Chapter 13

The Role of O-Linked β-N-Acetylglucosamine (GlcNAc) Modification in Cell Signaling

Paula Santoyo-Ramos, María Cristina Castañeda-Patlán and Martha Robles-Flores

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/47874

1. Introduction

The modification of serine or threonine residues of nuclear and cytoplasmic proteins by the monosaccharide, β-D-Nacetylglucosamine was discovered in the early 1980s by Torres and Hart [1]. However, relative development in this field has remained sluggish for nearly two decades, mainly due to the lack of tools and techniques for the identification and quantification of O-GlcNAc modification in proteins.

O-GlcNAc is a unique type of intracellular glycan attachment. Protein glycosylation traditionally refers to the covalent attachment of complex oligosaccharides to proteins in intraluminal compartments or cellular membranes, or in proteins that are destined for secretion. In contrast, O-GlcNAcylation is abundant in cytoplasmic and nuclear proteins, which are modified with a single β-*N*-acetylglucosamine monosaccharide moiety through an *O*-β-glycosydic attachment to serine and/or threonine side chains of the polypeptide backbone [2].

Because O-GlcNAc modification in protein occurs at serine/threonine residues, the potential for interplay between serine/threonine phosphorylation and O-GlcNAc modification has been realized very early on. However, unlike phosphorylation, which is regulated by hundreds of kinases and phosphatases, O-GlcNAc cycling has only two mediators: the enzymes O-GlcNAc transferase (OGT), and O-GlcNAc amidase (OGA) [2]. The addition and removal of O-GlcNAc is mediated by the concerted action of these two enzymes. Uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) is the sugar donor for the O-GlcNAc modification, for glycophosphatidylinositol lipids synthesis, N-glycosylation, and other cellular processes.

2. Synthesis of UDP-GlcNAc from glucose via the hexosamine biosynthetic pathway

The synthesis of UDP-GlcNAc from glucose occurs via the hexosamine biosynthetic pathway [3], as depicted in Figure 1. When glucose enters a cell, it becomes phosphorylated by hexokinase, and can be redirected from the main glycolytic/glycogen pathways to secondary pathways. About 2- 5% of intracellular glucose enters the hexosamine biosynthetic pathway (HBP); thus, the amount of protein GlcNAcylation is considered to be sensitive to the nutrient (i.e., glucose and/or glutamine). Glutamine/fructose aminotransferase (GFAT) commits glucose to this pathway, and represents the access to the HBP. This pathway links glycolytic metabolism with the amino acid metabolism via the requirement of glutamine to produce glucosamine-6-phosphate. The HBP pathway culminates with the formation of UDP-GlcNAc, the high-energy donor substrate for the O-GlcNAc transferase. As can be observed in Figure 1, the biosynthesis of UDP-GlcNAc is affected and regulated by nearly every metabolic pathway in the cell, and because OGT-catalyzed O-GlcNAcylation is sensitive to insulin, to nutrients, and to cellular stress, it has been proposed that O-GlcNAcylation serves primarily to modulate cellular signaling and transcription regulatory pathways in response to nutrients and stress [3- 6].

