Regulation of Protein Degradation by O-GlcNAcylation: Crosstalk with Ubiquitination*

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The post-translational modification of intracellular proteins by O-linked N-acetylglucosamine (O-GlcNAc) regulates essential cellular processes such as signal transduction, transcription, translation, and protein degradation. Misfolded, damaged, and unwanted proteins are tagged with a chain of ubiquitin moieties for degradation by the proteasome, which is critical for cellular homeostasis. In this review, we summarize the current knowledge of the interplay between O-GlcNAcylation and ubiquitination in the control of protein degradation. Understanding the mechanisms of action of O-GlcNAcylation in the ubiquitin-proteosome system shall facilitate the development of therapeutics for human diseases such as cancer, metabolic syndrome, and neurodegenerative diseases. ***Molecular & Cellular Proteomics 12: 10.1074/mcp.R113.029751, 3489–3497, 2013.***

THE UBIQUITIN-PROTEASOME SYSTEM

Ubiquitination is a post-translational process by which ubiquitin is covalently attached to the lysine residues of target proteins. Ubiquitin is an 8.5-kDa protein that exists in all eukaryotic cells (1). It is encoded in mammals by four genes. The UBB and UBC genes encode polyubiquitin precursors, whereas UBA52 and RPS27A genes encode fusion proteins composed of a ubiquitin and the ribosomal proteins L40 and S27a, respectively (2). The ubiquitination process consists a cascade of reactions: (i) activation of ubiquitin by the ubiquitin-activating enzyme (E1), (ii) transfer of ubiquitin from E1 to a ubiquitin-conjugating enzyme (E2), and (iii) recognizing target proteins and mediating transfer of ubiquitin from E2 to the target by a ubiquitin ligase (E3) (3).

Polyubiquitination (at least four subunits) through lysine 48 (K48) of ubiquitin normally marks target proteins for proteasomal degradation. The ubiquitin-proteasome system (UPS)[1] is the key machinery by which cells dispose of misfolded and damaged proteins in order to maintain cellular homeostasis. In addition, monoubiquitination through K48 or polyubiquitination through other lysine residues of ubiquitin regulates distinct cellular processes, including subcellular localization, endocytosis, and enzymatic activity (4, 5).

Crosstalk between different types of post-translational modifications (PTMs) encodes a wealth of biological information. It is known that ubiquitination and other forms of PTMs are mutually regulated. A large body of evidence shows that phosphorylation and ubiquitination are connected either positively or negatively (6, 7). Regulatory crosstalk between lysine acetylation and ubiquitination has been shown to control protein stability (8). Crosstalk between histone methylation and ubiquitination is involved in gene expression and protein stability (9).

O-GlcNAc Modification and Its Interplay with Other PTMs—Thousands of cytoplasmic and nuclear proteins are modified by a single O-linked β-N-acetylglucosamine (O-GlcNAc) moiety at serine (S) or threonine (T) residues, termed O-GlcNAcylation (10, 11). O-GlcNAcylation is catalyzed by O-GlcNAc transferase (OGT), whereas the reverse reaction is mediated by O-GlcNAcase (OGA, NCOAT, or MGEA5). UDP-GlcNAc, the donor substrate for O-GlcNAcylation, is derived from extracellular glucose through the hexosamine biosynthetic pathway. Because UDP-GlcNAc and protein O-GlcNAc levels in the cell fluctuate with the availability of glucose, free fatty acids, uridine, and the amino acid glutamine, O-GlcNAc is proposed as a nutrient sensor and metabolic regulator (12, 13). This dynamic and reversible modification is emerging as a key regulator of diverse cellular processes, such as signal transduction, transcription, translation, and cytoskeletal functions (14–16). Aberrant O-GlcNAcylation has been implicated in a spectrum of human diseases, including diabetes, cancer, cardiovascular disease, and Alzheimer disease.

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[1] The abbreviations used are: ChREBP, carbohydrate-response element-binding protein; CK2, casein kinase 2; CRTC2, cAMP-response element-binding-protein-regulated transcription coactivator; DUB, deubiquitinase; ERβ, estrogen receptor β; HCF-1, host cell factor C1; O-GlcNAc, O-linked β-N-acetylglucosamine; OGT, O-GlcNAc transferase; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator 1-α; PTM, post-translational modification; UPS, ubiquitin-proteasome system.

