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MINIREVIEW

The Molecular Mechanisms Regulating the KEAP1-NRF2 Pathway

Liam Baird,[a] Masayuki Yamamoto[a,b]

[a]Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan
[b]Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan

ABSTRACT The KEAP1-NRF2 pathway is the principal protective response to oxidative and electrophilic stresses. Under homeostatic conditions, KEAP1 forms part of an E3 ubiquitin ligase, which tightly regulates the activity of the transcription factor NRF2 by targeting it for ubiquitination and proteasome-dependent degradation. In response to stress, an intricate molecular mechanism facilitated by sensor cysteines within KEAP1 allows NRF2 to escape ubiquitination, accumulate within the cell, and translocate to the nucleus, where it can promote its antioxidant transcription program. Recent advances have revealed that KEAP1 contains multiple stress sensors and inactivation modalities, which together allow diverse cellular inputs, from oxidative stress and cellular metabolites to dysregulated autophagy, to regulate NRF2 activity. This integration of the KEAP1-NRF2 system into multiple cellular signaling and metabolic pathways places NRF2 activation as a critical regulatory node in many disease phenotypes and suggests that the pharmaceutical modulation of NRF2's cytoprotective activity will be beneficial for human health in a broad range of noncommunicable diseases.

KEYWORDS E3 ubiquitin ligase, KEAP1, NRF2, antioxidant, oxidative stress, stress response

Oxidative stress plays an important role in the initiation and progression of many chronic diseases, including diabetes, cancer, and neurodegenerative diseases (1–4). Through the regulation of cytoprotective gene expression, the KEAP1-NRF2 stress response pathway is the principal inducible defense against oxidative and electrophilic stresses (5). Disease models utilizing Nrf2 knockout (KO) mice have directly implicated NRF2 activity in a wide range of diseases, including metabolic syndrome, diabetic nephropathy, rheumatoid arthritis, and Alzheimer's disease (6–9). The inducible nature of the pathway means that, by clearly delineating the molecular mechanism of NRF2 activation, we can optimize the design and activity of NRF2 modulators in order to mitigate the deleterious effects of oxidative stress on human health. As such, a thorough understanding of the KEAP1-NRF2 pathway may lead to the development of novel treatments for a broad range of human diseases.

The molecular activation and cytoprotective activity of the KEAP1-NRF2 pathway consist of four distinct but interlinked components: (i) chemical inducers of NRF2 activity, (ii) KEAP1, the protein sensor of these inducers, (iii) the transcription factor NRF2, which modulates the transcriptional response to inducers and oxidative stress, and (iv) the target genes which provide the cytoprotective output of the pathway.

PHASE II INDUCERS AND OXIDATIVE STRESS

Prior to the discovery of KEAP1 or NRF2, the beneficial effects of compounds which would later be referred to as "NRF2 inducers" and their impact on the induction of

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Address correspondence to Masayuki Yamamoto, masiyamamoto@med.tohoku.ac.jp.

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TABLE 1 Inducers of NRF2 activity

Inducer	Reference(s)
Endogenous signaling compounds/metabolites	
H_2O_2	122, 124
Lipid peroxidation products	110
Nitric oxide	119, 122
8-Nitro-cGMP	192, 193
Hydrogen sulfide	123, 194
Methylglyoxal	195
Oncometabolites	
Fumarate	196, 197
Succinylacetone	198
Immunometabolite itaconate	199, 200
Dietary compounds	
Sulforaphane	201
Curcumin	202
Pharmaceuticals	
Dimethyl fumarate	156
Bardoxylone	203
Microorganisms	
Bacteria/lipopolysaccharide	204
Marburg virus	205, 206
Plasmodium infection	207
Extracellular inducers	
Heat	208
Laminar flow	209
UVA radiation	210
Exercise	211

cytoprotective gene expression were studied in the context of chemoprevention. These pioneering studies revealed that a wide variety of structurally diverse chemical compounds, including substituted phenols, coumarins, indoles, and sulfur compounds, were all able to protect rodents from the tumorigenic properties of carcinogens (10, 11). Despite their structural diversity, the chemopreventative compounds functioned through a common mechanism, namely, the upregulation of phase II drug-metabolizing enzyme gene expression, and were therefore collectively named "phase II inducers" (Table 1) (11–15).

Analyses of upstream enhancer elements of the genes encoding the phase II NAD[P]H:quinone acceptor oxidoreductase 1 (NQO1) and glutathione *S*-transferase (GST) enzymes identified the sequence 5′-TGACNNNGC-3′, which was shown to be responsible for the increased gene expression in response to inducers and oxidative stress (16–19). This enhancer sequence became known as the antioxidant response element (ARE) or electrophile response element (EpRE).

NRF2 MODULATES THE TRANSCRIPTIONAL RESPONSE TO PHASE II INDUCERS AND OXIDATIVE STRESS

The transcription factor NRF2 (NF-E2-related factor 2) was identified due to its ability to bind to the NF-E2 site in the β-globin gene cluster (20, 21). Analysis of the NRF2-coding region revealed that its DNA binding domain had significant homology to the NF-E2 p45 transcription factor (Fig. 1). This protein family is distinguished by the presence of a CNC homology domain, located at the N terminus of the basic leucine zipper (bZIP) domain, and includes the transcription factors NF-E2 p45, NRF1, NRF2, NRF3, BACH1, BACH2, and the founding member of the family, cap and collar (CNC).

Through the comparison of consensus DNA binding sites of the CNC and bZIP protein families, NRF2 was revealed to be the transcription factor responsible for

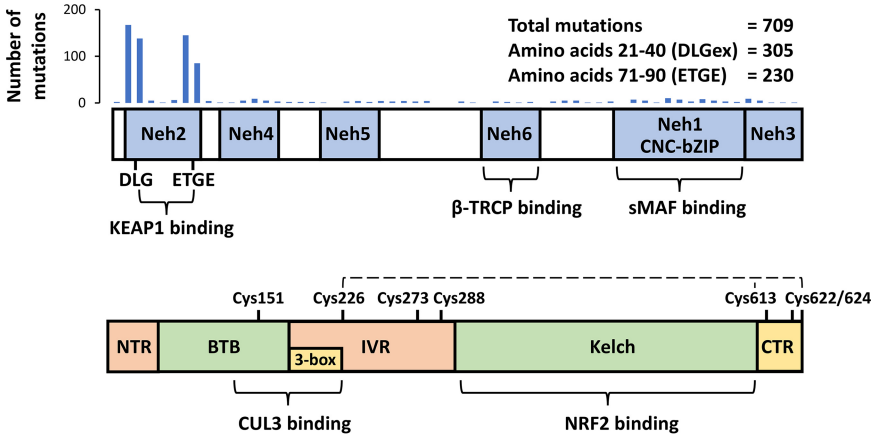

FIG 1 The domain architectures of the NRF2 and KEAP1 proteins. The frequency and spectrum of mutations in NRF2 in human tumors are shown above the domain scheme. For KEAP1, the locations of the stress sensors are shown, with a dashed line linking the three parts of the H_2O_2 sensor.

regulating the oxidative stress response. The first bZIP-specific DNA binding site to be identified was the T-MARE enhancer element, which was shown to be bound by the Maf oncogene either as a homodimer or as a heterodimer with members of the AP-1 transcription factor family (22). The similarity between the T-MARE and the consensus binding site of NF-E2 led to the discovery that the p18 small subunits of NF-E2 heterodimers are Maf family members and that together, the CNC factor p45 forms a heterodimer with one of the three p18 small Maf proteins, MAFF, MAFG, or MAFK, to form a functional NF-E2 complex (23). The chicken homologue of NRF2, ECH (erythroid cell-derived protein with CNC homology), was also found to dimerize with a small Maf protein to form a heterodimer which can similarly regulate gene expression through the NF-E2 consensus DNA motif (21).

As the T-MARE/NF-E2 consensus site was found in the enhancer regions of genes encoding the antioxidant proteins NQO1 and GSTa1 and had a sequence similar to that of the antioxidant response element, it was hypothesized that NRF2 may be responsible for the regulation of phase II enzyme gene expression (24). The treatment of Nrf2 KO mice with the phase II inducer butylated hydroxyanisole (BHA) validated this hypothesis, as the induction of the oxidative stress response was significantly diminished in the absence of NRF2, while Nrf2 KO mice also displayed increased sensitivity to a broad range of chemical insults (24–28). Furthermore, NRF2 was also shown to be responsible for the cytoprotective activity of phase II inducers, as the beneficial effects of oltipraz and sulforaphane are lost in Nrf2 KO mice in models of stomach, bladder, and skin carcinogenesis (26, 29–31).

The global analysis of gene expression in different model systems confirmed that NRF2 directly regulates both the basal and inducible expression of a broad range of genes involved in the oxidative stress response (Table 2) (24, 32–37). Furthermore, a comparison of chromatin immunoprecipitation sequencing (ChIP-Seq) analyses carried out on the CNC transcription factors revealed that although all family members bind to the same TGACNNNGC consensus motif, they mostly regulate the expression of non-overlapping sets of genes, confirming the specific function of NRF2 in the oxidative stress response (38–45).

Taken together, these complementary data from a wide variety of experimental models comprehensively show that NRF2 is the primary factor involved in the regulation of the antioxidant response.

KEAP1 IS THE PROTEIN SENSOR FOR PHASE II INDUCERS AND OXIDATIVE STRESS

It was observed that despite the structural diversity within the chemicals which elicit the oxidative stress response, they all have one common property: an electrophilic

TABLE 2 NRF2 target genes, focusing on the antioxidant and cytoprotective functions of NRF2

Target gene product	Reference(s)
Antioxidants/antioxidant-generating enzymes	
GCLC	33, 34
GCLM	33, 34
HO-1	32, 36
Glutathione reductase	34
Thioredoxin reductase 1	35, 36
Glutathione peroxidase 2	212
Thioredoxin	35, 213
Peroxiredoxin	32, 35
Sulfiredoxin	214, 215
Glutarodoxin	215, 216
Ferritin light chain	34
Ferritin heavy chain	214, 215
Xenobiotic-metabolizing enzymes	
NQO1	24, 33
GSTa1	24, 34
GSTa3	24, 33
Aldehyde dehydrogenase	34
Aldo-keto reductase 1b10	214
Carbonyl reductase	34
Pentose phosphate pathway/NADPH generation	
G6PDH	34, 215
6PGDH	34, 215
Transaldolase	34, 215
Transketolase	34, 215
Malic enzyme	34, 215

center that allows them to react with cysteine residues in proteins (15, 46). This realization led to the prescient prediction by Talalay and colleagues that a mechanism dependent on protein thiol modification may be responsible for the cytoprotective effects of phase II inducers (47).

The discovery that KEAP1 (Kelch-like ECH-associated protein 1) was the long-sought sensor protein of the oxidative stress response was made by analyzing a range of domain mutants of NRF2 (48). These experiments revealed that the Neh2 (Nrf2-ECH homology) domain of NRF2 was responsible for its negative regulation, and a yeast two-hybrid screen identified KEAP1 as the protein responsible for this negative regulation (48). Importantly, overexpression of KEAP1 was shown to repress the nuclear accumulation and transcriptional activity of NRF2, while the addition of phase II inducers was able to relieve this repression. Together, these findings provided the first evidence that KEAP1 was the sensor which could integrate into a single linear mechanism phase II inducers, NRF2 activity, and the regulation of cytoprotective gene expression (Fig. 2). This model was confirmed through the generation and analysis of Keap1 KO mice (49). In the absence of KEAP1, both NRF2 and its stress response target genes were constitutively activated, and they could not be further upregulated upon the addition of phase II inducers (49). These *in vivo* data conclusively showed that the KEAP1-NRF2 axis is responsible for the regulation of the oxidative stress response.

KEAP1 PROMOTES THE UBIQUITINATION AND PROTEASOME-DEPENDENT DEGRADATION OF NRF2

Early analysis of the mechanism regulating the oxidative stress response revealed that the transcript encoding NRF2 is not upregulated in response to stress, which implies that NRF2 activity is regulated through a posttranscriptional mechanism (32). The modulation of NRF2 protein levels through the use of translation and proteasome inhibitors revealed that the regulation of NRF2's protein stability was important for its cytoprotective function. In the absence of new translation, phase II inducers are not

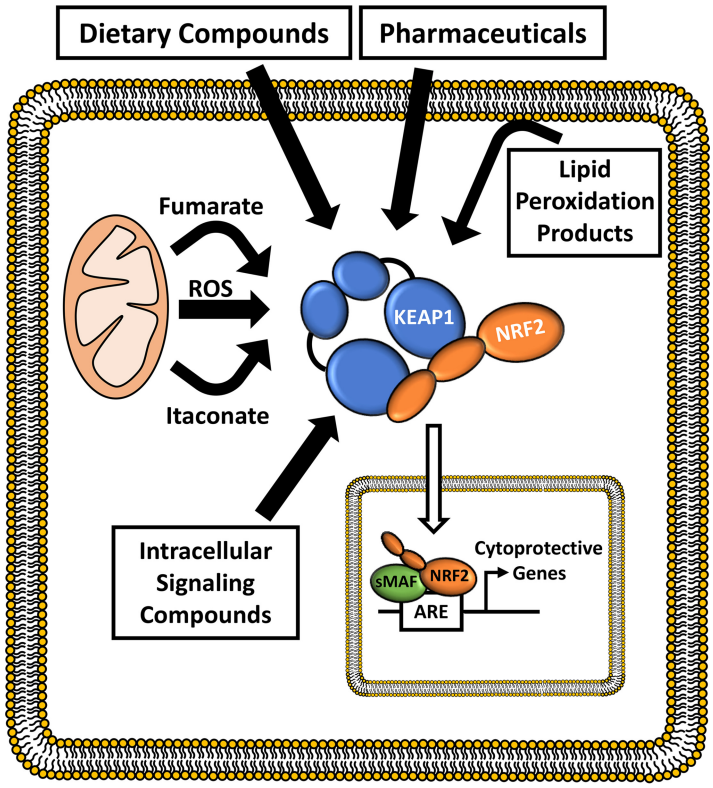

FIG 2 The KEAP1-NRF2 pathway integrates the sensing of a wide range of cellular stresses to the upregulation of cytoprotective gene expression. Endogenous and exogeneous stress molecules are able to directly bind to reactive cysteine residues within KEAP1, resulting in the stabilization of NRF2 and the upregulation of its cytoprotective transcription program.

able to upregulate NRF2 target genes, while inhibition of the 26S proteasome leads to a robust increase in NRF2 protein levels, suggesting that under basal conditions, NRF2 is subject to constitutive degradation (50).

This finding was consistent with an earlier observation showing that inhibition of the proteasome increased NRF2's binding to an ARE in the *GCLC* enhancer, which correlated with an increase in *GCLC* gene expression (51). Inducers were shown to liberate NRF2 from the proteasome-mediated degradation, resulting in the rapid stabilization of the NRF2 protein during oxidative stress (52–54). Together, these data revealed the crucial role that the proteasome-dependent degradation of NRF2 plays in the oxidative stress response.

The mechanism of NRF2 regulation was completed by the integration of KEAP1 into the NRF2 degradation pathway (55, 56). Together, these studies revealed that in the basal state, NRF2 is maintained at a low level due to its KEAP1-mediated proteasome-dependent degradation. In response to phase II inducers, the constitutive degradation of NRF2 is inhibited, which allows NRF2 to accumulate within the cell, translocate to the nucleus, and regulate cytoprotective gene expression.

