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O-GlcNAcylation, steroid hormone receptors and cancer

O-GlcNAcylacja, receptory dla steroidów i nowotwory

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Abstract

The addition of a single residue of O-linked N-acetylglucosamine (O-GlcNAc) to serine or threonine is a post-translational modification (O-GlcNAcylation) of proteins found in the cytoplasm or the nucleus. This dynamic modification is dependent on the environmental glucose concentration. O-GlcNAc modification is catalysed by a glycosyltransferase named O-linked N-acetyl-glucosaminyltransferase (OGT), and O-GlcNAc residue is removed by the antagonistic enzyme β-N-acetylglucosaminidase (O-GlcNAcase; OGA). Cytosolic O-GlcNAcylation is important for the proper transduction of signalling cascades, whereas nuclear O-GlcNAc is crucial for the transcriptional regulation. O-GlcNAcylation is also important in the regulation of the transcriptional activity of steroid hormone receptors. Both O-GlcNAc transferase and O-GlcNAcase are found in all tissues and have been shown to be essential for development in vertebrates, which underscores their fundamental roles in vital processes as well as in pathological conditions such as neoplastic transformation.

Key words: O-GlcNAcylation, steroid hormone receptors, cancer.

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Streszczenie

Glikozylacja polegająca na przyłączeniu pojedynczych reszt β-N-acetylo-D-glukozaminy do reszt seryny lub treoniny polipeptydu wiązaniem O-glikozydowym (O-GlcNAc), jest powszechną modyfikacją białek jądrowych i cytoplazmatycznych. Modyfikacja ta pozostaje w ścisłym związku z dostępnością glukozy. β-N-acetyloglukozaminylotransferaza (O-GlcNAc transferaza, OGT) jest enzymem odpowiedzialnym za katalityczne przyłączenie reszt O-GlcNAc, natomiast β-N-acetylo-D-glukozaminidaza (OGA) reszty te odłącza. Proces O-GlcNAcylacji zachodzący w cytozolu jest istotny z punktu widzenia transmisji sygnału komórkowego, natomiast przyłączanie reszt O-GlcNAc na terenie jądra wpływa na proces transkrypcji. Wykazano, że proces O-GlcNAcylacji moduluje regulację aktywności transkrypcyjnej receptorów hormonów steroidowych. Oba enzymy, O-GlcNAc transferaza i β-N-acetylo-D-glukozaminidaza wykrywane są we wszystkich tkankach i jak wykazano, niezbędne są dla prawidłowego rozwoju organizmu, jak również mogą uczestniczyć w procesie transformacji nowotworowej.

Słowa kluczowe: O-GlcNAcylacja, receptory dla steroidów, nowotwór

Introduction

Most cytoplasmic and nuclear proteins of multicellular eukaryotes are modified by a single O-GlcNAc moiety, termed O-GlcNAcylation [1]. These monosaccharide units are linked post-translationally to the hydroxyl groups of serine and threonine residues of proteins to form O-GlcNAc. The levels of intracellular O-GlcNAc are regulated by two enzymes that act oppositely. Uridine diphosphate N-acetylglucosamine:polypeptidyl transferase, known as O-GlcNAc transferase (OGT), uses uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) as a direct sugar donor [2]. OGT is enriched in the nucleus, but is also present in the cytosol of all cells as a multimer [3]. The catalytic activity of OGT is regulated by a wide range of intracellular concentrations of

UDP-GlcNAc that changes proportionally in response to the nutrients (glucose, glutamine, energy) through the hexosamine biosynthetic pathway [4-6]. Thus, the O-GlcNAcylation of substrates represents a 'metabolic sensor' that adjusts protein function according to the nutritional status of the cell [3]. A glycoside hydrolase, known as β-N-acetylglucosaminidase (O-GlcNAcase; OGA), catalyzes the cleavage of O-GlcNAc from proteins [7]. This enzyme is highly concentrated in the cytosol but is also present in the nucleus of all cell types [8, 9]. Figure 1 presents a scheme of the protein O-GlcNAcylation and the enzymes participating in this process.

