Finding the Missing Link Between the Unfolded Protein Response and O-GlcNAcylation in the Heart

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X-box Binding Protein 1 Couples the Unfolded Protein Response to Hexosamine Biosynthetic Pathway
Wang et al
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The endoplasmic reticulum (ER) stress–inducible transcription factor, x-box binding protein 1 (XBP1), which enhances protein glycosylation in the ER, was shown to also enhance protein glycosylation outside the ER, via a process called O-GlcNAcylation, which protected the heart from ischemia/reperfusion damage.

O-GlcNAcylation is a reversible post-translational modification that takes place outside of the ER and affects the functions of target proteins. In cardiac myocytes, O-GlcNAcylation increases acutely in response to a variety of conditions, including hypoxia, ischemia, ischemia/reperfusion, and oxidative stress, during which O-GlcNAcylation is generally protective. However, chronic increases in O-GlcNAcylation during diseases, such as diabetes mellitus, exacerbate cardiac dysfunction and damage. In contrast to O-GlcNAcylation, N-linked glycosylation (N-glycosylation) of proteins occurs in the ER, is relatively permanent, and is required for folding and trafficking of proteins in the ER and Golgi. N-glycosylation can be impaired by many of the same stresses that affect O-GlcNAcylation in the heart. Impaired N-glycosylation causes ER stress, subsequent activation of the unfolded protein response (UPR), and activation of the transcription factor, XBP1, which induces genes that restore N-glycosylation in the ER and promote adaptation to ER stress. A recent study, published in the journal *Cell,* showed that XBP1 also enhances O-GlcNAcylation, which protects the heart from ischemia/reperfusion damage.[1] Thus, in response to potentially damaging stress, XBP1 coordinates glycosylation inside and outside of the ER to confer protection.

The study by Wang et al[1] showed that XBP1 is the missing link between protein O-GlcNAcylation and the UPR (Figure [A]). In the heart, ischemia/reperfusion leads to ER stress, activation of the UPR and XBP1, which Wang et al determined to be a direct transcriptional activator of the gene encoding the rate-limiting step in the hexosamine biosynthetic pathway, which supplies the substrate required for O-GlcNAcylation, that is, uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). These findings reveal previously unappreciated links among the UPR, hexosamine biosynthesis, and O-GlcNAcylation that may serve protective roles in several tissues other than the heart.

The UPR is a conserved intracellular signaling system that is activated in response to an imbalance in ER protein homeostasis or ER proteostasis.[2] Conditions that impair protein folding in the ER, such as the lack of oxygen and nutrients in the ischemic heart, result in ER stress, which can lead to an imbalance in proteostasis because of the accumulation of potentially toxic misfolded proteins. One of the major ER stress signaling pathways is mediated by the ER-transmembrane protein, inositol-requiring protein-1. By way of mRNA splicing, inositol-requiring protein-1 converts the XBP1 mRNA from a transcript that encodes a protein that does not have transcriptional activity, XBP1 unspliced, to a transcript that encodes a form of XBP1 that is a potent transcription factor, XBP1 spliced ($XBP1_s$). $XBP1_s$-responsive genes encode proteins that, among other things, augment ER protein folding in various ways, including restoration and fortification of protein glycosylation in the ER lumen.

Protein glycosylation is a widespread post-translational modification that has substantial impact on the function of the heart. It can take place in the ER lumen, as well as in the cytosol, mitochondria, and nucleus (Figure [B]).[3] The O-linked protein glycosylation that takes place outside the ER is responsible for the post-translational modification of >1000 different proteins. O-linked glycosylation involves the post-translational addition of the monosaccharide, GlcNAc, to a serine or threonine in target proteins. In contrast to N-linked glycosylation, there is no known consensus sequence on target proteins that are modified by O-linked glycosylation outside the ER. O-linked glycosylation in the cytosol, nucleus, and mitochondria, also called O-GlcNAcylation, is mediated by O-GlcNAc transferase, which uses UDP-GlcNAc as the glycosyl donor (Figure [C]). N-glycosylation and O-GlcNAcylation share the intermediate, UDP-GlcNAc, which is generated from glucose by the hexosamine biosynthetic pathway (Figure [D]). The rate-limiting step in UDP-GlcNAc formation is the conversion of fructose-6-phosphate to glucosamine-6-phosphate by the enzyme, glutamine:fructose amidotransferase (GFAT).