Since its discovery in 1984, O-GlcNAcylation has been extensively studied in relationship with phosphorylation (10, 17). Interplay between O-GlcNAcylation and other PTMs is emerging as an important area of investigation. It has been shown that OGT overexpression alters the acetylation and methylation of histones and the activity of an arginine methyltransferase, CARM1 (18). Allison *et al.* show that O-GlcNAcylation of RelA at T305 is required in order for p300-mediated acetylation at K310 to fully activate NF-κB transcription (19). O-GlcNAcylation of a histone lysine methyltranferase, MLL5, promotes methylation of H3K4 to facilitate retinoic-acid-induced granulopoiesis (20). Recent studies reveal that the ten-eleven translocation proteins TET2 and TET3 form a complex with OGT that sustains H3K4 methylation through O-GlcNAcylating host cell factor C1 (HCF-1), a component of the H3K4 methyltransferase SET1/COMPASS complex (21). Meanwhile, a growing body of evidence demonstrates that O-GlcNAcylation regulates mono- and polyubiquitination, protein stability, and proteasome function, which is the focus of this review.

O-GlcNAcylation Regulates Protein Ubiquitination via Phosphorylation—Because O-GlcNAcylation can affect phosphorylation (10) and phosphorylation can regulate ubiquitination (6), it is conceivable that O-GlcNAcylation controls protein ubiquitination and stability through interplay with phosphorylation (Table I, Fig. 1*A*), as exemplified below.

p53—The expression of the tumor suppressor p53 is tightly controlled by proteasomal degradation so as to maintain low levels under normal conditions and rapidly accumulate upon DNA damage (22). The fate of p53 is dictated by a variety of PTMs, including phosphorylation, acetylation, methylation, ubiquitination, and O-GlcNAcylation (23). Cho and colleagues demonstrate that the treatment of MCF-7 cells with an OGA inhibitor increases the level of O-GlcNAcylated p53 and decreases cell viability (24). O-GlcNAcylation of p53 at S149 inhibits phosphorylation at T155 by the COP9 signalosome, thereby reducing p53 ubiquitination and degradation.

Δ-*lactoferrin*—Δ-lactoferrin is a transcription factor that induces cell cycle arrest by up-regulating the expression of genes including *Skp1*, *DcpS*, and *Bax* (25). Δ-lactoferrin expression is down-regulated in cancer cells, whereas its high-level expression is correlated with a good prognosis in human breast cancer (25). It has been shown that Δ-lactoferrin is reciprocally O-GlcNAcylated and phosphorylated at S10 (26, 27). O-GlcNAcylation stabilizes Δ-lactoferrin and retains a basal level of transcriptional activity. Upon activation, Δ-lactoferrin is phosphorylated at S10, which promotes transcription and subsequent degradation through K379 polyubiquitination (27). These studies point to the idea that protein functions can be precisely controlled by dynamic and coordinated changes in O-GlcNAcylation, phosphorylation, and ubiquitination.

Snail1—The zinc-finger protein Snail1 regulates epithelial-mesenchymal transition and tumor progression by repressing the transcription of E-cadherin, a major component of cell

