lett 561(1–3):44–50

Ficko-Blean E, Stubbs KA, Nemirovsky O, Vocadlo DJ, Boraston AB (2008) Structural and mechanistic insight into the basis of mucopolysaccharidosis IIIB. Proc Natl Acad Sci U S A 105(18):6560–6565

Finlay DR, Newmeyer DD, Price TM, Forbes DJ (1987) Inhibition of in vitro nuclear transport by a lectin that binds to nuclear pores. J Cell Biol 104(2):189–200

Forsythe ME, Love DC, Lazarus BD, Kim EJ, Prinz WA, Ashwell G, Krause MW, Hanover JA (2006) *Caenorhabditis elegans* ortholog of a diabetes susceptibility locus: oga-1 (O-GlcNAcase) knockout impacts *O*-GlcNAc cycling, metabolism, and dauer. Proc Natl Acad Sci U S A 103(32):11952–11957

Fujiki R, Hashiba W, Sekine H, Yokoyama A, Chikanishi T, Ito S, Imai Y, Kim J, He HH, Igarashi K, Kanno J, Ohtake F, Kitagawa H, Roeder RG, Brown M, Kato S (2011) GlcNAcylation of histone H2B facilitates its monoubiquitination. Nature 480(7378):557–560

Fulop N, Marchase RB, Chatham JC (2007a) Role of protein *O*-linked *N*-acetyl-glucosamine in mediating cell function and survival in the cardiovascular system. Cardiovasc Res 73(2):288–297

Fulop N, Zhang Z, Marchase RB, Chatham JC (2007b) Glucosamine cardioprotection in perfused rat hearts associated with increased *O*-linked *N*-acetylglucosamine protein modification and altered p38 activation. Am J Physiol Heart Circ Physiol 292(5):H2227–H2236

Gambetta MC, Oktaba K, Muller J (2009) Essential role of the glycosyltransferase sxc/Ogt in polycomb repression. Science 325(5936):93–96

Gandy JC, Rountree AE, Bijur GN (2006) Akt1 is dynamically modified with *O*-GlcNAc following treatments with PUGNAc and insulin-like growth factor-1. FEBS Lett 580(13):3051–3058

Gao Y, Miyazaki J, Hart GW (2003) The transcription factor PDX-1 is post-translationally modified by *O*-linked *N*-acetylglucosamine and this modification is correlated with its DNA binding activity and insulin secretion in min6 beta-cells. Arch Biochem Biophys 415(2):155–163

Gao Y, Parker GJ, Hart GW (2000) Streptozotocin-induced beta-cell death is independent of its inhibition of *O*-GlcNAcase in pancreatic Min6 cells. Arch Biochem Biophys 383(2):296–302

Gao Y, Wells L, Comer FI, Parker GJ, Hart GW (2001) Dynamic *O*-glycosylation of nuclear and cytosolic proteins—cloning and characterization of a neutral, cytosolic beta-*N*-acetylglucosaminidase from human brain. J Biol Chem 276(13):9838–9845

Gloster TM, Zandberg WF, Heinonen JE, Shen DL, Deng L, Vocadlo DJ (2011) Hijacking a biosynthetic pathway yields a glycosyltransferase inhibitor within cells. Nat Chem Biol 7(3):174–181

Goldberg H, Whiteside C, Fantus IG (2011) *O*-linked beta-*N*-acetylglucosamine supports p38 MAPK activation by high glucose in glomerular mesangial cells. Am J Physiol Endocrinol Metab 301(4):E713–E726

Goldenberg I, Grossman E, Jacobson KA, Shneyvays V, Shainberg A (2001) Angiotensin II-induced apoptosis in rat cardiomyocyte culture: a possible role of AT1 and AT2 receptors. J Hypertens 19(9):1681–1689

Golks A, Tran TT, Goetschy JF, Guerini D (2007) Requirement for *O*-linked *N*-acetylglucosaminyltransferase in lymphocytes activation. EMBO J 26(20):4368–4379

Greig KT, Antonchuk J, Metcalf D, Morgan PO, Krebs DL, Zhang JG, Hacking DF, Bode L, Robb L, Kranz C, de Graaf C, Bahlo M, Nicola NA, Nutt SL, Freeze HH, Alexander WS, Hilton DJ, Kile BT (2007) Agm1/Pgm3-mediated sugar nucleotide synthesis is essential for hematopoiesis and development. Mol Cell Biol 27(16):5849–5859

Greis KD, Gibson W, Hart GW (1994) Site-specific glycosylation of the human cytomegalovirus tegument basic phosphoprotein (UL32) at serine 921 and serine 952. J Virol 68(12):8339–8349

Griffith LS, Schmitz B (1999) *O*-linked *N*-acetylglucosamine levels in cerebellar neurons respond reciprocally to pertubations of phosphorylation. Eur J Biochem 262(3):824–831

Gross BJ, Kraybill BC, Walker S (2005) Discovery of *O*-GlcNAc transferase inhibitors. J Am Chem Soc 127(42):14588–14589

Guinez C, Losfeld ME, Cacan R, Michalski JC, Lefebvre T (2006) Modulation of HSP70 GlcNAc-directed lectin activity by glucose availability and utilization. Glycobiology 16(1):22–28

Guinez C, Mir AM, Dehennaut V, Cacan R, Harduin-Lepers A, Michalski JC, Lefebvre T (2008) Protein ubiquitination is modulated by *O*-GlcNAc glycosylation. FASEB J 22(8):2901–2911

Guinez C, Mir AM, Leroy Y, Cacan R, Michalski JC, Lefebvre T (2007) Hsp70-GlcNAc-binding activity is released by stress, proteasome inhibition, and protein misfolding. Biochem Biophys Res Commun 361(2):414–420

Guinez C, Mir AM, Martin N, Leprince D, Michalski JC, Vergoten G, Lefebvre T (2010) Arginine 469 is a pivotal residue for the Hsc70-GlcNAc-binding property. Biochem Biophys Res Commun 400(4):537–542

Gurcel C, Vercoutter-Edouart AS, Fonbonne C, Mortuaire M, Salvador A, Michalski JC, Lemoine J (2008) Identification of new *O*-GlcNAc modified proteins using a click-chemistry-based tagging. Anal Bioanal Chem 390(8):2089–2097

