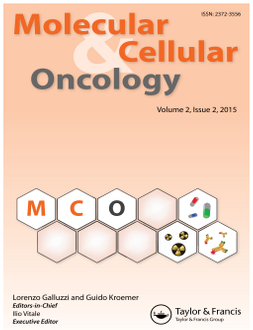

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AUTHOR'S VIEW

KEAP1 has a sweet spot: A new connection between intracellular glycosylation and redox stress signaling in cancer cells

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ABSTRACT
The KEAP1/NRF2 pathway is a master regulator of the redox stress response and is dysregulated in numerous human tumors. We discovered that NRF2 signaling is controlled by the site-specific glycosylation of KEAP1, revealing a potentially broad link among nutrient sensing, proteostasis and stress resistance in both normal and cancer cells.

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Recent years have brought renewed appreciation of the importance of cell metabolism in the etiology, progression and treatment of cancer.[1,2] Metabolic derangements are characteristic of most, if not all, human tumors, and methods of exploiting or suppressing these changes may open a therapeutic window to improve treatments.[1,2] In addition, tumor microenvironments often impose metabolic stresses, including hypoxia, lactic acidosis and glucose deprivation, which trigger gene expression and phenotypic changes during oncogenesis and cancer treatment. However, our understanding of how cancer cells sense and adapt to metabolic fluctuations or stresses remains incomplete.

One important nutrient-sensing mechanism in both normal and tumor tissues is the modification of nuclear and cytoplasmic proteins with O-linked *β-N* acetylglucosamine (O-GlcNAc), the sole form of intracellular glycosylation in mammals. O-GlcNAcylation reversibly decorates serines and threonines of thousands of nuclear, cytoplasmic and mitochondrial proteins.[3,4] In mammals, O-GlcNAc is added by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA), and O-GlcNAc cycling controls myriad processes, including cell metabolism, cell cycle progression and cell death.[3,4] UDP-GlcNAc, the nucleotide-sugar cofactor used by OGT, is biosynthesized from multiple essential metabolites, including glucose, glutamine, acetyl-coenzyme A, uridine and ATP.[4] Fluctuations in these nutrients affect the levels of O-GlcNAc, making it a sentinel for cell metabolism and nutrient status. Importantly, aberrant O-GlcNAcylation is also implicated in human diseases, especially cancer.[5] For example, numerous oncoproteins (e.g., MYC, AKT1) and tumor suppressors (e.g., TP53, AMPK) are O-GlcNAcylated, with consequences for tumor biology and anti-cancer chemotherapy.[3–5] In most cases, however, the OGT substrates that mediate signaling, and the biochemical effects that O-GlcNAc has on them, remain poorly characterized.

To elucidate the mechanisms and consequences of O-GlcNAc signaling in a cancer cell model, we profiled the global gene expression response of MDA-MB-231 cells to pharmacological inhibition of OGT or OGA.[6] Unexpectedly, we found that OGT blockade induced the expression of numerous known target genes of the transcription factor NRF2. NRF2 is a master regulator of the cellular response to oxidative stress and xenobiotics.[7,8] In unstressed cells, NRF2 associates with a complex comprising the cytoplasmic KEAP1 adaptor protein and CUL3 E3 ubiquitin ligase, which ubiquitinates NRF2, targeting it for proteasome-mediated destruction[7] (Fig. 1). During stress, various toxicants (e.g., electrophiles or oxidizing agents) covalently modify redox-active cysteines in KEAP1, impairing the ability of KEAP1/CUL3 to ubiquitinate NRF2.[7,8] Newly translated NRF2 then migrates to the nucleus, where it binds to promoters containing antioxidant response elements (ARE) and upregulates defense genes, such as the glutathione biosynthetic pathway, xenobiotic efflux pumps and drug-metabolizing enzymes.[7,8]