TABLE I
List of proteins for which stability is regulated by O-GlcNAc signaling

Protein	O-GlcNAc site	Expression and stability	Ubiquitination	Mechanism	Function	Reference
p53	S149	Increased by O-GlcNAc	Decreased by O-GlcNAc	Reduce phosphorylation at T155	Cancer	(24)
Δ-lactoferrin	S10	Increased by O-GlcNAc	Decreased by O-GlcNAc	Compete with phosphorylation at S10	Cell cycle arrest and apoptosis	(27)
Snail1	S112	Increased by O-GlcNAc	Decreased by O-GlcNAc	Block phosphorylation	Epithelial-mesenchymal transition	(30)
ERβ	S16	Increased by O-GlcNAc	N/D	Compete with phosphorylation at S16	Transcription	(32)
CK2α	S347	Reduced by O-GlcNAc	N/D	Reduce phosphorylation at T344	Cell proliferation	(34)
CRTC2	S70, S171	N/D	N/D	Compete with phosphorylation at S70, S171	Gluconeogenesis	(36, 37)
PGC-1α	S333	Increased by O-GlcNAc	Decreased by O-GlcNAc	Recruit BAP1	Gluconeogenesis	(13)
Clock	Site N/D	Increased by O-GlcNAc	Decreased by O-GlcNAc	Recruit BAP1	Circadian rhythm	(46)
Bmal1	S418	Increased by O-GlcNAc	Decreased by O-GlcNAc	Recruit BAP1	Circadian rhythm	(45, 46)
ChREBP	Site N/D	Increased by O-GlcNAc	Decreased by O-GlcNAc		Lipogenesis	(49, 50)
Keratins 8/18 (K8/18)	K8 (N/D) K18 (S29/S30/S48)	Reduced by O-GlcNAc	Increased by O-GlcNAc		Filament architecture	(53)
A20	Site N/D	Reduced by high glucose	Increased by high glucose		Atherosclerosis	(54)
β-catenin	Site N/D	Increased by HBP flux	N/D		Cell proliferation	(55)
p67eIF2	S60/T62/S63	Increased by O-GlcNAc	N/D		Protein synthesis	(56)
FoxM1	Not O-GlcNAcylated	Increased by OGT	N/D		Breast cancer	(57)
Plakoglobin	Site N/D	Increased by OGT	N/D		Cell–cell adhesion	(58)
Sp1	Site N/D	Reduced upon starvation	N/D		Transcription	(59)
Nkx2.5	Site N/D	Reduced by OGA inhibitors	N/D		Diabetic cardiomyopathy	(60)
SirT1	Not known whether O-GlcNAcylated	Cytosolic level decreased by HBP	N/D		β cell apoptosis	(61)

N/D, not determined.

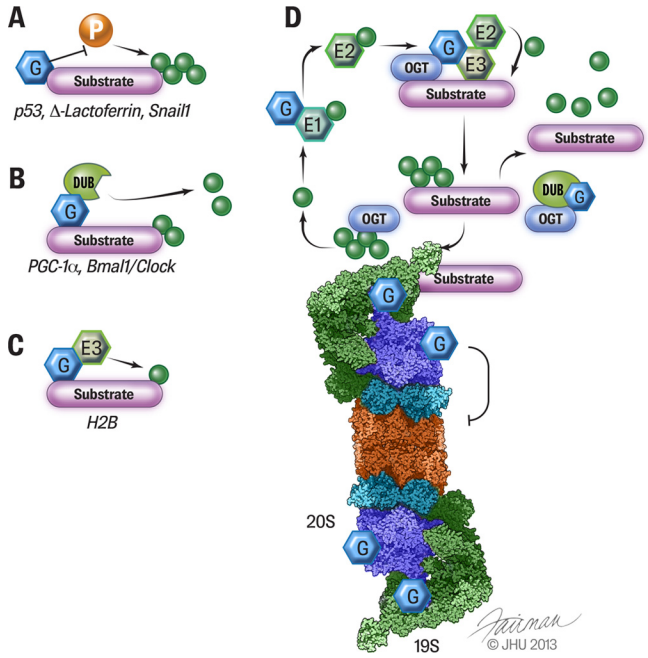

FIG. 1. **Modes of interaction between O-GlcNAcylation and ubiquitination.** *A*, O-GlcNAcylation of the substrate antagonizes phosphorylation at the same or adjacent site to control ubiquitination. *B*, O-GlcNAcylation provides a docking site for DUBs that deubiquitinate the substrate. *C*, O-GlcNAcylation of H2B recruits an E3 complex to monoubiquitinate H2B. *D*, targeting the ubiquitin-proteasome system. OGT physically interacts with ubiquitin precursors, E3s, and DUBs. O-GlcNAcylation has been found on E1s, E3s, DUBs, and 19S and 20S proteasomes.

adhesion junctions (28). It has been shown that phosphorylation of Snail1 by casein kinase 1 and glycogen synthase kinase-3β promotes the ubiquitination and proteasomal degradation of Snail1 (29). Park *et al.* show that O-GlcNAcylation of Snail1 at S112 decreases glycogen synthase kinase-3β-mediated phosphorylation and increases the stability of the protein (30). Consistently, the pharmacological inhibition of OGA by PUGNAc increases the half-life of Snail1 by inhibiting ubiquitination. In response to hyperglycemia, O-GlcNAc modification of Snail1 down-regulates E-cadherin transcription and therefore promotes cell migration and invasive programs (30).

