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Publication Date
2017-09-01

DOI
10.1016/j.freeradbiomed.2017.06.008

Free Radical Biology and Medicine 110 (2017) 196–205

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Nuclear factor-erythroid-2 related transcription factor-1 (Nrf1) is regulated by O-GlcNAc transferase

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ARTICLE INFO

Keywords:
Oxidative stress
O-GlcNAcylation
OGT
Transcription factor
Protein stability

ABSTRACT

The Nrf1 (Nuclear factor E2-related factor 1) transcription factor performs a critical role in regulating cellular homeostasis. Using a proteomic approach, we identified Host Cell Factor-1 (HCF1), a co-regulator of transcription, and O-GlcNAc transferase (OGT), the enzyme that mediates protein O-GlcNAcylation, as cellular partners of Nrf1a, an isoform of Nrf1. Nrf1a directly interacts with HCF1 through the HCF1 binding motif (HBM), while interaction with OGT is mediated through HCF1. Overexpression of HCF1 and OGT leads to increased Nrf1a protein stability. Addition of O-GlcNAc decreases ubiquitination and degradation of Nrf1a. Transcriptional activation by Nrf1a is increased by OGT overexpression and treatment with PUGNAc. Together, these data suggest that OGT can act as a regulator of Nrf1a.

1. Introduction

O-GlcNAc transferase (OGT) is a highly conserved enzyme that catalyzes the addition of GlcNAc moiety through a β-glycosidic O-linkage to serine and threonine residues of target proteins [1,2]. Unlike other forms of glycosylation in cells that occurs within the secretory pathway, O-GlcNAc modifications occur in the nucleus and cytoplasm [3]. O-GlcNAc glycosylation has been shown to play a role in regulating protein function by influencing enzymatic activity, stability and subcellular localization of proteins [4–7]. O-GlcNAcylation is sensitive to the nutritional state of the cell as components used for synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), the substrate for OGT, are generated from nutrients. O-GlcNAcylation also modulates regulatory pathways in response to cellular stress [8]. In addition, O-GlcNAcylation has been shown to mediate cell survival in response to increased ROS production [9]. OGT is able to associate with a diverse group of proteins through its TPR domain that mediates protein-protein interactions with numerous accessory proteins [10–13]. These accessory proteins include HCF1, TET proteins, TRAK1, BAP1, among others [14]. Interestingly, OGT also acts as a protease that catalyzes the cleavage and maturation of Host Cell Factor-1 (HCF1) [15].

HCF1 (also known as HCFC1) is a conserved protein originally identified as a required component for induction of immediate-early genes of herpes simplex virus type-1 [16,17]. However, HCF1 is now recognized as an essential co-factor for various transcription factors, and it is involved in regulating a plethora of cellular processes including cell-cycle progression and gene expression [18–21]. HCF1 is synthesized as a large 220-kDa precursor protein that undergoes site-specific proteolysis mediated by OGT into N- and C-terminal fragments of protein ranging in size from 68 to 180 kDa that remain stably associated as a complex [15]. HCF1 has no detectable DNA-binding or enzymatic activity. Instead, it contains multiple protein-protein interaction domains such as the six-blade β-propeller Kelch domain related to the *Drosophila* Kelch protein that acts as a scaffold for different proteins through a tetrapeptide consensus sequence (D/E)HYX termed the HCF-binding motif (HBM) present in target proteins [22]. HCF1 interacts with multiple transcriptional regulator proteins including, LZIP, Zhangfei, Krox20 and PGC1 [20,23–25]. HCF1 also associates with O-GlcNAc transferase, the BAP1 deubiquitinating enzyme, and various chromatin modifying enzymes [21,26–29].

Nrf1 (Nuclear factor erythroid-derived 2-related factor-1, and also known as NFE2L1) belongs to the Cap-N-Collar (CNC) family of transcription factors that includes, Nrf2, Nrf3 and p45NFE2 [30–33]. Nrf1 is essential for the maintenance of cellular homeostasis. The global knockout of Nrf1 in mice leads to embryonic lethality, whereas conditional knockout of Nrf1 in various tissues indicate Nrf1 is important in the regulation against development of steatohepatitis and neurodegeneration in mice [34–36]. Nrf1 regulates expression of genes that

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http://dx.doi.org/10.1016/j.freeradbiomed.2017.06.008
Received 21 April 2017; Received in revised form 12 June 2017; Accepted 14 June 2017
Available online 15 June 2017

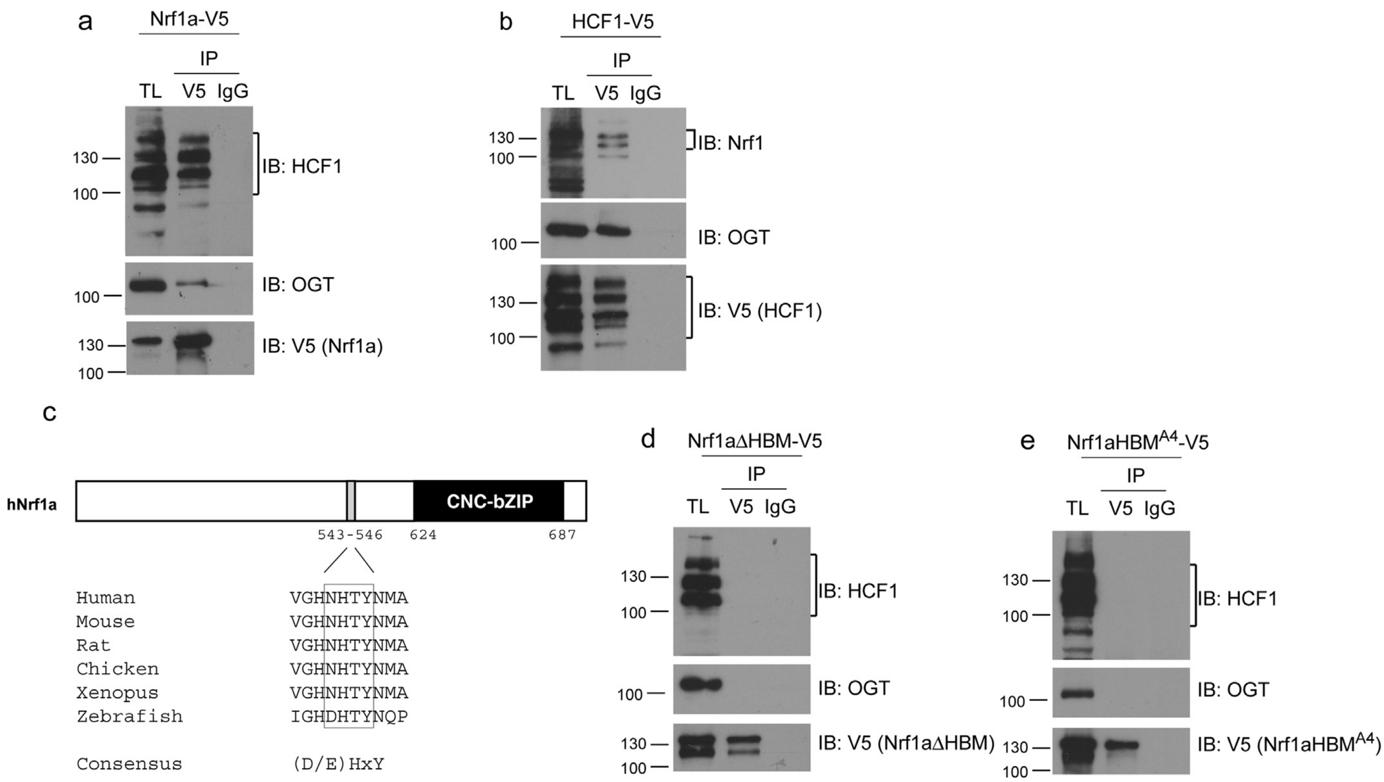

Fig. 1. Nrf1a interacts with HCF1 and OGT. The interaction between Nrf1a and HCF1 was tested by co-immunoprecipitation. (a) HEK293 cells were transfected with 10 µg V5-tagged Nrf1a, and cell lysate was immunoprecipitated using the anti-V5 mAb followed by western blotting using anti-HCF1 Ab and anti-OGT mAb. Western blotting with anti-V5 mAb was done as input control. (b) HEK293 cells were transfected with 10 µg V5-tagged HCF1, and cell lysate was immunoprecipitated using the anti-V5 mAb followed by western blotting using antibodies as described in panel-A. (c) Schematic diagram showing the amino acid sequence and location of HCF1 binding motif (HBM) on Nrf1a. HEK293 cells were transfected with (d) 10 µg Nrf1aΔHBM-V5, a mutant Nrf1a containing a deletion of the HBM, or (e) 10 µg Nrf1aHBM^{A4}-V5, a mutant Nrf1a in which the HBM sequence has been changed to four alanine residues, and Nrf1a was immunoprecipitated using the anti-V5 mAb followed by western blotting as described above. As previously reported, Nrf1a exists in two major forms that are detected here at approximately 130 and 110 kDa [45–47]. The p130 represents the ER-membrane embedded protein and the p110 is the soluble form (lacking the N-terminal peptide) that is processed from p130. OGT migrates at 110 kDa, and HCF1 is seen as multiple bands ranging in size from approximately 100 to 175 kDa.

encode antioxidant genes via cis-active sequences known as the antioxidant response element [37,38]. Nrf1 is also important in the proteotoxic stress response through its role in regulating expression of genes encoding the proteasome [35,39,40]. Aside from cellular stress response, Nrf1 has also been shown to regulate differentiation of osteoblast and odontoblast, and loss of Nrf1 function leads to genetic instability and the promotion of tumorigenesis [41–43].

