O-GlcNAcylation-independent OGT role in hypertonic stress regulation

Preview for "Urso, S., Comly, M., Hanover, J., & Lamitina, T. (2020). The O-GlcNAc transferase OGT is a conserved and essential regulator of the cellular and organismal response to hypertonic stress. *bioRxiv*."

By Lang Ding and Qian Zhao

Introduction

The intracellular protein glycosylation with O-linked N-Acetylglucosamine (O-GlcNAc) (O-GlcNAcylation) is regulated by O-GlcNAc transferase (OGT) for the addition and O-GlcNAcase (OGA) for the removal of O-GlcNAc to serine and threonine residues of proteins.[1] The donor of the OGT catalytic reaction is UDP-GlcNAc. O-GlcNAcylation is a known molecular sensor of intracellular metabolism and stress including insulin signaling, β-cell function, gluconeogenesis, as well as mediates the synaptic plasticity in adult mouse hippocampus, where is a particular vulnerable brain region.[2-7] OGT is present in the cytosol of all cells and is enriched in the nucleus. The length of the human *ogt* gene is around 43 kb at the Xq13.1 genomic locus. After alternative splicing, the *ogt* gene generates different isoforms including nucleocytoplasmic, mitochondrial, and short isoforms. OGT exists as a multimer with a C-terminal catalytic domain and a tetratricopeptide repeat (TPR)-rich N-terminal domain to mediate multimerization, protein-protein interactions for the selectivity of OGT. The number of TPRs is used to distinguish different isoforms, for example the OGT with 13 TPRs, 9 TPRs, and 3 TPRs is sorted as nucleocytoplasmic, mitochondrial, and short OGT, respectively.[8-10] OGT not only plays its biological role by its catalytic activity of O-GlcNAcylation, but also by its non-catalytic functions which are independent of O-GlcNAcylation. The OGT functions independent of O-GlcNAcylation are also broadly studied, such as inhibition of E-cadherin/catenin complex formation and neuron functions.[11, 12] Except yeast, all

metazoans express a single *ogt* gene. Using genetic knockout to study the functions of OGT is not feasible in most metazoans due to both the single cell and developmental level is lethal except *Caenorhabditis elegans*. The *ogt-1* null mutants of *C. elegans* are viable under standard cultivation conditions. Therefore, *C. elegans* is a good model to investigate the functions of OGT.[13, 14] In this paper, the authors introduced another different role of OGT independent of O-GlcNAcylation in hypertonic stress regulation in *C. elegans*.

Hypertonic stress in most living cells causes cell shrinkage or causes DNA damage and inhibits its repair as well as other toxic effect over cell metabolism and membrane function.[15, 16] To survive in hypertonic stress condition, cells generate organic osmolytes to be accumulated in the cytosol to track extracellular osmolarity to maintain intracellular water content and cell volume as an adaptive system.[17] Under hypertonic stress condition, *C. elegans* upregulates the biosynthetic enzyme glycerol-3-phosphate dehydrogenase (*gpdh-1*) to synthesize the organic osmolyte glycerol from glucose, which is essential for survival of the *C. elegans* under such condition. Some of the transcriptional mechanisms leading to upregulation of osmolyte accumulation genes and other genes during hypertonic stress were well studied.[18] However, the mechanism of post-transcriptional regulation for hypertonic stress is still unknown. Therefore, the authors conducted an unbiased forward genetic screen to find the key players.

Results

To identify novel regulators involving in osmolyte biosynthesis pathway, Urso et al. performed an unbiased F2 forward genetic screen for mutants that fail to up-regulate the glycerol, a primary osmolyte molecule, biosynthesis gene *gpdh-1*. The expression of gpdh-1 was visualized with *gpdh-1p::GFP* transcriptional reporter, while *col-12p:dsRed* reporter, whose expression is not affected by hypertonic stress was introduced as an internal control for non-specific effects. Two recessive alleles that genetically

fail to complement each other were identified. Subsequent sequencing-based cloning revealed that each allele contained a distinct nonsense mutation in the gene encoding the O-GlcNAc transferase *ogt-1*. Genetic complementation assay by CRISPR reversion as well as transgenic overexpression rescue the Nio (no induction of osmolyte biosynthesis gene expression) phenotype of *ogt-1*, suggesting that the *ogt-1* is required for hypertonic stress-induced up-regulation of *gpdh-1* expression.

