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Editorial

Protein O-GlcNAcylation

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Protein glycosylation is one of the most common posttranslational modifications occurring in essentially all living organisms. Glycopeptide bonds can be categorized into specific groups based on the nature of the carbohydrate-peptide bond and the oligosaccharide attached. There are 13 different monosaccharides and 8 amino acid residues participating in the carbohydrate-peptide linkages, resulting in at least 31 linkage types [1] with the three major groups being N-linked (attached to Asn residue), O-linked (attached to Ser/Thr residue), and C-linked (a mannose attached to Trp residue) glycosylation. The sugar groups found on O-glycoproteins include N- acetylgalactosamine (O-GalNAc), N-acetylglucosamine (O-GlcNAc), fucose, glucose, and mannose [2]. Among these modifications, protein O-GlcNAcylation is the process involving attachment of beta-N-acetylglucosamine (GlcNAc) to the -OH group of a Ser/Thr residue catalyzed by O-GlcNAc transferase (OGT) and this modification differs from other types of glycosylation by occurring exclusively within the nuclear and cytoplasmic compartments of the cell, with O-GlcNAc typically not forming complex structures and able to cycle rapidly multiple times similar to phosphorylation [3].

Recent proteomic studies suggest that more than 1500 proteins are modified by O-GlcNAc [4-7]. The Database of O-GlcNAcylated Proteins and Sites (dbOGAP) has curated experimental O- GlcNAcylation data from published literature for ~800 proteins. The O-GlcNAcylated proteins are primarily nucleocytoplasmic, including membrane- and non-membrane bounded organelle- associated proteins [8]. O-GlcNAcylated nuclear proteins identified include Nuclear pore proteins, RNA polymerase II, c-Myc oncoprotein, p53 tumor suppressor, and Estrogen receptors.

Other examples of O-GlcNAcylated proteins are cytokeratins, MAPs, tau, and rotavirus NS26 protein, et al [9]. Furthermore, the dbOGAP identifies ~400 O-GlcNAcylation sites on 172 proteins including Ser471 on Nuclear pore protein p62 [10], Thr58 on c-Myc oncoprotein [11], Ser149 on p53 tumor suppressor [12], and Ser10, Thr50, Thr575 on estrogen receptors [13-15].

Growing evidence supports critical roles for O-GlcNAcylation in cellular functions by regulating protein phosphorylation, altering protein degradation, adjusting the localization of proteins, modulating protein–protein interactions, and mediating transcription [16]. Extensive cross talk occurs between O-GlcNAcylation and phosphorylation with O-GlcNAc acting antagonistically to phosphorylation [17,18]. Phosphatases have been found to be associated with OGT [19] suggesting that the same complex can both remove phosphate and add an O-GlcNAc. This interplay is evident in tumorigenesis as phosphorylation of p53 and c-Myc leads to protein degradation, while O-GlcNAcylation blocks phosphorylation and potentially stabilizes the protein [20]. Altered OGT expression and O-GlcNAc modification levels have been implicated in a number of human diseases including cancer, diabetes, cardiovascular diseases and neurological disorders. Increased OGT expression and O-GlcNAc modifications have been found in breast cancer, prostate cancer, colon cancer and lung cancer [21-23]. The brains of Alzheimer's patients show significantly unregulated O-GlcNAcylation [24]. O-GlcNAcylation attenuates insulin signaling, worsens hyperglycemia, and directly participates in several diabetic complications [25]. On the other side, several studies have shown the roles of O-GlcNAcylation in cardioprotection by improving cell survival during acute stress [26].

The importance of protein O-GlcNAcylation is still coming to light as detection and screening methods continue to be developed and improved. Current detection methods include antibodies, lectin probes, radiolabeling, and mass spectrometry (MS). Anti-O-GlcNAc antibodies are available for specific detection of O-GlcNAc group on proteins with the ability to detect O- GlcNAc by western blot on 10 µg of OGT transfected HEK293 cell lysate using less than 1µg/mL of antibody. Site-specific O-GlcNAc antibodies have recently been developed for c-myc, Keratin, and vimentin, but are not commercially available [27]. However, developing site-specific O-GlcNAc antibodies can be costly and time consuming as most antibodies are unable to distinguish between the O-GlcNAcylated versus the non-O-GlcNAcylated protein. As an alternative to antibodies, the lectin probe, wheat germ agglutinin (WGA), is widely used for detection or enrichment of O-GlcNAcylated proteins. The limitations of WGA are that it has a higher affinity for sialic acid [28] and low affinity to monosaccharide O-GlcNAc. The dissociation constant for free GlcNAc binding to WGA is in the 10 millimolar range [5,29]. Radiolabeling of the GlcNAc group using β-1,4-galactosyltransferase (GalT) allows O-GlcNAcylated proteins to be analyzed by electrophoresis, chromatography, and MS. GalT transfers [3H]Gal from UDP-[3H]galactose to terminal GlcNAc groups, forming [3H]-βGal1-4βGlcNAc [30,31]. Other UDP-galactose analogues used for labeling O-GlcNAcylated