Multiple isoforms of Nrf1 exist; these protein isoforms include, TCF11, Nrf1a, p65NRF1 (also referred to as LCRF1/Nrf1β), Nrf1b, among others. TCF11, consisting of 772 amino acids, is the longest form of Nrf1 [44]. Nrf1a (742 aa) is generated through alternate splicing of exon 4 of the Nrf1 gene and lacks a Neh4 subdomain (aa 242–271 of TCF11) that contains a transactivation domain [44]. TCF11 and Nrf1a are anchored within the endoplasmic reticulum membrane through their N-terminal domain, and they undergo retro-translocation into the cytoplasm and processing prior to entry into the nucleus to promote gene expression [45–47]. Short isoforms of Nrf1 such as p65Nrf1 and Nrf1b lack the ER-membrane targeting domain. p65Nrf1 is a made up of 453 amino acids, and it has been reported to possess weak activation function, as well as trans-dominant negative effects on gene activation [48–50]. The Nrf1b isoform is generated from an alternative transcript, through an alternate promoter, that contains a unique N-terminus encoded by an alternate first exon. Nrf1b encodes for a protein of 583 amino acids and it is also capable of mediating gene activation [51].

Our previous work indicated that Nrf1a is a target for post-translational modification by ubiquitination. We hypothesize that stabilization of Nrf1a in response to stress arises due to its post-translational modification through deubiquitination. Through screening for the putative regulators of Nrf1a, we found that Nrf1a interacts with HCF1 and OGT. We show that O-GlcNAc modification by OGT leads to decreased ubiquitination and stabilization of Nrf1a, and HCF1 and OGT enhances transcriptional activation by Nrf1a.

2. Material and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), streptomycin, penicillin and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). Bradford protein assay reagent was from BioRad (Hercules, CA). BioT was purchased from Bioland Scientific (Paramount, CA). Dual Reporter Assay Kit was from Promega (Madison, WI). ImmunoPure streptavidin, and enhanced chemiluminescence substrate kit was from Pierce Biotechnology (Rockford, IL). Sequencing grade trypsin was purchased from Promega Corp. (Madison, WI), and chymotrypsin was from Roche (Palo Alto, CA). Tunicamycin and PUGNAc were purchased from Sigma-Aldrich (St. Louis, MO). All other general chemicals for buffers and culture media were purchased from ThermoFisher Scientific and/or Sigma-Aldrich. Primary antibodies against Tubulin-A (3873, mouse mAb), HCF1 (50708), OGT (24083, Rabbit mAb), Flag-Tag (14793, Rabbit mAb), Myc-Tag (2276, mAb), and HA-Tag (2367, mAb) were purchased from Cell Signaling Technology (Beverly, MA). Horseradish peroxidase linked anti-rabbit IgG (7074) and anti-mouse IgG (7076) antibodies were also from Cell Signaling Technology (Beverly, MA). Mouse monoclonal anti-V5 tag (MA5-15253) and RL2 antibodies (anti-O-GlcNAc; MA1-072) were from ThermoFischer. Antibodies from Cell Signaling Technology and ThermoFischer have been tested and validated on the lot level by

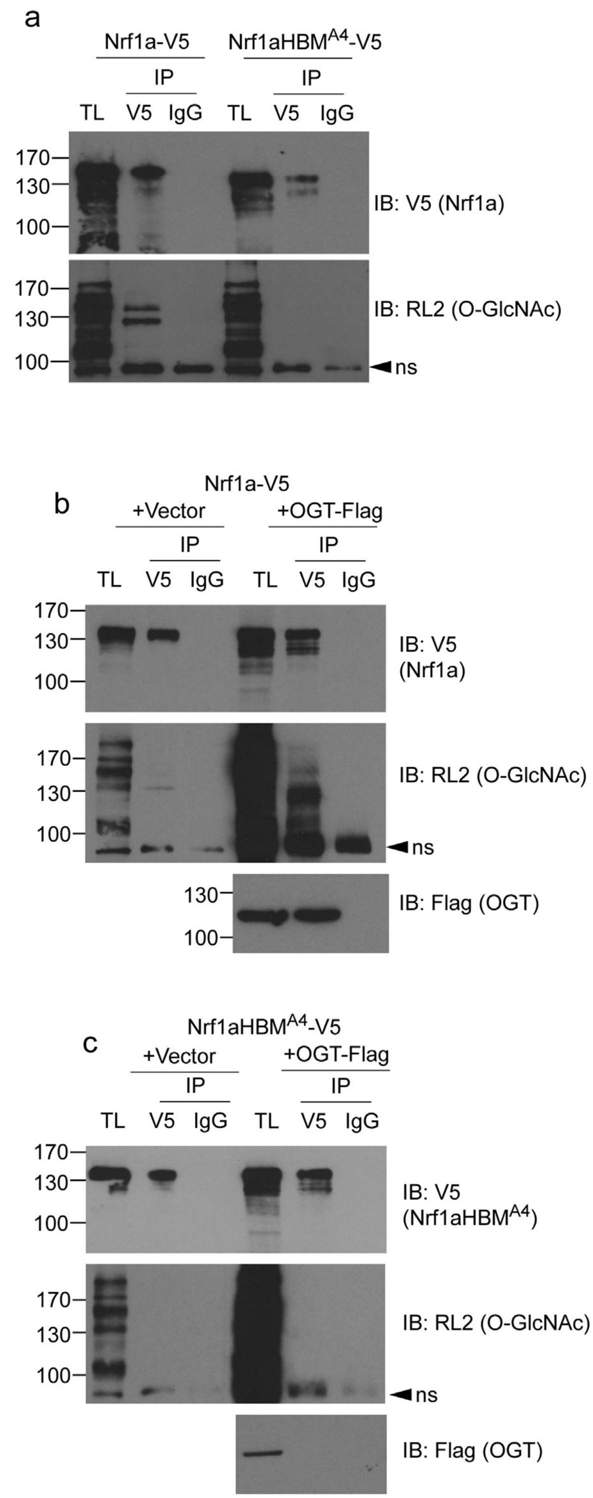

Fig. 2. Nrf1a is O-GlcNAc modified by OGT. O-GlcNAc modification of Nrf1a was examined by co-immunoprecipitation assays. (a) HEK293 cells were transfected with 10 μg V5-tagged Nrf1a or V5-tagged HBM^{A4} mutant form of Nrf1a, and Nrf1a immunoprecipitated using the anti-V5 mAb followed by western blotting with anti-V5 as input control, and the anti-O-GlcNAc mAb, RL2 to detect O-GlcNAcylation of Nrf1a. HEK293 cells were transfected with 2 μg (b) Nrf1a-V5 or (c) V5-tagged Nrf1aHBM^{A4}-V5 along with Flag-tagged OGT or control vector, Nrf1a was immunoprecipitated with anti-V5 (2 μg) and analyzed for O-GlcNAc modification by Western blotting with RL2. Immunoblotting against V5 and Flag was done as input controls.

manufacturer's in-house scientists for specificity, sensitivity, and reproducibility. Rabbit antibody against endogenous Nrf1 was previously described, and has been validated for specificity using Nrf1 knockout cells [47].

2.2. Plasmids

Nrf1a-HTBH was generated by cloning Nrf1a cDNA into the NotI and Pac1 sites of the HTBH expression plasmid, which contains two different affinity (streptavidin and hexahistidine) purification tags [52]. OGT-Flag and HCF1-Myc plasmids were from Addgene (Danvers, MA). HCF1-V5 was a gift from Dr. Thomas Kristie (NIH), and HA-tagged Ubiquitin expression plasmid was a gift from Dr. Peter Kaiser (University of California, Irvine). Nrf1a-V5 was previously described. Nrf1aΔHBM and Nrf1aHBM4^{A} were generated by inverse PCR using the Nrf1a-V5 as template with the primers Nrf1aΔHBM F/R and Nrf1aHBM4^{A} F/R, respectively (Nrf1aΔHBM-F: TACAACATGGCACCC-AGTGCC; Nrf1aΔHBM-R: CACGTGCTCCAGGT-AGGG; Nrf1aHBM4^{A}-F: GCAGCCAACATGGCACCCAG-TGCCCTG; Nrf1aHBM4^{A}-R: AGCTGCGT-GGCCCACGTGCTCC-AGGTA).

2.3. Tandem affinity purification and mass spectrometric analysis of Nrf1-interacting proteins

Purification and LC – MS/MS analysis of Nrf1 complex were done as previously described [52,53]. In brief, HEK293 cells stably expressing either Nrf1a-HTBH or the HTBH tag were grown to confluence, and collected by trysinization. Cell pellets were lysed in buffer A [100 mM sodium chloride, 50 mM sodium phosphate, 10% glycerol, 5 mM ATP, 1 mM DTT, 5 mM $MgCl_2$, 1 × protease inhibitor, 1 × phosphatase inhibitor, and 0.5% NP-40 (pH 7.5)]. Lysates were cleared by centrifugation at 13 ,000 rpm for 15 min, and the supernatant was incubated at 4 °C overnight with streptavidin resin. The streptavidin beads were then washed with 20 bed volumes of buffer A, followed by a final wash with 10 bed volumes of TEB buffer [50 mM Tris–HCl (pH 7.5)] containing 10% glycerol. Beads were then incubated in 2 bed volumes of TEB buffer with 1% TEV at 30 °C for 1 h. Nrf1a protein was eluted from the beads with TEB. The final eluate was concentrated and subjected to mass spectrometric analysis.

2.4. Western Blotting

Cells were lysed in cold RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1X Protease Inhibitor). Lysates were cleared by centrifugation for 15 min at 4 °C, and protein concentrations were determined by Bradford assay. An equal volume of 2 × SDS sample buffer (100 mM Tris, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromphenol blue, 10% 2-mercaptoethanol) was added to cell lysates, and the mixture was boiled for 5 min. Proteins were electrophoresed on SDS-PAGE gels and transferred onto nitrocellulose membranes. Membranes were then blocked in 5% skim milk in TBS-T (150 mM NaCl, 50 mM Tris-HCl pH 8.0, and 0.05% Tween 20) at room temperature for one hour, and then incubated with the indicated primary antibodies at 1:1000 dilution (unless otherwise indicated) overnight at 4 °C followed by a incubation with 1:2000 dilution of horseradish peroxidase-conjugated secondary anti-rabbit, or anti-mouse antibody. Antibody-antigen complexes on the blots were detected using chemiluminescent detection system. Densitometric analysis was performed using the Un-Scan-It Gel Analysis software (Orem, UT) for normalization.