Upon the identification of KEAP1, it was revealed to be a BTB-Kelch family protein (Fig. 1) (48). In 2003, contemporaneous with the elucidation of the mechanism of the KEAP1-dependent regulation of NRF2's protein stability, BTB proteins were discovered to function as adaptor proteins for cullin 3 (CUL3)-based E3 ubiquitin ligases (57–60). Based on this discovery, it was hypothesized that KEAP1 may regulate NRF2's degradation by forming a complex with CUL3 and RBX1 to form a functional E3 ubiquitin ligase (61). This hypothesis was validated by multiple groups, which confirmed that the KEAP1-CUL3 complex targets NRF2 for ubiquitination and proteasome-dependent degradation (61–64).

KEAP1 IS A COMPONENT OF AN E3 UBIQUITIN LIGASE

E3 ubiquitin ligases function in the final step of the three-step process of protein ubiquitination (reviewed in references 65 and 66). In the first step, E1 ubiquitin-activating enzymes form a covalent thioester bond between their catalytic cysteine residue and the C-terminal diglycine motif within ubiquitin. The E1 transfers the ubiquitin to the catalytic cysteine of an E2 ubiquitin-conjugating enzyme, forming an E2 ubiquitin thioester complex, which subsequently binds to a substrate-bound E3 ubiquitin ligase. The E3 ligase functions to correctly orientate the E2-loaded ubiquitin and the substrate protein and, in so doing, facilitates the ubiquitination of the substrate, in which an isopeptide bond is formed between ubiquitin and the ε-amino group of a lysine residue within the substrate. This monoubiquitinated substrate is then subject to additional successive rounds of ubiquitination, in which additional ubiquitin molecules are conjugated through Lys48 residues of the proceeding ubiquitin protein, producing a Lys48-polyubiquitinated chain attached to the substrate. Once the ubiquitin chain reaches four ubiquitins in length, the polyubiquitinated protein becomes a new substrate for degradation by the 26S proteasome (67). The human genome encodes two E1 enzymes, 38 E2s, and over 600 E3 ubiquitin ligases.

In the KEAP1-CUL3-RBX1 E3 ubiquitin ligase complex, KEAP1 functions as the substrate adaptor, RBX1 binds to the ubiquitin loaded E2-ubiquitin conjugating enzyme, and CUL3 provides the scaffold which joins KEAP1 and RBX1. Together, the complex functions to correctly orientate the NRF2-bound KEAP1 and the E2-bound RBX1 to facilitate ubiquitination of NRF2.

The first crystal structure of a complete RBX1-cullin-based E3 ubiquitin ligase, or cullin RING ligase (CRL), was solved for the CUL1-RBX1-SKP1-FBOX^{SKP2} quaternary complex (68). This structure revealed that the E3 ligase forms an elongated structure, with the SKP1-FBOX^{SKP2} substrate adaptor and RBX1 binding at opposite ends of the cullin protein. Posttranslational modification of cullin proteins by the ubiquitin-like protein NEDD8, through the process of neddylation, stimulates CRL activity (69–72). This cullin neddylation induces a conformational change in the cullin-RBX1 complex, such that the RING domain of RBX1 is "liberated" from an inhibitory interaction with the cullin protein (73, 74). This conformational change allows the RING-bound ubiquitin-loaded E2 to bridge the 50-Å distance between the catalytic site of the E2 and the target lysine residues within the substrate protein, and it therefore is critically required for protein ubiquitination to take place (68, 73, 75). When the protein level of the target protein is depleted, the substrate-free CRL is deneddylated, and thus inactivated, by the COP9 signalosome complex (76). The deneddylated CRL then binds to CAND1, which facilitates the formation of new substrate-bound CRL complexes (77). These novel CRL-substrate complexes then become targets for neddylation, and thus the cycle of CRL activation begins again.

A range of different experimental approaches together provide evidence to suggest that the ubiquitination of NRF2 by the KEAP1-CUL3-RBX1 E3 ubiquitin ligase complex occurs through the same intricate cycling mechanism common to all CRL E3 ubiquitin ligase complexes. Complete inhibition of CUL3 neddylation by the NEDD8-activating enzyme inhibitor MLN4924 results in the stabilization and cellular accumulation of NRF2, highlighting the fact that neddylation of CUL3 is required for NRF2 regulation (78). Consistent with this result, use of a complementary compound, CSN5i-3, which inhibits the COP9 deneddylation complex, has no impact on NRF2 degradation, presumably because the KEAP1-dependent CRL is locked in the active neddylated state (79). Interestingly, while the neddylation inhibitor NAcM-OPT results in a significant decrease in CUL3 neddylation, this does not result in the stabilization of NRF2, suggesting that even low levels of neddylated CUL3 are sufficient for the ubiquitination and degradation of NRF2 (80). Furthermore, both overexpression and small interfering RNA (siRNA)-mediated depletion of the recycling factor CAND1 result in an increase in NRF2 protein levels, implying that an optimum level of KEAP1-CUL3 complex cycling is required for ubiquitination of NRF2 (81). Together, these results are consistent with the

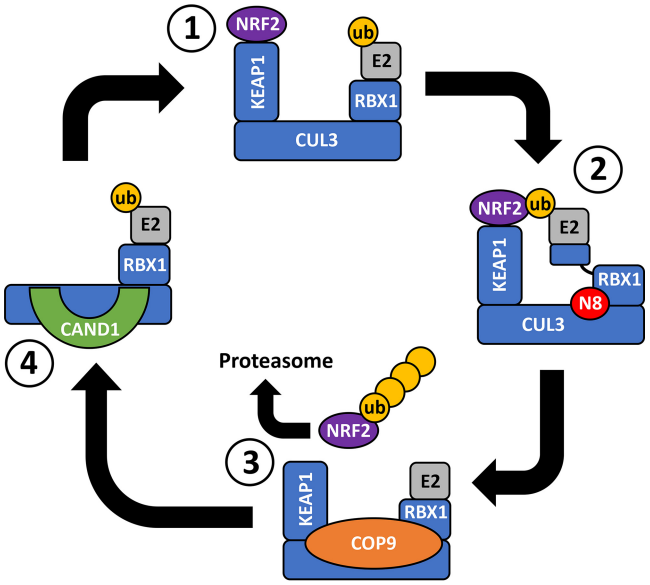

FIG 3 The cycle of cullin-RING ligase activation. 1, the KEAP1-dependent E3 ubiquitin ligase consisting of KEAP1, CUL3, and RBX1 binds to both the substrate, NRF2, and an E2 ubiquitin-conjugating enzyme. 2, neddylation by Nedd8 (N8) of CUL3 induces a conformational change in the complex such that the ubiquitin-bound E2 is able to transfer the ubiquitin to an acceptor lysine within NRF2. 3, after multiple rounds of ubiquitination, the polyubiquitinated NRF2 becomes a substrate for degradation by the proteasome. In the absence of NRF2, the KEAP1-dependent E3 ubiquitin ligase becomes a target for deneddylation, and thus inactivation, by COP9. 4, the deneddylated CUL3 is then bound by CAND1, which promotes the formation of new E3 ubiquitin ligase complexes, and the cycle of ubiquitination can begin again.

established cycling model of CRL activity and suggest that the activity of the KEAP1-CUL3-RBX1 CRL complex is consistent with the general model first proposed for SCF-CUL1-RBX1 complexes (Fig. 3).

In a variety of cellular signaling contexts, the output of ubiquitin conjugation reactions is fine-tuned by the parallel activity of deubiquitinating enzymes (DUBs) (82). In the context of NRF2 signaling, compared to the prodegradation function of the KEAP1-CUL3 complex, the relative importance of DUB activity for NRF2 stabilization is less well understood. That notwithstanding, the available data suggest that USP11 and DUB3 may be candidate DUBs for NRF2, although further studies are required to show robustly the physiological significance of DUB activity for NRF2 function (83, 84).

STRUCTURAL ANALYSIS OF THE KEAP1-NRF2-CUL3 COMPLEX

The original identification of KEAP1 revealed that its Kelch domain, along with the Neh2 domain of NRF2, is required for the negative regulation of the pathway (48). Based on these initial findings, a detailed understanding of the molecular interactions underpinning the regulation of NRF2 by KEAP1 began with the analysis of NRF2's Neh2 domain. Mutagenesis studies in zebrafish revealed that a short ETGE motif in the Neh2 domain is critical for binding to KEAP1 and for the negative regulation of NRF2's transcriptional activity (85). Furthermore, the analysis of evolutionarily conserved sequences in NRF2 identified a second sequence in the Neh2 domain, the DLG motif, to also be essential for the KEAP1-dependent regulation of NRF2 (86, 87). In a complementary parallel set of experiments, *in vitro* analysis of recombinant KEAP1 revealed that it forms a homodimer through its N-terminal BTB domain, while analysis of the stoichiometry of the KEAP1:NRF2 complex revealed the ratio of the proteins to be 2:1 (88–90). Together, these findings led to the hypothesis that a single NRF2 protein binds to the Kelch domain of one member of the KEAP1 dimer through its ETGE motif and to the second member of the KEAP1 dimer through its DLG motif (91, 92). Indeed, nuclear magnetic resonance (NMR) analysis of the KEAP1-NRF2 interaction revealed that the

TABLE 3 Binding affinities for the interactions between the Kelch domain of KEAP1 and NRF2 and p62

Motif	K_a (10^6 M^{-1})	Fold less than ETGE K_a	Reference
NRF2 ETGE	38.0	1.0	98
NRF2 DLGex	1.9	20.0	98
p62-KIR	0.3	126.7	146
Phosphorylated p62-KIR	8.5	4.5	146

ETGE and DLG motifs of NRF2 have overlapping binding sites in KEAP1, suggesting that one motif binds to a similar site on each member of the KEAP1 dimer (91). Furthermore, the NMR data also showed that the sequence between the DLG and ETGE motifs forms an α-helix in which six lysine residues, all potential targets for ubiquitination, are located on one side of the helix (91). This suggests that the utilization of a two-site binding mode positions NRF2's acceptor lysine residues in the correct orientation to facilitate ubiquitination by the KEAP1-dependent E3 ubiquitin ligase complex. The physiological significance of the two-site binding mode was confirmed through the analysis of NRF2 mutations in human cancer, as mutations in either the DLG or ETGE motif are sufficient to render NRF2 insensitive to KEAP1-mediated degradation (93).

Analysis of the crystal structure of the Kelch domain of KEAP1 revealed that it forms a six-bladed β-propeller structure with pseudo-6-fold symmetry, in which each of the six Kelch repeats forms a single blade of the propeller and where each blade comprises four antiparallel β-strands (94–96). The loops which connect the β-strands form a pocket on the bottom surface of the Kelch domain which provides the interface through which the ETGE motif (^{79}Glu-Thr-Gly-Glu^{82}) within the Neh2 domain of NRF2 binds to KEAP1 (95, 96). Asp77 to Glu82 of the Neh2 domain form a β-hairpin conformation, which allows the ETGE motif to bind tightly into the central pocket of the Kelch domain. All six blades of the Kelch propeller contribute to the interaction with NRF2, creating a total of 13 electrostatic interactions (95–97).

While the initial identification of the DLG motif confined it to residues Leu23 to Gly31, subsequent analysis based on the mutation spectrum of NRF2 in human tumors extended this sequence to Met17 to Gln51, which was designated the DLGex motif (Fig. 1) (98). Analysis of the DLGex motif, including its crystal structure in complex with the Kelch domain of KEAP1, revealed a number of significant differences compared to the shorter, original DLG motif (97, 98). For example, the larger DLGex motif contains three α-helices, and their interactions with one another form a U-shape structure which allows the DLGex motif to bind to the bottom surface of the Kelch domain (98). In contrast, the shorter DLG motif forms a four residue β-hairpin conformation, suggesting that the truncation of the true binding motif results in a significant change in its physical properties (97).

A comparison between the Kelch-DLGex crystal structure and the Kelch-ETGE crystal reveals that, while the binding interfaces for the two motifs partially overlap, the DLGex motif makes contact with a much larger surface of KEAP1 than the ETGE motif (780 Å^2 versus 550 Å^2), resulting in a greater number of electrostatic interactions in the Kelch-DLGex complex than in the Kelch-ETGE complex (14 versus 13) (98). Furthermore, differences in the thermodynamic interaction dynamics contribute to distinct binding kinetics for each of the two motifs. Thus, the binding of the ETGE motif involves a two-step process in which the formation of a transient intermediate occurs through a fast association and dissociation step, followed by a slower second step through which the final conformation of the complex is formed. In contrast, the binding of the DLGex motif follows a single fast association and fast dissociation modality (98). These kinetic differences result in the tighter binding of the ETGE motif than of the DLGex motif; however, the difference in affinity is only 20-fold, and not 100-fold as was previously reported for the shorter DLG motif (Table 3) (91, 98). Nevertheless, the differential binding modes for the two motifs allow for additional noncanonical signaling inputs to regulate NRF2's activity, as will be explained below.

Crystallization and structural analysis of the BTB dimerization domain of KEAP1 revealed that it consists of a three-stranded β-sheet coupled to six α-helices, which together form a structure similar to that of other BTB proteins (99–102). Cocrystallization studies with proteins related to KEAP1 have revealed that BTB proteins bind to the N terminus of CUL3 in a manner similar to the interaction between SKP1 and CUL1 (103–105). However, in addition to the SKP1-CUL1-like interface, BTB proteins also contain an additional pair of C-terminal α-helices, termed the "3-box," which are also required for binding to CUL3 (103–105). Sequence analysis of KEAP1 shows that it also contains a consensus 3-box motif (residues 180 to 212), suggesting that this conserved sequence is important for its function (Fig. 1).

Although high-resolution X-ray crystallography analysis of the full-length KEAP1 protein structure has not been performed, electron microscopy imaging of negatively stained recombinant KEAP1 revealed that the KEAP1 dimer forms a structure resembling a pair of cherries connected by their stalks (106). In this reconstruction of the KEAP1 dimer, each Kelch domain is contained within a cherry-like sphere, while the BTB dimerization domains form elongated forked-stem structures. The distance between the two binding pockets of the Kelch domains is 80 Å, which provides sufficient space to accommodate the binding of the DLGex and ETGE motifs, which are separated by approximately 98 Å, and therefore these structural data provide important complementary support for the two-site binding model (106). To complete the molecular characterization of the complete KEAP1-dependent E3 ubiquitin ligase complex, analysis of the stoichiometry of the KEAP1-CUL3 interaction revealed that KEAP1 and CUL3 bind in a 2:2 ratio, implying that each member of the KEAP1 dimer binds to a single CUL3-RBX1 protomer, which is consistent with other BTB-CUL3 complexes (103–105, 107).

THE CYSTEINE CODE OF OXIDATIVE STRESS SENSING

Prior to the discovery of KEAP1, the existence of a sensor protein containing reactive cysteine residues which could modulate the oxidative stress response was predicted based on the chemical properties of phase II inducers (47). The first direct evidence that KEAP1 contains stress sensors came from mass spectrometry studies using the electrophile dexamethasone 21-mesylate (89, 108). Through this approach, five residues in KEAP1 (Cys257, Cys273, Cys288, Cys297, and Cys613) were identified as having important roles in stress sensing. Subsequent work using cysteine point mutations found that Cys257 and Cys297 were not required for KEAP1's sensor function and also identified Cys151, which forms an additional stress sensor (109, 110). Subsequent mass spectrometry-based studies consistently found that a range of NRF2-activating compounds are able to covalently modify Cys151, Cys273, and Cys288, confirming their importance in the KEAP1-mediated stress response, as well as identifying other sensor residues, including Cys226 (111–114).