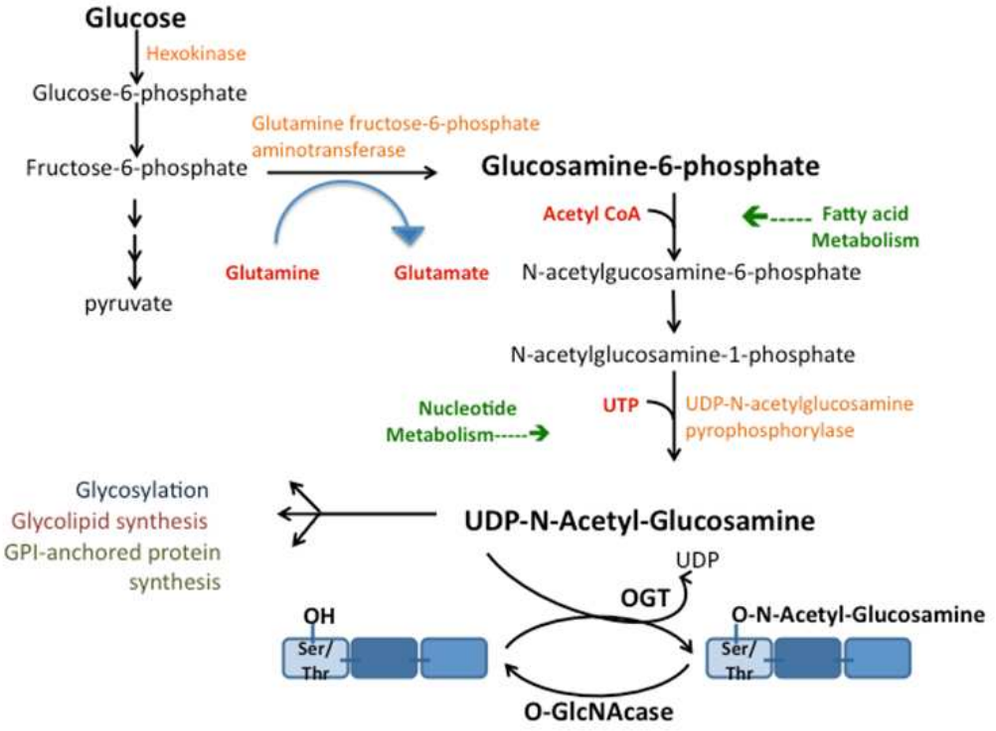

Figure 1. The synthesis of Uridine diphospho-β-N-acetylglucosamine (UDP-GlcNAc) from glucose (Hexosamine biosynthetic pathway [HBP]) and the O-GlcNAcylation cycle. Glucose is metabolized through the HBP to form the high-energy intermediate UDP-GlcNAc, which serves as the three-sugar donor for O-linked N-acetylglucosaminyl transferase (OGT). This enzyme catalyzes the transfer of GlcNAc from UDP-GlcNAc to the OH of serine or threonine residues of a protein substrate through β-glycosydic attachment. O-GlcNAcase hydrolyzes the glycosydic linkage to generate free GlcNAc and naked protein. The enzymes and the biosynthetic pathways involved in the process are depicted.

3. Enzymes that regulate O-GlcNAcylation cycle

The enzymes that catalyze the addition and removal of O-GlcNAc have been cloned and characterized. Similar to phosphorylation, O-linked -N-acetylglucosamine (O-GlcNAc) modification of nuclear and cytosolic proteins is an abundant, dynamic, and inducible posttranslational modification. However, in mammals, only one enzyme attends to the transference of O-GlcNAc: the Uridine diphospho-N-acetylglucosamine polypeptide β-N-acetylglucosaminyltransferase (OGT). This enzyme is regulated by binding partners as well as by posttranslational modification and alternative splicing. OGT interacts with several proteins that appear to target it to different locations inside the cell [4]. OGT is conserved from *Caenorhabditis elegans* to humans and it has been demonstrated that it is required for life because knock-out of the gene encoding OGT is lethal in embryonic mammalian stem cells [4]. The removal of O-GlcNAc is catalyzed by a neutral β-N-acetylglucosaminidase (O-GlcNAcase). The main characteristics of the enzymes involved in the O-GlcNAcylation cycle are the following:

3.1. OGT

The gene encoding OGT has been cloned from several organisms and is well conserved. There is 99% identity between rat and human OGT, and 61% identity between rat and *Caenorhabditis elegans* OGT. In mammalian cells, OGT is encoded by a single copy X-linked gene, while in plants, there are two homologs: spy, and secret agent [7-9].

OGT is expressed in all tissues studied, although it appears to be particularly rich in pancreas, brain, and thymus. OGT is a unique glycosyltransferase because it is a soluble protein rather than a type II membrane protein and has little or no homology to other glycosyltransferases. OGT contains two domains, an N-terminal Tetratricopeptide repeat (TPR) domain, and a C-terminal catalytic domain [7-9]. These domains are separated by a bipartite Nuclear localization sequence (NLS), thus, the enzyme is located predominantly within the nucleus. In mammalian cells, splicing of OGT mRNA leads to alternative transcripts, of which two are well characterized: mitochondrial OGT (mOGT), and nucleocytoplasmic OGT (ncOGT). mOGT has nine TPR repeats and an alternative N-terminus, which contains a mitochondrial targeting sequence.

ncOGT is the most widely studied of the known splice variants. It is a 1,037-amino-acid protein (110 kDa) and was thought to exist predominantly either as a homotrimer comprised of identical 110-kDa subunits or as a heterotrimer comprised of two identical 110-kDa subunits coupled with a 78-kDa subunit. ncOGT was originally isolated from rat liver cytosol, has a pH optimum near 6 (active pH range, 6–7.5), and, unlike many glycosyltransferases, it is not dependent on divalent cations [7].

3.2. O-GlcNAcase

O-GlcNAcase is a soluble, cytosolic β-N-acetylglucosaminidase expressed in all tissues examined and predominantly in brain. O-GlcNAcase is well conserved in mammals, with

97.8% identity between the human and the mouse gene and with 29% identity between the human and the C. elegans gene [10]. In contrast to lysosomal hexosaminidases (A and B), O-GlcNAcase is specific for β-N-acetylglucosamine, is not inhibited by N-acetylgalactosamine, and has a neutral optimum pH of 5.5–7 (lysosomal hexosaminidases have pH ~3.5–5.5). O-GlcNAcase co-purifies with a complex of proteins, is probably regulated by its interactions with other proteins, and is phosphorylated, suggesting an additional mechanism for its regulation [10]. O-GlcNAcase is efficiently inhibited by O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate (PUGNAc; Ki 54 nm). It is a 917-amino-acid protein, with at least two functional domains: an N-terminal hexosaminidase domain, and a C-terminal histone acetyltransferase (HAT) domain [11]. It has been shown that the C-terminal domain of O-GlcNAcase acetylates both free-core histones and nucleosomal histone proteins. Consistent with these data, OGT is known to associate with histone deacetylase complexes and to promote transcriptional silencing and dynamic modification of transcriptional complexes by O-GlcNAc and histone [11].