2.5. Transfection and luciferase assays

Cells were seeded onto a 24-well plate twenty fours before transfections. DNA transfections were done using BioT reagent according to the manufacturer's protocol. Cellular extracts were prepared 48 h after transfection, and Firefly- and Renilla-luciferase activities measured using Dual Reporter Assay Kit.

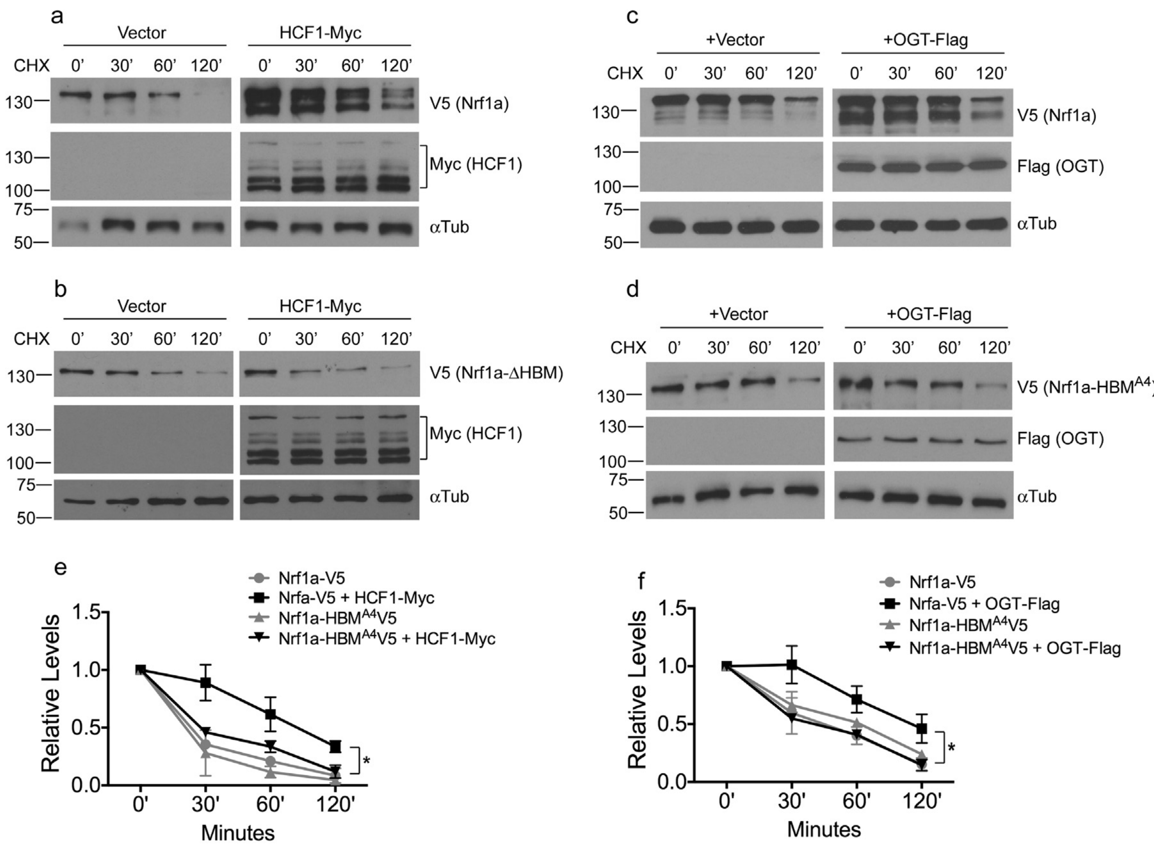

Fig. 3. Expression of HCF1 and OGT leads to increased Nrf1a stability. Cycloheximide chase was done to test the effects of HCF1 and OGT on Nrf1a stability. HEK293 cells were transfected with 1 µg (a) Nrf1a-V5 or (b) Nrf1aHBM^{A4}-V5 along with 1 µg HCF1-Myc or control vector. After 24hr, cells were treated with cycloheximide (50 µg/mL), and harvested at the indicated time points for western blotting using anti-V5 and anti-Myc antibodies. HEK293 cells transfected with 1 µg (c) Nrf1a-V5 or 1 µg (d) Nrf1aHBM^{A4}-V5 along with 1 µg OGT-Flag or control vector. After 24hr, cells were treated with cycloheximide (50 µg/mL), and harvested at the indicated time points for western blotting using anti-V5 and anti-Flag antibodies as described above. Loading of the lanes was determined by western blotting against alpha-tubulin. (e and f) Graphs show protein levels determined by densitometric quantification of band intensities in western blots normalized to alpha-tubulin. Each point represents the mean ± SEM of remaining protein for 3 independent experiments, *P value < 0.05, Student's *t*-test.

2.6. Co-Immunoprecipitation

Subconfluent HEK293T cells were transfected with 5–10 µg of expression vectors using BioT according to manufacturer's protocol. Cells were lysed in buffer containing 2% SDS, 150 mM NaCl, 10 mM Tris–HCl (pH 8.0), and 1 mM DTT, and then boiled for 5 min to disrupt protein-protein interactions. Lysates prepared in SDS-containing buffer were diluted fivefold with buffer lacking SDS. Diluted lysates were subjected to pre-clearing with protein-G Sepharose beads by incubation in the cold for 1-h. The protein samples were incubated with 2–5 µg of primary antibodies or IgG as a control overnight at 4 °C. After overnight incubation, protein-G Sepharose beads were added and incubated at 4 °C for 1 h. Beads were then collected by brief centrifugation and washed extensively with RIPA buffer. Proteins were eluted in 1 × SDS sample buffer and heated at 95 °C for 5 min. The samples were separated by SDS-PAGE and transferred onto a nitrocellulose membrane, followed by immunoblotting with indicated primary antibodies and horseradish peroxidase-conjugated secondary antibodies. Detection of peroxidase signal was performed using the chemiluminescence method.

2.7. Cycloheximide chase assays

HEK293T cells expressing various constructs indicated in the figures were incubated with 50 µg/mL cycloheximide in DMEM at 37 °C. At the times indicated in the figures, cells were harvested and lysates prepared for immunoblotting.

2.8. Statistical analysis

Data are expressed as means ± SEM. Statistical analyses using Student's *t*-test were done with Microsoft Excel (Redmond, WA). * indicates p values < 0.05, and considered significant.

3. Results

3.1. HCF and OGT interact with Nrf1a

To identify novel Nrf1-interacting proteins that may modulate Nrf1 functionality, streptavidin-tagged Nrf1a purified from HEK293 cells was analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). HCF1 and OGT were repeatedly identified in three independent affinity purifications and LC-MS/MS analysis (data not shown). To validate the mass spectrometry data, we conducted immunoprecipitation and Western blot analysis of lysates prepared from HEK293 cells expressing Nrf1a-V5 fusion protein. Both HCF1 and OGT were detected (Fig. 1a). Next, we conducted co-immunoprecipitation assays using HEK293 cells expressing V5-tagged HCF1. Western blot analysis detected both endogenous Nrf1a and OGT (Fig. 1b). Proteins that have shown to interact with HCF1 bind via a tetrapeptide sequence - (D/E)HXY, designated the HCF1-binding motif (HBM). Sequence analysis revealed a consensus HBM (NHTY) in human Nrf1a, and is conserved in other species (Fig. 1c). To determine the significance of the HBM in Nrf1a in HCF1 binding, we prepared two HBM mutants for co-immunoprecipitation assays. The deletion of the NHTY sequence (Nrf1a-V5ΔHBM) completely abrogated the interaction between Nrf1a and HCF1 (Fig. 1d). Similar results were obtained with