Haberhausen G, Schmitt I, Kohler A, Peters U, Rider S, Chelly J, Terwilliger JD, Monaco AP, Muller U (1995) Assignment of the dystonia-parkinsonism syndrome locus, DYT3, to a small region within a 1.8-Mb YAC contig of Xq13.1. Am J Hum Genet 57(3):644–650

Haltiwanger RS, Blomberg MA, Hart GW (1992) Glycosylation of nuclear and cytoplasmic proteins. Purification and characterization of a uridine diphospho-*N*-acetylglucosamine:polypeptide beta-*N*-acetylglucosaminyltransferase. J Biol Chem 267(13):9005–9013

Haltiwanger RS, Busby S, Grove K, Li S, Mason D, Medina L, Moloney D, Philipsberg G, Scartozzi R (1997) *O*-glycosylation of nuclear and cytoplasmic proteins: regulation analogous to phosphorylation? BiochemBiophysResCommun 231:237–242

Haltiwanger RS, Grove K, Philipsberg GA (1998) Modulation of *O*-linked *N*-acetylglucosamine levels on nuclear and cytoplasmic proteins in vivo using the peptide *O*-GlcNAc-beta-*N*-acetylglucosaminidase inhibitor O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-*N*-phenylcarbamate. J Biol Chem 273(6):3611–3617

Hamiel CR, Pinto S, Hau A, Wischmeyer PE (2009) Glutamine enhances heat shock protein 70 expression via increased hexosamine biosynthetic pathway activity. Am J Physiol Cell Physiol 297(6):C1509–C1519

Han I, Kudlow JE (1997) Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility. Mol Cell Biol 17(5):2550–2558

Hanover JA, Forsythe ME, Hennessey PT, Brodigan TM, Love DC, Ashwell G, Krause M (2005) A *Caenorhabditis elegans* model of insulin resistance: altered macronutrient storage and dauer formation in an OGT-1 knockout. Proc Natl Acad Sci U S A 102(32):11266–11271

Hanover JA, Yu S, Lubas WB, Shin SH, Ragano-Caracciola M, Kochran J, Love DC (2003) Mitochondrial and nucleocytoplasmic isoforms of *O*-linked GlcNAc transferase encoded by a single mammalian gene. Arch Biochem Biophys 409(2):287–297

Hardy J, Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 12(10):383–388

Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O (2011) Cross talk between *O*-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. Annu Rev Biochem 80:825–858

Heckel D, Comtesse N, Brass N, Blin N, Zang KD, Meese E (1998) Novel immunogenic antigen homologous to hyaluronidase in meningioma. Hum Mol Genet 7(12):1859–1872

Henchcliffe C, Beal MF (2008) Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol 4(11):600–609

Housley MP, Rodgers JT, Udeshi ND, Kelly TJ, Shabanowitz J, Hunt DF, Puigserver P, Hart GW (2008) *O*-GlcNAc regulates FoxO activation in response to glucose. J Biol Chem 283(24):16283–16292

Housley MP, Udeshi ND, Rodgers JT, Shabanowitz J, Puigserver P, Hunt DF, Hart GW (2009) A PGC-1alpha-*O*-GlcNAc transferase complex regulates FoxO transcription factor activity in response to glucose. J Biol Chem 284(8):5148–5157

Hsieh TJ, Lin T, Hsieh PC, Liao MC, Shin SJ (2012) Suppression of glutamine:fructose-6-phosphate amidotransferase-1 inhibits adipogenesis in 3T3-L1 adipocytes. J Cell Physiol 227(1):108–115

Hu Y, Belke D, Suarez J, Swanson E, Clark R, Hoshijima M, Dillmann WH (2005) Adenovirus-mediated overexpression of *O*-GlcNAcase improves contractile function in the diabetic heart. Circ Res 96(9):1006–1013

Huang JB, Clark AJ, Petty HR (2007) The hexosamine biosynthesis pathway negatively regulates IL-2 production by Jurkat T cells. Cell Immunol 245(1):1–6

Huddleston MJ, Bean MF, Carr SA (1993) Collisional fragmentation of glycopeptides by electrospray ionization LC/MS and LC/MS/MS: methods for selective detection of glycopeptides in protein digests. Anal Chem 65(7):877–884

Hunton DL, Zou L, Pang Y, Marchase RB (2004) Adult rat cardiomyocytes exhibit capacitative calcium entry. Am J Physiol Heart Circ Physiol 286(3):H1124–H1132

Hwang SY, Shin JH, Hwang JS, Kim SY, Shin JA, Oh ES, Oh S, Kim JB, Lee JK, Han IO (2010) Glucosamine exerts a neuroprotective effect via suppression of inflammation in rat brain ischemia/reperfusion injury. Glia 58(15):1881–1892

Ise H, Kobayashi S, Goto M, Sato T, Kawakubo M, Takahashi M, Ikeda U, Akaike T (2010) Vimentin and desmin possess GlcNAc-binding lectin-like properties on cell surfaces. Glycobiology 20(7):843–864

Jackson SP, Tjian R (1989) Purification and analysis of RNA polymerase II transcription factors by using wheat germ agglutinin affinity chromatography. Proc Natl Acad Sci U S A 86(6):1781–1785

Jacobsen KT, Iverfeldt K (2011) *O*-GlcNAcylation increases non-amyloidogenic processing of the amyloid-beta precursor protein (APP). Biochem Biophys Res Commun 404(3):882–886

Jacobsen SE, Binkowski KA, Olszewski NE (1996) SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction Arabidopsis. Proc Natl Acad Sci U S A 93(17):9292–9296

James LR, Fantus IG, Goldberg H, Ly H, Scholey JW (2000) Overexpression of GFAT activates PAI-1 promoter in mesangial cells. Am J Physiol Renal Physiol 279(4):F718–F727

Jarrar D, Chaudry IH, Wang P (1999) Organ dysfunction following hemorrhage and sepsis: mechanisms and therapeutic approaches. Int J Mol Med 4(6):575–583

Jiang M-S, Hart GW (1997) A subpopulation of estrogen receptors are modified by *O*-Linked *N*-acetylglucosamine. JBiolChem 272:2421–2498