4. O-GlcNAc-phosphorylation interplay

Glycomic analyses have shown that O-GlcNAcylation has extensive cross-talk with phosphorylation, where it serves as a nutrient/stress sensor to modulate signaling, transcription, and cytoskeletal functions. Nearly all *O*-GlcNAc-modified proteins are also phosphorylated, supporting the notion that interplay exists between the two modifications [3]. Indeed, it has been reported that *O*-GlcNAcylation and phosphorylation both occur in serine and threonine residues in many instances, and that the two modifications can compete for occupancy at the same site or at adjacent residues [12]. Therefore, it has been proposed that a Yin-Yang relationship does exist at the global level as well as at specific sites in several proteins. YinOYang prediction residues refer to S/T residues that can be either phosphorylated or modified by O-GlcNAc (http://www.cbs.dtu.dk/services/YinOYang/). A recently developed Website contains the most up-to-date list of published O-GlcNAc modification sites and an algorithm to predict whether a site might be O-GlcNAcylated (http://cbsb.lombardi.georgetown.edu/hulab/OGAP.html) [3]. However, subsequent studies have identified increasing numbers of O-GlcNAc modification sites that are adjacent or even distal to phosphorylation sites. In addition, pharmacologically inhibiting dephosphorylation does not decrease *O*-GlcNAcylation in all proteins, and inhibition of OGA or overexpression of OGT can produce increases in phosphorylation [13]. Thus, these data suggest a complex interplay between the two modifications. Therefore, although regulatory roles of O-GlcNAc interplay with phosphorylation occur, the binary Yin-Yang model is extremely simplistic and it is noteworthy that different types of interplay between these two posttranslational modifications can take place [12, 13].

Our understanding of the regulatory mechanisms involved in O-GlcNAc modification and its interplay with serine/threonine phosphorylation in proteins remains elusive. This is probably due to the fact that O-GlcNAcylation remained undetected until the early 1980s by commonly used analytical protein methods, including gel electrophoresis and the majority of forms of High-pressure liquid chromatography (HPLC) [3]. For example, addition of the

sugar does not generally affect migration of a polypeptide in gel electrophoresis, or upon isoelectric focusing, or even in high-resolution, two-dimensional gels. In addition, the sugar modification is rapidly hydrolyzed by cellular hexosaminidases upon cellular damage or during protein isolation if countermeasures are not employed [3]. A major breakthrough in the detection and site mapping of O-GlcNAc occurred first with the development of Fourier transform mass spectrometers capable of Electron capture dissociation (ECD), and subsequently with the development of ion-trap mass spectrometers, which could perform Electron transfer dissociation-Mass spectrometry (ETD-MS). These recent successes in O-GlcNAc modification-site mapping in proteins have revealed two important clues: first, nearly all O-GlcNAc modified proteins are known phospho-proteins, and second, the prevalence of tyrosine phosphorylation among O-GlcNAc- modified proteins is exceptionally higher (~68%) than its normal occurrence (~2%) alone [2]. In this respect, Mishra S et al [14] recently reported that tyrosine phosphorylation interacts with O-GlcNAc modification, a phenomenon not previously known [14]. Subsequently, two additional articles were published showing that O-GlcNAc modification of Insulin receptor substrate 1 (IRS1) occurs in close proximity of tyrosine phosphorylation sites and affects the tyrosine phosphorylation-dependent function of IRS1 [15,16].

The extensive cross-talk between O-GlcNAcylation and phosphorylation represents a new paradigm for cellular signaling. Accurate delineation of the complex cross-talk between O-GlcNAcylation and phosphorylation could elucidate the global complex regulatory mechanism vital for cellular functions, such as regulation of cell cycle and cell growth, proliferation, and apoptosis.

5. O-GlcNAcylation in cell signaling and cellular stress

A generalization with respect to the roles of O-GlcNAcylation in cellular signaling has emerged during the past two decades. The primary function of O-GlcNAcylation appears to be the modulation of cellular processes in response to nutrients and to cellular stress [3, 17]. Cells dynamically induce O-GlcNAc protein modification in response to numerous forms of cellular stress, and this appears to be a protective response of cells. For example, blocking the hexosamine biosynthetic pathway ablates glucose-mediated cellular protection [18]. Raising O-GlcNAc levels by either inhibition of O-GlcNAcase or overexpression of OGT rendered cells more tolerant to several forms of stress, such as exposition to ultraviolet (UV) irradiation, ethanol, and sodium arsenite [18].