Before the study by Wang et al, it had been shown that ischemia could activate the UPR and XBP1 in cultured cardiac myocytes and in infarcted mouse hearts, and that XBP1 served a protective role under these conditions (Figure [A]).[4] Moreover, it had been shown that ischemia/reperfusion can increase protein O-GlcNAcylation in cultured cardiac myocytes and in the

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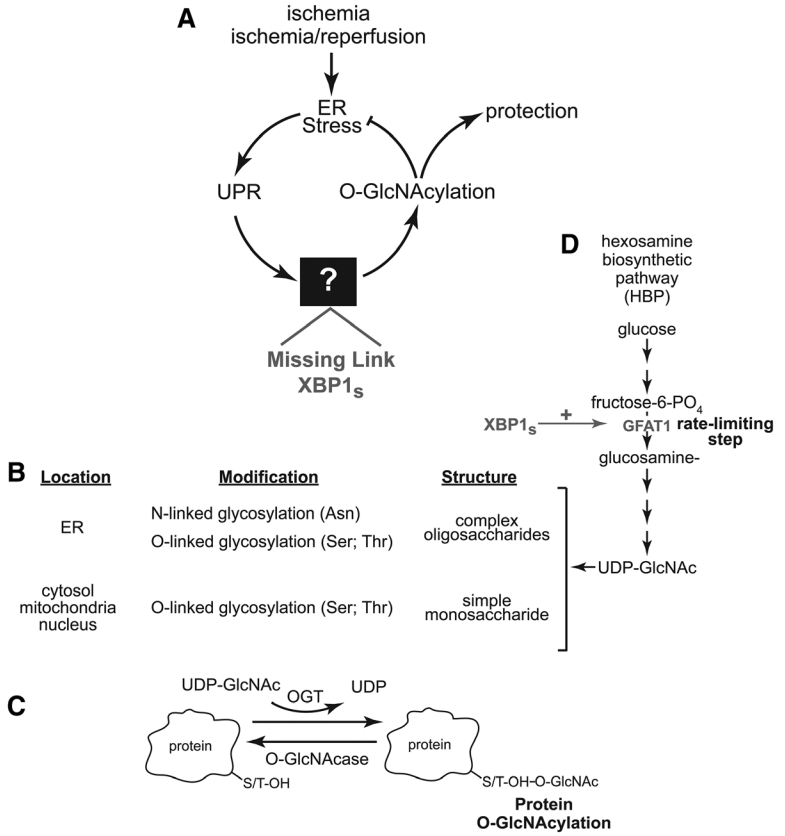

Figure. A, x-box binding protein 1 (XBP1) is the missing link between the unfolded protein response (UPR) and O-GlcNAcylation. The contributions made by Wang et al are shown in blue. B, Proteins are glycosylated in different cell compartments. C, A key substrate for O-GlcNAcylation, which is used by the enzyme O-GlcNAc transferase (OGT), is uridine diphosphate N-acetyl glucosamine (UDP-GlcNAc). D, UDP-GlcNAc is generated by the hexose biosynthetic pathway (HBP). O-GlcNAc groups can be removed from proteins by O-GlcNAcase. The contributions made by Wang et al are shown in blue. ER indicates endoplasmic reticulum; and GFAT, glutamine:fructose amidotransferase.

mouse heart, in vivo, and that O-GlcNAcylation protected the heart from ischemia/reperfusion damage.[5] However, the link between the UPR and O-GlcNAcylation remained unknown (Figure [A], black box). In pursuit of finding this missing link, Wang et al observed that, in the mouse heart, ischemia/reperfusion activated ER stress, XBP1, O-GlcNAcylation and GFAT1, as well as several other enzymes in the hexosamine biosynthetic pathway. They then postulated that enzymes responsible for hexosamine biosynthesis, in particular, GFAT1, which catalyzes the rate-limiting reaction, might be transcriptionally controlled by $XBP1_s$. This was a critical insight that led to the discovery of the missing link. A most insightful leap was when Wang et al went on to show that the promoter-proximal 5′-flanking region of the GFAT1 gene has sequence through which $XBP1_s$ enhanced GFAT1 transcription in cardiac myocytes. Moreover, using combinations of XBP1 gain and loss of function in the heart, in vivo, and in cultured cardiac myocytes, coupled with GFAT1 gain- and loss-of-function maneuvers, Wang et al provided clear mechanistic evidence supporting the hypothesis that $XBP1_s$ is the missing link between the UPR and protein O-GlcNAcylation. Moreover, they provided evidence supporting the idea that $XBP1_s$-mediated increases in O-GlcNAcylation can protect the heart from ischemia/reperfusion damage demonstrated by a reduction in infarct size.