Jinek M, Rehwinkel J, Lazarus BD, Izaurralde E, Hanover JA, Conti E (2004) The superhelical TPR-repeat domain of *O*-linked GlcNAc transferase exhibits structural similarities to importin alpha. Nat Struct Mol Biol 11(10):1001–1007

Jones SP, Zachara NE, Ngoh GA, Hill BG, Teshima Y, Bhatnagar A, Hart GW, Marban E (2008) Cardioprotection by *N*-acetylglucosamine linkage to cellular proteins. Circulation 117(9):1172–1182

Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, Sollott SJ (2004) Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest 113(11):1535–1549

Kajstura J, Cigola E, Malhotra A, Li P, Cheng W, Meggs LG, Anversa P (1997) Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. J Mol Cell Cardiol 29(3):859–870

Kang ES, Han D, Park J, Kwak TK, Oh MA, Lee SA, Choi S, Park ZY, Kim Y, Lee JW (2008) *O*-GlcNAc modulation at Akt1 Ser473 correlates with apoptosis of murine pancreatic beta cells. Exp Cell Res 314(11–12):2238–2248

Kang JE, Cirrito JR, Dong H, Csernansky JG, Holtzman DM (2007) Acute stress increases interstitial fluid amyloid-beta via corticotropin-releasing factor and neuronal activity. Proc Natl Acad Sci U S A 104(25):10673–10678

Kawauchi K, Araki K, Tobiume K, Tanaka N (2009) Loss of p53 enhances catalytic activity of IKKbeta through *O*-linked beta-*N*-acetyl glucosamine modification. Proc Natl Acad Sci U S A 106(9):3431–3436

Kazemi Z, Chang H, Haserodt S, McKen C, Zachara NE (2010) *O*-linked beta-*N*-acetylglucosamine (*O*-GlcNAc) regulates stress-induced heat shock protein expression in a GSK-3beta-dependent manner. J Biol Chem 285(50):39096–39107

Kearse KP, Hart GW (1991) Lymphocyte activation induces rapid changes in nuclear and cytoplasmic glycoproteins. Proc Natl Acad Sci U S A 88(5):1701–1705

Kelly WG, Dahmus ME, Hart GW (1993) RNA polymerase II is a glycoprotein. Modification of the COOH-terminal domain by *O*-GlcNAc. J Biol Chem 268(14):10416–10424

Kem DC, Johnson EI, Capponi AM, Chardonnens D, Lang U, Blondel B, Koshida H, Vallotton MB (1991) Effect of angiotensin II on cytosolic free calcium in neonatal rat cardiomyocytes. Am J Physiol 261(1 Pt 1):C77–C85

Khidekel N, Arndt S, Lamarre-Vincent N, Lippert A, Poulin-Kerstien KG, Ramakrishnan B, Qasba PK, Hsieh-Wilson LC (2003) A chemoenzymatic approach toward the rapid and sensitive detection of *O*-GlcNAc posttranslational modifications. J Am Chem Soc 125(52):16162–16163

Khidekel N, Ficarro SB, Clark PM, Bryan MC, Swaney DL, Rexach JE, Sun YE, Coon JJ, Peters EC, Hsieh-Wilson LC (2007) Probing the dynamics of *O*-GlcNAc glycosylation in the brain using quantitative proteomics. Nat Chem Biol 3(6):339–348

Khidekel N, Ficarro SB, Peters EC, Hsieh-Wilson LC (2004) Exploring the *O*-GlcNAc proteome: direct identification of *O*-GlcNAc-modified proteins from the brain. Proc Natl Acad Sci U S A 101(36):13132–13137

Kim C, Nam DW, Park SY, Song H, Hong HS, Boo JH, Jung ES, Kim Y, Baek JY, Kim KS, Cho JW, Mook-Jung I (2012) *O*-linked beta-*N*-acetylglucosaminidase inhibitor attenuates beta-amyloid plaque and rescues memory impairment. Neurobiol Aging 34(1):275–285

Kim EJ, Kang DO, Love DC, Hanover JA (2006a) Enzymatic characterization of *O*-GlcNAcase isoforms using a fluorogenic GlcNAc substrate. Carbohydr Res 341(8):971–982

Kim SJ, Sung JY, Um JW, Hattori N, Mizuno Y, Tanaka K, Paik SR, Kim J, Chung KC (2003) Parkin cleaves intracellular alpha-synuclein inclusions via the activation of calpain. J Biol Chem 278(43):41890–41899

Kim YH, Song M, Oh YS, Heo K, Choi JW, Park JM, Kim SH, Lim S, Kwon HM, Ryu SH, Suh PG (2006b) Inhibition of phospholipase C-beta1-mediated signaling by *O*-GlcNAc modification. J Cell Physiol 207(3):689–696

Knapp S, Abdo M, Ajayi K, Huhn RA, Emge TJ, Kim EJ, Hanover JA (2007) Tautomeric modification of GlcNAc-thiazoline. Org Lett 9(12):2321–2324

Konrad RJ, Zhang F, Hale JE, Knierman MD, Becker GW, Kudlow JE (2002) Alloxan is an inhibitor of the enzyme *O*-linked *N*-acetylglucosamine transferase. Biochem Biophys Res Commun 293(1):207–212

Kreppel LK, Blomberg MA, Hart GW (1997) Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique *O*-GlcNAc transferase with multiple tetratricopeptide repeats. J Biol Chem 272(14):9308–9315

Kreppel LK, Hart GW (1999) Regulation of a cytosolic and nuclear *O*-GlcNAc transferase. Role of the tetratricopeptide repeats. J Biol Chem 274(45):32015–32022

Ku NO, Toivola DM, Strnad P, Omary MB (2010) Cytoskeletal keratin glycosylation protects epithelial tissue from injury. Nat Cell Biol 12(9):876–885

Kuo M, Zilberfarb V, Gangneux N, Christeff N, Issad T (2008) *O*-GlcNAc modification of FoxO1 increases its transcriptional activity: a role in the glucotoxicity phenomenon? Biochimie 90(5):679–685

Kwak TK, Kim H, Jung O, Lee SA, Kang M, Kim HJ, Park JM, Kim SH, Lee JW (2010) Glucosamine treatment-mediated *O*-GlcNAc modification of paxillin depends on adhesion state of rat insulinoma INS-1 cells. J Biol Chem 285(46):36021–36031