Many transcription factors are modified by O-GlcNAcylation in response to physiological stimuli, cell cycle stage, and developmental stage, and this modification can modulate their function in different ways [19]. O-linked GlcNAc moieties on transcription factors may be recognized by several components of the transcriptional machinery, serve as a nuclear localization signal, antagonize the action of protein kinases by masking potential serine and threonine sites for phosphorylation, modulate DNA binding activity or half-life, and increase the stability of transcription factors in the cell [19]. Although quantitatively the majority of O-GlcNAc occurs in chromatin proteins, many cytosolic enzymes, including

kinases, glycolytic enzymes, the majority of cytoskeleton regulatory proteins, and the cytoskeleton proteins themselves are also modified [3]. In most cells, OGT is found mainly within the nucleus, and O-GlcNAcase is found mainly within the cytosol. In the following section, we will describe how O-GlcNAcylation affects cellular signaling, using as examples the following four proteins involved in carcinogenesis:

5.1. O-GlcNAc modification of protein kinase C (PKC)

PKC comprises a family of 10 lipid-dependent serine/threonine kinases that play key roles in proliferation and cell cycle progression, differentiation, tumorigenesis, apoptosis, cytoskeletal remodeling, ion-channel modulation, and secretion. Activation of PKC isozymes is dependent on tyrosine-kinase receptors and G-protein-coupled receptors. Based on differences in sequence homology and in biochemical properties, PKC isozymes have been classified into three subfamilies: the conventional PKC isozymes (cPKC) α, βI, βII, and γ, which are dependent on diacylglycerol (DAG) and Ca^{2+} for their activity and which respond to phorbol esters; the novel PKC (nPKC) δ, ε, η, and θ, which are insensitive to Ca^{2+} but DAG-dependent, and the atypical PKC (aPKC) ι/λ and ζ, which neither require Ca^{2+} nor respond to DAG or phorbol esters [20].

It has been reported that increased flux through hexosamine biosynthetic pathway affects the activity and translocation of certain PKC isoforms [21]. We reported for the first time that conventional, novel, and atypical PKC isozymes are all posttranslationally modified by O-GlcNAc [20]. Activation of PKC is generally correlated with its serine-theonine phosphorylation and translocation to cell membranes, but it has been also shown that other posttranslational modifications (PTM), such as tyrosine phosphorylation or tyrosine nitration, may modulate PKC activity [22, 23]

We have investigated the posttranslational modifications induced on PKC isozymes as result of their activation upon exposure of cells to a direct PKC activator (the phorbol ester Tetradecanoyl phorbol acetate [TPA]), or to an extracellular ligand known to activate PKC-dependent pathways [20]. Using freshly isolated rat hepatocytes, we studied the effect of epinephrine on PKC isoforms via activation of α1-adrenergic receptors in comparison with acute TPA treatment. The cells were incubated for 5 min in the absence (vehicle) or presence of 1 μM TPA or 10 μM epinephrine plus 10 μM propranolol (β-adrenergic antagonist). PKC isozymes were immunoprecipitated from cell extracts, and the posttranslational modifications produced on them were analyzed by Western blotting utilizing antibodies that recognizes O-linked GlcNAc in proteins (Affinity Bioreagents, Inc).

We found that posttranslational modifications other than Ser/Thr phosphorylation, such as tyrosine nitration and tyrosine phosphorylation, are commonly present in all PKC isozymes and that they change rapidly and dynamically in response to extracellular stimuli [20]. Our data demonstrated for the first time that all PKC isozymes are also dynamically modified by O-linked β-N-acetylglucosamine (O-GlcNAc); the presence of this modification was confirmed in part by Fourier transformation-Ion cyclotron resonance (FT-ICR) mass

spectrometry analysis, and interestingly, the O-GlcNAc modified Ser or Thr were mapped at similar positions in several PKC isozymes.

General analysis of the collected data showed several interesting things [20]. First, it was noteworthy that O-GlcNAc modification appeared at similar positions in all PKC isoforms, such as the middle part of the molecule. Second, despite the percentage of sequence identity varied between PKC isoforms, the O-GlcNAc-modification was found in different peptides with similar sequences shared by several isozymes, and third, in many instances, the probably modified residue found agreed with the predicted O-GlcNAc potential sites (http://www. cbs.dtu.dk/services/YinOYang/), with YinOYang predictions (S/T residues that can be either phosphorylated or modified by O-GlcNAc), and in the case of PKCε, matched exactly with T710 located at 'turn motif', which is known to be an autophosphorylation 'priming site'. In other instances, the modified residue found did not exactly match the predicted ones, but were mapped near the phosphorylation 'priming sites': T517 in PKC epsilon, located immediately prior to the beginning of 'activation loop', T689 and S690, present at the end of the 'hydrophobic motif' of PKCγ, or S670, present at 'turn motif' in PKCθ, immediately prior to the S676 'priming site'. Therefore, these findings suggest that O-GlcNAcylation and phosphorylation may modulate each other.