A few questions arise from the study by Wang et al, one of which concerns the other isoform of GFAT, GFAT2, which is considered to be the major isoform of GFAT in the heart.[6] Other studies have shown that it is GFAT2, and not GFAT1 that is regulated in the heart by pressure overload or exercise.[7–9] This leads to the question of whether GFAT2 could be regulated by $XBP1_s$? There is a sequence at −273 to −265 in the mouse GFAT2 promoter (TCACGTCT), which is close to the sequence and location of the $XBP1_s$ binding site that Wang et al found at −276 of the mouse GFAT1 promoter (CCACGTCA). Both elements have the core ACGT sequence, which was previously shown to be required for $XBP1_s$ binding.[10] Thus, as with GFAT1, $XBP1_s$ might bind to the GFAT2 promoter and increase GFAT2 transcription. Wang et al briefly investigated whether GFAT2 might also be regulated by XBP1, but found that, in contrast to GFAT1, GFAT2 expression was not increased by XBP1. These results are consistent with a previous study, which showed that GFAT1, but not GFAT2, was induced by $XBP1_s$.[11] Thus, although a putative $XBP1_s$ binding site exists in GFAT2, it seems that, in contrast to GFAT1, GFAT2 does not serve as an XBP1 target.

Another question that arises is how does O-GlcNAcylation protect the heart from ischemia/reperfusion damage? Answering this question will require knowledge of the proteins that are O-GlcNAcylated, as well as an understanding of how O-GlcNAcylation alters their functions. Recently, the identities of many O-GlcNAcylated cardiac proteins were identified, but just how O-GlcNAcylation affects their functions remains to be determined.[12]

Is O-GlcNAcylation always cardioprotective? Although O-GlcNAcylation protects the heart from ischemia/reperfusion damage, in other settings, including the diabetic heart, O-GlcNAcylation seems to contribute to cardiac dysfunction.[13] Driven mostly by elevated glucose and the resulting increase in flux through the hexosamine biosynthesis, O-GlcNAcylation

of several proteins increases in the diabetic heart. For example, Ca2+/calmodulin-dependent kinase II is O-GlcNAcylated in the diabetic heart, which leads to a hyperactivation of calmodulin-dependent kinase II, increased phosphorylation of the ryanodine receptor. Ryanodine receptor phosphorylation by calmodulin-dependent kinase II results in increased calcium leaks from the sarcoplasmic reticulum, which contributes to the arrhythmia observed in diabetic cardiomyopathy.[14]

What roles does O-GlcNAcylation play in other cardiac pathologies? O-GlcNAcylation is increased in mouse models of pathological cardiac hypertrophy and heart failure. In hypertrophy, nuclear factor of activated T-cells (NFAT) activation, which is a key driver of hypertrophic growth, is inhibited by blocking O-GlcNAcylation.[15] Moreover, NFAT is O-GlcNAcylated, leading to speculation that cardiac hypertrophy is evoked, at least partly, by the direct O-GlcNAcylation of NFAT.[16] In a mouse model of heart failure induced by myocardial infarction, O-GlcNAcylation increased during heart failure.[17] In this study, cardiac-specific deletion of OGA decreased O-GlcNAcylation, increased infarct size, and decreased survival, suggesting that O-GlcNAcylation is protective in this model of heart disease.

In summary, O-GlcNAcylation has major effects in the healthy and diseased heart. However, unlike protein phosphorylation, which governs the structure and function of a wide spectrum of hundreds of protein kinase substrates, O-GlcNAcylation addition and removal require only 2 enzymes; thus, the molecular mechanisms regulating the extent and determining the targets of O-GlcNAcylation must be different than those that regulate protein phosphorylation. The study by Wang et al has contributed significantly to our understanding of how O-GlcNAcylation can be regulated by the UPR and the transcription factor, XBP1, and that XBP1 protects the heart from ischemia/reperfusion damage, partly by increasing O-GlcNAcylation. As a result of their study, XBP1 is now also recognized for its roles as a regulator of O-glycosylation outside of the ER. These findings suggest that, by coordinating N- and O-glycosylation, XBP1 plays a pivotal role in most glycosylation events and, thus, potentially regulates the functions of a vast number of proteins. Further underscoring the potentially beneficial functions of XBP1 on an organismal level was a study that appeared in the same issue of *Cell* as the article by Wang et al, which demonstrated that XBP1-mediated increases in hexosamine biosynthesis in *Caenorhabditis elegans* extended lifespan.[18] Taken together, these paradigm-shifting studies significantly expand our understanding of the UPR, and specifically, XBP1 as central regulators of life and death decisions in cells.