Kwon NS, Lee SH, Choi CS, Kho T, Lee HS (1994) Nitric oxide generation from streptozotocin. FASEB J: Off Publ Fed Am Soc Exp Biol 8(8):529–533

Laczy B, Marsh SA, Brocks CA, Wittmann I, Chatham JC (2010) Inhibition of *O*-GlcNAcase in perfused rat hearts by NAG-thiazolines at the time of reperfusion is cardioprotective in an *O*-GlcNAc dependent manner. Am J Physiol Heart Circ Physiol 299(5):H1715–H1727

Lazarus MB, Nam Y, Jiang J, Sliz P, Walker S (2011) Structure of human *O*-GlcNAc transferase and its complex with a peptide substrate. Nature 469(7331):564–567

Lee RT, Lee YC (2000) Affinity enhancement by multivalent lectin-carbohydrate interaction. Glycoconj J 17(7–9):543–551

Lee TN, Alborn WE, Knierman MD, Konrad RJ (2006) Alloxan is an inhibitor of *O*-GlcNAc-selective *N*-acetyl-beta-D-glucosaminidase. Biochem Biophys Res Commun 350(4):1038–1043

Lefebvre T, Cieniewski C, Lemoine J, Guerardel Y, Leroy Y, Zanetta JP, Michalski JC (2001) Identification of *N*-acetyl-D-glucosamine-specific lectins from rat liver cytosolic and nuclear compartments as heat-shock proteins. Biochem J 360(Pt 1):179–188

Lim K, Chang HI (2010) *O*-GlcNAc inhibits interaction between Sp1 and sterol regulatory element binding protein 2. Biochem Biophys Res Commun 393(2):314–318

Lim KH, Chang HI (2006) *O*-linked *N*-acetylglucosamine suppresses thermal aggregation of Sp1. FEBS Lett 580(19):4645–4652

Lima VV, Spitler K, Choi H, Webb RC, Tostes RC (2012) *O*-GlcNAcylation and oxidation of proteins: is signalling in the cardiovascular system becoming sweeter? Clin Sci (Lond) 123(8):473–486

Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191

Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong CX (2004a) *O*-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. Proc Natl Acad Sci U S A 101(29):10804–10809

Liu HR, Gao F, Tao L, Yan WL, Gao E, Christopher TA, Lopez BL, Hu A, Ma XL (2004b) Antiapoptotic mechanisms of benidipine in the ischemic/reperfused heart. Br J Pharmacol 142(4):627–634

Liu J, Marchase RB, Chatham JC (2007) Increased *O*-GlcNAc levels during reperfusion lead to improved functional recovery and reduced calpain proteolysis. Am J Physiol Heart Circ Physiol 293(3):H1391–H1399

Liu J, Pang Y, Chang T, Bounelis P, Chatham JC, Marchase RB (2006) Increased hexosamine biosynthesis and protein *O*-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. J Mol Cell Cardiol 40(2):303–312

Liu K, Paterson AJ, Konrad RJ, Parlow AF, Jimi S, Roh M, Chin E Jr, Kudlow JE (2002) Streptozotocin, an *O*-GlcNAcase inhibitor, blunts insulin and growth hormone secretion. Mol Cell Endocrinol 194(1–2):135–146

Love DC, Ghosh S, Mondoux MA, Fukushige T, Wang P, Wilson MA, Iser WB, Wolkow CA, Krause MW, Hanover JA (2010) Dynamic *O*-GlcNAc cycling at promoters of *Caenorhabditis elegans* genes regulating longevity, stress, and immunity. Proc Natl Acad Sci U S A 107(16):7413–7418

Love DC, Kochan J, Cathey RL, Shin SH, Hanover JA (2003) Mitochondrial and nucleocytoplasmic targeting of *O*-linked GlcNAc transferase. J Cell Sci 116(Pt 4):647–654

Lubas WA, Frank DW, Krause M, Hanover JA (1997) *O*-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. J Biol Chem 272(14):9316–9324

Lubas WA, Hanover JA (2000) Functional expression of *O*-linked GlcNAc transferase. Domain structure and substrate specificity. J Biol Chem 275(15):10983–10988

Lundquist JJ, Toone EJ (2002) The cluster glycoside effect. Chem Rev 102(2):555–578

Macauley MS, Bubb AK, Martinez-Fleites C, Davies GJ, Vocadlo DJ (2008) Elevation of global *O*-GlcNAc levels in 3T3-L1 adipocytes by selective inhibition of *O*-GlcNAcase does not induce insulin resistance. J Biol Chem 283(50):34687–34695

Macauley MS, Whitworth GE, Debowski AW, Chin D, Vocadlo DJ (2005) *O*-GlcNAcase uses substrate-assisted catalysis: kinetic analysis and development of highly selective mechanism-inspired inhibitors. J Biol Chem 280(27):25313–25322

Machida M, Jigami Y (1994) Glycosylated DNA-binding proteins from filamentous fungus, *Aspergillus oryzae*: modification with *N*-acetylglucosamine monosaccharide through an *O*-glycosidic linkage. BiosciBiotechnolBiochem 58:344–348

Marotta NP, Cherwien CA, Abeywardana T, Pratt MR (2012) *O*-GlcNAc modification prevents peptide-dependent acceleration of alpha-synuclein aggregation. ChemBioChem 13(18):2665–2670

Marshall S, Bacote V, Traxinger RR (1991) Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. J Biol Chem 266(8):4706–4712

Marshall S, Duong T, Wu T, Hering MA, Yada J, Higgins S, Orbus RJ, Yan ZH, Rumberger JM (2003) Enhanced expression of uridine diphosphate-*N*-acetylglucosaminyl transferase (OGT) in a stable, tetracycline-inducible HeLa cell line using histone deacetylase inhibitors: kinetics of cytosolic OGT accumulation and nuclear translocation. Anal Biochem 319(2):304–313

Marshall S, Nadeau O, Yamasaki K (2004) Dynamic actions of glucose and glucosamine on hexosamine biosynthesis in isolated adipocytes: differential effects on glucosamine 6-phosphate, UDP-*N*-acetylglucosamine, and ATP levels. J Biol Chem 279(34):35313–35319