proteins contain chemical handles such as the azido [32] or ketone moiety [33], which can react with biotin-alkyne to form a stable triazole conjugate, allowing the GlcNAc group to be biotinylated. Subsequently, the biotinylated GlcNAc group can be enriched by streptavidin affinity chromatography and subjected to MS analysis, or to be detected by probing with streptavidin conjugated HRP in ELISA or by western blot. Invitrogen sells kits using this technology under the "Click-it" brand, and claims to detect and characterize O-GlcNAc–modified glycoproteins in low femtomole range. Advancements over the past several years have made MS an important tool for glycosylation analysis. In-gel trypsin digestion followed by LC-MS/MS analysis allowed the identification of around 1500 O-GlcNAc proteins from a single cell line [34]. Electron capture dissociation (ECD) and electron transfer dissociation (ETD) MS have greatly increased the speed and reliability of O-GlcNAc protein site assignments [35]. Comparing to ECD, ETD is more cost effective and therefore has found more applications in proteomic analysis of O-GlcNAc modifications.

While useful, the available tools for protein O-GlcNAcylation analysis have their limitations. Novel methods for rapid and sensitive identification of O-GlcNAcylated proteins are needed to expand our knowledge in this field. The development of high affinity reagents for site-specific detection of protein O-GlcNAcylation will be extremely important for future research in providing deeper insights into the biological and disease causing roles of protein O- GlcNAcylation.