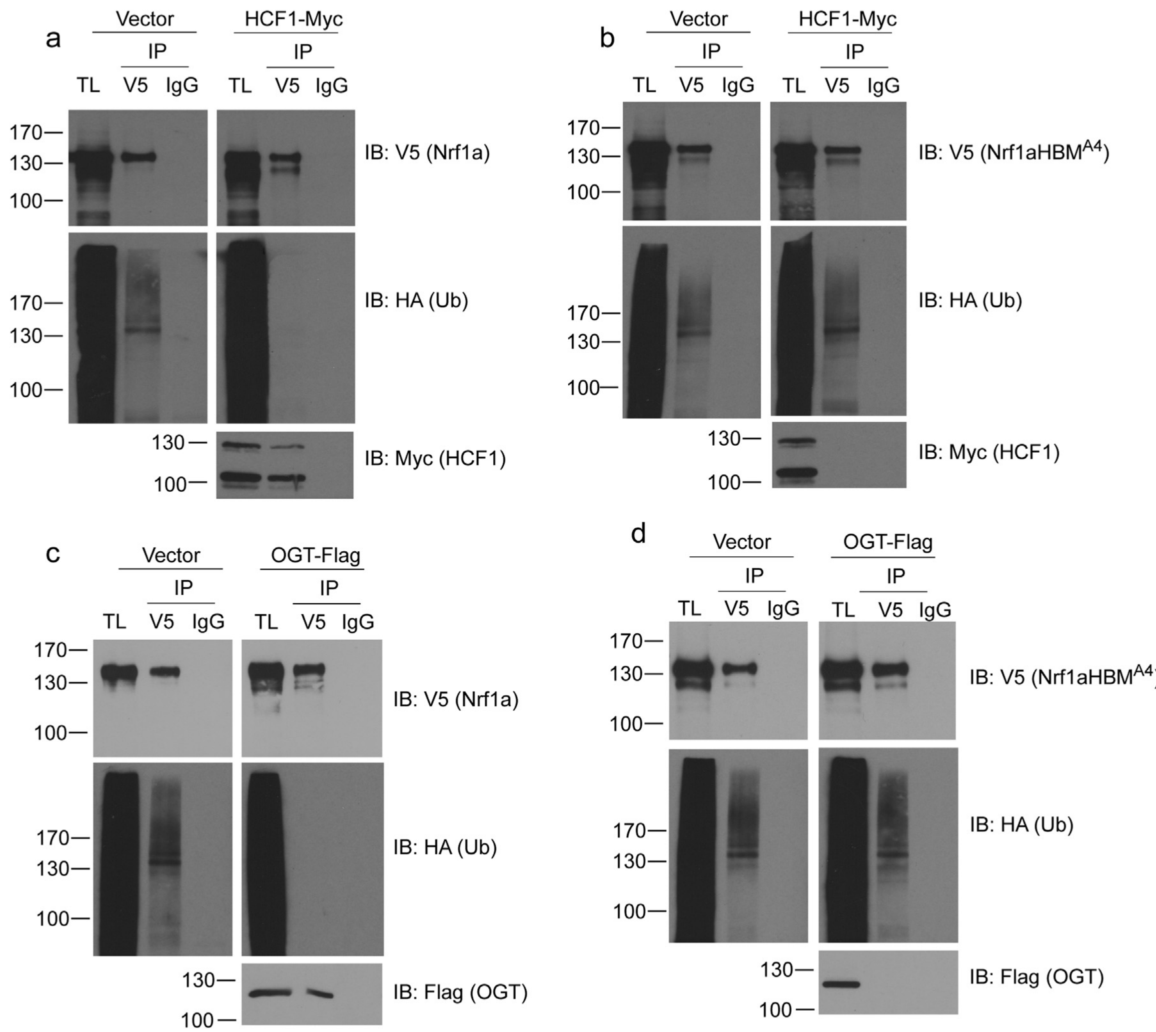

Fig. 4. Ubiquitination on Nrf1a is modulated by HCF1 and OGT. Investigation of the effects of HCF1 and OGT on Nrf1a ubiquitination was done by immunoprecipitation assays. HEK293 cells transfected with 1 µg HA-Ub and 2 µg (a and c) Nrf1a-V5 or 2 µg (b and d) Nrf1aHBM^{A4}-V5 along with (a and b) 5 µg HCF1-Myc or (c and d) 5 µg OGT-Flag. Cell extracts were then prepared 24 h after and immunoprecipitated with anti-V5 mAb and Nrf1a analyzed for their ubiquitin content by immunoblotting with an anti-HA Ab. Expression of HCF1 and OGT was verified by western blotting using anti-Myc and anti-Flag antibodies.

Nrf1a-V5HBM^{A4}, in which the NHTY sequence has been changed to four alanine residues (Fig. 1e). In addition, both HBM mutants failed to precipitate OGT (Fig. 1d and e), suggesting Nrf1a associates with OGT in vivo through its interaction with HCF1. Based on these results, we conclude that HCF1 and OGT interact with Nrf1a.

3.2. Nrf1a O-GlcNAcylation is increased by OGT over expression

To determine the significance of OGT-Nrf1a interaction, we examined if Nrf1a is O-GlcNAcylated. Nrf1a-V5 transfected into HEK293 cells was immunoprecipitated and probed with RL2, an O-GlcNAc-specific antibody. O-GlcNAc-reactive bands corresponding to Nrf1a were detected in the immunoprecipitate from cells expressing Nrf1a-V5, but not in the immunoprecipitate from cells expressing the HBM^{A4} mutant of Nrf1a (Fig. 2a). Next, we examined whether Nrf1a undergoes O-GlcNAcylation by OGT. HEK293 cells were transfected with OGT-Flag along with Nrf1a-V5 or Nrf1aHBM^{A4}-V5 mutant, and the effects of OGT on O-GlcNAcylation of Nrf1 was assessed by co-immunoprecipitation and immunoblotting. As shown in Fig. 2b, OGT overexpression significantly augmented levels of O-GlcNAc-reactive bands in cells transfected with wild type Nrf1a, but not HBM^{A4} mutant Nrf1a (Fig. 2c). From these results, we conclude that Nrf1 can undergo O-GlcNAcylation by OGT.

3.3. Expression of HCF1 and OGT increases the half-life of Nrf1a

Next, we sought to examine whether HCF1 modulates the stability of Nrf1a protein. HEK293 cells were transfected with Nrf1a-V5 along with Myc-tagged HCF1, or vector-control. Chase assays were then performed and Nrf1a stability was assessed over a period of 120 min in the presence of cycloheximide, an inhibitor of protein synthesis. Western blotting showed that while Nrf1a-V5 levels rapidly diminished in cells transfected with empty vector, the half-life of Nrf1a-V5 was increased from 30 min to approximately 90 min by HCF1-Myc (Fig. 3a, e). In contrast, expression of HCF1-Myc had no effect on Nrf1a-HBM^{A4}-V5 (Fig. 3b, e). We then assessed whether an excess of OGT would also affect Nrf1 stability. Cycloheximide chase assays were carried out on HEK293 cells transfected with Nrf1a-V5 along with OGT-Flag expression construct, or vector-control. OGT-Flag expression also increased the half-life of Nrf1a-V5 by approximately 3-fold in HEK293 cells (Fig. 3c, f). However, Western blotting showed that OGT-Flag overexpression did not alter the half-life of Nrf1a-HBM^{A4}-V5 (Fig. 3d, f). It is also interesting to note that both the membrane-bound and processed form of Nrf1a is stabilized by HCF1 and OGT. Based on these results, we conclude that the Nrf1a protein is stabilized by HCF1 and OGT.

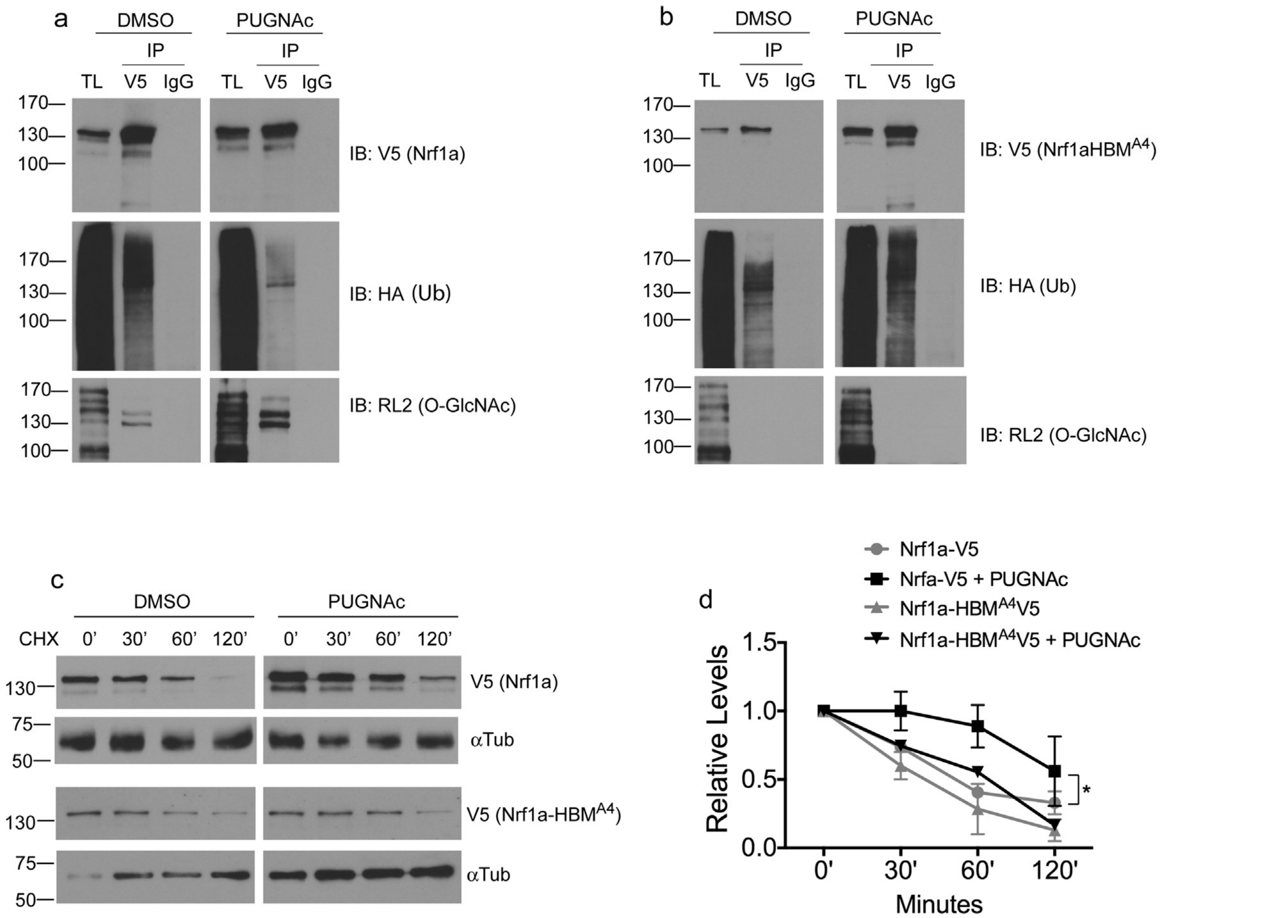

Fig. 5. The half-life of Nrf1a is increased by PUGNAc. HEK293 cells were transfected with 5 µg (a) Nrf1a-V5 or (b) Nrf1aHBM^{A4}-V5 along 1 µg HA-Ub. After 24 h, cells were treated with 100 µM PUGNAc for 24 h to inhibit OGA-mediated removal of O-GlcNAc from proteins in cells. Cell extracts were then prepared and immunoprecipitated with an anti-V5 mAb and ubiquitin content on Nrf1a was analyzed by immunoblotting with anti-HA antibody. O-GlcNac on Nrf1a was analyzed by immunoblotting with RL2. (c) HEK293 cells were transfected with 1 µg Nrf1a-V5 or 1 µg Nrf1aHBM^{A4}-V5, and after 24hr, cells were treated with 100 µM PUGNAc for 24hr and followed by cycloheximide (50 µg/mL). Cells were then harvested at the indicated time points for western blotting using anti-V5 mAb. Loading in each lane was determined by immunoblotting against alpha-tubulin. (d) Graph shows protein levels determined by densitometric quantification of band intensities in western blots normalized to alpha-tubulin. Each point represents the mean ± SEM of remaining protein for 3 independent experiments, *P value < 0.05, Student's *t*-test.