The biochemical meaning of these posttranslational modifications for PKC alpha and PKCδ activity was investigated [20]. The results obtained suggested that while Ser/Thr phosphorylation at C terminus and tyrosine phosphorylation status appear to regulate the activation states of both PKCα and PKCδ isozymes, only PKCα activity appears to be regulated as well, in this case, in a negative manner, by tyrosine nitration and O-GlcNAc posttranslational modifications. Thus, our data indicated that phosphorylation status, both in Ser/Thr and in Tyr residues, may regulate the activity of all PKC isozymes, but the biochemical consequences of tyrosine nitration and O-GlcNAc modifications may be different for each PKC isozyme.

5.2. O-GlcNAc modification of c-Myc

c-Myc (v-myc myelocytomatosis viral oncogene homolog), a helix-loop-helix leucine zipper transcription factor, contributes to the development of several malignancies. In a normal resting cell, c-Myc levels are very low. However, upon mitogenic stimulation, c-myc expression levels are highly increased, as well as upon canonical Wnt signaling stimulation.

C-myc heterodimerizes with Max to regulate transcription of proliferation and cell differentiation genes [24]. Cell proliferation requires the coordinated activity of cytosolic and mitochondrial metabolic pathways to provide ATP and building blocks for DNA, RNA, and protein synthesis. Many metabolic pathway genes are targets of the c-myc oncogene and cell-cycle regulator. Morris F et al [25] demonstrated that Myc expression also increased global O-linked N-acetylglucosamine protein modification, and inhibition of hexosamine biosynthesis selectively reduced growth of Myc-expressing cells, suggesting its importance in Myc-induced proliferation.

c-Myc can be phosphorylated by casein kinase II [26] and by Mitogen-activated protein kinase (MAP kinase) [24]. It also has been reported that can be modified by O-GlcNAc [28, 29]. Phosphorylation at Thr-58 and/or Ser-62 in the N-terminal transcription activation/malignant transformation domain (TAD) of c-Myc may modulate transactivation and co-transformation by c-Myc [29].

The O-GlcNAc modification of c-Myc has been identified both in mammalian (rabbit reticulocyte lysate and Chinese hamster ovary [CHO]) cell line and in insect cell systems [19]. This modification of c-Myc was shown by three different methods as follows: (i) demonstration of lectin- binding to in vitro translated protein using a protein-protein interaction mobility-shift assay; (ii) glycosidase or glycosyltransferase treatment of in vitro translated protein analyzed by lectin-affinity chromatography, and (iii) direct characterization of the sugar moieties on purified recombinant protein overexpressed in either insect cells or CHO cells [29]. O-GlcNAc-modified sites within c-Myc were originally found to be located near the transcriptional activation domain [28]. A later study identified Thr-58, an in vivo phosphorylation site in the transactivation domain, as the major site of O-GlcNAc modification [29]. This suggests a mutually exclusive modification of c-Myc by either phosphorylation or O-GlcNAc modification. The transactivation domain of c-Myc associates with the tumor suppressor Retinoblastoma protein Rb and the Rb-related protein p107 in vitro [19, 30]. Thus, the presence of O-GlcNAc modification on c-Myc may result in altered interaction with Rb and Rb-related protein p107, thereby interfering with transactivation by c-Myc. Interestingly, Thr-58 is located within a mutational hot spot in lymphomas, suggesting that this region is associated with increased tumorigenicity [19].

5.3. O-GlcNAc modification of β-catenin

The Wnt pathway plays an important role in development and in regulation of adult stem-cell systems. Deregulation of Wnt signaling causes developmental defects and cancer. Canonical Wnt signaling operates through regulating the phosphorylation and degradation of the transcription co-activator β-catenin [31]. Without stimulation by Wnt, β-catenin is assembled into the so-called “destruction complex” in which Adenomatous polyposis coli (APC) plays a central role, and includes Axin, Glycogen synthase kinase 3 (GSK3)β, and Casein kinase 1 (CK1). This complex directs a series of phosphorylation events on β-catenin that targets it for ubiquitination and subsequent proteolysis via the proteasome. Stimulation by Wnt leads to inhibition of β-catenin breakdown, allowing β-catenin to accumulate, enter the nucleus, and activate a Wnt target gene program [31, 32].

The phosphorylation of β-catenin is key in the regulation of its intracellular levels, hence in its transcriptional activity. Nuclear β-catenin is the hallmark of activated canonical Wnt signaling. Thus, nuclear import/export of β-catenin represents a crucial step in regulating signaling competent β-catenin levels and serves as an attractive target for pharmacological interventions in cancer and other diseases associated with altered Wnt signaling. However, the mechanisms that regulate the nuclear localization of β-catenin remain unclear. β-catenin contains no recognizable NLS; thus, it has been proposed that it is imported by a piggy-back

mechanism. However, it has been demonstrated that β-catenin nuclear import can occur in the absence of transport factors, such as importins or the Ran GTPase [33]. Moreover, β-catenin was found to compete with importin-β for docking to components of the nuclear pore complex. Indeed, the central arm repeats are required for β-catenin import, and these are structurally related with the importin-β HEAT (Huntington, Elongation factor 3, PR65/A, TOR) repeats that bind the nuclear pore complex [34]. With respect to the β-catenin nuclear export, although the precise mechanism is not completely understood, several distinct β-catenin nuclear export pathways have been reported to date.