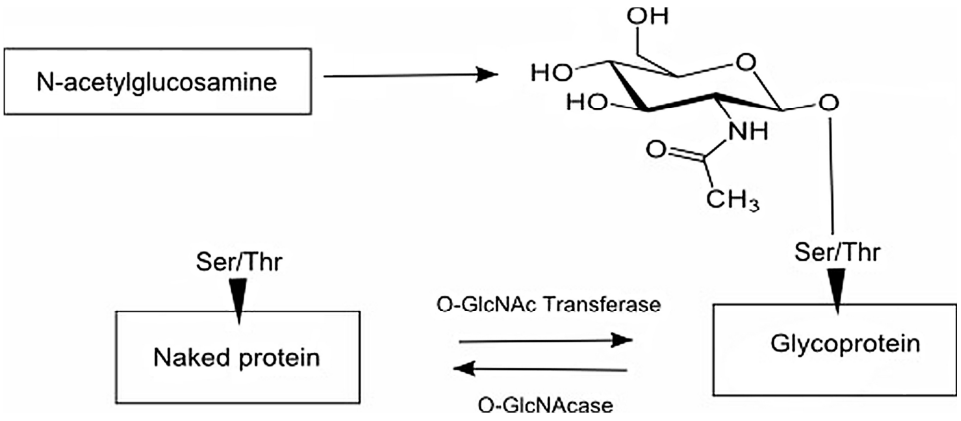

Fig. 1. O-GlcNAcylation scheme and the enzymes participating in this process.

The O-GlcNAc modification shares a complex relationship with phosphorylation, as both modifications are capable of mutually inhibiting the occupation of each other on the same or nearby amino acid residue [10]. O-GlcNAcylation and protein phosphorylation are convertible in many aspects, and a crosstalk between GlcNAcylation and phosphorylation exists. Over 600 proteins have been reported to be O-GlcNAcylated since it was first identified in

1984 , many of which were identified in recent years as a result of improved mass spectrometry technologies [11, 12]. The 'Yin-Yang hypothesis', proposing that O-GlcNAc shows the interplay with protein phosphorylation seems to be of particular interest.. More than 50% of the known O-GlcNAc sites have a Pro-Val-Ser motif similar to that recognised by proline-directed kinases, and in some cases O-GlcNAc is reciprocal with phosphorylation on some well-studied proteins. For example, reciprocal O-GlcNAcylation and phosphorylation at the same Ser16 of murine estrogen receptor β (ERβ) modulate the degradation of ERβ by stabilizing or destabilizing the protein, respectively [11, 13]. A disturbing phosphorylation events affects O-GlcNAcylation levels and vice versa. All O-GlcNAcylated proteins can also be modified by phosphorylation. It is believed that O-GlcNAcylation may regulate kinases or phosphatases involved in the specific signalling, or alternatively, phosphorylation may affect the functions of OGT and O-GlcNAcase [10, 14].

O-GlcNAcylation and epigenetics

Histone modifications regulate many different physiological processes, and the deregulation of these processes leads to various complications and diseases. Histone modifications contribute to silence tumor suppressor genes or destabilize the genome, thereby facilitating further chromosomal rearrangement and deregulation and leading to cancer [15].

Acetylation, methylation, and phosphorylation are among the best known histones post-translational modifications. The first two modifications are involved in the activation of chromatin and the third in both activation and repression. Histones can also be modified by ADP-Ribosylation, SUMOylation, ubiquitination, and O-GlcNAcylation [1, 16, 17].

Sakabe et al. [18] found that all four core nucleosomal histones are modified with O-GlcNAc. The researchers specify three O-GlcNAcylation sites on H2A, H2B, and H4 at Thr101, Ser36, and Ser47, respectively. Histone H2B tail is also O-GlcNAcylated at Ser112, and this modification favoures Lys120 monoubiquitination, in which the GlcNAc moiety can serve as an anchor for a histone H2B ubiquitin ligase [19] Further research has identified Ser10 and threonine 32 as another O-GlcNAc site on the H3 tail. It was also shown that increasing the intracellular level of UDP-GlcNAc enhanced histone O-GlcNAcylation and partially suppressed phosphorylation of histone H3 at Ser10. Histone O-GlcNAcylation is related to other modifications associated with active or inactive chromatin states. The covalent linkage of histones by O-GlcNAc changes along the cell cycle, and was detected at higher levels during interphase than mitosis, which inversely correlated with phosphorylation [20]. In addition, OGT and OGA were found to be physically associated with Aurora B and type1 protein phosphatase (PP1), a kinase and phosphatase that phosphorylate and dephosphorylate H3 serine 10 and serine 28 during mitosis [21]. Fardini et al [17] hypothesize that the regulation of histone assembly/disassembly is managed in part by a heterotetrameric complex made of Aurora B, PP1, OGT, and OGA.