REFERENCES

1. Spiro RG. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology. 2002; 12: 43R-56R.
2. Van den Steen P, Rudd PM, Dwek RA, Opdenakker G. Concepts and principles of O-linked glycosylation. Crit Rev Biochem Mol Biol. 1998; 33: 151-208.
3. Hart GW, Akimoto Y. The O-GlcNAc Modification. New York: Cold Spring Harbor; 2009.
4. Nandi A, Sprung R, Barma DK, Zhao Y, Kim SC, Falck JR, et al. Global identification of O-GlcNAc-modified proteins. Anal Chem. 2006; 78: 452-458.
5. Vosseller K, Trinidad JC, Chalkley RJ, Specht CG, Thalhammer A, Lynn AJ, et al. O-linked N-acetylglucosamine proteomics of postsynaptic density preparations using lectin weak affinity chromatography and mass spectrometry. Mol Cell Proteomics. 2006; 5: 923-934.
6. Khidekel N, Ficarro SB, Clark PM, Bryan MC, Swaney DL, Rexach JE, et al. Probing the dynamics of O-GlcNAc glycosylation in the brain using quantitative proteomics. Nat Chem Biol. 2007; 3: 339-348.
7. Wang Z, Gucek M, Hart GW. Cross-talk between GlcNAcylation and phosphorylation: site-specific phosphorylation dynamics in response to globally elevated O-GlcNAc. Proc Natl Acad Sci U S A. 2008; 105: 13793-13798.
8. Wang J, Torii M, Liu H, Hart GW, Hu ZZ. dbOGAP - an integrated bioinformatics resource for protein O-GlcNAcylation. BMC Bioinformatics. 2011; 12: 91.
9. Varki A, Cummings R, Esko J, et al. editors. Essentials of Glycobiology. Chapter 14, The O-GlcNAc Modification. New York: Cold Spring Harbor; 1999.
10. D'Onofrio M, Starr CM, Park MK, Holt GD, Haltiwanger RS, Hart GW, et al. Partial cDNA sequence encoding a nuclear pore protein modified by O-linked N-acetylglucosamine. Proc Natl Acad Sci U S A. 1988; 85: 9595-9599.
11. Chou TY, Hart GW, Dang CV. c-Myc is glycosylated at threonine 58, a known phosphorylation site and a mutational hot spot in lymphomas. J Biol Chem. 1995; 270: 18961-18965.
12. Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, Kim YS, et al. Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability. Nat Cell Biol. 2006; 8: 1074-1083.
13. Cheng X, Hart GW. Glycosylation of the murine estrogen receptor-alpha. J Steroid Biochem Mol Biol. 2000; 75: 147-158.
14. Cheng X, Cole RN, Zaia J, Hart GW. Alternative O-glycosylation/O-phosphorylation of the murine estrogen receptor beta. Biochemistry. 2000; 39: 11609-11620.
15. Jiang MS, Hart GW. A subpopulation of estrogen receptors are modified by O-linked N-acetylglucosamine. J Biol Chem. 1997; 272: 2421-2428.
16. Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! Biochim Biophys Acta. 2006; 1761: 599-617.
17. Wang Z, Pandey A, Hart GW. Dynamic interplay between O-linked N-acetylglucosaminylation and glycogen synthase kinase-3-dependent phosphorylation. Mol Cell Proteomics. 2007; 6: 1365-1379.
18. Wang Z, Udeshi ND, Slawson C, Compton PD, Sakabe K, Cheung WD, et al. Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates cytokinesis. Sci Signal. 2010; 3: ra2.
19. Wells L, Kreppel LK, Comer FI, Wadzinski BE, Hart GW. O-GlcNAc transferase is in a functional complex with protein phosphatase 1 catalytic subunits. J Biol Chem. 2004; 279: 38466-38470.
20. Slawson C, Copeland RJ, Hart GW. O-GlcNAc signaling: a metabolic link between diabetes and cancer? Trends Biochem Sci. 2010; 35: 547-555.
21. Gu Y, Mi W, Ge Y, Liu H, Fan Q, Han C, et al. GlcNAcylation plays an essential role in breast cancer metastasis. Cancer Res. 2010; 70: 6344-6351.
22. Lynch TP, Reginato MJ. O-GlcNAc transferase: a sweet new cancer target. Cell Cycle. 2011; 10: 1712-1713.
23. Mi W, Gu Y, Han C, Liu H, Fan Q, Zhang X, et al. O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy. Biochim Biophys Acta. 2011; 1812: 514-519.
24. Griffith LS, Schmitz B. O-linked N-acetylglucosamine is upregulated in Alzheimer brains. Biochem Biophys Res Commun. 1995; 213: 424-431.
25. Issad T, Masson E, Pagesy P. O-GlcNAc modification, insulin signaling and diabetic complications. Diabetes Metab. 2010; 36: 423-435.
26. Ngoh GA, Facundo HT, Zafir A, Jones SP. O-GlcNAc signaling in the cardiovascular system. Circ Res. 2010; 107: 171-185.
27. Zachara NE, Vosseller K, Hart GW. Detection and analysis of proteins modified by O-linked N-acetylglucosamine. Curr Protoc Protein Sci. 2011; Chapter 17: Unit 17.6.-
28. Bhavanandan VP, Katlic AW. The interaction of wheat germ agglutinin with sialoglycoproteins. The role of sialic acid. J Biol Chem. 1979; 254: 4000-4008.
29. Leickt L, Bergström M, Zopf D, Ohlson S. Bioaffinity chromatography in the 10 mM range of Kd. Anal Biochem. 1997; 253: 135-136.
30. Greis KD, Hayes BK, Comer FI, Kirk M, Barnes S, Lowary TL, et al. Selective detection and site-analysis of O-GlcNAc-modified

glycopeptides by beta-elimination and tandem electrospray mass spectrometry. Anal Biochem. 1996; 234: 38-49.

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

32. Wang Z, Park K, Comer F, Hsieh-Wilson LC, Saudek CD, Hart GW. Site-specific GlcNAcylation of human erythrocyte proteins: potential biomarker(s) for diabetes. Diabetes. 2009; 58: 309-317.

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

34. Hahne H, Sobotzki N, Nyberg T, Helm D, Borodkin VS, van Aalten DM, et al. Proteome wide purification and identification of O-GlcNAc-modified proteins using click chemistry and mass spectrometry. J Proteome Res. 2013; 12: 927-936.

35. Myers SA, Daou S, Affar el B, Burlingame A. Electron transfer dissociation (ETD): the mass spectrometric breakthrough essential for O-GlcNAc protein site assignments-a study of the O-GlcNAcylated protein host cell factor C1. Proteomics. 2013; 13: 982-991.

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