Sayet et al [35] demonstrated that O-GlcNAcylation of β-catenin negatively regulates its levels in the nucleus. TCF reporter plasmid (TOPflash) reporter assays and mRNA expression of β-catenin's target genes indicated that O-GlcNAcylation of β-catenin results in a decrease in its transcriptional activity. The authors showed also that this novel modification of β-catenin regulates its nuclear localization and transcriptional function. Their results indicated that O-GlcNAcylation of β-catenin is inversely related to its nuclear localization and transcriptional function. This finding highlights O-GlcNAcylation as a new level of regulation of β-catenin transcriptional function.

5.4. O-GlcNAc modification of p53

The tumor suppressor protein p53 is a cell cycle regulator, and mutations in p53 cause cancer. This protein is considered the major 'gatekeeper' of genomic stability. Under conditions of cellular stress, environmental damage or genetic catastrophe, p53 expression and stability increase, followed by the induction of genes that promote cell-cycle arrest, apoptosis, and autophagy [13].

Owing to the pivotal role of p53 in maintaining genomic integrity, p53 is tightly regulated by proteolytic degradation. Normally, p53 levels are low due to continuous degradation by ubiquitin-dependent proteolysis. In unstressed cells, p53 interacts with Mdm2, which acts as an ubiquitin ligase, leading to degradation of p53 by the proteasome [36, 37]. The stability of p53 is also affected by phosphorylation. The amino-terminal domain of the tumor suppressor contains the transactivation domain and several known phosphorylation sites. Phosphorylation at Ser18 and Ser23 promotes p53 stability and tumor suppression, and phosphorylation of p53 at Thr155 (which resides in the DNA-binding domain) promotes p53 degradation by the COP9 signalosome [38].

The mechanism by which O-GlcNAc modification enhances p53 stability has been recently established by the identification of the O-GlcNAc residue within p53 employing mass spectrometry (MS). Activation of p53 by stress involves the O-GlcNAc modification of Ser-149, and modification of this site interferes with phosphorylation at Thr-155 by means of the COP9-associated kinases. Lowering phosphorylation at Thr-155 weakens the interaction of p53 with Mdm2 and decreases p53 ubiquitination/proteolysis, resulting in higher stability of the p53 protein [39]. However, mutating Ser-149 to Ala does not significantly decrease O-GlcNAc modification of p53 [39], suggesting that in addition to Ser-149, there are other residues within p53 that become O-GlcNAc-modified.

6. O-GlcNAcylation and cancer

Altered O-linked GlcNAc modification has been linked with several human diseases, including cardiovascular disease, neurodegenerative disorders, diabetes mellitus, and cancer [13]. Given the extensive cross-talk between O-GlcNAcylation and phosphorylation and the known roles of phosphorylation in mechanisms underlying cancer, it is not surprising that O-GlcNAcylation is also involved in the etiology of cancer.

The unique metabolism of tumors was described many years ago by Otto Warburg, who identified tumor cells with increased glycolysis and decreased mitochondrial activity [40]. Warburg found that normal tissues used mitochondrial oxidation to account for 90% of ATP production, with glycolysis accounting for 10%. However, tumors used less of the highly efficient oxidative phosphorylation, producing < 50% of ATP from oxidation and >50% from glycolysis. This shift was thought to occur even though there was sufficient oxygen to support mitochondrial function and is called ''aerobic glycolysis'' [40].

Aerobic glycolysis is just one component of the metabolic transformation. In order to engage in replicative division, a cell must duplicate its genome, proteins and lipids and assemble the components into daughter cells; in short, it must become a factory for macromolecular biosynthesis. Thus, enhanced biosynthetic capacity is a key feature of the metabolic transformation of tumor cells. Synthesis of nucleotides and fatty acids and consumption of glucose and glutamine are widespread among tumors and tumor cell lines. It appears likely that these activities, particularly the use of glutamine as a source of both reductive power and anaplerosis, are general characteristics of tumor cell growth and proliferation [41]. Glutamine contributes essentially to every core metabolic task of proliferating tumor cells: it participates in bioenergetics, supports cell defenses against oxidative stress, and complements glucose metabolism in the production of macromolecules [41]. Interest in glutamine metabolism has been further heightened by recent findings that c-myc controls glutamine uptake and degradation, and that glutamine itself exerts influence over a number of signaling pathways that contribute to tumor growth [13, 41]. Because HBP flux and UDP-GlcNAc availability are directly affected by many different nutrients, such as glucose, fatty acids, and amino acids, it is possible that in cancer cells, the HBP, together with O-GlcNAc modification, serves as a glucose/nutrient sensor that links metabolism to the activation of many oncogenic signaling pathways within the cell.