The structure of O-GlcNAcase consists of N-terminal glycosidase domain and C-terminal region with homology to histone acetyltransferases (HATs) [22, 23]. This HAT domain has both active and inactive states. HATs and histone deacetylases (HDACs) act competitively within large multiprotein complexes that recruit them to their nucleosomal substrates on DNA and give them the ability to contribute to the activation or repression of gene expression, respectively [24].

The Polycomb group (PcG) system represses the transcription of important developmental regulators from early embryogenesis through birth to adulthood. PcG proteins form several complexes that are thought to collaborate to repress gene transcription. PcG functions by recruiting additional chromatin factors to PcG-regulated loci or by downregulating PcG genes themselves. These types of PcG system modulation allow the context-dependent induction of genes during development, in cancer, and in response to the changes in the environment [25, 26].

In 2009 protein glycosylation was included in the group of posttranslational modifications involved in the regulation of PcG repression [27]. Gambetta et al. [28] show that the Drosophila PcG gene super sex comb (sxc) encodes O-linked N-acetylglucosamine transferase and polycomb response elements that control promoters at a distance via chromatin structure and interference with RNA polymerase II. The sxc/Ogt mutants that lack OGT expression or express truncated proteins with no catalytic activity lose all cellular O-GlcNAcylation and show the severe derepression of multiple PcG target genes. On this basis it has been concluded that O-GlcNAcylation is essential for PcG repression [27-29].

The ten-eleven translocation family of enzymes (TET1, TET2, and TET3) catalyses the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, an intermediate form possibly involved in DNA demethylation [30-32]. TET1 have been linked to epigenetic repression complexes, especially mSin3A and Prc2, and O-GlcNAcylation can positively regulate TET1 protein concentration [33-36]. TET2 and TET3 interact directly with O-GlcNAc transferase. OGT does not appear to influence hmC activity, rather TET2 and TET3 support OGT activity. TET2/3 and OGT are located at active promoters enriched

for H3K4me3. Reducing the activity of one of these proteins results in the decrease in H3K4me3 and in the decreased transcription. TET2/3-OGT complex O-GlcNAcylates the H3K4 methyltransferase SET1/COMPASS and promote its binding to chromatin [37].

O-GlcNAcylation and steroid hormone receptors

Nuclear hormone receptors are a superfamily of transcription factors that associate with conserved regions on gene promoters to regulate the transcription of specific genes. This family includes steroid and thyroid receptors, retinoic acid and vitamin D3 receptors, peroxisomal activators and developmental regulators, and a number of orphan receptors of unknown function [38]. In the case of steroid hormone receptors the binding of ligand by a steroid receptor induces a cascade of molecular events which result in the activation of the transcription activity of the steroid receptor. This process includes phosphorylation, conformational changes and receptor dimerization. The activated receptor is capable of binding to hormone responsive elements (HREs) in the promoters of hormone-regulated genes ,thereby affecting the transcription of these genes [39]. Steroid receptors are known as ligand-regulated transcription factors, but some evidence indicates that steroid receptors can be activated in the absence of cognate hormone [38].

In the hormone-independent activation of steroid recepors, the signals emanating from membrane receptors through cell signalling cascades alter the activities of kinases and/or phosphatases resulting in the altered phosphorylation of steroid receptors and/or proteins interacting with them [40-41].

Bowe et al. [42] show that a subtle increase in O-GlcNAc levels inhibits the activation of nuclear hormone receptors. The increased levels of O-GlcNAc

impair estrogen receptor activation and cause a decrease loss of progesterone receptors. The increased O-GlcNAc levels suppress the transcriptional expression of coactivators and of the nuclear hormone receptors themselves. Also, the increased O-GlcNAc levels are associated with the increased transcription of genes encoding corepressor proteins NCoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoid and thyroid receptors). The association of OGT with nuclear hormone corepressors contributes to its ability to specifically repress nuclear hormone signaling pathways. The researchers proposed a hypothetical model which clarifies the relationship between O-GlcNAcylation and hormone receptor cell signal transmission (Figure 2). During the transcriptional repression O-linked N-acetylglucosaminyl-transferase interacts with corepressors (NCoR/SMRT, mSin3a) on nuclear hormone receptors and this complex is targeted to sites of transcriptional repression. O-GlcNAc modification of critical elements of the transcriptome and the proteasome contributes to transcriptional repression. These may include Sp1 and RNA polymerase II (Pol II). But when there is the activation of the hormone receptor, NCOAT (O-GlcNAcase and acetyltransferase) activity is required to remove O-GlcNAc residues from critical parts of the transcriptome and the proteasome. The removal of O-GlcNAc residues enables the degradation of corepressors and proteolytic recycling of coactivators.