Interestingly, Cys151, Cys226, Cys273, Cys288, and Cys613 are all located adjacent to basic amino acids, which are predicted to lower the pK_a of the cysteine residue by stabilizing the thiolate anion (Fig. 4) (115). This lowering of the pK_a will increase the reactivity of the cysteine, making it ideally suited to carry out a sensor function. Consistent with this idea, X-ray crystallography analyses revealed that within its three-dimensional environment, Cys151 is surrounded by a cluster of positively charged amino acids (His129, Lys131, Arg135, Lys150, and His154), mutation of which destroys the sensor function (102, 116, 117). Together, these findings show that in addition to the cysteine residues themselves, the surrounding basic residues also play an important functional role in KEAP1's stress sensing.

The physiological importance of Cys151 was confirmed through the generation of C151S complementation rescue mice, in which Cys151 was mutated into Ser151 (118). These mice were viable but were unable to respond to the inducer *tert*-butylhydroquinone (tBHQ), confirming the importance of Cys151 *in vivo* for the oxidative stress response (118). Analysis of the sensor function of Cys273 and Cys288 was hindered by the fact that mutating the residues into either serine or alanine produces a nonfunctional KEAP1 protein which is unable to regulate NRF2's activity (89, 109, 118).

Cysteine 151

Zebrafish A		VGETCVLH	
Zebrafish B		VGEKCVIH	
Xenopus		VGEKCVIH	
Chicken		VGERCVLH	
Mouse		VGEKCVLH	
Human	147	MGEKCVLH	154

Cysteine 273

Zebrafish A		NAVHIYAL	
Zebrafish B		QAVRCHSL	
Xenopus		RAVRCHSL	
Chicken		RAVRCHAL	
Mouse		RAVRCHAL	
Human	269	RAVRCHSL	276

Cysteine 288

Zebrafish A		QLQSCPIL	
Zebrafish B		QLEHF---	
Xenopus		QLQRCEIL	
Chicken		QLQKCELL	
Mouse		QLQKCEIL	
Human	284	QLQKCEIL	291

Cysteine 226/613/622/624

Zebrafish A		SLSHCQLLEL	
Zebrafish B		TLSHCQLVTL	
Xenopus		RVNPCESVNC	
Chicken		NLSHCQLATL	
Mouse		NLSHCQLATL	
Human	222	NLSHCQLVTL	608

VTMEPCPGILP------EEEEEVDEEM---		
VTMEPCHKELI----------PCQC		
ITMEPCRKQPCGGGLFPERVKDGSHDKKKC		
ITMEPCKPGQRKPGQ---------RHECPC		
VTMEPCRKQID----------QQNCTC		
VTMEPCRKQID----------QQNCTC	624	

FIG 4 The conservation of KEAP1's four main stress sensors within vertebrates. The sensor cysteine residues are highlighted in red, with the adjacent positively charged amino acids underlined and in bold. Zebrafish contain two copies of KEAP1, with the stress sensors distributed between the paralogues.

These data show that in addition to their putative sensor function, Cys273 and Cys288 also play an important role in KEAP1's basal function for the ubiquitination of NRF2. The generation of novel C273W and C288E mutants of KEAP1, which retain the ability to target NRF2 for ubiquitination in the basal state, allowed for the uncoupling of the basal and sensor functions of Cys273 and Cys288 and conclusively showed that Cys273 and Cys288 function as stress sensors (119).

The existence of multiple sensors within KEAP1 is a salient feature of the oxidative stress pathway (Fig. 5). The relative importance of each sensor for distinct stressors was revealed through the study of the antioxidant response in zebrafish, which contain two related KEAP1 proteins, with the sensors differentially distributed across the paralogues (120). Thus, analysis of the oxidative stress response in zebrafish, and subsequently confirmed in mammals, revealed that NRF2 inducers can be divided into Cys151-dependent and Cys151-independent compounds (117, 120, 121). Utilization of the functional Cys273 and Cys288 mutants allowed the Cys151-independent inducers to be further subdivided based on their requirements for the Cys273 or Cys288 sensor motifs (119). Together, these data revealed that the different sensors within KEAP1 have distinct physiological functions in response to different forms of stress.

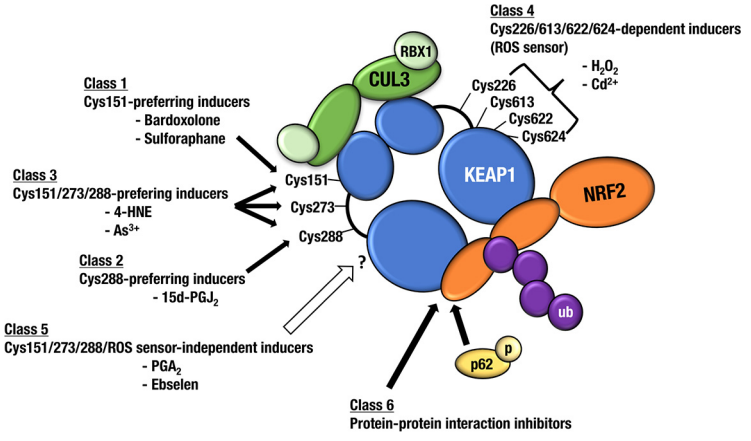

FIG 5 The classification of NRF2 inducers based on their specificity for different stress sensors within KEAP1. Inducers of NRF2 activity can be divided into five categories based on their preference for the Cys151, Cys273, Cys288 or the Cys226, Cys613, Cys622/624 ROS sensor within KEAP1. The sixth class of inducers function independently of the stress sensors by directly inhibiting NRF2's binding to KEAP1.

Despite its physiological importance, the sensor for H_2O_2 could not be attributed to either Cys151, Cys273, or Cys288, suggesting that KEAP1 contains a fourth stress sensor (119). Analysis of the redox state of KEAP1 suggested that Cys226 and Cys613 play an important role in sensing H_2O_2; however, in mice, knock-in mutations at either locus did not abolish the response to H_2O_2 (122–124). These data suggested that the sensor for H_2O_2 is more complex than those previously identified for electrophiles. The generation and analysis of additional mutant mouse lines, including both single and compound mutations, revealed that the sensor for H_2O_2 consists of four residues within KEAP1: Cys226, Cys613, Cys622, and Cys624 (124). Together, these residues function in a redundant fashion such that the loss of a single residue does not inactivate the sensor. This elaborate fail-safe mechanism is distinct from that used by the previously identified sensors at Cys151, Cys273, and Cys288, revealing that KEAP1 uses multiple sensing mechanisms to coordinate the NRF2-dependent oxidative stress response.

To date, four distinct cysteine sensors have been identified within KEAP1. The analysis of knock-in mice and stable clones of complemented mouse embryo fibroblasts (MEFs) has resulted in the classification of NRF2 inducers into six distinct groups based on their specificities for different sensor motifs (Fig. 5). Class I inducers, including the medically relevant bardoxolone, specifically target the Cys151 sensor (120, 125). Class II inducers, which target the Cys288 sensor, currently consist of only prostaglandin 15d-PGJ_2 (110, 119). The third class of inducers, including arsenite and 4-hydroxynonenal (4-HNE), function in a more promiscuous fashion, as they are able to bind to either Cys151, Cys273, or Cys288 (117, 119). The most recently delineated class of inducers, class IV, which includes cadmium and the physiologically important H_2O_2, bind to the oxidative stress sensor consisting of Cys226, Cys613, Cys622, and Cys624 (117, 124). Class V consists of compounds which inactivate KEAP1 but function independently of Cys151, Cys226, Cys273, Cys288, Cys613, Cys622, and Cys624. The fact that class V compounds, including prostaglandin A_2 (PGA_2), cannot stabilize NRF2 in an 11-cysteine mutant of KEAP1 suggests that they function through an as-yet-unidentified sensor. This additional sensor may lie within Cys257, Cys319, Cys434, or Cys489, as they are all also mutated in the 11-cysteine-less KEAP1 mutant (124). The final class of compounds includes nonelectrophilic protein-protein interaction inhibitors (PPIs), which directly inhibit the interaction between KEAP1 and NRF2 and thus function independently of KEAP1's stress sensors (discussed in detail below).

INACTIVATION OF KEAP1 BY NRF2 INDUCERS

The idea that NRF2 inducers function by inhibiting the KEAP1-dependent ubiquitination of NRF2 is well established (62, 126). However, robust experimental support for the mechanism through which KEAP1's E3 ubiquitin ligase activity is inactivated is currently lacking. Furthermore, the existence of multiple sensors within KEAP1 allows for the possibility that different classes of inducers inactivate KEAP1 through distinct mechanisms.

One mechanism through which Cys151-dependent inducers may function is by promoting the dissociation of the KEAP1-CUL3 complex. As Cys151 is located within the CUL3-binding BTB domain of KEAP1, it has been suggested that inducer binding may disrupt the interaction between KEAP1 and CUL3, which would result in the stabilization of NRF2 as it could no longer be targeted for ubiquitination by the complete KEAP1-CUL3-RBX1 E3 ubiquitin ligase complex (62, 102, 107, 127–129). However, the data related to inducer-mediated inhibition of KEAP1-CUL3 binding are conflicting, with multiple reports suggesting that inducers do not dissociate KEAP1 from CUL3 (107, 130–133). In some of the experiments in which inducers were able to dissociate KEAP1 from CUL3, relatively high concentrations of the compounds were used, which has been shown to exhibit nonspecific effects in certain contexts (62, 102). While the mutation of Cys151 to tryptophan, which mimics the reaction of Cys151 with inducers due to the bulky physical properties of the tryptophan side chain, is able to inhibit KEAP1's ubiquitin ligase function, the general applicability of these results to the wild-type protein is unclear, as C151W is a loss-of-function mutation (120, 134).

Nevertheless, there remains the possibility that the addition of bulky inducers is able to dissociate KEAP1 from CUL3 (107).

Where observed, the dissociation between KEAP1 and CUL3 takes place over a time scale of hours, whereas NRF2 is stabilized by inducers within 15 minutes (54, 56). Importantly, this means that the causality required for this mechanism (KEAP1 first dissociates from CUL3, resulting in stabilization of NRF2) has not been demonstrated. Furthermore, structural analysis of three different inducers bound to Cys151 of KEAP1, as well as the C151W mutant, provided no evidence that inducer binding to Cys151 causes a conformation change in the BTB domain of KEAP1 which would affect CUL3 binding (102, 116). Indeed, the most recent crystal structure of the BTB domain of KEAP1 bound to CUL3 suggests that Cys151 is not located in close proximity to the CUL3 binding interface (Protein Data Bank ID 5NLB). Together, the available data suggest that while Cys151-dependent inducers may be able to dissociate KEAP1 from CUL3 under certain circumstances, there is currently insufficient evidence to suggest that this is the main mechanism through which they stabilize NRF2. Similarly, cysteine-reactive inducers are not able to dissociate NRF2 from KEAP1, which implies that inducers modify the activity but not the composition of the KEAP1-CUL3-NRF2 complex (90, 135).

The fact that oxidative stress is not able to dissociate NRF2 from KEAP1 implies that when inducers bind to and inactivate KEAP1, NRF2 is neither ubiquitinated nor released from the KEAP1 dimer, which is an idea which is supported by the study of inactivating mutations in KEAP1. In cancer, mutations in KEAP1 often occur which do not impair KEAP1's ability to bind to NRF2 but do result in the stabilization of NRF2, which shows that the interaction between KEAP1 and NRF2 is not sufficient to promote NRF2's ubiquitination (136–139). Therefore, the data suggest that in response to oxidative stress, NRF2 functions as a suicide substrate, as it occupies KEAP1's binding sites but cannot be targeted for ubiquitination and degradation. Importantly, in the absence of protein translation, NRF2 is not stabilized by inducers, which suggests that newly translated NRF2 is required for the upregulation of ARE-dependent gene expression (50, 55).

Additional insights into the molecular mechanism of KEAP1's regulation of NRF2 can be obtained from the mutation spectrum of KEAP1 in human tumors. For example, mutations in close proximity to the stress sensor motifs in the IVR domain of KEAP1 (C252W, R272C, R272L, and Q284L) result in the inactivation of KEAP1, as do most mutations in either Cys273 or Cys288, which implies that the secondary structure of the sensor-rich IVR domain is critical for KEAP1's E3 ubiquitin ligase function (119, 136, 137, 139). This hypothesis is supported by the fact that inducers have been shown to promote a conformational change in KEAP1 upon binding to its sensor motifs (127, 140).

Taken together, the current data suggest that activation of KEAP1's stress sensors leads to a conformational change in the E3 ligase complex such that NRF2 is no longer correctly orientated with the RBX1-bound E2-ubiquitin conjugating enzyme and therefore is not ubiquitinated (Fig. 6). Under these stressed conditions, the NRF2 binding sites of the inactivated KEAP1 become saturated with "old" NRF2, which allows newly translated NRF2 to bypass KEAP1 binding, translocate to the nucleus, and activate ARE-dependent gene expression (135).

REGULATION OF NRF2 ACTIVITY BY p62 THROUGH THE HINGE-AND-LATCH MECHANISM

In parallel with the experimental approaches focusing on the oxidative stress-induced regulation of NRF2, a cDNA expression screen for activators of NRF2-dependent gene expression identified the autophagy adaptor protein p62 as a novel regulator of ARE gene expression (141). Concomitantly, data from an autophagy-deficient Atg7 conditional knockout mouse revealed that *in vivo*, the accumulation of p62 could also activate the NRF2-dependent stress response (142). Importantly, the p62-mediated regulation of NRF2 was not dependent on the cellular redox state, which

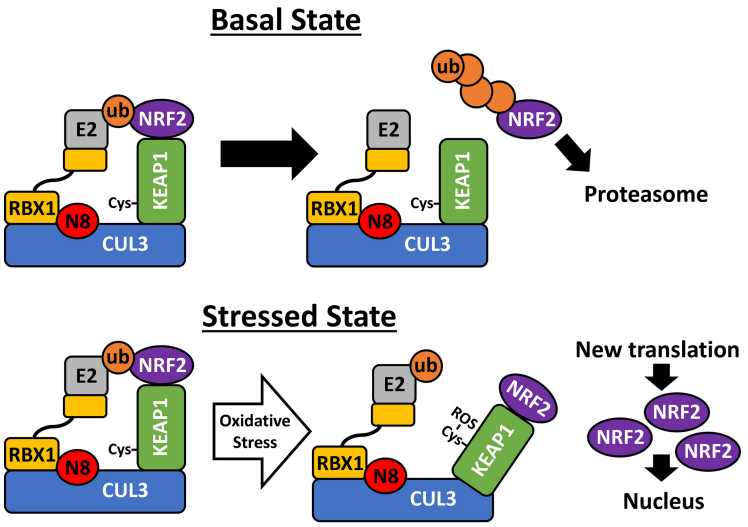

FIG 6 The mechanism of NRF2 activation by oxidative stress. In the basal nonstressed state, NRF2 is targeted for ubiquitination and proteasome-dependent degradation by the KEAP1-dependent E3 ubiquitin ligase. In response to oxidative stress, the direct binding of stressors to reactive cysteine residues results in a conformation change in KEAP1, which inhibits the ubiquitination of NRF2. As NRF2 is not release by KEAP1, it saturates all of KEAP1's binding sites, allowing newly translated NRF2 to bypass KEAP1, translocate to the nucleus, and upregulate cytoprotective gene expression.

suggested that it functioned through a novel, cysteine sensor-independent mechanism (143). Taken together, these complementary results suggested that a noncanonical mechanism may integrate NRF2 activity into the autophagy response pathway.