Western blot methods showed decreased *O*-GlcNAc levels in some tumor samples compared with controls. However, other studies suggested that *O*-GlcNAc levels increase in some cancers. There are potential sources of confusion from these studies. Crucial tumorigenic roles of specific O-GlcNAcylated proteins might be masked on analyzing only global cellular O-GlcNAcylation. In addition, cellular levels of O-GlcNAc, OGT, and OGA have a tight relationship with multiple cellular activities. Despite conflicting data in primary tumor samples, increased O-GlcNAcylation does appear to be a general characteristic of cancer cells. Histological sections from breast, lung, and colon tumors demonstrated increased O-GlcNAcylation compared with matched adjacent tissue [42]. In the case of the

lung and colon tissue, both OGT and OGA expression appeared to increase. Similarly, in patients with chronic lymphocytic leukemia, the O-GlcNAcylation of proteins was increased when compared with normal lymphocytes [43]. Furthermore, breast cancer cells in which OGT was knocked down and that were transplanted into nude mice formed fewer tumors compared with controls [13].

Chemotherapeutics targeting OGT or OGA inhibition could potentially alter tumor function or could render tumors more susceptible to other chemotherapeutic agents; however, which adverse affects OGT or OGA inhibition might have on normal cells is unknown.

7. Conclusions

Many cytoplasmic and nuclear proteins are dynamically modified by O-linked β-N-acetylglucosamine (O-GlcNAc). However, the precise function that it carries out on each protein remains unknown. Interestingly, O-GlcNAc shares many common traits with O-phosphate. They are both dynamic modifications processed by specific enzymes that modify serine/threonine residues and that rapidly respond to extracellular stimuli. Posttranslational modifications are essential devices to generate the tremendous diversity, complexity, and heterogeneity of gene products. Determination of the in vivo roles that they play is one of the main challenges in proteomics and signal transduction research.

Author details

Paula Santoyo-Ramos, María Cristina Castañeda-Patlán and Martha Robles-Flores*
Department of Biochemistry, Faculty of Medicine, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico

8. References

[1]. 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): 3308-17.

[2] Mishra S, Ande SR, Salter NW (2011). O-GlcNAc modification: why so intimately associated with phosphorylation? Cell Communication and Signaling 9:1-4.

[3] Hart GW, Slawson C, Ramirez-Correa G, and Lagerlof O (2011). Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. Annu. Rev. Biochem 80:825–58.

[4] Ngoh GA, Jones SP (2008). New insights into metabolic signaling and cell survival: the role of beta-O-linkage of N-acetylglucosamine. J Pharmacol Exp Ther. 327(3):602-9.

[5] 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.

* Corresponding Author

[6] 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):16283-92.

[7] Nolte D, Müller U (2002). Human O-GlcNAc transferase (OGT): genomic structure, analysis of splice variants, fine mapping in Xq13.1. Mamm Genome. 13(1):62-4.

[8] 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):9308-15.

[9] 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):9316-24.

[10] Wells L, Gao Y, Mahoney JA, Vosseller K, Chen C, Rosen A, Hart GW (2002). Dynamic O-glycosylation of nuclear and cytosolic proteins further characterization of the nucleocytoplasmic beta-N-acetylglucosaminidase. J. Biol. Chem. 277:1755-61.

[11] Toleman C, Paterson AJ, Whisenhunt TR, Kudlow JE (2004). Characterization of the histone acetyltransferase (HAT) domain of a bifunctional protein with activable OGlcNAcase and HAT activities. J Biol Chem 279: 53665-53673.

[12] Hart GW, Housley MP, Slawson C (2007). Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. Nature 446 (7139):1017-22.

[13] Slawson C, Hart GW (2011). O-GlcNAc signaling: implications for cancer cell biology. Nat Rev Cancer 11: 678-684.

[14] Ande SR, Moulik S, Mishra S (2009). Interaction between O-GlcNAc modification and tyrosine phosphorylation of prohibitin: implication for a novel binary switch. PLoS ONE, 4(2):e4586.

[15] Whelan SA, Dias WB, Lakshmanan T, Lane MD, Hart GW (2010). Regulation of insulin receptor 1 (IRS-1)/AKT kinase mediated insulin signaling by O-linked-{beta}-N-acetylglucosamine (O-GlcNAc) in 3T3-L1 adipocytes. J Biol Chem 285: 5204-11.

[16] Klein A, Berkaw MN, Buse MG, Ball LE (2009). O-GlcNAc modification of insulin receptor substrate-1 (IRS-1) occurs in close proximity to multiple SH2 domain binding motifs. Mol Cell Proteomics 8:2733-45.

[17] 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):39096-107.

[18] Chatham JC, Marchase RB (2010). Protein OGlcNAcylation: a critical regulator of the celular response to stress. Curr Signal Transduct Ther. 5(1):49-59.