Estrogen Receptor β—The nuclear receptor estrogen receptor β (ERβ) mediates many aspects of estrogen action, including reproduction, inflammation, behavior, and energy metabolism (31). Cheng and Hart demonstrate that ERβ is reciprocally modified by O-GlcNAcylation and phosphorylation at S16. The S16A mutant devoid of both modifications reduces ERβ turnover, whereas the S16E mutant mimicking constitutive phosphorylation has an increased turnover rate (32). A simple interpretation of these results is that O-GlcNAcylation protects the S16 site from phosphorylation and Pro-Glu-Ser-Thr region–mediated proteasomal degradation. However, whether this process involves the ubiquitination of ERβ remains unclear.

TABLE II
Putative OGT-binding proteins involved in ubiquitination

Family	Symbol	Name
Ubiquitin	RPS27A	Ubiquitin and ribosomal protein S27a precursor
Ubiquitin	UBA52	Ubiquitin and ribosomal protein L40 precursor
Ubiquitin	UBB	Ubiquitin B precursor
Ubiquitin	UBC	UBC ubiquitin C
E3[a]	DDB1	DNA damage-binding protein 1
E3	HUWE1	HECT, UBA, and WWE domain containing 1, E3 ubiquitin-protein ligase
E3	MYCBP2	MYC binding protein 2, E3 ubiquitin-protein ligase
E3	PRPF19	Pre-mRNA-processing factor 19
DUB[b]	BAP1	BRCA1 associated protein 1
DUB	OTUD4	OTU domain-containing protein 4
DUB	PRPF8	Pre-mRNA-processing-splicing factor 8
DUB	USP9X	Ubiquitin specific protease 9

Data from Ruan *et al.* (13).
[a] Putative E3s are annotated by Li *et al.* (85).
[b] The inventory of DUBs is described by Nijman *et al*. (86).

Casein Kinase 2-α—Casein kinase 2 (CK2) is a serine/threonine protein kinase that has been implicated in cell proliferation, DNA repair, circadian rhythm, and other cellular processes (33). Tarrant *et al.* demonstrate that phosphorylation of the catalytic subunit of CK2α at T344 increases protein stability by promoting the interaction with Pin1 (34). Moreover, the proximal S347 is modified by O-GlcNAc, which antagonizes T344 phosphorylation and leads to proteasomal degradation of CK2α (34). It will be interesting to explore the role of ubiquitination of CK2α in this context.

cAMP-response Element-binding-protein-regulated Transcription Coactivator 2—cAMP-response element-binding-protein-regulated transcription coactivator 2 (CRTC2) is a transcriptional coactivator for cAMP-response element-binding protein and an important regulator of gluconeogenesis in the liver (35). Under fasting conditions, glucagon induces dephosphorylation of CRTC2 at S171, resulting in CRTC2 translocation into the nucleus to activate the transcription of gluconeogenic genes (35). During feeding, insulin activates the Ser/Thr kinase SIK2 to phosphorylate CRTC2 at S171. Subsequently, phosphorylated CRTC2 translocates to the cytoplasm and undergoes ubiquitination-dependent degradation (36). Dentin *et al.* reveal that O-GlcNAcylation of CRTC2 at S70 and S171 competes with phosphorylation to suppress cytoplasmic sequestration, thereby contributing to hyperglycemia-induced hepatic gluconeogenesis (37). Whether O-GlcNAcylation has a direct effect on CRTC2 ubiquitination and stability warrants further investigation.