Subsequent analysis revealed that p62 was able to directly bind to KEAP1 due to the presence of a ^{349}DPSTGE^{354} motif, which is similar to the high-affinity ETGE motif within NRF2 (143, 144). Structural analysis of the interaction between KEAP1 and p62 found that the binding interface overlaps significantly with the NRF2 binding sites in the Kelch domain, suggesting that p62 may function as a competitive inhibitor of NRF2 binding (143). Interestingly, due to the differential binding affinities of the ETGE and DLG motifs of NRF2 for KEAP1, a "hinge-and-latch mechanism" had previously been proposed, in which the release of the weakly binding DLG motif would result in the inhibition of NRF2's ubiquitination (97, 145). The discovery of the p62-dependent pathway allowed for the direct testing of this model of NRF2 activation. Importantly, overexpression of p62 results in a significant decrease in the ubiquitination of NRF2 and the upregulation of ARE-dependent gene expression, confirming that p62 functions as a competitive inhibitor of NRF2 binding and validating the "hinge-and-latch mechanism" of NRF2 activation (143, 144). Furthermore, phosphorylation of Ser351 within p62, which is observed in both human hepatocellular carcinoma and autophagy-deficient mouse liver, enables p62 to interact with two additional residues within the Kelch domain, which further enhances its ability to compete with NRF2 for KEAP1 binding (Table 3) (146, 147). Together, these data suggest that the noncanonical regulation of NRF2 by p62 is physiologically important for human health and disease.

Interestingly, in addition to competing with NRF2 for binding to KEAP1, data from a range of knockout mouse models suggest that p62 is also able to directly promote the degradation of KEAP1 through selective autophagy (148, 149). This p62-dependent degradation of KEAP1 further integrates NRF2 activation into the autophagy pathway. Furthermore, in response to electrophilic stimuli, the half-life of KEAP1 is reduced (from 12.7 h to as low as 3.4 h), which suggests that under conditions of sustained stress, degradation of KEAP1 may also contribute to the NRF2-dependent oxidative stress response (148).

Although evidence has been provided to suggest that other proteins can also utilize the hinge-and-latch mechanism to inhibit the interaction between KEAP1 and NRF2, to

date, robust experimental support has been provided only for the p62 pathway (143, 144, 146–152).

The validation of the hinge-and-latch model suggested that a protein-protein inhibitor (PPI)-based approach could be utilized to pharmaceutically promote NRF2 activity. This novel modulation modality may provide an advantage over the more common electrophile-based inducers due to the added degree of pathway specificity which can be achieved. For example, the potent NRF2 inducers based on the triterpenoid scaffold, like CDDO, can bind to over 500 cellular proteins, including mTORC1, AMP-activated protein kinase (AMPK), and IKK, which may result in pleiotropic effects in cells (140, 153). Similarly, the dietary compound sulforaphane and its derivatives are also able to bind to histone deacetylase 6 (HDAC6), peroxiredoxins, and thioredoxin and therefore may have a broad effect on the cellular redox state, while the electrophilic NRF2 inducer dimethyl fumarate also modulates glycolysis through its interaction with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (154–157). Together, these studies suggest that, as many of the electrophile inducers of NRF2 also have other cellular targets beyond KEAP1, a complementary PPI-based approach may be beneficial in certain contexts.

To this end, high-throughput screening, fragment-based discovery, and structure-based virtual screening have all been used to identify compounds which can compete with NRF2 for binding with KEAP1, resulting in the stabilization of NRF2 and the upregulation of its ARE-dependent gene signature (158–164). One such PPI compound, named compound 7, is able to induce NQO1 expression with a half-maximal effective concentration (EC_{50}) of 12 nM, which is comparable to those of potent electrophile inducers; this therefore suggests that the utilization of a PPI-based approach may be a viable complementary strategy for NRF2 activation (162).

ADDITIONAL REGULATORS OF NRF2 ACTIVITY

In the absence of the KEAP1-binding Neh2 domain, NRF2 is still a relatively unstable protein, which suggests that it is subject to additional modes of regulation (86). This observation led to the identification of a second degron in the Neh6 domain, which revealed that NRF2's activity can be regulated by an additional degradation pathway (86). This KEAP1-independent regulation of NRF2 is dependent on the E3 ubiquitin ligase β-TRCP and requires prior phosphorylation by glycogen synthase kinase 3β (GSK-3β) and a priming kinase, for which a candidate is c-Jun N-terminal kinase (JNK) (165–171).

In comparison with KEAP1, a number of important observations suggest that the β-TRCP pathway plays a secondary role in the regulation of NRF2. In all mouse models in which NRF2 activity is increased (Keap1$^{F/-}$, Keap1$^{-/-}$ Nrf2$^{+/-}$, caNrf2, and NEKO) the mice are distinguished from their wild-type littermates due to their small size (172–175). In contrast, in mice deficient for both isoforms of β-TRCP, a small-size phenotype was not observed, which suggests that, because KEAP1 is active in the β-TRCP KO mice, NRF2 activity is not increased (176). Furthermore, in Keap1 knockout mice, β-TRCP activity is unable to rescue the NRF2-dependent juvenile lethal phenotype (49). Taken together, the *in vivo* data suggest that β-TRCP functions as a supplementary pathway for NRF2 regulation.

Interestingly, data from human cancer analysis point to an important physiological function for the β-TRCP-dependent regulation of NRF2 in human disease. While mutations inactivating the Neh6 degron in NRF2 are rare in human tumors (Fig. 1), presumably due to the dominance of KEAP1, detailed analysis has revealed that activating mutations in the KEAP1-NRF2 and phosphatidylinositol 3-kinase (PI3K)–AKT pathways together are the most common combination of mutations between signaling pathways in human tumors (177). Activation of the PI3K-AKT pathway results in the phosphorylation and inhibition of GSK-3β and thus the inactivation of β-TRCP-mediated degradation of NRF2. This suggests that the supplementary inactivation of the β-TRCP pathway, in concert with KEAP1 inactivation, is physiologically important for NRF2 function in human cancer. This finding is supported by mouse models, where in

TABLE 4 Additional mechanisms of NRF2 activation, focusing on pathways which are supported by multiple studies

Pathway	Mechanism	References
β-TRCP	The Neh6 domain of NRF2 contains an additional degron which is ubiquitinated by β-TRCP	86, 166
Protein kinase C	In response to inducers like phorbol myristate acetate, PKC can phosphorylate NRF2 on Ser40	184, 185
PERK	In response to ER stress, PERK can phosphorylate NRF2 on Thr80	217–219
Acetylation of NRF2	Acetylation of NRF2 by p300/CBP enhances DNA binding, which can be inhibited by ARF or directly deacetylated by SIRT1 and SIRT2	220–223
Transcription of NRF2	Increased transcription of NRF2 overloads KEAP1's repressor activity, resulting in an increase in antioxidant gene expression	174, 224
MicroRNAs	High levels of microRNA 200a result in an increase in NRF2-dependent gene expression	225, 226
KEAP1 promoter hypermethylation	KEAP1 promoter hypermethylation results in a decrease in KEAP1 protein levels and a concomitant increase in NRF2 protein levels	227, 228
Cross talk with p53	Mutant forms of p53, but not the wild type, modulate the transcriptional output of NRF2 signaling	229, 230
Cross talk with AMPK	Activation of AMPK signaling can result in increased NRF2-dependent gene expression	183, 231

the absence of KEAP1, NRF2 levels can be further upregulated by the additional loss of PTEN activity, which inactivates GSK-3β through the PTEN–PI3K–AKT–GSK-3β cascade (178, 179).

In addition to β-TRCP, a number of additional mechanisms, posttranslational modifications, and cross talk with other signaling pathways have been proposed to play a role in the regulation of NRF2's activity (Table 4). In most cases, and in contrast to the KEAP1-mediated mode of regulation, the physiological significance of these additional mechanisms is currently unclear, as they mostly lack support from knockout mouse models.

Similar to the case of β-TRCP, as Keap1 knockout mice are juvenile lethal due to hyperactivation of NRF2, it is suggested that any additional modes of regulation are unable to adequately control NRF2 activity in the absence of KEAP1 and therefore are limited to a minor role in the oxidative stress response (49). In addition, in cancer, mutations in NRF2 are highly localized within the KEAP1 binding ETGE and DLGex motifs, which provides additional support from human disease that KEAP1 is the main regulator of NRF2 (Fig. 1) (93). Furthermore, the analysis of the contributions of additional signaling pathways to NRF2 activation is complicated by the fact that any experimental manipulations which result in a change in the cellular redox state, or generation of secondary messengers, may activate NRF2 through the canonical KEAP1 pathway. For example, inhibition of p38α generates reactive oxygen species (ROS), while activation of KRAS induces the generation of the NRF2 activator 15d-PGJ_2, both of which could stabilize NRF2 through KEAP1 inactivation (180, 181). Thus, the wide variety of mechanism through which KEAP1 can be inactivated may complicate the interpretation of experiments carried out using small-molecule activators or inhibitors which display pleiotropic effects.

A number of reports have suggested that NRF2 is phosphorylated by a range of cellular kinases, with mass spectrometry analysis frequently identifying phosphorylation of Ser215, Ser408, and Ser577 within NRF2 (182, 183). However, mutation of these phosphorylated residues has almost no impact on NRF2's stability or transcriptional output, and therefore their physiological significance is currently unclear (182, 183). This uncertainty of the importance of NRF2 phosphorylation is exemplified by the study of Ser40 phosphorylation by protein kinase C (PKC) (Table 4) (184, 185). Ser40 lies within the DLGex motif, which is required for KEAP1 binding, and therefore is ideally located to play an important role in NRF2 activation. However, *in vitro* analysis revealed that phosphorylation of Ser40 has a minimal effect on the binding of NRF2 to KEAP1, and therefore the importance of this modification is currently unclear (98). As the experiments related to the PKC-NRF2 axis were carried out before it was revealed that NRF2 is regulated primarily at the level of protein stability by KEAP1, their significance should be interpreted with caution. Based on the current literature, PKC activity is not required for the NRF2-dependent oxidative stress response.

CONCLUSION

The upregulation of the NRF2-dependent transcription program is observed during almost all life span extension interventions, which clearly highlights the beneficial effects of NRF2's myriad of cytoprotective activities (186). A comprehensive understanding of the mechanism and cellular functions of the KEAP1-NRF2 pathway has allowed the field to progress to the point at which the pharmaceutical modulation of NRF2's activity can be used to improve human health.

A prominent example of this is the NRF2 inducer dimethyl fumarate (Tecfidera), which is currently used as a treatment for relapsing multiple sclerosis, where its modulation of neuroinflammation allows it to function in a neuroprotective manner (156, 187). In addition, while the phase 3 chronic kidney disease BEACON clinical trial of the NRF2 inducer bardoxolone (a derivative of the potent NRF2 inducer CDDO) was prematurely terminated due to safety concerns related to an increase in cardiovascular events, the valuable knowledge gained from this trial, including the identification of risk factors, is currently being applied in ongoing clinical trials for patients with Alport syndrome, chronic kidney disease (Tsubaki study and Ayame study), and pulmonary hypertension (125, 188–191).

As oxidative stress is a key contributor to the development of a broad range of noncommunicable diseases, the pleiotropic outputs of NRF2's transcriptional program, coupled with the ease with which its activity can be upregulated by small molecules, demonstrate the exciting potential that modulating NRF2's activity has for the treatment of human diseases.

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REFERENCES

1. Sies H, Berndt C, Jones DP. 2017. Oxidative stress. Annu Rev Biochem 86:715–748. https://doi.org/10.1146/annurev-biochem-061516-045037.
2. Brownlee M. 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820. https://doi.org/10.1038/414813a.
3. Gorrini C, Harris IS, Mak TW. 2013. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12:931–947. https://doi.org/10.1038/nrd4002.
4. Barnham KJ, Masters CL, Bush AI. 2004. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 3:205–214. https://doi.org/10.1038/nrd1330.
5. Yamamoto M, Kensler TW, Motohashi H. 2018. The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 98:1169–1203. https://doi.org/10.1152/physrev.00023.2017.
6. Xue P, Hou Y, Chen Y, Yang B, Fu J, Zheng H, Yarborough K, Woods CG, Liu D, Yamamoto M, Zhang Q, Andersen ME, Pi J. 2013. Adipose deficiency of Nrf2 in ob/ob mice results in severe metabolic syndrome. Diabetes 62:845–854. https://doi.org/10.2337/db12-0584.
7. Jiang T, Huang Z, Lin Y, Zhang Z, Fang D, Zhang DD. 2010. The protective role of Nrf2 in streptozotocin-induced diabetic nephropathy. Diabetes 59:850–860. https://doi.org/10.2337/db09-1342.
8. Maicas N, Ferrándiz ML, Brines R, Ibáñez L, Cuadrado A, Koenders MI, van den Berg WB, Alcaraz MJ. 2011. Deficiency of Nrf2 accelerates the effector phase of arthritis and aggravates joint disease. Antioxid Redox Signal 15:889–901. https://doi.org/10.1089/ars.2010.3835.
9. Rojo AI, Pajares M, Rada P, Nuñez A, Nevado-Holgado AJ, Killik R, Van Leuven F, Ribe E, Lovestone S, Yamamoto M, Cuadrado A. 2017. NRF2 deficiency replicates transcriptomic changes in Alzheimer's patients and worsens APP and TAU pathology. Redox Biol 13:444–451. https://doi.org/10.1016/j.redox.2017.07.006.
10. Wattenberg LW. 1972. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J Natl Cancer Inst 48:1425–1430.
11. Wattenberg LW. 1985. Chemoprevention of cancer. Cancer Res 45:1–8.
12. Benson AM, Batzinger RP, Ou SY, Bueding E, Cha YN, Talalay P. 1978. Elevation of hepatic glutathione S-transferase activities and protection against mutagenic metabolites of benzo(a)pyrene by dietary antioxidants. Cancer Res 38:4486–4495.
13. Benson AM, Cha YN, Bueding E, Heine HS, Talalay P. 1979. Elevation of extrahepatic glutathione S-transferase and epoxide hydratase activities by 2(3)-tert-butyl-4-hydroxyanisole. Cancer Res 39:2971–2977.
14. Benson AM, Hunkeler MJ, Talalay P. 1980. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. Proc Natl Acad Sci U S A 77:5216–5220. https://doi.org/10.1073/pnas.77.9.5216.
15. Prochaska HJ, De Long MJ, Talalay P. 1985. On the mechanisms of induction of cancer-protective enzymes: a unifying proposal. Proc Natl Acad Sci U S A 82:8232–8236. https://doi.org/10.1073/pnas.82.23.8232.
16. Friling RS, Bensimon A, Tichauer Y, Daniel V. 1990. Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element. Proc Natl Acad Sci U S A 87:6258–6262. https://doi.org/10.1073/pnas.87.16.6258.
17. Rushmore TH, Pickett CB. 1990. Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. Characterization of a

xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. J Biol Chem 265:14648–14653.
18. Rushmore TH, Morton MR, Pickett CB. 1991. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J Biol Chem 266:11632–11639.
19. Favreau LV, Pickett CB. 1991. Transcriptional regulation of the rat NAD(P)H:quinone reductase gene. Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. J Biol Chem 266:4556–4561.
20. Moi P, Chan K, Asunis I, Cao A, Kan YW. 1994. 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 U S A 91:9926–9930. https://doi.org/10.1073/pnas.91.21.9926.
21. Itoh K, Igarashi K, Hayashi N, Nishizawa M, Yamamoto M. 1995. Cloning and characterization of a novel erythroid cell-derived CNC family transcription factor heterodimerizing with the small Maf family proteins. Mol Cell Biol 15:4184–4193. https://doi.org/10.1128/mcb.15.8.4184.
22. Kataoka K, Noda M, Nishizawa M. 1994. Maf nuclear oncoprotein recognizes sequences related to an AP-1 site and forms heterodimers with both Fos and Jun. Mol Cell Biol 14:700–712. https://doi.org/10.1128/mcb.14.1.700.
23. Igarashi K, Kataoka K, Itoh K, Hayashi N, Nishizawa M, Yamamoto M. 1994. Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins. Nature 367:568–572. https://doi.org/10.1038/367568a0.
24. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 236:313–322. https://doi.org/10.1006/bbrc.1997.6943.
25. Chan K, Kan YW. 1999. Nrf2 is essential for protection against acute pulmonary injury in mice. Proc Natl Acad Sci U S A 96:12731–12736. https://doi.org/10.1073/pnas.96.22.12731.
26. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, Kensler TW. 2001. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc Natl Acad Sci U S A 98:3410–3415. https://doi.org/10.1073/pnas.051618798.
27. Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, Harada T, Yamamoto M. 2001. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci 59:169–177. https://doi.org/10.1093/toxsci/59.1.169.
28. Aoki Y, Sato H, Nishimura N, Takahashi S, Itoh K, Yamamoto M. 2001. Accelerated DNA adduct formation in the lung of the Nrf2 knockout mouse exposed to diesel exhaust. Toxicol Appl Pharmacol 173:154–160. https://doi.org/10.1006/taap.2001.9176.
29. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, Lozniewski A. 2002. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci U S A 99:7610–7615. https://doi.org/10.1073/pnas.112203099.
30. Iida K, Itoh K, Kumagai Y, Oyasu R, Hattori K, Kawai K, Shimazui T, Akaza H, Yamamoto M. 2004. Nrf2 is essential for the chemopreventive efficacy of oltipraz against urinary bladder carcinogenesis. Cancer Res 64:6424–6431. https://doi.org/10.1158/0008-5472.CAN-04-1906.
31. Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, Conney AH, Kong AN. 2006. Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer Res 66:8293–8296. https://doi.org/10.1158/0008-5472.CAN-06-0300.
32. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, Yamamoto M. 2000. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. J Biol Chem 275:16023–16029. https://doi.org/10.1074/jbc.275.21.16023.
33. McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, Wolf CR, Cavin C, Hayes JD. 2001. The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. Cancer Res 61:3299–3307.
34. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. 2002. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res 62:5196–5203.
35. Kwak MK, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M, Kensler TW. 2003. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. J Biol Chem 278:8135–8145. https://doi.org/10.1074/jbc.M211898200.
36. Lee JM, Calkins MJ, Chan K, Kan YW, Johnson JA. 2003. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. J Biol Chem 278:12029–12038. https://doi.org/10.1074/jbc.M211558200.
37. Yates MS, Tran QT, Dolan PM, Osburn WO, Shin S, McCulloch CC, Silkworth JB, Taguchi K, Yamamoto M, Williams CR, Liby KT, Sporn MB, Sutter TR, Kensler TW. 2009. Genetic versus chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression profiles between Keap1 knockout and triterpenoid-treated mice. Carcinogenesis 30:1024–1031. https://doi.org/10.1093/carcin/bgp100.
38. Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, Shyr C, Wakabayashi N, Kensler TW, Wasserman WW, Biswal S. 2010. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic Acids Res 38:5718–5734. https://doi.org/10.1093/nar/gkq212.
39. Warnatz HJ, Schmidt D, Manke T, Piccini I, Sultan M, Borodina T, Balzereit D, Wruck W, Soldatov A, Vingron M, Lehrach H, Yaspo ML. 2011. The BTB and CNC homology 1 (BACH1) target genes are involved in the oxidative stress response and in control of the cell cycle. J Biol Chem 286:23521–23532. https://doi.org/10.1074/jbc.M111.220178.
40. Hirotsu Y, Katsuoka F, Funayama R, Nagashima T, Nishida Y, Nakayama K, Engel JD, Yamamoto M. 2012. Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic Acids Res 40:10228–10239. https://doi.org/10.1093/nar/gks827.
41. Fujita R, Takayama-Tsujimoto M, Satoh H, Gutiérrez L, Aburatani H, Fujii S, Sarai A, Bresnick EH, Yamamoto M, Motohashi H. 2013. NF-E2 p45 is important for establishing normal function of platelets. Mol Cell Biol 33:2659–2670. https://doi.org/10.1128/MCB.01274-12.
42. Roychoudhuri R, Clever D, Li P, Wakabayashi Y, Quinn KM, Klebanoff CA, Ji Y, Sukumar M, Eil RL, Yu Z, Spolski R, Palmer DC, Pan JH, Patel SJ, Macallan DC, Fabozzi G, Shih H-Y, Kanno Y, Muto A, Zhu J, Gattinoni L, O'Shea JJ, Okkenhaug K, Igarashi K, Leonard WJ, Restifo NP. 2016. BACH2 regulates CD8(+) T cell differentiation by controlling access of AP-1 factors to enhancers. Nat Immunol 17:851–860. https://doi.org/10.1038/ni.3441.
43. Otsuki A, Suzuki M, Katsuoka F, Tsuchida K, Suda H, Morita M, Shimizu R, Yamamoto M. 2016. Unique cistrome defined as CsMBE is strictly required for Nrf2-sMaf heterodimer function in cytoprotection. Free Radic Biol Med 91:45–57. https://doi.org/10.1016/j.freeradbiomed.2015.12.005.
44. Baird L, Tsujita T, Kobayashi EH, Funayama R, Nagashima T, Nakayama K, Yamamoto M. 2017. A homeostatic shift facilitates endoplasmic reticulum proteostasis through transcriptional integration of proteostatic stress response pathways. Mol Cell Biol 37:e00439-16. https://doi.org/10.1128/MCB.00439-16.
45. Katsuoka F, Otsuki A, Takahashi M, Ito S, Yamamoto M. 2019. Direct and specific functional evaluation of the Nrf2 and MafG heterodimer by introducing a tethered dimer into small Maf-deficient cells. Mol Cell Biol 39:e00273-19. https://doi.org/10.1128/MCB.00273-19.
46. Talalay P, De Long MJ, Prochaska HJ. 1988. Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. Proc Natl Acad Sci U S A 85:8261–8265. https://doi.org/10.1073/pnas.85.21.8261.
47. Prestera T, Holtzclaw WD, Zhang Y, Talalay P. 1993. Chemical and molecular regulation of enzymes that detoxify carcinogens. Proc Natl Acad Sci U S A 90:2965–2969. https://doi.org/10.1073/pnas.90.7.2965.
48. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 13:76–86. https://doi.org/10.1101/gad.13.1.76.
49. Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, Takahashi S, Imakado S, Kotsuji T, Otsuka F, Roop DR, Harada T, Engel JD, Yamamoto M. 2003. Keap1-null mutation leads to postnatal lethality

due to constitutive Nrf2 activation. Nat Genet 35:238–245. https://doi.org/10.1038/ng1248.
50. Kwak MK, Itoh K, Yamamoto M, Kensler TW. 2002. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. Mol Cell Biol 22:2883–2892. https://doi.org/10.1128/mcb.22.9.2883-2892.2002.
51. Sekhar KR, Soltaninassab SR, Borrelli MJ, Xu ZQ, Meredith MJ, Domann FE, Freeman ML. 2000. Inhibition of the 26S proteasome induces expression of GLCLC, the catalytic subunit for gamma-glutamylcysteine synthetase. Biochem Biophys Res Commun 270:311–317. https://doi.org/10.1006/bbrc.2000.2419.
52. Sekhar KR, Yan XX, Freeman ML. 2002. Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. Oncogene 21:6829–6834. https://doi.org/10.1038/sj.onc.1205905.
53. Stewart D, Killeen E, Naquin R, Alam S, Alam J. 2003. Degradation of transcription factor Nrf2 via the ubiquitin-proteasome pathway and stabilization by cadmium. J Biol Chem 278:2396–2402. https://doi.org/10.1074/jbc.M209195200.
54. Nguyen T, Sherratt PJ, Huang HC, Yang CS, Pickett CB. 2003. Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. J Biol Chem 278:4536–4541. https://doi.org/10.1074/jbc.M207293200.
55. Itoh K, Wakabayashi N, Katoh Y, Ishii T, O'Connor T, Yamamoto M. 2003. Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. Genes Cells 8:379–391. https://doi.org/10.1046/j.1365-2443.2003.00640.x.
56. McMahon M, Itoh K, Yamamoto M, Hayes JD. 2003. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. J Biol Chem 278:21592–21600. https://doi.org/10.1074/jbc.M300931200.
57. Pintard L, Willis JH, Willems A, Johnson JL, Srayko M, Kurz T, Glaser S, Mains PE, Tyers M, Bowerman B, Peter M. 2003. The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature 425:311–316. https://doi.org/10.1038/nature01959.
58. Xu L, Wei Y, Reboul J, Vaglio P, Shin TH, Vidal M, Elledge SJ, Harper JW. 2003. BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature 425:316–321. https://doi.org/10.1038/nature01985.
59. Geyer R, Wee S, Anderson S, Yates J, Wolf DA. 2003. BTB/POZ domain proteins are putative substrate adaptors for cullin 3 ubiquitin ligases. Mol Cell 12:783–790. https://doi.org/10.1016/s1097-2765(03)00341-1.
60. Furukawa M, He YJ, Borchers C, Xiong Y. 2003. Targeting of protein ubiquitination by BTB-Cullin 3-Roc1 ubiquitin ligases. Nat Cell Biol 5:1001–1007. https://doi.org/10.1038/ncb1056.
61. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, Igarashi K, Yamamoto M. 2004. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol 24:7130–7139. https://doi.org/10.1128/MCB.24.16.7130-7139.2004.
62. Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M. 2004. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. Mol Cell Biol 24:10941–10953. https://doi.org/10.1128/MCB.24.24.10941-10953.2004.
63. Cullinan SB, Gordan JD, Jin J, Harper JW, Diehl JA. 2004. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol Cell Biol 24:8477–8486. https://doi.org/10.1128/MCB.24.19.8477-8486.2004.
64. Furukawa M, Xiong Y. 2005. BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase. Mol Cell Biol 25:162–171. https://doi.org/10.1128/MCB.25.1.162-171.2005.
65. Deshaies RJ, Joazeiro CA. 2009. RING domain E3 ubiquitin ligases. Annu Rev Biochem 78:399–434. https://doi.org/10.1146/annurev.biochem.78.101807.093809.
66. Buetow L, Huang DT. 2016. Structural insights into the catalysis and regulation of E3 ubiquitin ligases. Nat Rev Mol Cell Biol 17:626–642. https://doi.org/10.1038/nrm.2016.91.
67. Pierce NW, Kleiger G, Shan SO, Deshaies RJ. 2009. Detection of sequential polyubiquitylation on a millisecond timescale. Nature 462:615–619. https://doi.org/10.1038/nature08595.
68. Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, Chu C, Koepp DM, Elledge SJ, Pagano M, Conaway RC, Conaway JW, Harper JW, Pavletich NP. 2002. Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature 416:703–709. https://doi.org/10.1038/416703a.
69. Read MA, Brownell JE, Gladysheva TB, Hottelet M, Parent LA, Coggins MB, Pierce JW, Podust VN, Luo RS, Chau V, Palombella VJ. 2000. Nedd8 modification of cul-1 activates SCF(beta(TrCP))-dependent ubiquitination of IkappaBalpha. Mol Cell Biol 20:2326–2333. https://doi.org/10.1128/mcb.20.7.2326-2333.2000.
70. Podust VN, Brownell JE, Gladysheva TB, Luo RS, Wang C, Coggins MB, Pierce JW, Lightcap ES, Chau V. 2000. A Nedd8 conjugation pathway is essential for proteolytic targeting of p27Kip1 by ubiquitination. Proc Natl Acad Sci U S A 97:4579–4584. https://doi.org/10.1073/pnas.090465597.
71. Morimoto M, Nishida T, Honda R, Yasuda H. 2000. Modification of cullin-1 by ubiquitin-like protein Nedd8 enhances the activity of SCF-(skp2) toward p27(kip1). Biochem Biophys Res Commun 270:1093–1096. https://doi.org/10.1006/bbrc.2000.2576.
72. Wu K, Chen A, Pan ZQ. 2000. Conjugation of Nedd8 to CUL1 enhances the ability of the ROC1-CUL1 complex to promote ubiquitin polymerization. J Biol Chem 275:32317–32324. https://doi.org/10.1074/jbc.M004847200.
73. Duda DM, Borg LA, Scott DC, Hunt HW, Hammel M, Schulman BA. 2008. Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. Cell 134:995–1006. https://doi.org/10.1016/j.cell.2008.07.022.
74. Baek K, Krist DT, Prabu JR, Hill S, Klügel M, Neumaier LM, von Gronau S, Kleiger G, Schulman BA. 2020. NEDD8 nucleates a multivalent cullin-RING-UBE2D ubiquitin ligation assembly. Nature 578:461–466. https://doi.org/10.1038/s41586-020-2000-y.
75. Saha A, Deshaies RJ. 2008. Multimodal activation of the ubiquitin ligase SCF by Nedd8 conjugation. Mol Cell 32:21–31. https://doi.org/10.1016/j.molcel.2008.08.021.
76. Cavadini S, Fischer ES, Bunker RD, Potenza A, Lingaraju GM, Goldie KN, Mohamed WI, Faty M, Petzold G, Beckwith RE, Tichkule RB, Hassiepen U, Abdulrahman W, Pantelic RS, Matsumoto S, Sugasawa K, Stahlberg H, Thomä NH. 2016. Cullin-RING ubiquitin E3 ligase regulation by the COP9 signalosome. Nature 531:598–603. https://doi.org/10.1038/nature17416.
77. Pierce NW, Lee JE, Liu X, Sweredoski MJ, Graham RL, Larimore EA, Rome M, Zheng N, Clurman BE, Hess S, Shan SO, Deshaies RJ. 2013. Cand1 promotes assembly of new SCF complexes through dynamic exchange of F box proteins. Cell 153:206–215. https://doi.org/10.1016/j.cell.2013.02.024.
78. Soucy TA, Smith PG, Milhollen MA, Berger AJ, Gavin JM, Adhikari S, Brownell JE, Burke KE, Cardin DP, Critchley S, Cullis CA, Doucette A, Garnsey JJ, Gaulin JL, Gershman RE, Lublinsky AR, McDonald A, Mizutani H, Narayanan U, Olhava EJ, Peluso S, Rezaei M, Sintchak MD, Talreja T, Thomas MP, Traore T, Vyskocil S, Weatherhead GS, Yu J, Zhang J, Dick LR, Claiborne CF, Rolfe M, Bolen JB, Langston SP. 2009. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature 458:732–736. https://doi.org/10.1038/nature07884.
79. Schlierf A, Altmann E, Quancard J, Jefferson AB, Assenberg R, Renatus M, Jones M, Hassiepen U, Schaefer M, Kiffe M, Weiss A, Wiesmann C, Sedrani R, Eder J, Martoglio B. 2016. Targeted inhibition of the COP9 signalosome for treatment of cancer. Nat Commun 7:13166. https://doi.org/10.1038/ncomms13166.
80. Scott DC, Hammill JT, Min J, Rhee DY, Connelly M, Sviderskiy VO, Bhasin D, Chen Y, Ong SS, Chai SC, Goktug AN, Huang G, Monda JK, Low J, Kim HS, Paulo JA, Cannon JR, Shelat AA, Chen T, Kelsall IR, Alpi AF, Pagala V, Wang X, Peng J, Singh B, Harper JW, Schulman BA, Guy RK. 2017. Blocking an N-terminal acetylation-dependent protein interaction inhibits an E3 ligase. Nat Chem Biol 13:850–857. https://doi.org/10.1038/nchembio.2386.
81. Lo SC, Hannink M. 2006. CAND1-mediated substrate adaptor recycling is required for efficient repression of Nrf2 by Keap1. Mol Cell Biol 26:1235–1244. https://doi.org/10.1128/MCB.26.4.1235-1244.2006.
82. Komander D, Clague MJ, Urbé S. 2009. Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol 10:550–563. https://doi.org/10.1038/nrm2731.
83. Sowa ME, Bennett EJ, Gygi SP, Harper JW. 2009. Defining the human deubiquitinating enzyme interaction landscape. Cell 138:389–403. https://doi.org/10.1016/j.cell.2009.04.042.