3.4. Ubiquitination of Nrf1a is decreased by expression of HCF1 and OGT

Because Nrf1a has been previously demonstrated to be a short-lived protein degraded through the ubiquitin–proteasome pathway (56), we speculated that HCF1 and OGT may promote stabilization by inhibition of ubiquitination of Nrf1a. To test this idea, in vivo ubiquitination assays were done. HEK293 cells were transfected with HA-tagged ubiquitin expression vector along with expression constructs encoding Myc-tagged HCF1 and Nrf1a-V5. Lysates were immunoprecipitated with anti-V5 antibody followed by immunoblotting using anti-HA antibody. Levels of ubiquitinated Nrf1a were markedly reduced by expression of HCF1 (Fig. 4a, compare middle panels). Next, we examined the effects the HBM mutation of Nrf1a on deubiquitination in vivo. Western blotting of lysates immunoprecipitated from HEK293 cells transfected with Nrf1a-HBM^{A4}-V5 showed that levels of ubiquitinated Nrf1a was not diminished by HCF1 expression (Fig. 4b, compare middle panels). We then assessed the effects of OGT overexpression on Nrf1a ubiquitination. HEK293 cells were transfected with Ub-HA and Nrf1a-V5 along with OGT-Flag, or empty vector-control. Lysates were then immunoprecipitated with anti-V5 antibody followed by western blotting with anti-HA antibody. Similar to the effects of HCF1, a reduction in ubiquitinated Nrf1a levels was observed in cells transfected with OGT-Flag (Fig. 4c). However, this effect was not seen in the absence of HCF1 binding site when the HBM^{A4} mutant form of Nrf1a-V5 was used (Fig. 4d). From these results, we conclude that Nrf1 ubiquitination is reduced by HCF1 and OGT.

3.5. O-GlcNAcylation promotes stabilization of Nrf1

Next, we asked whether the O-GlcNAc modification of Nrf1a is of functional significance. We tested the hypothesis that O-GlcNAcylation stabilizes the Nrf1a protein. To do this, we first examined the effects of PUGNAc, an inhibitor of O-GlcNAcase (OGA) enzyme that catalyzes removal of O-GlcNAc from proteins, on Nrf1a ubiquitination. To determine whether O-GlcNAcylation modulates Nrf1a ubiquitination, we carried out an in vivo ubiquitination assay. HA-tagged ubiquitin expression vector was transfected into HEK293 cells along with Nrf1a-V5 or Nrf1aHBM^{A4}-V5. Cells were then treated with DMSO-control, or PUGNAc, and lysates were prepared for immunoprecipitation and immunoblot analysis. A marked decrease in ubiquitinated Nrf1a-V5 levels was observed with PUGNAc treatment (Fig. 5a). However, ubiquitination of the HBM^{A4} mutant of Nrf1a was not diminished by PUGNAc-mediated inhibition of OGA (Fig. 5b). Next, we examined the effects of PUGNAc on Nrf1a-V5 protein levels in HEK293 cells. Cycloheximide chase analysis revealed that the half-life of Nrf1a-V5 protein was increased from 30 min in controls to approximately 120 min in PUGNAc-treated cells (Fig. 5c). By contrast, PUGNAc treatment had no effect on the half-life of Nrf1aHBM^{A4}-V5 (Fig. 5c). From these results, we conclude that O-GlcNAc levels modulate the ubiquitination and stabilization of Nrf1 protein.

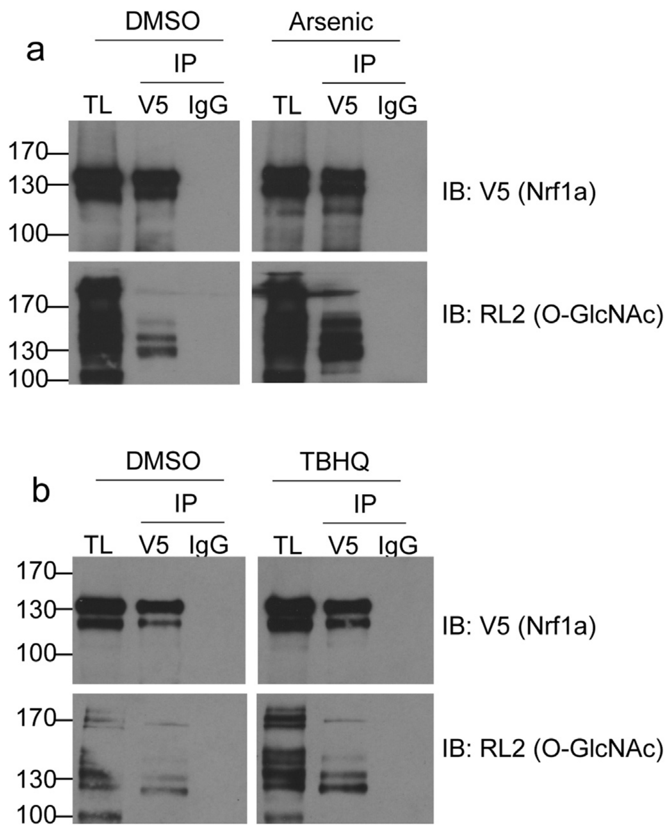

Fig. 6. Oxidative stress stimulates O-GlcNAc modification of Nrf1a. HEK293 cells were transfected with Nrf1a-V5, and 24 h after transfection, cells were treated with (a) arsenic (5 μM), (b) TBHQ (100 μM) or DMSO for 24 h. Cell lysates were then prepared and Nrf1a was immunoprecipitated with anti-V5 and analyzed for O-GlcNAc modification by western blotting with RL2. As input control, immunoprecipitates were subjected to immunoblotting against V5-tag.

3.6. O-GlcNAcylation of Nrf1 is elevated by oxidative stress

Induction of oxidative stress by arsenic and tert-butylhydroquinone (TBHQ) has been shown to up-regulate Nrf1a protein expression [54]. Hence, we wondered whether oxidative stress affected O-GlcNAc modifications of Nrf1a. To do this, Nrf1a-V5 transfected HEK293 cells were treated with arsenic and TBHQ, and whole cell lysates were subjected to immunoprecipitation with anti-V5 antibody, and followed by examination of O-GlcNAc modification of Nrf1a-V5. As shown in Fig. 6, western blot analysis of cellular extracts from treated and untreated cells with RL2, an O-GlcNAc-specific antibody, indicates that total O-GlcNAc modified protein content was not substantially altered by arsenic or TBHQ. Next, immunoprecipitates were western blotted against V5, and similar amounts of Nrf1a-V5 were detected in treated and untreated cells indicating that expression of transfected Nrf1a-V5 was not altered by drug treatment. In contrast however, western blots showed O-GlcNAc modification of immunoprecipitated Nrf1a-V5 was increased by TBHQ, and especially by arsenic. Taken together, these findings suggest that O-GlcNAc modification of Nrf1a is enhanced by arsenic and TBHQ.

3.7. Nrf1 mediated transactivation of ARE-genes is enhanced by OGT

Our results indicate that OGT plays a role in modulating the turnover rate of Nrf1a. To determine the biological implication of this finding, we examined the effects of OGT on Nrf1a-mediated activation of antioxidant response element (ARE)-driven targets. To test this, Nrf1a and OGT were co-expressed in HEK293 cells, and their effects on the ARE-driven luciferase reporter were analyzed. Co-expression of OGT potentiated Nrf1a-mediated activation of luciferase reporter (Fig. 7a). In contrast, activation of luciferase expression by Nrf1aΔHBM-V5, which is incapable of interacting with OGT, was not enhanced by co-transfection of OGT expression plasmid. Next, we evaluated the effect of PUGNAc-mediated elevation of O-GlcNAcylation on Nrf1-dependent luciferase reporter expression. As shown in Fig. 7b, PUGNAc treatment increased the reporter activation by Nrf1a-V5, but not by Nrf1aΔHBM-V5. To further confirm the role of protein O-GlcNAcylation on Nrf1a-mediated transcriptional activation, we examined the effects of PUGNAc treatment on expression of endogenous Nrf1-target genes in cells. Nrf1-/- MEF cells were transduced with Nrf1a or empty vector. Cells were then treated with DMSO, or PUGNAc, and mRNA levels of various known Nrf1-regulated genes were measured using RT-qPCR. As shown in Fig. 7c, PUGNAc treatment resulted in a 1.5–2-fold induction of GCLC, GCLM, GSTA4, PSMB2, PSMC5, and PSMD12 in cells expressing Nrf1a. In contrast, induction was not observed in vector transduced Nrf1-/- cells treated with PUGNAc. Together, these results suggest that O-GlcNAcylation can modulate ARE-mediated gene transcription in cells through Nrf1a.

4. Discussion

The Nrf1 transcription factor regulates expression of cytoprotective and detoxification genes, and genes involved in development and other cellular processes. In this study, we identified OGT and HCF1 as cellular partners of Nrf1a, and OGT interacts with Nrf1a through HCF1. We provide evidence that Nrf1a undergoes O-GlcNAc modification by OGT, and O-GlcNAcylation enhances the stability and transcriptional activity of Nrf1a.

A growing body of evidence demonstrates that O-GlcNAcylation occurs in response to diverse forms of damaging agents resulting from normal metabolic processes, as well as environmental factors, suggesting that O-GlcNAc modifications of protein is a component of the stress response program in cells [9,55–58]. For instance, oxidative stress induced by hydrogen peroxide treatment leads to increased FOXO4 O-GlcNAcylation and enhanced transcriptional activity [59]. The FOXO1 transcription factor, which also has an important role in the oxidative stress response, is also known to undergo O-GlcNAc modification [59,60]. Our data shows that O-GlcNAc modification increases Nrf1a stability, and treatment to induce oxidative stress promotes O-GlcNAc modification of Nrf1a. Furthermore, PUGNAc treatment, which increases O-GlcNAc modification of proteins, resulted in increased reporter gene activation by Nrf1a. These findings suggest that O-GlcNAc also regulates ARE-mediated gene expression by Nrf1a. While our findings here suggest that increased activation by Nrf1a takes place as a result of increased protein stability, we cannot rule out from the current studies that O-GlcNAcylation may also affect the cellular localization or activity of Nrf1a. Additional experimentation is required to address these possibilities.