Martinez-Fleites C, Macauley MS, He Y, Shen DL, Vocadlo DJ, Davies GJ (2008) Structure of an *O*-GlcNAc transferase homolog provides insight into intracellular glycosylation. Nat Struct Mol Biol 15(7):764–765

McClain DA (2002) Hexosamines as mediators of nutrient sensing and regulation in diabetes. J Diabetes Complications 16(1):72–80

Meldrum DR, Cleveland JC Jr, Mitchell MB, Sheridan BC, Gamboni-Robertson F, Harken AH, Banerjee A (1996) Protein kinase C mediates Ca2(+)-induced cardioadaptation to ischemia–reperfusion injury. Am J Physiol 271(3 Pt 2):R718–R726

Mikesh LM, Ueberheide B, Chi A, Coon JJ, Syka JE, Shabanowitz J, Hunt DF (2006) The utility of ETD mass spectrometry in proteomic analysis. Biochim Biophys Acta 1764(12):1811–1822

Mio T, Yamada-Okabe T, Arisawa M, Yamada-Okabe H (1999) Saccharomyces cerevisiae GNA1, an essential gene encoding a novel acetyltransferase involved in UDP-*N*-acetylglucosamine synthesis. J Biol Chem 274(1):424–429

Miura T, Miki T (2009) GSK-3beta, a therapeutic target for cardiomyocyte protection. Circ J 73(7):1184–1192

Monsigny M, Roche AC, Sene C, Maget-Dana R, Delmotte F (1980) Sugar-lectin interactions: how does wheat-germ agglutinin bind sialoglycoconjugates? Eur J Biochem / FEBS 104(1):147–153

Murphy E, Imahashi K, Steenbergen C (2005) Bcl-2 regulation of mitochondrial energetics. Trends Cardiovasc Med 15(8):283–290

Musicki B, Kramer MF, Becker RE, Burnett AL (2005) Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by *O*-GlcNAc in diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A 102(33):11870–11875

Myers SA, Panning B, Burlingame AL (2011) Polycomb repressive complex 2 is necessary for the normal site-specific *O*-GlcNAc distribution in mouse embryonic stem cells. Proc Natl Acad Sci U S A 108(23):9490–9495

Nagy T, Champattanachai V, Marchase RB, Chatham JC (2006) Glucosamine inhibits angiotensin II-induced cytoplasmic Ca2+ elevation in neonatal cardiomyocytes via protein-associated *O*-linked *N*-acetylglucosamine. Am J Physiol Cell Physiol 290(1):C57–C65

Nandi A, Sprung R, Barma DK, Zhao Y, Kim SC, Falck JR (2006) Global identification of *O*-GlcNAc-modified proteins. Anal Chem 78(2):452–458

Nathan C (2002) Points of control in inflammation. Nature 420(6917):846–852

Ngoh GA, Facundo HT, Hamid T, Dillmann W, Zachara NE, Jones SP (2009a) Unique hexosaminidase reduces metabolic survival signal and sensitizes cardiac myocytes to hypoxia/reoxygenation injury. Circ Res 104(1):41–49

Ngoh GA, Facundo HT, Zafir A, Jones SP (2010) *O*-GlcNAc signaling in the cardiovascular system. Circ Res 107(2):171–185

Ngoh GA, Hamid T, Prabhu SD, Jones SP (2009b) *O*-GlcNAc signaling attenuates ER stress-induced cardiomyocyte death. Am J Physiol Heart Circ Physiol 297(5):H1711–H1719

Ngoh GA, Watson LJ, Facundo HT, Dillmann W, Jones SP (2008) Non-canonical glycosyltransferase modulates post-hypoxic cardiac myocyte death and mitochondrial permeability transition. J Mol Cell Cardiol 45(2):313–325

Ngoh GA, Watson LJ, Facundo HT, Jones SP (2011) Augmented *O*-GlcNAc signaling attenuates oxidative stress and calcium overload in cardiomyocytes. Amino Acids 40(3):895–911

Nollen EA, Morimoto RI (2002) Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. J Cell Sci 115(Pt 14):2809–2816

Not LG, Brocks CA, Vamhidy L, Marchase RB, Chatham JC (2010) Increased *O*-linked beta-*N*-acetylglucosamine levels on proteins improves survival, reduces inflammation and organ damage 24 hours after trauma-hemorrhage in rats. Crit Care Med 38(2):562–571

Not LG, Marchase RB, Fulop N, Brocks CA, Chatham JC (2007) Glucosamine administration improves survival rate after severe hemorrhagic shock combined with trauma in rats. Shock 28(3):345–352

O'Donnell N, Zachara NE, Hart GW, Marth JD (2004) Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. Mol Cell Biol 24(4):1680–1690

Okuyama R, Yachi M (2001) Cytosolic *O*-GlcNAc accumulation is not involved in beta-cell death in HIT-T15 or Min6. Biochem Biophys Res Commun 287(2):366–371

Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 1(5):376–386

Ong SE, Kratchmarova I, Mann M (2003) Properties of 13C-substituted arginine in stable isotope labeling by amino acids in cell culture (SILAC). J Proteome Res 2(2):173–181

Ozcan S, Andrali SS, Cantrell JE (2010) Modulation of transcription factor function by *O*-GlcNAc modification. Biochim Biophys Acta 1799(5–6):353–364

Pang Y, Hunton DL, Bounelis P, Marchase RB (2002) Hyperglycemia inhibits capacitative calcium entry and hypertrophy in neonatal cardiomyocytes. Diabetes 51(12):3461–3467

Pathak S, Borodkin VS, Albarbarawi O, Campbell DG, Ibrahim A, van Aalten DM (2012) *O*-GlcNAcylation of TAB1 modulates TAK1-mediated cytokine release. EMBO J 31(6):1394–1404

Patti ME, Virkamaki A, Landaker EJ, Kahn CR, Yki-Jarvinen H (1999) Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. Diabetes 48(8):1562–1571

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276(5321):2045–2047

Rengifo J, Gibson CJ, Winkler E, Collin T, Ehrlich BE (2007) Regulation of the inositol 1,4,5-trisphosphate receptor type I by *O*-GlcNAc glycosylation. J Neurosci 27(50):13813–13821