84. Zhang Q, Zhang ZY, Du H, Li SZ, Tu R, Jia YF, Zheng Z, Song XM, Du RL, Zhang XD. 2019. DUB3 deubiquitinates and stabilizes NRF2 in chemotherapy resistance of colorectal cancer. Cell Death Differ 26:2300–2313. https://doi.org/10.1038/s41418-019-0303-z.
85. Kobayashi M, Itoh K, Suzuki T, Osanai H, Nishikawa K, Katoh Y, Takagi Y, Yamamoto M. 2002. Identification of the interactive interface and phylogenic conservation of the Nrf2-Keap1 system. Genes Cells 7:807–820. https://doi.org/10.1046/j.1365-2443.2002.00561.x.
86. McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD. 2004. Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. J Biol Chem 279:31556–31567. https://doi.org/10.1074/jbc.M403061200.
87. Katoh Y, Iida K, Kang MI, Kobayashi A, Mizukami M, Tong KI, McMahon M, Hayes JD, Itoh K, Yamamoto M. 2005. Evolutionary conserved N-terminal domain of Nrf2 is essential for the Keap1-mediated degradation of the protein by proteasome. Arch Biochem Biophys 433:342–350. https://doi.org/10.1016/j.abb.2004.10.012.
88. Zipper LM, Mulcahy RT. 2002. The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. J Biol Chem 277:36544–36552. https://doi.org/10.1074/jbc.M206530200.
89. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, Talalay P. 2004. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. Proc Natl Acad Sci U S A 101:2040–2045. https://doi.org/10.1073/pnas.0307301101.
90. Eggler AL, Liu G, Pezzuto JM, van Breemen RB, Mesecar AD. 2005. Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. Proc Natl Acad Sci U S A 102:10070–10075. https://doi.org/10.1073/pnas.0502402102.
91. Tong KI, Katoh Y, Kusunoki H, Itoh K, Tanaka T, Yamamoto M. 2006. Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. Mol Cell Biol 26:2887–2900. https://doi.org/10.1128/MCB.26.8.2887-2900.2006.
92. McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD. 2006. Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. J Biol Chem 281:24756–24768. https://doi.org/10.1074/jbc.M601119200.
93. Shibata T, Ohta T, Tong KI, Kokubu A, Odogawa R, Tsuta K, Asamura H, Yamamoto M, Hirohashi S. 2008. Cancer related mutations in NRF2 impair its recognition by Keap1-Cul3 E3 ligase and promote malignancy. Proc Natl Acad Sci U S A 105:13568–13573. https://doi.org/10.1073/pnas.0806268105.
94. Li X, Zhang D, Hannink M, Beamer LJ. 2004. Crystal structure of the Kelch domain of human Keap1. J Biol Chem 279:54750–54758. https://doi.org/10.1074/jbc.M410073200.
95. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, Kang MI, Kobayashi A, Yokoyama S, Yamamoto M. 2006. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell 21:689–700. https://doi.org/10.1016/j.molcel.2006.01.013.
96. Lo SC, Li X, Henzl MT, Beamer LJ, Hannink M. 2006. Structure of the Keap1:Nrf2 interface provides mechanistic insight into Nrf2 signaling. EMBO J 25:3605–3617. https://doi.org/10.1038/sj.emboj.7601243.
97. Tong KI, Padmanabhan B, Kobayashi A, Shang C, Hirotsu Y, Yokoyama S, Yamamoto M. 2007. Different electrostatic potentials define ETGE and DLG motifs as hinge and latch in oxidative stress response. Mol Cell Biol 27:7511–7521. https://doi.org/10.1128/MCB.00753-07.
98. Fukutomi T, Takagi K, Mizushima T, Ohuchi N, Yamamoto M. 2014. Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. Mol Cell Biol 34:832–846. https://doi.org/10.1128/MCB.01191-13.
99. Ahmad KF, Engel CK, Privé GG. 1998. Crystal structure of the BTB domain from PLZF. Proc Natl Acad Sci U S A 95:12123–12128. https://doi.org/10.1073/pnas.95.21.12123.
100. Ahmad KF, Melnick A, Lax S, Bouchard D, Liu J, Kiang CL, Mayer S, Takahashi S, Licht JD, Privé GG. 2003. Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. Mol Cell 12:1551–1564. https://doi.org/10.1016/s1097-2765(03)00454-4.
101. Stogios PJ, Downs GS, Jauhal JJ, Nandra SK, Privé GG. 2005. Sequence and structural analysis of BTB domain proteins. Genome Biol 6:R82. https://doi.org/10.1186/gb-2005-6-10-r82.
102. Cleasby A, Yon J, Day PJ, Richardson C, Tickle IJ, Williams PA, Callahan JF, Carr R, Concha N, Kerns JK, Qi H, Sweitzer T, Ward P, Davies TG. 2014. Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. PLoS One 9:e98896. https://doi.org/10.1371/journal.pone.0098896.
103. Zhuang M, Calabrese MF, Liu J, Waddell MB, Nourse A, Hammel M, Miller DJ, Walden H, Duda DM, Seyedin SN, Hoggard T, Harper JW, White KP, Schulman BA. 2009. Structures of SPOP-substrate complexes: insights into molecular architectures of BTB-Cul3ubiquitin ligases. Mol Cell 36:39–50. https://doi.org/10.1016/j.molcel.2009.09.022.
104. Canning P, Cooper CD, Krojer T, Murray JW, Pike AC, Chaikuad A, Keates T, Thangaratnarajah C, Hojzan V, Ayinampudi V, Marsden BD, Gileadi O, Knapp S, von Delft F, Bullock AN. 2013. Structural basis for Cul3 protein assembly with the BTB-Kelch family of E3 ubiquitin ligases. J Biol Chem 288:7803–7814. https://doi.org/10.1074/jbc.M112.437996.
105. Ji AX, Privé GG. 2013. Crystal structure of KLHL3 in complex with Cullin3. PLoS One 8:e60445. https://doi.org/10.1371/journal.pone.0060445.
106. Ogura T, Tong KI, Mio K, Maruyama Y, Kurokawa H, Sato C, Yamamoto M. 2010. Keap1 is a forked-stem dimer structure with two large spheres enclosing the intervening, double glycine repeat, and C-terminal domains. Proc Natl Acad Sci U S A 107:2842–2847. https://doi.org/10.1073/pnas.0914036107.
107. Iso T, Suzuki T, Baird L, Yamamoto M. 2016. Absolute amounts and status of the Nrf2-Keap1-Cul3 complex within cells. Mol Cell Biol 36:3100–3112. https://doi.org/10.1128/MCB.00389-16.
108. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. 2002. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proc Natl Acad Sci U S A 99:11908–11913. https://doi.org/10.1073/pnas.172398899.
109. Zhang DD, Hannink M. 2003. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol Cell Biol 23:8137–8151. https://doi.org/10.1128/mcb.23.22.8137-8151.2003.
110. Levonen AL, Landar A, Ramachandran A, Ceaser EK, Dickinson DA, Zanoni G, Morrow JD, Darley-Usmar VM. 2004. Cellular mechanisms of redox cell signalling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. Biochem J 378:373–382. https://doi.org/10.1042/BJ20031049.
111. Hong F, Sekhar KR, Freeman ML, Liebler DC. 2005. Specific patterns of electrophile adduction trigger Keap1 ubiquitination and Nrf2 activation. J Biol Chem 280:31768–31775. https://doi.org/10.1074/jbc.M503346200.
112. Hong F, Freeman ML, Liebler DC. 2005. Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. Chem Res Toxicol 18:1917–1926. https://doi.org/10.1021/tx0502138.
113. Luo Y, Eggler AL, Liu D, Liu G, Mesecar AD, van Breemen RB. 2007. Sites of alkylation of human Keap1 by natural chemoprevention agents. J Am Soc Mass Spectrom 18:2226–2232. https://doi.org/10.1016/j.jasms.2007.09.015.
114. Copple IM, Goldring CE, Jenkins RE, Chia AJ, Randle LE, Hayes JD, Kitteringham NR, Park BK. 2008. The hepatotoxic metabolite of acetaminophen directly activates the Keap1-Nrf2 cell defense system. Hepatology 48:1292–1301. https://doi.org/10.1002/hep.22472.
115. Snyder GH, Cennerazzo MJ, Karalis AJ, Field D. 1981. Electrostatic influence of local cysteine environments on disulfide exchange kinetics. Biochemistry 20:6509–6519. https://doi.org/10.1021/bi00526a001.
116. Huerta C, Jiang X, Trevino I, Bender CF, Ferguson DA, Probst B, Swinger KK, Stoll VS, Thomas PJ, Dulubova I, Visnick M, Wigley WC. 2016. Characterization of novel small-molecule NRF2 activators: structural and biochemical validation of stereospecific KEAP1 binding. Biochim Biophys Acta 1860:2537–2552. https://doi.org/10.1016/j.bbagen.2016.07.026.
117. McMahon M, Lamont DJ, Beattie KA, Hayes JD. 2010. Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals. Proc Natl Acad Sci U S A 107:18838–18843. https://doi.org/10.1073/pnas.1007387107.
118. Yamamoto T, Suzuki T, Kobayashi A, Wakabayashi J, Maher J, Motohashi H, Yamamoto M. 2008. Physiological significance of reactive

cysteine residues of Keap1 in determining Nrf2 activity. Mol Cell Biol 28:2758–2770. https://doi.org/10.1128/MCB.01704-07.
119. Saito R, Suzuki T, Hiramoto K, Asami S, Naganuma E, Suda H, Iso T, Yamamoto H, Morita M, Baird L, Furusawa Y, Negishi T, Ichinose M, Yamamoto M. 2016. Characterizations of three major cysteine sensors of Keap1 in stress response. Mol Cell Biol 36:271–284. https://doi.org/10.1128/MCB.00868-15.
120. Kobayashi M, Li L, Iwamoto N, Nakajima-Takagi Y, Kaneko H, Nakayama Y, Eguchi M, Wada Y, Kumagai Y, Yamamoto M. 2009. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. Mol Cell Biol 29:493–502. https://doi.org/10.1128/MCB.01080-08.
121. Takaya K, Suzuki T, Motohashi H, Onodera K, Satomi S, Kensler TW, Yamamoto M. 2012. Validation of the multiple sensor mechanism of the Keap1-Nrf2 system. Free Radic Biol Med 53:817–827. https://doi.org/10.1016/j.freeradbiomed.2012.06.023.
122. Fourquet S, Guerois R, Biard D, Toledano MB. 2010. Activation of NRF2 by nitrosative agents and H2O2 involves KEAP1 disulfide formation. J Biol Chem 285:8463–8471. https://doi.org/10.1074/jbc.M109.051714.
123. Hourihan JM, Kenna JG, Hayes JD. 2013. The gasotransmitter hydrogen sulfide induces nrf2-target genes by inactivating the keap1ubiquitin ligase substrate adaptor through formation of a disulfide bond between cys-226 and cys-613. Antioxid Redox Signal 19:465–481. https://doi.org/10.1089/ars.2012.4944.
124. Suzuki T, Muramatsu A, Saito R, Iso T, Shibata T, Kuwata K, Kawaguchi S-I, Iwawaki T, Adachi S, Suda H, Morita M, Uchida K, Baird L, Yamamoto M. 2019. Molecular mechanism of cellular oxidative stress sensing by Keap1. Cell Rep 28:746–758. https://doi.org/10.1016/j.celrep.2019.06.047.
125. de Zeeuw D, Akizawa T, Audhya P, Bakris GL, Chin M, Christ-Schmidt H, Goldsberry A, Houser M, Krauth M, Lambers Heerspink HJ, McMurray JJ, Meyer CJ, Parving HH, Remuzzi G, Toto RD, Vaziri ND, Wanner C, Wittes J, Wrolstad D, Chertow GM, BEACON Trial Investigators. 2013. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. N Engl J Med 369:2492–2503. https://doi.org/10.1056/NEJMoa1306033.
126. Kobayashi A, Kang MI, Watai Y, Tong KI, Shibata T, Uchida K, Yamamoto M. 2006. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol Cell Biol 26:221–229. https://doi.org/10.1128/MCB.26.1.221-229.2006.
127. Gao L, Wang J, Sekhar KR, Yin H, Yared NF, Schneider SN, Sasi S, Dalton TP, Anderson ME, Chan JY, Morrow JD, Freeman ML. 2007. Novel n-3 fatty acid oxidation products activate Nrf2 by destabilizing the association between Keap1 and Cullin3. J Biol Chem 282:2529–2537. https://doi.org/10.1074/jbc.M607622200.
128. Rachakonda G, Xiong Y, Sekhar KR, Stamer SL, Liebler DC, Freeman ML. 2008. Covalent modification at Cys151 dissociates the electrophile sensor Keap1 from the ubiquitin ligase CUL3. Chem Res Toxicol 21:705–710. https://doi.org/10.1021/tx700302s.
129. Kansanen E, Bonacci G, Schopfer FJ, Kuosmanen SM, Tong KI, Leinonen H, Woodcock SR, Yamamoto M, Carlberg C, Ylä-Herttuala S, Freeman BA, Levonen AL. 2011. Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism. J Biol Chem 286:14019–14027. https://doi.org/10.1074/jbc.M110.190710.
130. Ichikawa T, Li J, Meyer CJ, Janicki JS, Hannink M, Cui T. 2009. Dihydro-CDDO-trifluoroethyl amide (dh404), a novel Nrf2 activator, suppresses oxidative stress in cardiomyocytes. PLoS One 4:e8391. https://doi.org/10.1371/journal.pone.0008391.
131. Li Y, Paonessa JD, Zhang Y. 2012. Mechanism of chemical activation of Nrf2. PLoS One 7:e35122. https://doi.org/10.1371/journal.pone.0035122.
132. Baird L, Dinkova-Kostova AT. 2013. Diffusion dynamics of the Keap1-Cullin3 interaction in single live cells. Biochem Biophys Res Commun 433:58–65. https://doi.org/10.1016/j.bbrc.2013.02.065.
133. McMahon M, Swift SR, Hayes JD. 2018. Zinc-binding triggers a conformational-switch in the cullin-3 substrate adaptor protein KEAP1 that controls transcription factor NRF2. Toxicol Appl Pharmacol 360:45–57. https://doi.org/10.1016/j.taap.2018.09.033.
134. Eggler AL, Small E, Hannink M, Mesecar AD. 2009. Cul3-mediated Nrf2 ubiquitination and antioxidant response element (ARE) activation are dependent on the partial molar volume at position 151 of Keap1. Biochem J 422:171–180. https://doi.org/10.1042/BJ20090471.
135. Baird L, Llères D, Swift S, Dinkova-Kostova AT. 2013. Regulatory flexibility in the Nrf2-mediated stress response is conferred by conformational cycling of the Keap1-Nrf2 protein complex. Proc Natl Acad Sci U S A 110:15259–15264. https://doi.org/10.1073/pnas.1305687110.
136. Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, Herman JG, Baylin SB, Sidransky D, Gabrielson E, Brock MV, Biswal S. 2006. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS Med 3:e420. https://doi.org/10.1371/journal.pmed.0030420.
137. Ohta T, Iijima K, Miyamoto M, Nakahara I, Tanaka H, Ohtsuji M, Suzuki T, Kobayashi A, Yokota J, Sakiyama T, Shibata T, Yamamoto M, Hirohashi S. 2008. Loss of Keap1 function activates Nrf2 and provides advantages for lung cancer cell growth. Cancer Res 68:1303–1309. https://doi.org/10.1158/0008-5472.CAN-07-5003.
138. Hast BE, Cloer EW, Goldfarb D, Li H, Siesser PF, Yan F, Walter V, Zheng N, Hayes DN, Major MB. 2014. Cancer-derived mutations in KEAP1 impair NRF2 degradation but not ubiquitination. Cancer Res 74:808–817. https://doi.org/10.1158/0008-5472.CAN-13-1655.
139. Saigusa D, Motoike IN, Saito S, Zorzi M, Aoki Y, Kitamura H, Suzuki M, Katsuoka F, Ishii H, Kinoshita K, Motohashi H, Yamamoto M. 2020. Impacts of NRF2 activation in non-small-cell lung cancer cell lines on extracellular metabolites. Cancer Sci 111:667–678. https://doi.org/10.1111/cas.14278.
140. Dinkova-Kostova AT, Holtzclaw WD, Wakabayashi N. 2005. Keap1, the sensor for electrophiles and oxidants that regulates the phase 2 response, is a zinc metalloprotein. Biochemistry 44:6889–6899. https://doi.org/10.1021/bi047434h.
141. Liu Y, Kern JT, Walker JR, Johnson JA, Schultz PG, Luesch H. 2007. A genomic screen for activators of the antioxidant response element. Proc Natl Acad Sci U S A 104:5205–5210. https://doi.org/10.1073/pnas.0700898104.
142. Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiyama Y, Kominami E, Tanaka K. 2007. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131:1149–1163. https://doi.org/10.1016/j.cell.2007.10.035.
143. Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, Sou YS, Ueno I, Sakamoto A, Tong KI, Kim M, Nishito Y, Iemura S, Natsume T, Ueno T, Kominami E, Motohashi H, Tanaka K, Yamamoto M. 2010. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol 12:213–223. https://doi.org/10.1038/ncb2021.
144. Lau A, Wang XJ, Zhao F, Villeneuve NF, Wu T, Jiang T, Sun Z, White E, Zhang DD. 2010. A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. Mol Cell Biol 30:3275–3285. https://doi.org/10.1128/MCB.00248-10.
145. Tong KI, Kobayashi A, Katsuoka F, Yamamoto M. 2006. Two-site substrate recognition model for the Keap1-Nrf2 system: a hinge and latch mechanism. Biol Chem 387:1311–1320. https://doi.org/10.1515/BC.2006.164.
146. Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, Saito T, Yang Y, Kouno T, Fukutomi T, Hoshii T, Hirao A, Takagi K, Mizushima T, Motohashi H, Lee MS, Yoshimori T, Tanaka K, Yamamoto M, Komatsu M. 2013. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. Mol Cell 51:618–631. https://doi.org/10.1016/j.molcel.2013.08.003.
147. Saito T, Ichimura Y, Taguchi K, Suzuki T, Mizushima T, Takagi K, Hirose Y, Nagahashi M, Iso T, Fukutomi T, Ohishi M, Endo K, Uemura T, Nishito Y, Okuda S, Obata M, Kouno T, Imamura R, Tada Y, Obata R, Yasuda D, Takahashi K, Fujimura T, Pi J, Lee MS, Ueno T, Ohe T, Mashino T, Wakai T, Kojima H, Okabe T, Nagano T, Motohashi H, Waguri S, Soga T, Yamamoto M, Tanaka K, Komatsu M. 2016. p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. Nat Commun 7:12030. https://doi.org/10.1038/ncomms12030.
148. Taguchi K, Fujikawa N, Komatsu M, Ishii T, Unno M, Akaike T, Motohashi H, Yamamoto M. 2012. Keap1 degradation by autophagy for the maintenance of redox homeostasis. Proc Natl Acad Sci U S A 109:13561–13566. https://doi.org/10.1073/pnas.1121572109.
149. Bae SH, Sung SH, Oh SY, Lim JM, Lee SK, Park YN, Lee HE, Kang D, Rhee SG. 2013. Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and prevent oxidative liver damage. Cell Metab 17:73–84. https://doi.org/10.1016/j.cmet.2012.12.002.
150. Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, Watanabe S, Ando J, Iwadate M, Yamamoto M, Lee MS, Tanaka K, Komatsu M.