The control of protein stability plays an important role in regulation of numerous transcription factors. We have previously shown Nrf1a is a short-lived protein degraded through the ubiquitin–proteasome pathway [61]. We show that overexpression of HCF1 and OGT, as well as PUGNAc treatment decreases steady-state ubiquitination of Nrf1a. Based on this data, we surmise that O-GlcNAcylation modulates the ubiquitination and subsequent degradation of the Nrf1a protein. Although the mechanism by which this occurs is not known currently, O-GlcNAcylation exerting an effect on ubiquitination has also been reported for other proteins [62–65]. One possibility is that O-GlcNAc modified residues serves as docking sites for deubiquitinase enzymes that then removes ubiquitin from the protein. For instance, O-GlcNAcylation of PGC-1a has been suggested to recruit BRCA1 Associated Protein-1 (BAP1), a ubiquitin hydrolase, which cleaves ubiquitin from PGC-1a, thus rescuing the protein from UPS-mediated degradation [66]. It would be of interest to investigate whether O-GlcNAcylation of Nrf1 also promotes interaction with deubiquitinating enzyme such as BAP1, or other ubiquitin hydrolases, and to study how such interactions

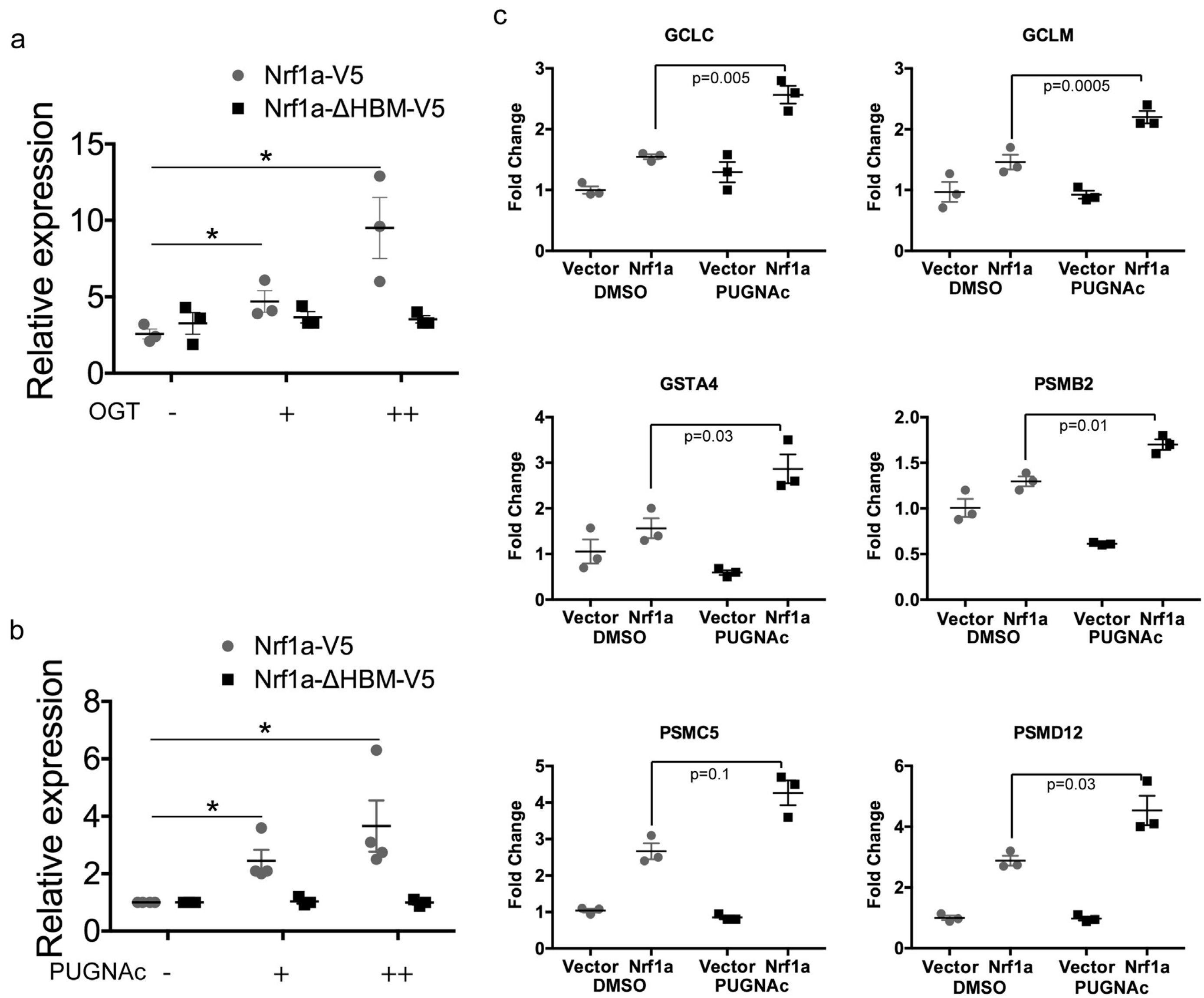

Fig. 7. Nrf1a mediated transactivation of ARE-genes is enhanced by O-GlcNAcylation. (a) Luciferase activity in HEK293 cells transfected with 10 ng GCLM-luciferase (a luciferase reporter driven by the human GCLM gene promoter that contains an antioxidant response element), and 10 ng Nrf1a-V5, or Nrf1aΔHBM-V5 expression vector, along with 10 ng or 20 ng OGT expression vector and 0.2 ng RLTK control. Firefly luciferase activity was normalized to Renilla luciferase activity. Luciferase activity in the absence of OGT expression plasmid was set as 1-fold activation. Data is presented as means ± SEM for 3 independent experiments each containing 3 replicates. *P value < 0.05, Student's *t*-test. (b) HEK293 cells were transfected with 10 ng GCLM-luciferase, along with 10 ng Nrf1a-V5, or Nrf1aΔHBM-V5 and 0.2 ng RLTK control. After 24hr, cells were treated with PUGNAc (100 µM or 200 µM) or DMSO vehicle control, and after overnight incubation, luciferase activity was normalized to Renilla luciferase activity. Data represents means ± SEM of three independent experiments. *P value < 0.05 (Student's *t*-test) relative to DMSO treated cells. (c) Nrf1-/- MEF cells transduced with control, or Nrf1a retrovirus was cultured with DMSO or PUGNAc (100 µM) for 24 h; and analyzed for expression of known Nrf1 target genes. Dot-plots depict relative expression of indicated genes. P values were calculated by *t*-test (n = 3).

might function in gene regulation events during cellular stress response.

O-GlcNAc modification is a dynamic process that responds to various cellular cues. O-GlcNAc sites on proteins can also undergo phosphorylation, and it is accepted that there is extensive crosstalk between these two forms of modifications in the regulation of cell signaling pathways [67,68]. The phosphorylation site can be at the same location as the O-GlcNAcylation site, or at nearby serine and threonine residues in the protein. Several studies have shown that Nrf1 is regulated by phosphorylation. Glycogen Synthase Kinase 3 (GSK3) was previously demonstrated to negatively regulate Nrf1a stability [69]. Phosphorylation of Nrf1a facilitated by GSK3 was shown to recruit Fbw7, the F-box containing ubiquitin ligase, for proteasomal degradation of Nrf1a. Future studies aimed at determining if O-GlcNAcylation by OGT plays a role in modulating GSK3-dependent, FBX7-mediated Nrf11 degradation are warranted.

While this manuscript was in preparation, another group reported that OGT negatively regulates expression of another Nrf1 isoform, TCF11 [70]. In their analysis, they observed that inhibition of OGT function by alloxan and siRNA-mediated knockdown led to increased TCF11 expression and function as measured by reporter gene activation. These findings contrast sharply with our results on Nrf1a. The reason for this discrepancy is unclear. The conflicting results observed could conceivably be explained by some contribution due to the technical nature of the different experiments. Although we do not know the possible reasons for the discrepancy about the consequence of OGT knockdown, the use of a pharmacological inhibitor of OGT may not be selective, as alloxan has also been shown to be more potent in inhibiting OGA than OGT [71]. It is also conceivable that OGT may regulate Nrf1a differently than TCF11. Nrf1a is generated through alternate splicing of exon 4 of the Nrf1 gene and lacks a Neh4 subdomain (aa 242–271 of TCF11). TCF11 expression in mammals is speculated to be a relatively recent evolutionary event as TCF11 expression has only been identified in human cells thus far. Contrarily, Nrf1a is expressed in nearly all vertebrates. Therefore, we speculate that TCF11 somehow has attained an opposite OGT mediated regulatory mechanism as it began to be expressed later in phylogeny. In this regard, it is interesting that SKN1, an Nrf1 ortholog present in *C. elegans*, was recently shown to be positively regulated by an OGT interaction similar to that with Nrf1a shown in our investigation [72]. As of now however, there are no studies elucidating the functional difference between TCF11 and Nrf1a. Nevertheless, this data hints that TCF11 may have acquired novel regulatory mechanisms that oppose previous mechanisms controlling Nrf1a stability. OGT-mediated regulation of TCF11 may have diverged from that of Nrf1a due to the presence of an additional transactivation domain in Neh4 [49]. Such a possibility of differential regulation of protein isoforms is not without precedence. For example, the ARD1 (Arrest defective 1 protein) gene encodes two isoforms—ARD1^{235} and ARD1^{225} are expressed in mouse, while ARD1^{235} is expressed only in humans [73]. It was shown that both of these isoforms are regulated by different mechanisms in a species-specific manner suggesting that they have different functions in the cell.

In summary, our present study reports that Nrf1 is O-GlcNAcylated, and this modification occurs in response to oxidative stress, thereby regulating Nrf1 stability and Nrf1-mediated gene activation. These findings suggest that post-translational modification via addition of O-GlcNAc plays a role in stress responses of Nrf1a.