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Disclosures

None.

References

1. Wang ZV, Deng Y, Gao N, et al. Spliced X-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell*. 2014;156:1179–1192.
2. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011;334:1081–1086.
3. Ngoh GA, Facundo HT, Zafir A, Jones SP. O-GlcNAc signaling in the cardiovascular system. *Circ Res*. 2010;107:171–185.
4. Thuerauf DJ, Marcinko M, Gude N, Rubio M, Sussman MA, Glembotski CC. Activation of the unfolded protein response in infarcted mouse heart and hypoxic cultured cardiac myocytes. *Circ Res*. 2006;99:275–282.
5. Ngoh GA, Watson LJ, Facundo HT, Jones SP. Augmented O-GlcNAc signaling attenuates oxidative stress and calcium overload in cardiomyocytes. *Amino Acids*. 2011;40:895–911.
6. Oki T, Yamazaki K, Kuromitsu J, Okada M, Tanaka I. cDNA cloning and mapping of a novel subtype of glutamine:fructose-6-phosphate amidotransferase (GFAT2) in human and mouse. *Genomics*. 1999;57:227–234.
7. Lunde IG, Aronsen JM, Kvaløy H, Qvigstad E, Sjaastad I, Tønnessen T, Christensen G, Grønning-Wang LM, Carlson CR. Cardiac O-GlcNAc signaling is increased in hypertrophy and heart failure. *Physiol Genomics*. 2012;44:162–172.
8. Young ME, Yan J, Razeghi P, Cooksey RC, Guthrie PH, Stepkowski SM, McClain DA, Tian R, Taegtmeyer H. Proposed regulation of gene expression by glucose in rodent heart. *Gene Regul Syst Bio*. 2007;1:251–262.
9. Belke DD. Swim-exercised mice show a decreased level of protein O-GlcNAcylation and expression of O-GlcNAc transferase in heart. *J Appl Physiol (1985)*. 2011;111:157–162.
10. Kanemoto S, Kondo S, Ogata M, Murakami T, Urano F, Imaizumi K. XBP1 activates the transcription of its target genes via an ACGT core sequence under ER stress. *Biochem Biophys Res Commun*. 2005;331:1146–1153.
11. Acosta-Alvear D, Zhou Y, Blais A, Tsikitis M, Lents NH, Arias C, Lennon CJ, Kluger Y, Dynlacht BD. XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. *Mol Cell*. 2007;27:53–66.
12. Zachara NE. The roles of O-linked β-N-acetylglucosamine in cardiovascular physiology and disease. *Am J Physiol Heart Circ Physiol*. 2012;302:H1905–H1918.
13. Fricovsky ES, Suarez J, Ihm SH, Scott BT, Suarez-Ramirez JA, Banerjee I, Torres-Gonzalez M, Wang H, Ellrott I, Maya-Ramos L, Villarreal F, Dillmann WH. Excess protein O-GlcNAcylation and the progression of diabetic cardiomyopathy. *Am J Physiol Regul Integr Comp Physiol*. 2012;303:R689–R699.
14. Erickson JR, Pereira L, Wang L, Han G, Ferguson A, Dao K, Copeland RJ, Despa F, Hart GW, Ripplinger CM, Bers DM. Diabetic hyperglycaemia activates CaMKII and arrhythmias by O-linked glycosylation. *Nature*. 2013;502:372–376.
15. Facundo HT, Brainard RE, Watson LJ, Ngoh GA, Hamid T, Prabhu SD, Jones SP. O-GlcNAc signaling is essential for NFAT-mediated transcriptional reprogramming during cardiomyocyte hypertrophy. *Am J Physiol Heart Circ Physiol*. 2012;302:H2122–H2130.
16. Dassanayaka S, Jones SP. O-GlcNAc and the cardiovascular system. *Pharmacol Ther*. 2014;142:62–71.
17. Watson LJ, Facundo HT, Ngoh GA, Ameen M, Brainard RE, Lemma KM, Long BW, Prabhu SD, Xuan YT, Jones SP. O-linked β-N-acetylglucosamine transferase is indispensable in the failing heart. *Proc Natl Acad Sci USA*. 2010;107:17797–17802.
18. Denzel MS, Storm NJ, Gutschmidt A, Baddi R, Hinze Y, Jarosch E, Sommer T, Hoppe T, Antebi A. Hexosamine pathway metabolites enhance protein quality control and prolong life. *Cell*. 2014;156:1167–1178.