2011. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. J Cell Biol 193:275–284. https://doi.org/10.1083/jcb.201102031.
151. Inoue D, Suzuki T, Mitsuishi Y, Miki Y, Suzuki S, Sugawara S, Watanabe M, Sakurada A, Endo C, Uruno A, Sasano H, Nakagawa T, Satoh K, Tanaka N, Kubo H, Motohashi H, Yamamoto M. 2012. Accumulation of p62/SQSTM1 is associated with poor prognosis in patients with lung adenocarcinoma. Cancer Sci 103:760–766. https://doi.org/10.1111/j.1349-7006.2012.02216.x.
152. Baird L, Swift S, Llères D, Dinkova-Kostova AT. 2014. Monitoring Keap1-Nrf2 interactions in single live cells. Biotechnol Adv 32:1133–1144. https://doi.org/10.1016/j.biotechadv.2014.03.004.
153. Yore MM, Kettenbach AN, Sporn MB, Gerber SA, Liby KT. 2011. Proteomic analysis shows synthetic oleanane triterpenoid binds to mTOR. PLoS One 6:e22862. https://doi.org/10.1371/journal.pone.0022862.
154. Gibbs A, Schwartzman J, Deng V, Alumkal J. 2009. Sulforaphane destabilizes the androgen receptor in prostate cancer cells by inactivating histone deacetylase 6. Proc Natl Acad Sci U S A 106:16663–16668. https://doi.org/10.1073/pnas.0908908106.
155. Ahn YH, Hwang Y, Liu H, Wang XJ, Zhang Y, Stephenson KK, Boronina TN, Cole RN, Dinkova-Kostova AT, Talalay P, Cole PA. 2010. Electrophilic tuning of the chemoprotective natural product sulforaphane. Proc Natl Acad Sci U S A 107:9590–9595. https://doi.org/10.1073/pnas.1004104107.
156. Linker RA, Lee DH, Ryan S, van Dam AM, Conrad R, Bista P, Zeng W, Hronowsky X, Buko A, Chollate S, Ellrichmann G, Brück W, Dawson K, Goelz S, Wiese S, Scannevin RH, Lukashev M, Gold R. 2011. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain 134:678–692. https://doi.org/10.1093/brain/awq386.
157. Kornberg MD, Bhargava P, Kim PM, Putluri V, Snowman AM, Putluri N, Calabresi PA, Snyder SH. 2018. Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. Science 360:449–453. https://doi.org/10.1126/science.aan4665.
158. Hu L, Magesh S, Chen L, Wang L, Lewis TA, Chen Y, Khodier C, Inoyama D, Beamer LJ, Emge TJ, Shen J, Kerrigan JE, Kong AN, Dandapani S, Palmer M, Schreiber SL, Munoz B. 2013. Discovery of a small-molecule inhibitor and cellular probe of Keap1-Nrf2 protein-protein interaction. Bioorg Med Chem Lett 23:3039–3043. https://doi.org/10.1016/j.bmcl.2013.03.013.
159. Marcotte D, Zeng W, Hus JC, McKenzie A, Hession C, Jin P, Bergeron C, Lugovskoy A, Enyedy I, Cuervo H, Wang D, Atmanene C, Roecklin D, Vecchi M, Vivat V, Kraemer J, Winkler D, Hong V, Chao J, Lukashev M, Silvian L. 2013. Small molecules inhibit the interaction of Nrf2 and the Keap1 Kelch domain through a non-covalent mechanism. Bioorg Med Chem 21:4011–4019. https://doi.org/10.1016/j.bmc.2013.04.019.
160. Zhuang C, Narayanapillai S, Zhang W, Sham YY, Xing C. 2014. Rapid identification of Keap1-Nrf2 small-molecule inhibitors through structure-based virtual screening and hit-based substructure search. J Med Chem 57:1121–1126. https://doi.org/10.1021/jm4017174.
161. Bertrand HC, Schaap M, Baird L, Georgakopoulos ND, Fowkes A, Thiollier C, Kachi H, Dinkova-Kostova AT, Wells G. 2015. Design, synthesis, and evaluation of triazole derivatives that induce Nrf2 dependent gene products and inhibit the Keap1-Nrf2 protein-protein interaction. J Med Chem 58:7186–7194. https://doi.org/10.1021/acs.jmedchem.5b00602.
162. Davies TG, Wixted WE, Coyle JE, Griffiths-Jones C, Hearn K, McMenamin R, Norton D, Rich SJ, Richardson C, Saxty G, Willems HM, Woolford AJ, Cottom JE, Kou JP, Yonchuk JG, Feldser HG, Sanchez Y, Foley JP, Bolognese BJ, Logan G, Podolin PL, Yan H, Callahan JF, Heightman TD, Kerns JK. 2016. Monoacidic inhibitors of the Kelch-like ECH-associated protein 1: nuclear factor erythroid 2-related factor 2 (KEAP1:NRF2) protein-protein interaction with high cell potency identified by fragment-based discovery. J Med Chem 59:3991–4006. https://doi.org/10.1021/acs.jmedchem.6b00228.
163. Tran KT, Pallesen JS, Solbak SMØ, Narayanan D, Baig A, Zang J, Aguayo-Orozco A, Carmona RMC, Garcia AD, Bach A. 2019. A comparative assessment study of known small-molecule Keap1-Nrf2 protein-protein interaction inhibitors: chemical synthesis, binding properties, and cellular activity. J Med Chem 62:8028–8052. https://doi.org/10.1021/acs.jmedchem.9b00723.
164. Lazzara PR, David BP, Ankireddy A, Richardson BG, Dye K, Ratia KM, Reddy SP, Moore TW. 2019. Isoquinoline Kelch-like ECH-associated protein 1-nuclear factor (erythroid-derived 2)-like 2 (KEAP1-NRF2) inhibitors with high metabolic stability. J Med Chem https://doi.org/10.1021/acs.jmedchem.9b01074.
165. Salazar M, Rojo AI, Velasco D, de Sagarra RM, Cuadrado A. 2006. Glycogen synthase kinase-3 β inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J Biol Chem 281:14841–14851. https://doi.org/10.1074/jbc.M513737200.
166. Rada P, Rojo AI, Chowdhry S, McMahon M, Hayes JD, Cuadrado A. 2011. SCF β-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol Cell Biol 31:1121–1133. https://doi.org/10.1128/MCB.01204-10.
167. Rojo AI, Medina-Campos ON, Rada P, Zúñiga-Toalá A, López-Gazcón A, Espada S, Pedraza-Chaverri J, Cuadrado A. 2012. Signaling pathways activated by the phytochemical nordihydroguaiaretic acid contribute to a Keap1-independent regulation of Nrf2 stability: role of glycogen synthase kinase-3. Free Radic Biol Med 52:473–487. https://doi.org/10.1016/j.freeradbiomed.2011.11.003.
168. Rada P, Rojo AI, Evrard-Todeschi N, Innamorato NG, Cotte A, Jaworski T, Tobón-Velasco JC, Devijver H, García-Mayoral MF, Van Leuven F, Hayes JD, Bertho G, Cuadrado A. 2012. Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/β-TrCP axis. Mol Cell Biol 32:3486–3499. https://doi.org/10.1128/MCB.00180-12.
169. Chowdhry S, Zhang Y, McMahon M, Sutherland C, Cuadrado A, Hayes JD. 2013. Nrf2 is controlled by two distinct β-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene 32:3765–3781. https://doi.org/10.1038/onc.2012.388.
170. Kim TY, Siesser PF, Rossman KL, Goldfarb D, Mackinnon K, Yan F, Yi X, MacCoss MJ, Moon RT, Der CJ, Major MB. 2015. Substrate trapping proteomics reveals targets of the βTrCP2/FBXW11 ubiquitin ligase. Mol Cell Biol 35:167–181. https://doi.org/10.1128/MCB.00857-14.
171. Chen Y, Liu K, Zhang J, Hai Y, Wang P, Wang H, Liu Q, Wong CC, Yao J, Gao Y, Liao Y, Tang X, Wang XJ. 2020. JNK phosphorylates the Neh6 domain of Nrf2 and downregulates cytoprotective genes in acetaminophen-induced liver injury. Hepatology https://doi.org/10.1002/hep.31116.
172. Taguchi K, Maher JM, Suzuki T, Kawatani Y, Motohashi H, Yamamoto M. 2010. Genetic analysis of cytoprotective functions supported by graded expression of Keap1. Mol Cell Biol 30:3016–3026. https://doi.org/10.1128/MCB.01591-09.
173. Schäfer M, Farwanah H, Willrodt AH, Huebner AJ, Sandhoff K, Roop D, Hohl D, Bloch W, Werner S. 2012. Nrf2 links epidermal barrier function with antioxidant defense. EMBO Mol Med 4:364–379. https://doi.org/10.1002/emmm.201200219.
174. Suzuki T, Shibata T, Takaya K, Shiraishi K, Kohno T, Kunitoh H, Tsuta K, Furuta K, Goto K, Hosoda F, Sakamoto H, Motohashi H, Yamamoto M. 2013. Regulatory nexus of synthesis and degradation deciphers cellular Nrf2 expression levels. Mol Cell Biol 33:2402–2412. https://doi.org/10.1128/MCB.00065-13.
175. Suzuki T, Seki S, Hiramoto K, Naganuma E, Kobayashi EH, Yamaoka A, Baird L, Takahashi N, Sato H, Yamamoto M. 2017. Hyperactivation of Nrf2 in early tubular development induces nephrogenic diabetes insipidus. Nat Commun 8:14577. https://doi.org/10.1038/ncomms14577.
176. Kanarek N, Horwitz E, Mayan I, Leshets M, Cojocaru G, Davis M, Tsuberi BZ, Pikarsky E, Pagano M, Ben-Neriah Y. 2010. Spermatogenesis rescue in a mouse deficient for the ubiquitin ligase SCFβ-TrCP by single substrate depletion. Genes Dev 24:470–477. https://doi.org/10.1101/gad.551610.
177. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, Dimitriadoy S, Liu DL, Kantheti HS, Saghafinia S, Chakravarty D, Daian F, Gao Q, Bailey MH, Liang WW, Foltz SM, Shmulevich I, Ding L, Heins Z, Ochoa A, Gross B, Gao J, Zhang H, Kundra R, Kandoth C, Bahceci I, Dervishi L, Dogrusoz U, Zhou W, Shen H, Laird PW, Way GP, Greene CS, Liang H, Xiao Y, Wang C, Iavarone A, Berger AH, Bivona TG, Lazar AJ, Hammer GD, Giordano T, Kwong LN, McArthur G, Huang C, Tward AD, Frederick MJ, McCormick F, Meyerson M, Cancer Genome Atlas Research Network, Van Allen EM, Cherniack AD, Ciriello G, Sander C, Schultz N. 2018. Oncogenic signaling pathways in the Cancer Genome Atlas. Cell 173:321–337. https://doi.org/10.1016/j.cell.2018.03.035.
178. Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H, Yamamoto M, Motohashi H. 2012. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell 22:66–79. 2012 https://doi.org/10.1016/j.ccr.2012.05.016.
179. Taguchi K, Hirano I, Itoh T, Tanaka M, Miyajima A, Suzuki A, Motohashi