Rexach JE, Clark PM, Hsieh-Wilson LC (2008) Chemical approaches to understanding *O*-GlcNAc glycosylation in the brain. Nat Chem Biol 4(2):97–106

Rexach JE, Rogers CJ, Yu SH, Tao J, Sun YE, Hsieh-Wilson LC (2010) Quantification of *O*-glycosylation stoichiometry and dynamics using resolvable mass tags. Nat Chem Biol 6(9):645–651

Rissman RA, Lee KF, Vale W, Sawchenko PE (2007) Corticotropin-releasing factor receptors differentially regulate stress-induced tau phosphorylation. J Neurosci 27(24):6552–6562

Rissman RA, Staup MA, Lee AR, Justice NJ, Rice KC, Vale W, Sawchenko PE (2012) Corticotropin-releasing factor receptor-dependent effects of repeated stress on tau phosphorylation, solubility, and aggregation. Proc Natl Acad Sci U S A 109(16):6277–6282

Roos MD, Xie W, Su K, Clark JA, Yang X, Chin E, Paterson AJ, Kudlow JE (1998) Streptozotocin, an analog of *N*-acetylglucosamine, blocks the removal of *O*-GlcNAc from intracellular proteins. Proc Assoc Am Physicians 110(5):422–432

Roquemore EP, Chou TY, Hart GW (1994) Detection of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) on cytoplasmic and nuclear proteins. Methods Enzymol 230:443–460

Ross SA, Chen X, Hope HR, Sun S, McMahon EG, Broschat K, Gulve EA (2000) Development and comparison of two 3T3-L1 adipocyte models of insulin resistance: increased glucose flux vs glucosamine treatment. Biochem Biophys Res Commun 273(3):1033–1041

Ryu IH, Do SI (2011) Denitrosylation of *S*-nitrosylated OGT is triggered in LPS-stimulated innate immune response. Biochem Biophys Res Commun 408(1):52–57

Sakabe K, Wang Z, Hart GW (2010) Beta-*N*-acetylglucosamine (*O*-GlcNAc) is part of the histone code. Proc Natl Acad Sci U S A 107(46):19915–19920

Sakurai H, Miyoshi H, Mizukami J, Sugita T (2000) Phosphorylation-dependent activation of TAK1 mitogen-activated protein kinase kinase kinase by TAB1. FEBS Lett 474(2–3):141–145

Sayat R, Leber B, Grubac V, Wiltshire L, Persad S (2008) *O*-GlcNAc-glycosylation of beta-catenin regulates its nuclear localization and transcriptional activity. Exp Cell Res 314(15):2774–2787

Shafi R, Lyer SPN, Ellies LG, O'Donnell N, Marek KW, Chui D, Hart GW, Marth JD (2000) The *O*-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc Natl Acad Sci USA 97(11):5735–5739

Shao Q, Saward L, Zahradka P, Dhalla NS (1998) Ca2+ mobilization in adult rat cardiomyocytes by angiotensin type 1 and 2 receptors. Biochem Pharmacol 55(9):1413–1418

Shen A, Kamp HD, Grundling A, Higgins DE (2006) A bifunctional *O*-GlcNAc transferase governs flagellar motility through anti-repression. Genes Dev 20(23):3283–3295

Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. Science 293(5528):263–269

Shin SH, Love DC, Hanover JA (2011) Elevated *O*-GlcNAc-dependent signaling through inducible mOGT expression selectively triggers apoptosis. Amino Acids 40(3):885–893

Shrikhande GV, Scali ST, da Silva CG, Damrauer SM, Csizmadia E, Putheti P, Matthey M, Arjoon R, Patel R, Siracuse JJ, Maccariello ER, Andersen ND, Monahan T, Peterson C, Essayagh S, Studer P, Guedes RP, Kocher O, Usheva A,

Veves A, Kaczmarek E, Ferran C (2010) *O*-glycosylation regulates ubiquitination and degradation of the anti-inflammatory protein A20 to accelerate atherosclerosis in diabetic ApoE-null mice. PLoS One 5(12):e14240

Sinclair DA, Syrzycka M, Macauley MS, Rastgardani T, Komljenovic I, Vocadlo DJ, Brock HW, Honda BM (2009) Drosophila *O*-GlcNAc transferase (OGT) is encoded by the Polycomb group (PcG) gene, super sex combs (sxc). Proc Natl Acad Sci U S A 106(32):13427–13432

Singleton KD, Wischmeyer PE (2008) Glutamine induces heat shock protein expression via *O*-glycosylation and phosphorylation of HSF-1 and Sp1. JPEN J Parenter Enteral Nutr 32(4):371–376

Slawson C, Copeland RJ, Hart GW (2010) *O*-GlcNAc signaling: a metabolic link between diabetes and cancer? Trends Biochem Sci 35(10):547–555

Slawson C, Lakshmanan T, Knapp S, Hart GW (2008) A mitotic GlcNAcylation/phosphorylation signaling complex alters the posttranslational state of the cytoskeletal protein vimentin. Mol Biol Cell 19(10):4130–4140

Slawson C, Zachara NE, Vosseller K, Cheung WD, Lane MD, Hart GW (2005) Perturbations in *O*-linked beta-*N*-acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. J Biol Chem 280(38):32944–32956

Snow CM, Senior A, Gerace L (1987) Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. J Cell Biol 104(5):1143–1156

Sobott F, Watt SJ, Smith J, Edelmann MJ, Kramer HB, Kessler BM (2009) Comparison of CID versus ETD based MS/MS fragmentation for the analysis of protein ubiquitination. J Am Soc Mass Spectrom 20(9):1652–1659

Soesanto YA, Luo B, Jones D, Taylor R, Gabrielsen JS, Parker G, McClain DA (2008) Regulation of Akt signaling by *O*-GlcNAc in euglycemia. Am J Physiol Endocrinol Metab 295(4):E974–E980

Sohn KC, Lee KY, Park JE, Do SI (2004) OGT functions as a catalytic chaperone under heat stress response: a unique defense role of OGT in hyperthermia. Biochem Biophys Res Commun 322(3):1045–1051

Song M, Kim HS, Park JM, Kim SH, Kim IH, Ryu SH, Suh PG (2008) *o*-GlcNAc transferase is activated by CaMKIV-dependent phosphorylation under potassium chloride-induced depolarization in NG-108-15 cells. Cell Signal 20(1):94–104