O-GlcNAcylation Stabilizes Proteins by Recruiting Deubiquitinase—Using a proteomic approach, Ruan *et al.* recently identified a large number of putative OGT-binding proteins. Many proteins in the ubiquitination pathway, including ubiq-

uitin precursors, E3 ubiquitin ligases, and deubiquitinases (DUBs), are enriched (Table II). Although the functions of these interactions have not been determined, these findings raise the possibility that O-GlcNAc signaling directly modulates the ubiquitin system (13).

Peroxisome Proliferator-activated Receptor Gamma Co-activator 1-α—Peroxisome proliferator-activated receptor gamma co-activator 1-α (PGC-1α) is a key transcriptional cofactor that promotes mitochondrial biogenesis and hepatic gluconeogenesis. It integrates multiple metabolic signals and is extensively regulated by PTMs, including phosphorylation, methylation, acetylation, ubiquitination, and O-GlcNAcylation (38). Ruan *et al.* demonstrate that OGT forms a glucose-sensitive complex with HCF-1 (13). As a scaffold protein, HCF-1 recruits OGT to O-GlcNAcylate PGC-1α at S333. BAP1 is a DUB known to interact with HCF-1 through an HCF-1 binding motif (39, 40). Ruan *et al.* further show that O-GlcNAcylation of PGC-1α facilitates the recruitment of BAP1 that deubiquitinates and stabilizes PGC-1α. This is the first demonstration that O-GlcNAcylation regulates protein ubiquitination and stability through a DUB. Diabetic animals have increased levels of HCF-1 and BAP1 in the liver, which is associated with increased PGC-1α levels and gluconeogenesis. Knockdown of OGT and HCF-1 improves glucose metabolism in diabetic *db*/*db* mice (13). This study elucidates the role of the antagonism between O-GlcNAcylation and ubiquitination as a regulatory mechanism governing metabolic homeostasis.

BMAL1/CLOCK—The circadian clock functions to align physiological and behavioral processes with daily environmental cycles (41). The molecular clock involves a transcriptional feedback loop in which BMAL1 and CLOCK activate the *Period* (*Per1*, *-2*, and *-3*) and *Cryptochrome* (*Cry1* and *-2*) genes. PERs and CRYs accumulate rhythmically and form an inhibitory complex against BMAL1/CLOCK to repress their own transcription (42). The pace of the clock is controlled by various regulatory mechanisms, including PTMs of core clock proteins (43).

The core clock proteins, including BMAL1, CLOCK, PER, and CRY, have been shown to be modified by O-GlcNAc in *Drosophila* and mammals (44–47). BMAL1 and CLOCK are rhythmically modified and stabilized by O-GlcNAcylation (45, 46). Li *et al.* further demonstrate that the OGT–BAP1 complex O-GlcNAcylates and deubiquitinates BMAL1 and CLOCK to control the amplitude of circadian oscillation in response to nutrient availability. Disruption of O-GlcNAc signaling in mouse liver perturbs the diurnal rhythm of glucose metabolism (46).

Taken together, the studies by Ruan *et al.* and Li *et al.* define the OGT–HCF-1–BAP1 complex as a key modulator of ubiquitination, suggesting a novel mechanism by which O-GlcNAcylation controls protein stability (13, 46) (Fig. 1*B*).

O-GlcNAcylation Regulates Protein Ubiquitination via Unknown Mechanisms—

Carbohydrate-response Element-binding Protein—Carbohydrate-response element-binding protein (ChREBP) is a basic helix-loop-helix leucine zipper transcription factor that regulates glucose and lipid metabolism in a glucose-dependent manner (48). Under low glucose conditions, phosphorylated, inactive ChREBP primarily resides in the cytoplasm. High glucose levels trigger ChREBP dephosphorylation at S196 by PP2A and translocation into the nucleus, followed by dephosphorylation at T666 to induce the transcriptional activity of ChREBP. However, dephosphorylation at these sites is not sufficient for the constitutive activation of ChREBP, suggesting additional layers of regulation (48). Guinez *et al.* show that ChREBP is modified by O-GlcNAc, which stabilizes ChREBP to increase the transcription of lipogenic genes (49). OGT overexpression induces lipogenesis by increasing ChREBP levels, whereas OGA overexpression prevents hepatic steatosis in *db*/*db* mice (49). Recently, another study showed that FoxO1 reduces ChREBP stability by inhibiting O-GlcNAcylation and promoting ubiquitination of the protein (50). How O-GlcNAcylation of ChREBP affects its ubiquitination is not known.