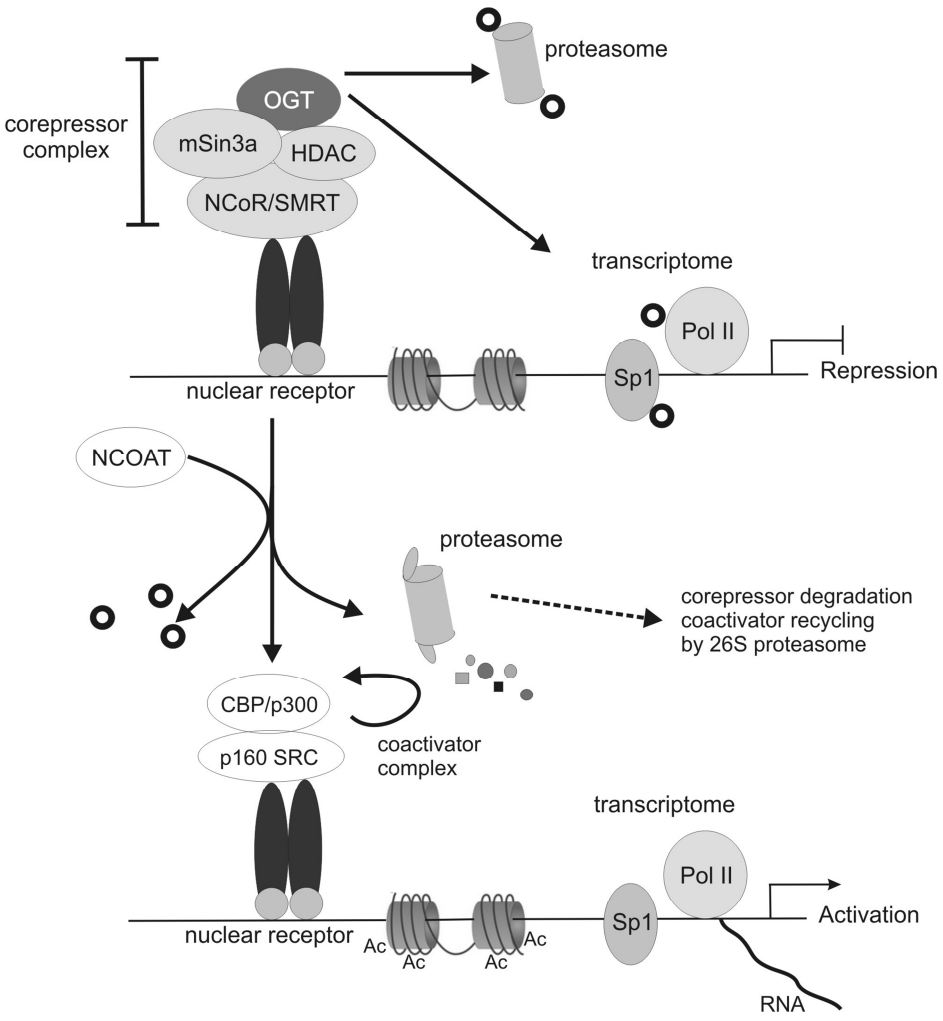

Fig. 2 Hypothetical model joining O-GlcNAcylation and nuclear hormone receptor signal transduction.

O-GlcNAcylation and cancer

Nutritional conditions modulate the availability of nutrients important for the growth and proliferation of cancer cells. Neoplastic cells proliferate intensively and, therefore, must use carbon and nitrogen rich nutrients to biosynthesize metabolites needed for cell proliferation [43].