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388(6645):839–840

Sprung R, Nandi A, Chen Y, Kim SC, Barma D, Falck JR, Zhao Y (2005) Tagging-via-substrate strategy for probing *O*-GlcNAc modified proteins. J Proteome Res 4(3):950–957

St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K, Lin J, Yang W, Simon DK, Bachoo R, Spiegelman BM (2006) Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 127(2):397–408

Sumegi M, Hunyadi-Gulyas E, Medzihradszky KF, Udvardy A (2003) 26S proteasome subunits are *O*-linked *N*-acetylglucosamine-modified in *Drosophila melanogaster*. Biochem Biophys Res Commun 312(4):1284–1289

Syka JE, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF (2004) Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc Natl Acad Sci U S A 101(26):9528–9533

Tarrant MK, Rho HS, Xie Z, Jiang YL, Gross C, Culhane JC, Yan G, Qian J, Ichikawa Y, Matsuoka T, Zachara N, Etzkorn FA, Hart GW, Jeong JS, Blackshaw S, Zhu H, Cole PA (2012) Regulation of CK2 by phosphorylation and *O*-GlcNAcylation revealed by semisynthesis. Nat Chem Biol 8(3):262–269

Teo CF, Ingale S, Wolfert MA, Elsayed GA, Not LG, Chatham JC, Wells L, Boons GJ (2010) Glycopeptide-specific monoclonal antibodies suggest new roles for *O*-GlcNAc. Nat Chem Biol 6(5):338–343

Toleman C, Paterson AJ, Shin R, Kudlow JE (2006) Streptozotocin inhibits *O*-GlcNAcase via the production of a transition state analog. Biochem Biophys Res Commun 340(2):526–534

Torres CR, Hart GW (1984) Topography and polypeptide distribution of terminal *N*-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for *O*-linked GlcNAc. J Biol Chem 259(5):3308–3317

Traxinger RR, Marshall S (1991) Coordinated regulation of glutamine:fructose-6-phosphate amidotransferase activity by insulin, glucose, and glutamine. Role of hexosamine biosynthesis in enzyme regulation. J Biol Chem 266(16):10148–10154

Tsujimoto Y (2003) Cell death regulation by the Bcl-2 protein family in the mitochondria. J Cell Physiol 195(2):158–167

Tsujimoto Y, Nakagawa T, Shimizu S (2006) Mitochondrial membrane permeability transition and cell death. Biochim Biophys Acta 1757(9–10):1297–1300

Turner JR, Tartakoff AM, Greenspan NS (1990) Cytologic assessment of nuclear and cytoplasmic *O*-linked *N*-acetylglucosamine distribution by using anti-streptococcal monoclonal antibodies. Proc Natl Acad Sci U S A 87(15):5608–5612

Udeshi ND, Compton PD, Shabanowitz J, Hunt DF, Rose KL (2008) Methods for analyzing peptides and proteins on a chromatographic timescale by electron-transfer dissociation mass spectrometry. Nat Protoc 3(11):1709–1717

Udeshi ND, Shabanowitz J, Hunt DF, Rose KL (2007) Analysis of proteins and peptides on a chromatographic timescale by electron-transfer dissociation MS. FEBS J 274(24):6269–6276

Vocadlo DJ, Hang HC, Kim EJ, Hanover JA, Bertozzi CR (2003) A chemical approach for identifying *O*-GlcNAc-modified proteins in cells. Proc Natl Acad Sci U S A 100(16):9116–9121

Vosseller K, Trinidad JC, Chalkley RJ, Specht CG, Thalhammer A, Lynn AJ, Snedecor JO, Guan S, Medzihradszky KF, Maltby DA, Schoepfer R, Burlingame AL (2006) *O*-linked *N*-acetylglucosamine proteomics of postsynaptic density preparations using lectin weak affinity chromatography and mass spectrometry. Mol Cell Proteomics 5(5):923–934

Walgren JL, Vincent TS, Schey KL, Buse MG (2003) High glucose and insulin promote *O*-GlcNAc modification of proteins, including alpha-tubulin. Am J Physiol Endocrinol Metab 284(2):E424–E434

Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ (2001) TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 412(6844):346–351

Wang J, Torii M, Liu H, Hart GW, Hu ZZ (2011) dbOGAP—an integrated bioinformatics resource for protein O-GlcNAcylation. BMC Bioinforma 12:91

Wang Z, Gucek M, Hart GW (2008) Cross-talk between GlcNAcylation and phosphorylation: site-specific phosphorylation dynamics in response to globally elevated *O*-GlcNAc. Proc Natl Acad Sci U S A 105(37):13793–13798

Wang Z, Pandey A, Hart GW (2007) Dynamic interplay between *O*-linked *N*-acetylglucosaminylation and glycogen synthase kinase-3-dependent phosphorylation. Mol Cell Proteomics 6(8):1365–1379

Wang Z, Udeshi ND, O'Malley M, Shabanowitz J, Hunt DF, Hart GW (2010a) Enrichment and site mapping of *O*-linked *N*-acetylglucosamine by a combination of chemical/enzymatic tagging, photochemical cleavage, and electron transfer dissociation mass spectrometry. Mol Cell Proteomics 9(1):153–160

Wang Z, Udeshi ND, Slawson C, Compton PD, Sakabe K, Cheung WD, Shabanowitz J, Hunt DF, Hart GW (2010b) Extensive crosstalk between *O*-GlcNAcylation and phosphorylation regulates cytokinesis. Sci Signal 3(104):ra2

Wells L, Gao Y, Mahoney JA, Vosseller K, Chen C, Rosen A, Hart GW (2002a) Dynamic *O*-glycosylation of nuclear and cytosolic proteins: further characterization of the nucleocytoplasmic beta-*N*-acetylglucosaminidase, *O*-GlcNAcase. J Biol Chem 277(3):1755–1761

Wells L, Kreppel LK, Comer FI, Wadzinski BE, Hart GW (2004) *O*-GlcNAc transferase is in a functional complex with protein phosphatase 1 catalytic subunits. J Biol Chem 279(37):38466–38470