Keratins 8 and 18—Type I and II keratin proteins are expressed in specific pairs in various tissues during development and differentiation (51). Keratin pair 8/18 is widely studied in terms of the regulation of protein interaction, ubiquitination, and filament organization by phosphorylation (52). Keratins 8 and 18 are also highly O-GlcNAcylated (53). O-GlcNAcylation increases keratin 8/18 solubility, ubiquitination, and proteasomal degradation. O-GlcNAc-deficient keratin 18 is more stable and causes changes in the filament architecture. There does not seem to be a reciprocal relationship between O-GlcNAcylation and phosphorylation of keratin 8/18 *in vivo*. The mechanism by which O-GlcNAcylation increases ubiquitination is still a mystery (53).

A20—The zinc-finger protein A20 is a negative regulator of NF-κB signaling that has been shown to suppress apoptosis and inflammation. Shrikhande *et al.* reported that hyperglycemia promotes the O-GlcNAcylation, ubiquitination, and degradation of A20, which accelerate atherosclerosis in diabetic mice (54). Interestingly, A20 has both ubiquitin ligase and deubiquitinase activities, suggesting that regulation of the A20 protein level by O-GlcNAcylation is a control point for the ubiquitination of A20 target proteins.

As listed in Table I, the hexosamine/O-GlcNAc pathway can modulate the stability of many other proteins either positively or negatively. For example, O-GlcNAc signaling increases the stability of β-catenin (55), p67eIF2 (56), FoxM1 (57), plakoglobin (58), and Sp1 (59) but decreases the stability of Nkx2.5 (60) and cytosolic SirT1 (61). However, whether the UPS is involved in the regulation of these proteins has not been determined.

O-GlcNAcylation Facilitates Monoubiquitination—Monoubiquitination is a dynamic and reversible PTM involved in nonproteolytic functions (62). Histones are well-known targets

of monoubiquitination. H2B monoubiquitination at K120 has been shown to regulate transcription initiation and elongation (62). Several recent studies suggest that O-GlcNAc is also part of the histone code (63–67). Therefore, it is appealing to determine the interactions between O-GlcNAcylation and other histone markers. Fujiki *et al.* have demonstrated that O-GlcNAcylation of H2B at S112, which is sensitive to glucose availability, promotes K120 monoubiquitination by anchoring the BRE1A/1B complex, an E3 ligase (Fig. 1*C*) (64). Genome-wide analysis reveals that H2B S112 O-GlcNAcylation is associated with transcribed gene loci, many of which overlap with K120 monoubiquitination (64). It will be interesting to determine whether O-GlcNAcylation regulates the monoubiquitination of non-histone proteins.

O-GlcNAcylation Regulates the Ubiquitination Process—The preceding sections outline individual proteins that are covalently modified and regulated by O-GlcNAcylation and ubiquitination. There is also evidence that O-GlcNAcylation modulates global ubiquitination (68). Thermal stress induces a rapid increase in both O-GlcNAcylation and ubiquitination. Increasing O-GlcNAc levels via glucosamine or PUGNAc promotes ubiquitination, whereas decreasing O-GlcNAc levels via forskolin, glucose deprivation, or OGT knockdown reduces ubiquitination (68). However, increasing ubiquitination levels via the proteasome inhibitor has no obvious effect on global O-GlcNAcylation. These results suggest that O-GlcNAcylation affects ubiquitination, but not vice versa. The authors also found that the E1 enzyme Uba1 is O-GlcNAcylated, suggesting the possible regulation of protein ubiquitination by E1 O-GlcNAcylation (68).