Author contribution

JYC conceived and wrote the paper. JLV and JWH performed the studies in Figs. 1 through 7. CSL and DVH generated the Nrf1 expression construct and purified the protein for mass spec analysis by XW and LH. HMK provided technical assistance and contributed to the preparation of figures. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgements

The authors have no conflicts of interest to disclose. This research was supported by NIH grants CA091907 to JYC, and GM074830 to LH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

[1] R.S. Haltiwanger, M.A. Blomberg, G.W. Hart, Glycosylation of nuclear and cytoplasmic proteins. Purification and characterization of a uridine diphospho-N-acetylglucosamine: polypeptide beta-N-acetylglucosaminyltransferase, J. Biol. Chem. 267 (13) (1992) 9005–9013.
[2] R.S. Haltiwanger, G.D. Holt, G.W. Hart, Enzymatic addition of O-GlcNAc to nuclear and cytoplasmic proteins. Identification of a uridine diphospho-N-acetylglucosamine: peptide beta-N-acetylglucosaminyltransferase, J. Biol. Chem. 265 (5) (1990) 2563–2568.
[3] G.W. Hart, R.S. Haltiwanger, G.D. Holt, W.G. Kelly, Glycosylation in the nucleus and cytoplasm, Annu. Rev. Biochem. 58 (1989) 841–874.
[4] C. Guinez, A.M. Mir, V. Dehennaut, R. Cacan, A. Harduin-Lepers, J.C. Michalski, T. Lefebvre, Protein ubiquitination is modulated by O-GlcNAc glycosylation, FASEB J. 22 (8) (2008) 2901–2911.
[5] H. Ise, S. Kobayashi, M. Goto, T. Sato, M. Kawakubo, M. Takahashi, U. Ikeda, T. Akaike, Vimentin and desmin possess GlcNAc-binding lectin-like properties on cell surfaces, Glycobiology 20 (7) (2010) 843–864.
[6] K. Lim, H.I. Chang, O-GlcNAc inhibits interaction between Sp1 and sterol regulatory element binding protein 2, Biochem. Biophys. Res. Commun. 393 (2) (2010) 314–318.
[7] J. Rengifo, C.J. Gibson, E. Winkler, T. Collin, B.E. Ehrlich, Regulation of the inositol 1,4,5-trisphosphate receptor type I by O-GlcNAc glycosylation, J. Neurosci. 27 (50) (2007) 13813–13821.
[8] J.A. Groves, A. Lee, G. Yildirir, N.E. Zachara, Dynamic O-GlcNAcylation and its roles in the cellular stress response and homeostasis, Cell Stress Chaperon-. 18 (5) (2013) 535–558.
[9] N.E. Zachara, N. O'Donnell, W.D. Cheung, J.J. Mercer, J.D. Marth, G.W. Hart, Dynamic O-GlcNAc modification of nucleocytoplasmic proteins in response to stress. A survival response of mammalian cells, J. Biol. Chem. 279 (29) (2004) 30133–30142.
[10] R.J. Copeland, J.W. Bullen, G.W. Hart, Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glucose toxicity, Am. J. Physiol. Endocrinol. Metab. 295 (1) (2008) E17–E28.
[11] S.P. Iyer, G.W. Hart, Roles of the tetratricopeptide repeat domain in O-GlcNAc transferase targeting and protein substrate specificity, J. Biol. Chem. 278 (27) (2003) 24608–24616.
[12] P. Marz, J. Stetefeld, K. Bendfeldt, C. Nitsch, J. Reinstein, R.L. Shoeman, B. Dimitriades-Schmutz, M. Schwager, D. Leiser, S. Ozcan, U. Otten, S. Ozbek, Ataxin-10 interacts with O-linked beta-N-acetylglucosamine transferase in the brain, J. Biol. Chem. 281 (29) (2006) 20263–20270.
[13] X. Yang, F. Zhang, J.E. Kudlow, Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein O-GlcNAcylation to transcriptional repression, Cell 110 (1) (2002) 69–80.
[14] Z.G. Levine, S. Walker, The biochemistry of O-GlcNAc transferase: which functions make it essential in mammalian cells? Annu. Rev. Biochem. 85 (2016) 631–657.
[15] F. Capotosti, S. Guernier, F. Lammers, P. Waridel, Y. Cai, J. Jin, J.W. Conaway, R.C. Conaway, W. Herr, O-GlcNAc transferase catalyzes site-specific proteolysis of HCF-1, Cell 144 (3) (2011) 376–388.
[16] T.M. Kristie, Y. Liang, J.L. Vogel, Control of alpha-herpesvirus IE gene expression by HCF-1 coupled chromatin modification activities, Biochim. Biophys. Acta 1799 (3–4) (2010) 257–265.
[17] J. Wysocka, W. Herr, The herpes simplex virus VP16-induced complex: the makings of a regulatory switch, Trends Biochem. Sci. 28 (6) (2003) 294–304.
[18] H. Goto, S. Motomura, A.C. Wilson, R.N. Freiman, Y. Nakabeppu, K. Fukushima, M. Fujishima, W. Herr, T. Nishimoto, A single-point mutation in HCF causes temperature-sensitive cell-cycle arrest and disrupts VP16 function, Genes Dev. 11 (6) (1997) 726–737.
[19] E. Julien, W. Herr, Proteolytic processing is necessary to separate and ensure proper cell growth and cytokinesis functions of HCF-1, EMBO J. 22 (10) (2003) 2360–2369.
[20] R.L. Luciano, A.C. Wilson, HCF-1 functions as a coactivator for the zinc finger protein Krox20, J. Biol. Chem. 278 (51) (2003) 51116–51124.
[21] S. Tyagi, A.L. Chabes, J. Wysocka, W. Herr, E2F activation of S phase promoters via association with HCF-1 and the MLL family of histone H3K4 methyltransferases, Mol. Cell 27 (1) (2007) 107–119.
[22] J. Knez, D. Piluso, P. Bilan, J.P. Capone, Host cell factor-1 and E2F4 interact via multiple determinants in each protein, Mol. Cell Biochem. 288 (1–2) (2006) 79–90.
[23] R.N. Freiman, W. Herr, Viral mimicry: common mode of association with HCF by VP16 and the cellular protein LZIP, Genes Dev. 11 (23) (1997) 3122–3127.
[24] J. Lin, P. Puigserver, J. Donovan, P. Tarr, B.M. Spiegelman, Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor, J. Biol. Chem. 277 (3) (2002) 1645–1648.
[25] R. Lu, V. Misra, Zhangfei: a second cellular protein interacts with herpes simplex virus accessory factor HCF in a manner similar to Luman and VP16, Nucleic Acids Res. 28 (12) (2000) 2446–2454.
[26] Y. Dou, T.A. Milne, A.J. Tackett, E.R. Smith, A. Fukuda, J. Wysocka, C.D. Allis, B.T. Chait, J.L. Hess, R.G. Roeder, Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF, Cell 121 (6) (2005) 873–885.
[27] Y.J. Machida, Y. Machida, A.A. Vashisht, J.A. Wohlschlegel, A. Dutta, The deubiquitinating enzyme BAP1 regulates cell growth via interaction with HCF-1, J. Biol. Chem. 284 (49) (2009) 34179–34188.
[28] J. Wysocka, M.P. Myers, C.D. Laherty, R.N. Eisenman, W. Herr, Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1, Genes Dev. 17 (7) (2003) 896–911.
[29] H. Yu, N. Mashtalir, S. Daou, I. Hammond-Martel, J. Ross, G. Sui, G.W. Hart, F.J. Rauscher 3rd, E. Drobetsky, E. Milot, Y. Shi, B. Affar el, The ubiquitin carboxyl hydrolase BAP1 forms a ternary complex with YY1 and HCF-1 and is a critical regulator of gene expression, Mol. Cell Biol. 30 (21) (2010) 5071–5085.
[30] N.C. Andrews, H. Erdjument-Bromage, M.B. Davidson, P. Tempst, S.H. Orkin, Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein, Nature 362 (6422) (1993) 722–728.
[31] J.Y. Chan, X.L. Han, Y.W. Kan, Cloning of Nrf1, an NF-E2-related transcription factor, by genetic selection in yeast, Proc. Natl. Acad. Sci. USA 90 (23) (1993) 11371–11375.
[32] A. Kobayashi, E. Ito, T. Toki, K. Kogame, S. Takahashi, K. Igarashi, N. Hayashi, M. Yamamoto, Molecular cloning and functional characterization of a new Cap'n' collar family transcription factor Nrf3, J. Biol. Chem. 274 (10) (1999) 6443–6452.
[33] P. Moi, K. Chan, I. Asunis, A. Cao, Y.W. Kan, Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region, Proc. Natl. Acad. Sci. USA 91 (21) (1994) 9926–9930.
[34] J.Y. Chan, M. Kwong, R. Lu, J. Chang, B. Wang, T.S. Yen, Y.W. Kan, Targeted disruption of the ubiquitous CNC-bZIP transcription factor, Nrf-1, results in anemia and embryonic lethality in mice, EMBO J. 17 (6) (1998) 1779–1787.
[35] C.S. Lee, C. Lee, T. Hu, J.M. Nguyen, J. Zhang, M.V. Martin, M.P. Vawter, E.J. Huang, J.Y. Chan, Loss of nuclear factor E2-related factor 1 in the brain leads to dysregulation of proteasome gene expression and neurodegeneration, Proc. Natl. Acad. Sci. USA 108 (20) (2011) 8408–8413.
[36] Z. Xu, L. Chen, L. Leung, T.S. Yen, C. Lee, J.Y. Chan, Liver-specific inactivation of the Nrf1 gene in adult mouse leads to nonalcoholic steatohepatitis and hepatic neoplasia, Proc. Natl. Acad. Sci. USA 102 (11) (2005) 4120–4125.
[37] M. Kwong, Y.W. Kan, J.Y. Chan, The CNC basic leucine zipper factor, Nrf1, is