Glucose and glutamine are the two most abundant extracellular nutrients. They contribute carbons to the synthesis of nucleic acids, lipids, and proteins in proliferating tumor cells. In addition, glutamine also donates nitrogen to nucleotide and amino acid synthesis. Apart from the function in neoplastic transformation, glucose and glutamine metabolism intervene in protein O-GlcNAcylation which controls protein subcellular localization, stability, or activity according to the nutritional environment [17]. UDP-GlcNAc, a donor substrate, is a nutrient sensor as its intracellular concentration directly correlates with fluctuating glucose concentrations [44].

Approximately 2-5% of total glucose is shunted into a hexosamine biosynthetic pathway to produce UDP-GlcNAc. The HBP integrates the nutrient status of the cell by utilizing glucose, acetyl-CoA, glutamine, and UTP to produce UDP-GlcNAc. The rate-limiting step is the next enzyme in the pathway, glutamine fructose aminotransferase which uses fructose-6-phosphate and glutamine to synthesize glucosamine-6-P [17, 29].

The excess of nutrients intake is powered by the HBP and intensifies abnormally elevated O-GlcNAcylation of signalling molecules and transcription factors. This modification has been proposed to play an important role in the alteration associated with neoplastic transformation [17].

O-GlcNAcylation and protein phosphorylation are congruent in many aspects. Some disturbing phosphorylation events affect GlcNAcylation levels

and vice versa. Both these modifications are highly responsive to nutrients, and, their donor substrates are high-energy products of cellular metabolism. All O-GlcNAc modified proteins can also be modified by phosphorylation. It is speculated that GlcNAcylation may regulate kinases or phosphatases involved in the specific signaling, or alternatively, phosphorylation may affect the functions of OGT and O-GlcNAcase [14]. For example, a proto-oncogene c-Myc modulating the transcription of genes involved in cell metabolism and proliferation can be modified by phosphorylation and GlcNAcylation at Thr 58. This site is most often mutated in Burkitt lymphoma [45, 46].

O-GlcNAc level and OGT expression are elevated in cancers [47]. Increased protein O-GlcNAcylation and increased OGT expression have been detected in different types of cancer e.g. breast, endometrial, prostate, colon, lung, liver, bladder, and chronic lymphocytic leukemia cells [17]. While, in some studies ambiguous results were obtained for OGA level. There was a decrease [48, 49], no change [50], and increase in OGA levels [17]. Most likely, these differences are due to the fact that OGA expression itself is upregulated upon the increased O-GlcNAcylation level in the cell [51]. On the other hand, it also cannot be excluded that the changes in OGA level are associated with the activity of OGT as a Polycomb group protein (PcG). The PcG proteins act on the chromatin as a cooperative, mulitmeric complex, and OGT was identified as the gene encoded by super sex combs (sxc), an essential component of PcG complexes in Drosophila [28, 52]. On the basis of the information collected by Love and coworkers [53], in vertebrates, OGT plays a key role in the ability of the PcG proteins to repress genes appropriately, presumably acting downstream of PRC2 (PcG repressive complex 2) binding and H3K27 trimethylation. The OGA gene is embedded within the highly

conserved cluster of Nk homeobox genes regulated by PcG repression. It appears that neoplastic cells display both the increased uptake of nutriments involved in O-GlcNAc biosynthesis and the increased capacity to O-GlcNAcylate proteins. The alteration in O-GlcNAcylation may directly affect important steps in tumorigenesis [17]. Increasing evidence suggests that OGT plays a critical role during oncogenesis and tumor progression in numerous cancers [48, 50, 54].

The knockdown of O-GlcNAcase in liver cancer cells increases total OGlcNAcylation levels and the migration and invasion of these cells. In addition, the O-GlcNAcase gene is localized in frequently altered chromosomal regions, critical in the pathogenesis of breast and ovarian cancer. Thus, the increased OGT and the decreased O-GlcNAcase levels in cancers may lead to a general increase in O-GlcNAc cycling that has diverse tumor-promoting functions [55, 56].

The data presented by Lynch et al [55] show a role for the elevated expression of OGT and O-GlcNAcylation observed in prostate cancer in the invasion, angiogenesis, and metastasis of cancer cells.

Conclusion

Neoplastic cells upregulate glycolysis, increasing glucose uptake to meet energy needs. O-GlcNAcylation as a metabolic sensor may be a key factor involved in cancer growth and invasion. Thus O-linked N-acetylglucosaminyltransferase may represent a novel therapeutic target for neoplastic transformation.

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