Based on the assumption that the fluctuation of *gpdh-1* expression is an indicator of osmolyte biosynthesis pathway, Urso et al. looked into the expression levels of several mRNAs that are induced by osmotic stress. Interestingly, the expression level of these genes are still up-regulated by hypertonic stress in *ogt-1* background, and so does the GFP mRNA derived from the *gpdh-1p::GFP* reporter. Since the protein level of GFP is strongly reduced, Urso et al deduce that OGT-1 may regulate osmosensitive gene expression at post-transcriptional level. Indeed, GPDH-1::GFP protein is not induced by hypertonic stress in *ogt-1* mutants, while the mRNA is still induced to wild type level.
Further hypotonic stress assay shows that even the loss of function of ogt-1 has no effects on acute survival rate, it disrupts the ability of worms to adapt to mild hypertonic stress induced by either physiological exposures or genetically loss of function. For instance, mutants of genes encode secreted extracellular matrix (ECM) proteins such as *osm-8* and *osm-11* exhibit constitutively elevated *gpdh-1p::GFP* expression under isotonic conditions. However, the up regulation of *gpdh-1p::GFP* levels were significantly suppressed in *osm-8;ogt-1* and *osm-11*;*ogt-1* double mutants.

Tissue specific rescue assay shows the expression of *ogt-1* from a hypodermal specific promoter was sufficient to rescue *gpdh-1* induction by hypertonic stress in ogt-1 mutants, suggesting that *ogt-1* acts cell autonomously in the hypoderm to regulate osmosensitive protein expression. More interestingly, as an evolutionarily conserved protein, overexpression of a human OGT cDNA driven by *C. elegans ogt-1*

promoter partially rescued *gpdh-1p::GFP* induction by hypertonic stress, even with catalytically deficient human OGT (OGT H498A). In conclusion, a non-catalytic function of OGT-1 acts in the hypodermis and regulates osmosensitive protein induction by hypertonic stress at post-transcriptional level.

Discussion

This paper was the first time to demonstrate that OGT has a specifically conserved role independent of O-GlcNAcylation in the essential process of osmoregulation. Interestingly, the levels of the *ogt-1* encoded proteins are significantly reduced while no change of mRNAs level under the hypertonicity induced upregulation of stress. Even though the *ogt-1* is not essential in *C. elegans* as the ogt-1 knockout *C. elegans* is viable, when comes to the hypertonic stress condition related to cell volume regulation, *ogt-1* becomes essential because *ogt-1* mutants are completely unable to adapt and reproduce. Therefore, it will be worth exploring the OGT role in cell volume regulation in other organisms. In addition, the mechanism of *C. elegans* with ogt-1 knockout is viable, which is different from other organisms, remains unexplored. Apart from the O-GlcNAcylation dependent OGT functions, the wide O-GlcNAcylation independent OGT functions are still waiting to be investigated by engineering missense alleles of *ogt-1*. The authors tested the function of OGT in regulation of hypertonic stress is independent of the catalytic domain. However, the downstream targets and mechanisms of OGT-1 required in response to hypertonic stress should be illustrated. In addition, a profile change of O-GlcNAcylation of the hypertonic stress mediators should be displayed. And opposite to the OGT enzymatic activity, OGA activity or expression change also should be explored.

References

1. Varki, A. et al. Essentials of Glycobiology, *3rd edition*. *Cold Spring Harbor (NY)* (Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, (2015).
2. Ruan, H. Bin *et al.* O-GlcNAc transferase/host cell factor C1 complex regulates gluconeogenesis by modulating PGC-1α stability. *Cell Metab.* **16**, 226–237 (2012).