essential for cell survival in response to oxidative stress-inducing agents. Role for Nrf1 in gamma-gcs(l) and gss expression in mouse fibroblasts, J. Biol. Chem. 274 (52) (1999) 37491–37498.
[38] M.C. Myhrstad, C. Husberg, P. Murphy, O. Nordstrom, R. Blomhoff, J.O. Moskaug, A.B. Kolsto, TCF11/Nrf1 overexpression increases the intracellular glutathione level and can transactivate the gamma-glutamylcysteine synthetase (GCS) heavy subunit promoter, Biochim. Biophys. Acta 1517 (2) (2001) 212–219.
[39] C.S. Lee, D.V. Ho, J.Y. Chan, Nuclear factor-erythroid 2-related factor 1 regulates expression of proteasome genes in hepatocytes and protects against endoplasmic reticulum stress and steatosis in mice, FEBS J. 280 (15) (2013) 3609–3620.
[40] S.K. Radhakrishnan, C.S. Lee, P. Young, A. Beskow, J.Y. Chan, R.J. Deshaies, Transcription factor Nrf1 mediates the proteasome recovery pathway after proteasome inhibition in mammalian cells, Mol. Cell 38 (1) (2010) 17–28.
[41] J. Kim, W. Xing, J. Wergedal, J.Y. Chan, S. Mohan, Targeted disruption of nuclear factor erythroid-derived 2-like 1 in osteoblasts reduces bone size and bone formation in mice, Physiol. Genom. 40 (2) (2010) 100–110.
[42] K. Narayanan, A. Ramachandran, M.C. Peterson, J. Hao, A.B. Kolsto, A.D. Friedman, A. George, The CCAAT enhancer-binding protein (C/EBP)beta and Nrf1 interact to regulate dentin sialophosphoprotein (DSPP) gene expression during odontoblast differentiation, J. Biol. Chem. 279 (44) (2004) 45423–45432.
[43] D.H. Oh, D. Rigas, A. Cho, J.Y. Chan, Deficiency in the nuclear-related factor erythroid 2 transcription factor (Nrf1) leads to genetic instability, FEBS J. 279 (22) (2012) 4121–4130.
[44] L. Luna, N. Skammelsrud, O. Johnsen, K.J. Abel, B.L. Weber, H. Prydz, A.B. Kolsto, Structural organization and mapping of the human TCF11 gene, Genomics 27 (2) (1995) 237–244.
[45] S.K. Radhakrishnan, W. den Besten, R.J. Deshaies, p97-dependent retrotranslocation and proteolytic processing govern formation of active Nrf1 upon proteasome inhibition, Elife 3 (2014) e01856.
[46] J. Steffen, M. Seeger, A. Koch, E. Kruger, Proteasomal degradation is transcriptionally controlled by TCF11 via an ERAD-dependent feedback loop, Mol. Cell 40 (1) (2010) 147–158.
[47] W. Wang, J.Y. Chan, Nrf1 is targeted to the endoplasmic reticulum membrane by an N-terminal transmembrane domain. Inhibition of nuclear translocation and transacting function, J. Biol. Chem. 281 (28) (2006) 19676–19687.
[48] J.J. Caterina, D. Donze, C.W. Sun, D.J. Ciavatta, T.M. Townes, Cloning and functional characterization of LCR-F1: a bZIP transcription factor that activates erythroid-specific, human globin gene expression, Nucleic Acids Res. 22 (12) (1994) 2383–2391.
[49] C. Husberg, P. Murphy, E. Martin, A.B. Kolsto, Two domains of the human bZIP transcription factor TCF11 are necessary for transactivation, J. Biol. Chem. 276 (21) (2001) 17641–17652.
[50] W. Wang, A.M. Kwok, J.Y. Chan, The p65 isoform of Nrf1 is a dominant negative inhibitor of ARE-mediated transcription, J. Biol. Chem. 282 (34) (2007) 24670–24678.
[51] E.K. Kwong, K.M. Kim, P.J. Penalosa, J.Y. Chan, Characterization of Nrf1b, a novel isoform of the nuclear factor-erythroid-2 related transcription factor-1 that activates antioxidant response element-regulated genes, PLoS One 7 (10) (2012) e48404.
[52] X. Wang, C.F. Chen, P.R. Baker, P.L. Chen, P. Kaiser, L. Huang, Mass spectrometric characterization of the affinity-purified human 26S proteasome complex, Biochemistry 46 (11) (2007) 3553–3565.
[53] X. Wang, L. Huang, Identifying dynamic interactors of protein complexes by quantitative mass spectrometry, Mol. Cell Proteom. 7 (1) (2008) 46–57.
[54] R. Zhao, Y. Hou, P. Xue, C.G. Woods, J. Fu, B. Feng, D. Guan, G. Sun, J.Y. Chan, M.P. Waalkes, M.E. Andersen, J. Pi, Long isoforms of NRF1 contribute to arsenic-induced antioxidant response in human keratinocytes, Environ. Health Perspect. 119 (1) (2011) 56–62.
[55] C.M. Ferrer, T.P. Lynch, V.L. Sodi, J.N. Falcone, L.P. Schwab, D.L. Peacock, D.J. Vocadlo, T.N. Seagroves, M.J. Reginato, O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway, Mol. Cell 54 (5) (2014) 820–831.
[56] G.D. Liu, C. Xu, L. Feng, F. Wang, The augmentation of O-GlcNAcylation reduces glyoxal-induced cell injury by attenuating oxidative stress in human retinal microvascular endothelial cells, Int. J. Mol. Med. 36 (4) (2015) 1019–1027.
[57] G.A. Ngoh, T. Hamid, S.D. Prabhu, S.P. Jones, O-GlcNAc signaling attenuates ER stress-induced cardiomyocyte death, Am. J. Physiol. Heart Circ. Physiol. 297 (5) (2009) H1711–H1719.
[58] T.T. Peternelj, S.A. Marsh, N.A. Strobel, A. Matsumoto, D. Briskey, V.J. Dalbo, P.S. Tucker, J.S. Coombes, Glutathione depletion and acute exercise increase O-GlcNAc protein modification in rat skeletal muscle, Mol. Cell Biochem. 400 (1–2) (2015) 265–275.
[59] S.R. Ho, K. Wang, T.R. Whisenhunt, P. Huang, X. Zhu, J.E. Kudlow, A.J. Paterson, O-GlcNAcylation enhances FOXO4 transcriptional regulation in response to stress, FEBS Lett. 584 (1) (2010) 49–54.
[60] M.P. Housley, J.T. Rodgers, N.D. Udeshi, T.J. Kelly, J. Shabanowitz, D.F. Hunt, P. Puigserver, G.W. Hart, O-GlcNAc regulates FoxO activation in response to glucose, J. Biol. Chem. 283 (24) (2008) 16283–16292.
[61] M. Biswas, D. Phan, M. Watanabe, J.Y. Chan, The Fbw7 tumor suppressor regulates nuclear factor E2-related factor 1 transcription factor turnover through proteasome-mediated proteolysis, J. Biol. Chem. 286 (45) (2011) 39282–39289.
[62] S.F. Baldini, C. Wavelet, I. Hainault, C. Guinez, T. Lefebvre, The nutrient-dependent O-GlcNAc modification controls the expression of liver fatty acid synthase, J. Mol. Biol. (2016).
[63] M.D. Li, H.B. Ruan, M.E. Hughes, J.S. Lee, J.P. Singh, S.P. Jones, M.N. Nitabach, X. Yang, O-GlcNAc signaling entrains the circadian clock by inhibiting BMAL1/CLOCK ubiquitination, Cell Metab. 17 (2) (2013) 303–310.
[64] H.B. Ruan, Y. Nie, X. Yang, Regulation of protein degradation by O-GlcNAcylation: crosstalk with ubiquitination, Mol. Cell Proteom. 12 (12) (2013) 3489–3497.
[65] W.H. Yang, J.E. Kim, H.W. Nam, J.W. Ju, H.S. Kim, Y.S. Kim, J.W. Cho, Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability, Nat. Cell Biol. 8 (10) (2006) 1074–1083.
[66] H.B. Ruan, X. Han, M.D. Li, J.P. Singh, K. Qian, S. Azarhoush, L. Zhao, A.M. Bennett, V.T. Samuel, J. Wu, J.R. Yates 3rd, X. Yang, O-GlcNAc transferase/host cell factor C1 complex regulates gluconeogenesis by modulating PGC-1alpha stability, Cell Metab. 16 (2) (2012) 226–237.
[67] G.W. Hart, C. Slawson, G. Ramirez-Correa, O. Lagerlof, Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease, Annu. Rev. Biochem. 80 (2011) 825–858.
[68] P. Hu, S. Shimoji, G.W. Hart, Site-specific interplay between O-GlcNAcylation and phosphorylation in cellular regulation, FEBS Lett. 584 (12) (2010) 2526–2538.
[69] M. Biswas, E.K. Kwong, E. Park, P. Nagra, J.Y. Chan, Glycogen synthase kinase 3 regulates expression of nuclear factor-erythroid-2 related transcription factor-1 (Nrf1) and inhibits pro-survival function of Nrf1, Exp. Cell Res. 319 (13) (2013) 1922–1931.
[70] J. Chen, X. Liu, F. Lu, X. Liu, Y. Ru, Y. Ren, L. Yao, Y. Zhang, Transcription factor Nrf1 is negatively regulated by its O-GlcNAcylation status, FEBS Lett. 589 (18) (2015) 2347–2358.
[71] A.W. Lee, T.N. Knierman, M.D. Konrad, R.J. Alloxan, is an inhibitor of O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase, Biochem. Biophys. Res. Commun. 350 (1) (2006) 1038–1043.
[72] H. Li, X. Liu, D. Wang, L. Su, T. Zhao, Z. Li, C. Lin, Y. Zhang, B. Huang, J. Lu, X. Li, O-GlcNAcylation of SKN-1 modulates the lifespan and oxidative stress resistance in Caenorhabditis elegans, Sci. Rep. 7 (2017) 43601.
[73] K.H. Chun, S.J. Cho, J.S. Choi, S.H. Kim, K.W. Kim, S.K. Lee, Differential regulation of splicing, localization and stability of mammalian ARD1235 and ARD1225 isoforms, Biochem. Biophys. Res. Commun. 353 (1) (2007) 18–25.