[19] Özcan S, Andrali SS, Cantrell JEL (2010). Modulation of transcription factor function by O-GlcNAc modification. Biochim Biophys Acta 1799: 353–364

[20] Robles-Flores M, Meléndez L, García W, Mendoza-Hernández G, Lam TT, Castañeda-Patlán C, González-Aguilar H (2008). Posttranslational modifications on protein kinase C isozymes. Effects of Epinephrine and phorbol esters. BBA Mol Cell Res 1783: 695-712

[21] Matthews JA, Acevedo-Duncan M, Potter RL (2005). Selective decrease of membrane-associated PKC-α and PKC-ε in response to elevated intracellular O-GlcNAc levels in transformed human glial cells, Biochim. Biophys. Acta 1743: 305–315.

[22] Konishi H, Tanaka M, Takemura Y, Matsuzaki H, Ono Y, Kikkawa U, Nishizuka Y. (1997). Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2, Proc. Natl. Acad. Sci. U. S. A. 94: 11233–11237.

[23] Balafanova Z, Bolli R, Zhang J, Zheng Y, Pass JM, Bhatnagar A, Tang XL, Wang O, Cardwell E, Ping P (2002). Nitric oxide (NO) induces nitration of protein kinase Cε, facilitating PKCε translocation via enhanced PKCε-RACK2 interactions, (PKCε), facilitating PKCε J Biol Chem 277 (17) 15021-27.

[24] Meyer N., Penn LZ (2008). Reflecting on 25 years with MYC, Nat Rev Cancer 8: 976–990.

[25] Morrish F, Isern N, Sadilek M, Jeffrey M, Hockenbery DM (2009). c-Myc activates multiple metabolic networks to generate substrates for cell-cycle entry. Oncogene 28, 2485–2491

[26] Luscher B, Kuenzel EA, Krebs EG, Eisenman RN (1989). Myc oncoproteins are phosphorylated by casein kinase II, EMBO J. 8: 1111–1119.

[27] Seth A, Alvarez E, Gupta S, Davis RJ (1991). A phosphorylation site located in the NH2-terminal domain of c-Myc increases transactivation of gene expression, J Biol Chem 266: 23521–23524.

[28] Chou TY, Dang CV, Hart GW (1995). Glycosylation of the c-Myc transactivation domain, Proc. Natl. Acad. Sci. U. S. A. 92: 4417–4421.

[29] Chou TY, Hart GW and Dang CV (1995). C-Myc is glycosylated at Threonine 58, a known phosphorylaltion site and a mutational hot spot in lymphomas. J Biol Chem 270(32): 18961-18965.

[30] Gu W, Bhatia K, Magrath IT, Dang CV, Dalla-Favera R (1994). Binding and suppression of the Myc transcriptional activation domain by p107, Science 264: 251–254.

[31] Cadigan KM and Peifer M (2009). Wnt signaling from development to disease: insights from model systems. (Abstract) Cold Spring Harb Perspect Biol 1:a002881

[32] Wend P, Holland JD, Ziebold U, Birchmeier W (2010). Wnt signaling in stem and cancer stem cells. Semin Cell Dev Biol 21: 855–863.

[33] Fagotto F, Gluck U and Gumbiner BM (1998). Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of β-catenin. Curr Biol 8:181-190.

[34] Kulay U, Izaurralde E, Bischoff FR, Mattaj IW and Gorlich D (1997). Dominant-negative mutants of importin-β block multiple pathways of import and export through the nuclear pore complex. EMBO J 16: 1153-1163.

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

[36] Haupt Y, Maya R, Kazaz A, Oren M (1997). Mdm2 promotes the rapid degradation of p53, Nature 387: 296–299.

[37] Kubbutat MH, Jones SN, Vousden KH (1997). Regulation of p53 stability by Mdm2, Nature 387: 299–303.

[38] Bech-Otschir D, Kraft R, Huang X, Henklein P, Kapelari B, Pollmann C, Dubiel W (2001). COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system, EMBO J. 20: 1630–1639.

[39] Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, Kim YS, Cho JW (2006). Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability. Nat. Cell Biol. 8: 1074–1083.
[40] Warburg O (1956). On respiratory impairment in cancer cells. Science 124: 269–270.
[41] DeBerardinis RJ, Sayed N, Ditsworth D, and Thompson CB (2008). Brick by brick: metabolism and tumor cell growth. Curr Opin Genet Dev. 18(1): 54–61.
[42] Mi W, Gu Y, Han C, Liu H, Fan Q, Zhang X, Cong Q, Yu W (2011). O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy. Biochim Biophys Acta 1812: 514–519
[43] Shi Y, Tomic J, Wen F, Shaha S, Bahlo A, Harrison R, Dennis JW, Williams R, Gross BJ, Walker S, Zuccolo J, Deans JP, Hart GW, Spaner DE (2010). Aberrant O-GlcNAcylation characterizes chronic lymphocytic leukemia. Leukemia 24: 1588-1598