A large number of O-GlcNAcylated proteins/peptides and O-GlcNAcylation sites have been discovered using proteomic approaches. We searched published O-GlcNAcylated protein datasets for those involved in the ubiquitination process including ubiquitin precursors, E1s, E2s, E3s, and DUBs, and we found that many of theses proteins are modified by O-GlcNAc (Table III). Although the functions of O-GlcNAcylation of these proteins have not been fully characterized, it is conceivable that O-GlcNAcylation regulates protein ubiquitination through multiple nodes of the UPS (Fig. 1*D*).

O-GlcNAc Signaling Modulates Proteasome Activity—The 26S proteasome is responsible for the destruction of polyubiquitinated proteins. In addition to its roles in regulating protein ubiquitination, O-GlcNAc has long been proposed to directly modulate proteasome activity. Both the 20S catalytic particle and the 19S regulatory particle of the proteasome are known to be O-GlcNAcylated (69). Zhang *et al.* demonstrated that O-GlcNAcylation of Rpt2 ATPase in the 19S proteasome inhibits proteasome function, which may serve as a mechanism controlling cellular levels of amino acids in response to metabolic changes such as starvation and nutrient overload (70) (Fig. 1*D*). It should be noted that many of the studies listed in Table I were performed using indiscriminate approaches (*i.e.* OGT overexpression or OGA inhibition) that affect global O-GlcNAc levels. Thus, the stabilization of certain proteins by O-GlcNAc signaling could be, at least in part, attributed to proteasomal inhibition (Table I).

The short form of O-GlcNAcase (OGA-S), which lacks the histone acetyltransferase domain, accumulates on the surface of lipid droplets (71). Selective knockdown of OGA-S results in global proteasome inhibition and increased levels of perilipin-2 and -3. These findings suggest that proteasomal modulation links O-GlcNAc signaling to lipid droplet maturation (71).

O-GlcNAcylation and Ubiquitination in Physiology and Pathogenesis—During starvation, increased proteasomal degradation in the muscle supplies amino acids to the liver for *de novo* glucose synthesis (gluconeogenesis). In the starved muscle, reduced O-GlcNAc signaling promotes the ability of the proteasome to degrade polyubiquitinated proteins (72). Meanwhile, O-GlcNAc signaling in the liver promotes gluconeogenesis by deubiquitinating and stabilizing PGC-1α and CRTC2 (13, 37, 73). Thus, O-GlcNAcylation acts in concert with ubiquitination in multiple organs to control glucose metabolism.

Obesity and diabetes are associated with increased O-GlcNAcylation in multiple tissues (73). Glucotoxicity, which is caused by diabetic hyperglycemia, leads to hepatic steatosis, β cell failure, cardiomyopathy, and atherosclerosis. As shown in Table I, O-GlcNAcylation regulates the ubiquitination and/or stability of ChREBP, SirT1, Nkx2.5, and A20. Therefore, glucotoxcity may contribute to metabolic syndrome through aberrant O-GlcNAcylation and ubiquitination of those proteins in various tissues.

Many neurodegenerative diseases are associated with the formation of ubiquitin-conjugated protein aggregates in pathological inclusion bodies. For example, hyperphosphorylated Tau is the main component of neurofibrillary tangles, and amyloid-β is the major component of senile plaques in Alzheimer disease. α-synuclein aggregates form Lewy bodies in Parkinson disease, mutant Huntington proteins form inclusion bodies in Huntington disease, and skein-like inclusions exist in amyotrophic lateral sclerosis (74–77). The common cellular mechanism for these pathological conditions is the defective UPS. O-GlcNAc is also involved in many neurodegenerative diseases (10, 78, 79). O-GlcNAc signaling modulates phosphorylation of Tau and the processing of the amyloid-β precursor protein, and treatment of the OGA inhibitor hinders the progression of Alzheimer disease (78, 80). A recent study shows that O-GlcNAc cycling influences proteasome and autophagy pathways in *C. elegans* models of neurodegenerative diseases (81). Further studies should define the casual role of O-GlcNAcylation and ubiquitination in neurodegeneration, ideally using murine models and human patient samples.