Consistent with our gene expression data, we found that OGT inhibition triggers NRF2 protein accumulation in the nuclei of diverse cancer cell lines.[6] Furthermore, OGT inhibition also reduced NRF2 polyubiquitination, implicating NRF2 degradation as the relevant event. Next, we used a chemical biology approach to identify OGT substrate(s) that influence NRF2 ubiquitination and activation, and found that KEAP1 (but not CUL3 or NRF2 itself) is O-GlcNAcylated.[6] Mass spectrometry-based site-mapping revealed 11 candidate O-GlcNAcylated residues in human KEAP1.[6] Among these sites, we showed that Ser104 is critical for KEAP1's function in mediating NRF2 ubiquitination, as judged by transcriptional and reactive oxygen species (ROS) readouts of NRF2 activity.[6] Interestingly, glycosylation of KEAP1 Ser104 is required for its optimal interaction with CUL3, providing a likely molecular mechanism to explain these phenotypic observations[6] (Fig. 1). Finally, we found that glucose starvation phenocopies OGT inhibition, leading to KEAP1 deglycosylation, reduced KEAP1/CUL3 interaction, and induction of the NRF2 pathway in

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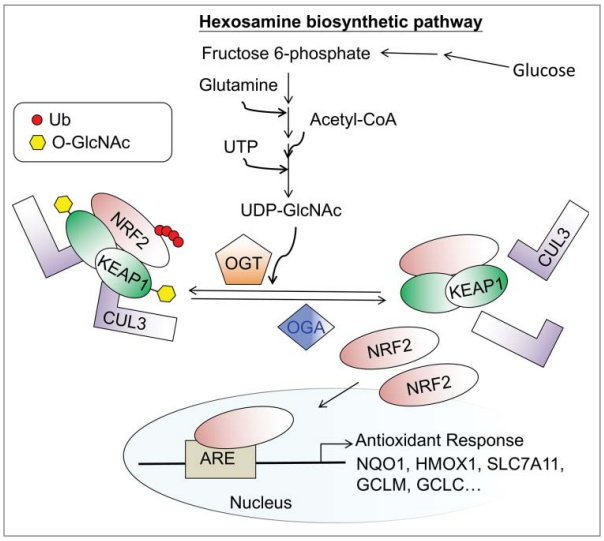

Figure 1. KEAP1 glycosylation regulates the NRF2 pathway. O-GlcNAc is a single monosaccharide post-translational modification of intracellular proteins. O-GlcNAc is added to substrates by the glycosyltransferase OGT using the nucleotide-sugar donor UDP-GlcNAc, and is removed by the glycoside hydrolase OGA. UDP-GlcNAc is synthesized by the hexosamine biosynthetic pathway from glucose, glutamine, acetyl-CoA and UTP, making O-GlcNAcylation a sentinel for several key metabolites. We found that O-GlcNAcylation of the adaptor protein KEAP1 is required for its optimal interaction with the ubiquitin E3 ligase CUL3, and for its ability to mediate the ubiquitination and destruction of the transcription factor NRF2, a master regulator of the cellular antioxidant stress response. The NRF2 pathway is inappropriately activated in many human tumors, conferring a growth advantage and treatment resistance. Our results suggest that it may be possible to suppress NRF2 signaling through the pharmacological enforcement of KEAP1 glycosylation, thereby sensitizing cancer cells to chemotherapies or radiation. (ARE: antioxidant response element; Ub: ubiquitin).

cultured cells. Notably, the gene expression signatures of low OGT activity and high NRF2 signaling are strongly correlated in various human breast tumor transcriptomic data sets.[6] Therefore, control of NRF2 signaling by KEAP1 O-GlcNAcylation is likely to be pathophysiologically significant, perhaps especially in poorly perfused, hypoglycemic/hypoxic solid tumors.

The NRF2 pathway has a well-established role in cancer etiology and treatment.[8] In particular, NRF2 is often constitutively activated in established tumors, where it confers proliferative and survival advantages in the face of ROS and chemotherapeutic drugs.[7,8] NRF2 activity is increased in human lung, breast, head and neck, ovarian and endometrial carcinomas, and high NRF2 correlates with both poor responses to chemo- and radiotherapies, and poor overall clinical outcome.[8] While many agents activate NRF2, it has been difficult to identify clinically useful NRF2 inhibitors.[7,8] Our studies demonstrated that pharmacological inhibition of KEAP1 deglycosylation, via a small molecule antagonist of OGA, prevented the induction of NRF2 by glucose starvation in cancer cells.[6] Because several tumor types rely on NRF2 for growth and survival, and because OGA inhibitors are well tolerated in animal models, our results raise the possibility that the NRF2 pathway could be suppressed by pharmacologically enforcing KEAP1 O-GlcNAcylation, potentially sensitizing tumor cells to chemo- or radiotherapies.