Wells L, Vosseller K, Cole RN, Cronshaw JM, Matunis MJ, Hart GW (2002b) Mapping sites of *O*-GlcNAc modification using affinity tags for serine and threonine post-translational modifications. Mol Cell Proteomics 1(10):791–804

Whelan SA, Dias WB, Thiruneelakantapillai L, Lane MD, Hart GW (2010) Regulation of insulin receptor substrate 1 (IRS-1)/AKT kinase-mediated insulin signaling by *O*-linked beta-*N*-acetylglucosamine in 3T3-L1 adipocytes. J Biol Chem 285(8):5204–5211

Whelan SA, Lane MD, Hart GW (2008) Regulation of the *O*-linked beta-*N*-acetylglucosamine transferase by insulin signaling. J Biol Chem 283(31):21411–21417

Whitworth GE, Macauley MS, Stubbs KA, Dennis RJ, Taylor EJ, Davies GJ, Greig IR, Vocadlo DJ (2007) Analysis of PUGNAc and NAG-thiazoline as transition state analogues for human *O*-GlcNAcase: mechanistic and structural insights into inhibitor selectivity and transition state poise. J Am Chem Soc 129(3):635–644

Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB (2001) Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. Shock 16(5):398–402

Wrabl JO, Grishin NV (2001) Homology between *O*-linked GlcNAc transferases and proteins of the glycogen phosphorylase superfamily. J Mol Biol 314(3):365–374

Wu SL, Huhmer AF, Hao Z, Karger BL (2007) On-line LC-MS approach combining collision-induced dissociation (CID), electron-transfer dissociation (ETD), and CID of an isolated charge-reduced species for the trace-level characterization of proteins with post-translational modifications. J Proteome Res 6(11):4230–4244

Xie W, Chung KK (2012) Alpha-synuclein impairs normal dynamics of mitochondria in cell and animal models of Parkinson's disease. J Neurochem 122(3):404–414

Xing D, Feng W, Not LG, Miller AP, Zhang Y, Chen YF, Majid-Hassan E, Chatham JC, Oparil S (2008) Increased protein *O*-GlcNAc modification inhibits inflammatory and neointimal responses to acute endoluminal arterial injury. Am J Physiol Heart Circ Physiol 295(1):H335–H342

Xu SL, Chalkley RJ, Wang ZY, Burlingame AL (2012) Identification of *O*-linked beta-D-*N*-acetylglucosamine-modified proteins from *Arabidopsis*. Methods Mol Biol 876:33–45

Yang S, Zou LY, Bounelis P, Chaudry I, Chatham JC, Marchase RB (2006a) Glucosamine administration during resuscitation improves organ function after trauma hemorrhage. Shock 25(6):600–607

Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, Kim YS, Cho JW (2006b) Modification of p53 with *O*-linked *N*-acetylglucosamine regulates p53 activity and stability. Nat Cell Biol 8(10):1074–1083

Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, So WV, Kudlow JE, Michell RH, Olefsky JM, Field SJ, Evans RM (2008) Phosphoinositide signalling links *O*-GlcNAc transferase to insulin resistance. Nature 451(7181):964–969

Yang YR, Song M, Lee H, Jeon Y, Choi EJ, Jang HJ, Moon HY, Byun HY, Kim EK, Kim DH, Lee MN, Koh A, Ghim J, Choi JH, Lee-Kwon W, Kim KT, Ryu SH, Suh PG (2012) *O*-GlcNAcase is essential for embryonic development and maintenance of genomic stability. Aging cell 11(3):439–448

Yuzwa SA, Macauley MS, Heinonen JE, Shan X, Dennis RJ, He Y, Whitworth GE, Stubbs KA, McEachern EJ, Davies GJ, Vocadlo DJ (2008) A potent mechanism-inspired *O*-GlcNAcase inhibitor that blocks phosphorylation of tau in vivo. Nat Chem Biol 4(8):483–490

Yuzwa SA, Shan X, Macauley MS, Clark T, Skorobogatko Y, Vosseller K, Vocadlo DJ (2012) Increasing *O*-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. Nat Chem Biol 8(4):393–399

Zachara NE, Cheung WD, Hart GW (2004a) Nucleocytoplasmic glycosylation, *O*-GlcNAc: identification and site mapping. Methods Mol Biol 284:175–194

Zachara NE, Molina H, Wong KY, Pandey A, Hart GW (2011a) The dynamic stress-induced "*O*-GlcNAc-ome" highlights functions for *O*-GlcNAc in regulating DNA damage/repair and other cellular pathways. Amino Acids 40(3):793–808

Zachara NE, O'Donnell N, Cheung WD, Mercer JJ, Marth JD, Hart GW (2004b) Dynamic *O*-GlcNAc modification of nucleocytoplasmic proteins in response to stress. A survival response of mammalian cells. J Biol Chem 279(29):30133–30142

Zachara NE, Vosseller K, Hart GW (2011b) Detection and analysis of proteins modified by *O*-linked N-acetylglucosamine. Curr Protoc Mol Biol Chapter 17:Unit 17 16. doi:10.1002/0471142727.mb1706s95

Zachara NE, Vosseller K, Hart GW (2011c) Detection and analysis of proteins modified by *O*-linked *N*-acetylglucosamine. Curr Protoc Mol Biol Chapter 12:Unit 8. doi:10.1002/0471140864.ps1208s66.

Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE (2003) *O*-GlcNAc modification is an endogenous inhibitor of the proteasome. Cell 115(6):715–725

Zhang T, Miyamoto S, Brown JH (2004) Cardiomyocyte calcium and calcium/calmodulin-dependent protein kinase II: friends or foes? Recent Prog Horm Res 59:141–168

Zou L, Yang S, Champattanachai V, Hu S, Chaudry IH, Marchase RB, Chatham JC (2009) Glucosamine improves cardiac function following trauma-hemorrhage by increased protein *O*-GlcNAcylation and attenuation of NF-{kappa}B signaling. Am J Physiol Heart Circ Physiol 296(2):H515–H523

Zou L, Yang S, Hu S, Chaudry IH, Marchase RB, Chatham JC (2007) The protective effects of PUGNAc on cardiac function after trauma-hemorrhage are mediated via increased protein *O*-GlcNAc levels. Shock 27(4):402–408