3. Durning, S. P., Flanagan-Steet, H., Prasad, N. & Wells, L. O-linked β-N-acetylglucosamine (O-GlcNAc) acts as a glucose sensor to epigenetically regulate the insulin gene in pancreatic beta cells. *J. Biol. Chem.* **291**, 2107–2118 (2016).
4. Whelan, S. A., Dias, W. B., Thiruneelakantapillai, L., Daniel Lane, M. & Hart, G. W. Regulation of insulin receptor substrate 1 (IRS-1)/AKT kinase-mediated insulin signaling by O-linked β-N-acetylglucosamine in 3T3-L1 adipocytes. *J. Biol. Chem.* **285**, 5204–5211 (2010).
5. Tallent, M. K. *et al.* In vivo modulation of O-GIcNAc levels regulates hippocampal synaptic plasticity through interplay with phosphorylation. *J. Biol. Chem.* **284**, 174–181 (2009).
6. Lagerlöf, O., Hart, G. W. & Huganir, R. L. O-GlcNAc transferase regulates excitatory synapse maturity. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 1684–1689 (2017).
7. Trinidad, J. C. *et al.* Global identification and characterization of both O-GlcNAcylation and phosphorylation at the murine synapse. *Mol. Cell. Proteomics* **11**, 215–229 (2012).
8. Kreppel, L. K. and Hart, G. W. Regulation of a cytosolic and nuclear O-GlcNAc transferase. Role of the tetratricopeptide repeats. *J. Biol. Chem*. **274**, 32015-32022 (1999).
9. Hanover J. A., Yu S., Lubas W. B., Shin S. H., Ragano-Caracciola M., Kochran J., Love D. C. Mitochondrial and nucleocytoplasmic isoforms of O-linked GlcNAc transferase encoded by a single mammalian gene. *Arch. Biochem. Biophys.* **409**, 287–297 (2003).
10. Love D. C., Kochan J., Cathey R. L., Shin S. H., Hanover J. A. Mitochondrial and nucleocytoplasmic targeting of O-linked GlcNAc transferase. *J. Cell. Sci*. **116**, 647–654 (2003).
11. Liu H., Gu Y., Qi J., et al. Inhibition of E-cadherin/catenin complex formation by O-linked N-acetylglucosamine transferase is partially independent of its catalytic activity. *Mol Med Rep*. **13**(2),1851-1860 (2016).
12. Giles, A. C., Desbois, M., Opperman, K. J., Tavora, R., Maroni, M. J., & Grill, B. A complex containing the O-GlcNAc transferase OGT-1 and the ubiquitin ligase EEL-1 regulates GABA neuron function. *J. of Biol. Chem.* ***294***(17), 6843-6856 (2019).
13. Kreppel L. K., Blomberg M. A., Hart G. W. 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 (1997).
14. Lubas W. A., Frank D. W., Krause M., Hanover J. A. O-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. *J. Biol. Chem*. **272**(14), 9316-24 (1997).
15. Alfieri, R. R., & Petronini, P. G. Hyperosmotic stress response: comparison with other cellular stresses. *Pflügers Archiv-European Journal of Physiology*, **454**(2), 173-185 (2007).
16. Brocker C., Thompson D. C., Vasiliou V. The role of hyperosmotic stress in inflammation and disease. *Biomol. Concepts*. **3**(4):345-364 (2012).
17. Yancey P. H. Compatible and counteracting solutes: protecting cells from the Dead Sea to the deep sea. *Sci Prog.* **87**(Pt 1):1-24 (2004).
18. Rohlfing A. K., Miteva Y., Hannenhalli S., Lamitina T. Genetic and physiological activation of osmosensitive gene expression mimics transcriptional signatures of pathogen infection in *C. elegans*. *PLoS One*. **5**(2): e9010 (2010).