The defective UPS causes the accumulation of misfolded or mutated proteins, which in turn contribute to tumor formation and progression (82). E3 ligases such as Cbl and DUBs such as BAP1 and A20 have been implicated in cancer development (82, 83). Many drugs that target the UPS machinery have

TABLE III
O-GlcNAcylated proteins involved in ubiquitination

Family	Symbol	Description	Species	Site	Reference
E1	Uba1	Ubiquitin activating enzyme 1	Human, Drosophila	N/D	(68, 87, 88)
E3[a]	Cbl	Casitas B-lineage lymphoma proto-oncogene	Human	S601	(89)
	Cnot4	CCR4-NOT transcription complex subunit 4	Mouse	S316, T331, T573	(90, 91)
	Fbxo2	F-box protein 2	Rat	N/D	(92)
	Fbxo41	F-box protein 41	Mouse	S386, T387	(90)
	Hecd1	HECT domain-containing protein 1	Mouse	N/D	(91)
	Hectd1	HECT domain containing 1	Mouse	S1350	(90)
	Herc1	HECT domain and RCC1-like domain-containing protein 1	Mouse	S3011	(90)
	Hic1	Hypermethylated in cancer 1	Human	N/D	(93)
	Huwe1	HECT, UBA, and WWE domain containing 1	Rat	N/D	(92)
	Kcmf1	Differentially expressed in branching tubulogenesis 91	Mouse	S262	(91)
	Nedd4	Neural precursor cell expressed, developmentally down-regulated 4	Mouse	S371, T375	(90, 91, 94)
	Prpf19	Pre-mRNA-processing factor 19	Human	T169	(95)
	Rc3h2	Ring finger and CCCH-type zinc finger domains 2	Mouse	T471, T472, S592, T841 or S844, S901	(90, 91)
	Ring1	RING finger protein 1	Human	N/D	(96)
	Rnf2	RING finger protein 2	Human	N/D	(96)
	Rnf123	Ring finger protein 123	Mouse	S1078	(90)
	Sf3b3	Splicing factor 3b, subunit 3	Human	N/D	(97)
	Sh3rf1	SH3 domain-containing RING finger protein 1	Mouse	T512, T91, T92, S526, T527	(90, 91)
	Trim33	Tripartite motif-containing 33	Mouse	S650	(90)
	Tulp4	Tubby like protein 4	Mouse	T943	(90)
	Ubr4	Ubiquitin protein ligase E3 component n-recognin 4	Mouse	S2577	(90)
	Unkl	RING finger protein unkempt-like	Mouse	S451, T459	(90, 91)
	Zbtb20	Zinc finger and BTB domain containing 20	Mouse	S268, T465, T480	(90)
	Zfp598	Zinc finger protein 598	Mouse	One site among S560, T563, and T564	(90)
DUB[b]	Bap1	BRCA1 associated protein 1	Human	N/D	(96)
	Usp11	Ubiquitin specific peptidase 11	Mouse	Two sites between S617 and S619	(90)
	Usp24	Ubiquitin specific peptidase 24	Mouse	S3	(90)
	Usp31	Ubiquitin specific peptidase 31	Mouse	One site between T1163 and T1165	(90)
	Usp5	Ubiquitin specific peptidase 5	Rat	N/D	(92)
	Usp8	Ubiquitin specific peptidase 8	Mouse	S218 and one site among S227/T231/S233	(90)
	Vcpip1	Valosin containing protein (p97)/p47 complex interacting protein 1	Mouse	T1072, S1075	(90, 91)

[a] Putative E3s are annotated by Li *et al.* (85).
[b] The inventory of DUBs is described by Nijman *et al.* (86).

shown promise in clinical trials of cancer therapy (82). Notably, the oncogene Cbl and the tumor suppressor BAP1 are O-GlcNAcylated (Table III). O-GlcNAc has also been implicated in cancer biology by targeting transcription factors such as p53, c-Myc, Sp1, and NF-κB (73, 84). Many proteins involved in cell proliferation, apoptosis, and adhesion, such as p53, Δ-lactoferrin, Snail1, β-catenin, FoxM1, and Sp1, are regulated by O-GlcNAcylation and ubiquitination (Table I). A better understanding of the regulation of the UPS by O-GlcNAcylation will provide crucial insight into cancer diagnosis and therapy.

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