Finally, these observations may have implications beyond NRF2 signaling. KEAP1 is a prototypical member of the Kelch-like (KLHL) family of proteins, which has 42 human members.[9] The physiologic functions of most KLHL proteins are poorly characterized, but their importance is demonstrated by their dysregulation in a wide range of human diseases, including prostate and colon cancers (KLHL20), giant axonal neuropathy (GAN1, also called KLHL16), familial hyperkalemic hypertension (KLHL3), retinitis pigmentosa (KLHL7), and distal myopathy (KLHL9).[9] Most KLHL proteins are thought to act analogously to KEAP1, binding to CUL3 to control the ubiquitination of specific target proteins. Indeed, aberrant KLHL protein function can perturb proteostasis and lead to disease. For example, KLHL20 promotes the CUL3-mediated degradation of the tumor suppressor proteins promyelocytic leukemia and death-associated protein kinase, and inappropriate KLHL20 activity contributes to the hypoxia resistance and progression of multiple cancer types.[10] Remarkably, the critical Ser104 glycosylation site that we identified in KEAP1 is conserved in 37 of the 42 human KLHL proteins, and we have found that other family members are also glycosylated.[6] Therefore, the site-specific O-GlcNAcylation of KLHL proteins may represent a general, previously unappreciated link between nutrient sensing, proteostasis and downstream signaling. It will be interesting to delineate the full physiologic and pathological effects of KLHL protein (mis)glycosylation in future work.

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References

1. Pavlova NN, Thompson, CB. The Emerging Hallmarks of Cancer Metabolism. Cell Metab. 2016;23:27-47. doi:10.1016/j.cmet.2015.12.006. PMID:26771115
2. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: Glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16:619-634. doi:10.1038/nrc.2016.71. PMID:27492215
3. Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. Cross talk between O-GlcNAcylation and phosphorylation: Roles in signaling, transcription, and chronic disease. Annu Rev Biochem. 2011;80:825-858. doi:10.1146/annurev-biochem-060608-102511. PMID:21391816
4. Bond MR, Hanover JA. A little sugar goes a long way: The cell biology of O-GlcNAc. J Cell Biol. 2015;208:869-880. doi:10.1083/jcb.201501101. PMID:25825515
5. Ferrer CM, Sodi VL, Reginato MJ. O-GlcNAcylation in Cancer Biology: Linking Metabolism and Signaling. J Mol Biol. 2016;428:3282-3294. doi:10.1016/j.jmb.2016.05.028. PMID:27343361
6. Chen PH, Smith TJ, Wu J, Siesser PF, Bisnett BJ, Khan F, Hogue M, Soderblom E, Tang F, Marks JR, et al. Glycosylation of KEAP1 links nutrient sensing to redox stress signaling. EMBO J. 2017;36(15):2233-2250. doi:10.15252/embj.201696113. PMID:28663241
7. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. Genes Dev. 2013;27:2179-2191. doi:10.1101/gad.225680.113. PMID:24142871
8. Menegon S, Columbano A, Giordano S. The Dual Roles of NRF2 in Cancer. Trends Mol Med. 2016;22:578-593. doi:10.1016/j.molmed.2016.05.002. PMID:27263465
9. Dhanoa BS, Cogliati T, Satish AG, Bruford, EA, Friedman JS. Update on the Kelch-like (KLHL) gene family. Hum Genomics. 2013;7:13. doi:10.1186/1479-7364-7-13. PMID:23676014
10. Yuan WC, Lee YR, Huang SF, Lin YM, Chen TY, Chung HC, Tsai CH, Chen HY, Chiang CT, Lai CK, et al. A Cullin3-KLHL20 Ubiquitin ligase-dependent pathway targets PML to potentiate HIF-1 signaling and prostate cancer progression. Cancer Cell. 2011;20:214-228. doi:10.1016/j.ccr.2011.07.008. PMID:21840486