H, Yamamoto M. 2014. Nrf2 enhances cholangiocyte expansion in Pten-deficient livers. Mol Cell Biol 34:900–913. https://doi.org/10.1128/MCB.01384-13.
180. Dolado I, Swat A, Ajenjo N, De Vita G, Cuadrado A, Nebreda AR. 2007. p38alpha MAP kinase as a sensor of reactive oxygen species in tumorigenesis. Cancer Cell 11:191–205. https://doi.org/10.1016/j.ccr.2006.12.013.
181. Grabocka E, Bar-Sagi D. 2016. Mutant KRAS enhances tumor cell fitness by upregulating stress granules. Cell 167:1803–1813. https://doi.org/10.1016/j.cell.2016.11.035.
182. Sun Z, Huang Z, Zhang DD. 2009. Phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution in modulating the Nrf2-dependent antioxidant response. PLoS One 4:e6588. https://doi.org/10.1371/journal.pone.0006588.
183. Matzinger M, Fischhuber K, Pölöske D, Mechtler K, Heiss EH. 2020. AMPK leads to phosphorylation of the transcription factor Nrf2, tuning transactivation of selected target genes. Redox Biol 29:101393. https://doi.org/10.1016/j.redox.2019.101393.
184. Huang HC, Nguyen T, Pickett CB. 2000. Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. Proc Natl Acad Sci U S A 97:12475–12480. https://doi.org/10.1073/pnas.220418997.
185. Huang HC, Nguyen T, Pickett CB. 2002. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J Biol Chem 277:42769–42774. https://doi.org/10.1074/jbc.M206911200.
186. Tyshkovskiy A, Bozaykut P, Borodinova AA, Gerashchenko MV, Ables GP, Garratt M, Khaitovich P, Clish CB, Miller RA, Gladyshev VN. 2019. Identification and application of gene expression signatures associated with lifespan extension. Cell Metab 30:573–593. https://doi.org/10.1016/j.cmet.2019.06.018.
187. Gold R, Kappos L, Arnold DL, Bar-Or A, Giovannoni G, Selmaj K, Tornatore C, Sweetser MT, Yang M, Sheikh SI, Dawson KT, DEFINE Study Investigators. 2012. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. N Engl J Med 367:1098–1107. https://doi.org/10.1056/NEJMoa1114287.
188. Chin MP, Reisman SA, Bakris GL, O'Grady M, Linde PG, McCullough PA, Packham D, Vaziri ND, Ward KW, Warnock DG, Meyer CJ. 2014. Mechanisms contributing to adverse cardiovascular events in patients with type 2 diabetes mellitus and stage 4 chronic kidney disease treated with bardoxolone methyl. Am J Nephrol 39:499–508. https://doi.org/10.1159/000362906.
189. Chin MP, Wrolstad D, Bakris GL, Chertow GM, de Zeeuw D, Goldsberry A, Linde PG, McCullough PA, McMurray JJ, Wittes J, Meyer CJ. 2014. Risk factors for heart failure in patients with type 2 diabetes mellitus and stage 4 chronic kidney disease treated with bardoxolone methyl. J Card Fail 20:953–958. https://doi.org/10.1016/j.cardfail.2014.10.001.
190. Chin MP, Bakris GL, Block GA, Chertow GM, Goldsberry A, Inker LA, Heerspink HJL, O'Grady M, Pergola PE, Wanner C, Warnock DG, Meyer CJ. 2018. Bardoxolone methyl improves kidney function in patients with chronic kidney disease stage 4 and type 2 diabetes: post-hoc analyses from Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes Study. Am J Nephrol 47:40–47. https://doi.org/10.1159/000486398.
191. Toto RD. 2018. Bardoxolone—the phoenix? J Am Soc Nephrol 29:360–361. https://doi.org/10.1681/ASN.2017121317.
192. Sawa T, Zaki MH, Okamoto T, Akuta T, Tokutomi Y, Kim-Mitsuyama S, Ihara H, Kobayashi A, Yamamoto M, Fujii S, Arimoto H, Akaike T. 2007. Protein S-guanylation by the biological signal 8-nitroguanosine 3′,5′-cyclic monophosphate. Nat Chem Biol 3:727–735. https://doi.org/10.1038/nchembio.2007.33.
193. Fujii S, Sawa T, Ihara H, Tong KI, Ida T, Okamoto T, Ahtesham AK, Ishima Y, Motohashi H, Yamamoto M, Akaike T. 2010. The critical role of nitric oxide signaling, via protein S-guanylation and nitrated cyclic GMP, in the antioxidant adaptive response. J Biol Chem 285:23970–23984. https://doi.org/10.1074/jbc.M110.145441.
194. Yang G, Zhao K, Ju Y, Mani S, Cao Q, Puukila S, Khaper N, Wu L, Wang R. 2013. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. Antioxid Redox Signal 18:1906–1919. https://doi.org/10.1089/ars.2012.4645.
195. Bollong MJ, Lee G, Coukos JS, Yun H, Zambaldo C, Chang JW, Chin EN, Ahmad I, Chatterjee AK, Lairson LL, Schultz PG, Moellering RE. 2018. A metabolite-derived protein modification integrates glycolysis with KEAP1-NRF2 signalling. Nature 562:600–604. https://doi.org/10.1038/s41586-018-0622-0.
196. Adam J, Hatipoglu E, O'Flaherty L, Ternette N, Sahgal N, Lockstone H, Baban D, Nye E, Stamp GW, Wolhuter K, Stevens M, Fischer R, Carmeliet P, Maxwell PH, Pugh CW, Frizzell N, Soga T, Kessler BM, El-Bahrawy M, Ratcliffe PJ, Pollard PJ. 2011. Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. Cancer Cell 20:524–537. https://doi.org/10.1016/j.ccr.2011.09.006.
197. Ooi A, Wong JC, Petillo D, Roossien D, Perrier-Trudova V, Whitten D, Min BW, Tan MH, Zhang Z, Yang XJ, Zhou M, Gardie B, Molinié V, Richard S, Tan PH, Teh BT, Furge KA. 2011. An antioxidant response phenotype shared between hereditary and sporadic type 2 papillary renal cell carcinoma. Cancer Cell 20:511–523. https://doi.org/10.1016/j.ccr.2011.08.024.
198. Yang F, Li J, Deng H, Wang Y, Lei C, Wang Q, Xiang J, Liang L, Xia J, Pan X, Li X, Long Q, Chang L, Xu P, Huang A, Wang K, Tang N. 2019. GSTZ1-1 deficiency activates NRF2/IGF1R axis in HCC via accumulation of oncometabolite succinylacetone. EMBO J 38:e101964. https://doi.org/10.15252/embj.2019101964.
199. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, Zaslona Z, Jedrychowski MP, Costa ASH, Higgins M, Hams E, Szpyt J, Runtsch MC, King MS, McGouran JF, Fischer R, Kessler BM, McGettrick AF, Hughes MM, Carroll RG, Booty LM, Knatko EV, Meakin PJ, Ashford MLJ, Modis LK, Brunori G, Sévin DC, Fallon PG, Caldwell ST, Kunji ERS, Chouchani ET, Frezza C, Dinkova-Kostova AT, Hartley RC, Murphy MP, O'Neill LA. 2018. Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature 556:113–117. https://doi.org/10.1038/nature25986.
200. Bambouskova M, Gorvel L, Lampropoulou V, Sergushichev A, Loginicheva E, Johnson K, Korenfeld D, Mathyer ME, Kim H, Huang LH, Duncan D, Bregman H, Keskin A, Santeford A, Apte RS, Sehgal R, Johnson B, Amarasinghe GK, Soares MP, Satoh T, Akira S, Hai T, de Guzman Strong C, Auclair K, Roddy TP, Biller SA, Jovanovic M, Klechevsky E, Stewart KM, Randolph GJ, Artyomov MN. 2018. Electrophilic properties of itaconate and derivatives regulate the IκBζ-ATF3 inflammatory axis. Nature 556:501–504. https://doi.org/10.1038/s41586-018-0052-z.
201. Zhang Y, Talalay P, Cho CG, Posner GH. 1992. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. Proc Natl Acad Sci U S A 89:2399–2403. https://doi.org/10.1073/pnas.89.6.2399.
202. Dinkova-Kostova AT, Massiah MA, Bozak RE, Hicks RJ, Talalay P. 2001. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. Proc Natl Acad Sci U S A 98:3404–3409. https://doi.org/10.1073/pnas.051632198.
203. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, Suh N, Williams C, Risingsong R, Honda T, Gribble GW, Sporn MB, Talalay P. 2005. Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. Proc Natl Acad Sci U S A 102:4584–4589. https://doi.org/10.1073/pnas.0500815102.
204. Rushworth SA, Chen X-L, Mackman N, Ogborne RM, O'Connell MA. 2005. Lipopolysaccharide-induced heme oxygenase-1 expression in human monocytic cells is mediated via Nrf2 and protein kinase C. J Immunol 175:4408–4415. https://doi.org/10.4049/jimmunol.175.7.4408.
205. Edwards MR, Johnson B, Mire CE, Xu W, Shabman RS, Speller LN, Leung DW, Geisbert TW, Amarasinghe GK, Basler CF. 2014. The Marburg virus VP24 protein interacts with Keap1 to activate the cytoprotective antioxidant response pathway. Cell Rep 6:1017–1025. https://doi.org/10.1016/j.celrep.2014.01.043.
206. Page A, Volchkova VA, Reid SP, Mateo M, Bagnaud-Baule A, Nemirov K, Shurtleff AC, Lawrence P, Reynard O, Ottmann M, Lotteau V, Biswal SS, Thimmulappa RK, Bavari S, Volchkov VE. 2014. Marburgvirus hijacks nrf2-dependent pathway by targeting nrf2-negative regulator keap1. Cell Rep 6:1026–1036. https://doi.org/10.1016/j.celrep.2014.02.027.
207. Ramos S, Carlos AR, Sundaram B, Jeney V, Ribeiro A, Gozzelino R, Bank C, Gjini E, Braza F, Martins R, Ademolue TW, Blankenhaus B, Gouveia Z, Faísca P, Trujillo D, Cardoso S, Rebelo S, Del Barrio L, Zarjou A, Bolisetty S, Agarwal A, Soares MP. 2019. Renal control of disease tolerance to malaria. Proc Natl Acad Sci U S A 116:5681–5686. https://doi.org/10.1073/pnas.1822024116.

208. Glory A, Averill-Bates DA. 2016. The antioxidant transcription factor Nrf2 contributes to the protective effect of mild thermotolerance (40°C) against heat shock-induced apoptosis. Free Radic Biol Med 99:485–497. https://doi.org/10.1016/j.freeradbiomed.2016.08.032.
209. Hosoya T, Maruyama A, Kang MI, Kawatani Y, Shibata T, Uchida K, Warabi E, Noguchi N, Itoh K, Yamamoto M. 2005. Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells. J Biol Chem 280:27244–27250. https://doi.org/10.1074/jbc.M502551200.
210. Hirota A, Kawachi Y, Itoh K, Nakamura Y, Xu X, Banno T, Takahashi T, Yamamoto M, Otsuka F. 2005. Ultraviolet A irradiation induces NF-E2-related factor 2 activation in dermal fibroblasts: protective role in UVA-induced apoptosis. J Invest Dermatol 124:825–832. https://doi.org/10.1111/j.0022-202X.2005.23670.x.
211. Done AJ, Traustadóttir T. 2016. Nrf2 mediates redox adaptations to exercise. Redox Biol 10:191–199. https://doi.org/10.1016/j.redox.2016.10.003.
212. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tuder RM, Biswal S. 2004. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. J Clin Invest 114:1248–1259. https://doi.org/10.1172/JCI200421146.
213. Kim YC, Masutani H, Yamaguchi Y, Itoh K, Yamamoto M, Yodoi J. 2001. Hemin-induced activation of the thioredoxin gene by Nrf2. A differential regulation of the antioxidant responsive element by a switch of its binding factors. J Biol Chem 276:18399–18406. https://doi.org/10.1074/jbc.M100103200.
214. MacLeod AK, McMahon M, Plummer SM, Higgins LG, Penning TM, Igarashi K, Hayes JD. 2009. Characterization of the cancer chemopreventive NRF2-dependent gene battery in human keratinocytes: demonstration that the KEAP1-NRF2 pathway, and not the BACH1-NRF2 pathway, controls cytoprotection against electrophiles as well as redox-cycling compounds. Carcinogenesis 30:1571–1580. https://doi.org/10.1093/carcin/bgp176.
215. Wu KC, Cui JY, Klaassen CD. 2011. Beneficial role of Nrf2 in regulating NADPH generation and consumption. Toxicol Sci 123:590–600. https://doi.org/10.1093/toxsci/kfr183.
216. Agyeman AS, Chaerkady R, Shaw PG, Davidson NE, Visvanathan K, Pandey A, Kensler TW. 2012. Transcriptomic and proteomic profiling of KEAP1 disrupted and sulforaphane-treated human breast epithelial cells reveals common expression profiles. Breast Cancer Res Treat 132:175–187. https://doi.org/10.1007/s10549-011-1536-9.
217. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. 2003. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol 23:7198–7209. https://doi.org/10.1128/mcb.23.20.7198-7209.2003.
218. Cullinan SB, Diehl JA. 2004. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. J Biol Chem 279:20108–20117. https://doi.org/10.1074/jbc.M314219200.
219. Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, Zhang F, Ye J, Koumenis C, Cavener D, Diehl JA. 2010. PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. Oncogene 29:3881–3895. https://doi.org/10.1038/onc.2010.153.
220. Sun Z, Chin YE, Zhang DD. 2009. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol Cell Biol 29:2658–2672. https://doi.org/10.1128/MCB.01639-08.
221. Kawai Y, Garduño L, Theodore M, Yang J, Arinze IJ. 2011. Acetylation-deacetylation of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) regulates its transcriptional activity and nucleocytoplasmic localization. J Biol Chem 286:7629–7640. https://doi.org/10.1074/jbc.M110.208173.
222. Yang X, Park SH, Chang HC, Shapiro JS, Vassilopoulos A, Sawicki KT, Chen C, Shang M, Burridge PW, Epting CL, Wilsbacher LD, Jenkitkasemwong S, Knutson M, Gius D, Ardehali H. 2017. Sirtuin 2 regulates cellular iron homeostasis via deacetylation of transcription factor NRF2. J Clin Invest 127:1505–1516. https://doi.org/10.1172/JCI88574.
223. Chen D, Tavana O, Chu B, Erber L, Chen Y, Baer R, Gu W. 2017. NRF2 is a major target of ARF in p53-independent tumor suppression. Mol Cell 68:224–232. https://doi.org/10.1016/j.molcel.2017.09.009.
224. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, Scrimieri F, Winter JM, Hruban RH, Iacobuzio-Donahue C, Kern SE, Blair IA, Tuveson DA. 2011. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 475:106–109. https://doi.org/10.1038/nature10189.
225. Eades G, Yang M, Yao Y, Zhang Y, Zhou Q. 2011. miR-200a regulates Nrf2 activation by targeting Keap1 mRNA in breast cancer cells. J Biol Chem 286:40725–40733. https://doi.org/10.1074/jbc.M111.275495.
226. Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Y, Mariani O, Nicolas A, Meyniel JP, Cottu P, Sastre-Garau X, Mechta-Grigoriou F. 2011. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. Nat Med 17:1627–1635. https://doi.org/10.1038/nm.2512.
227. Hanada N, Takahata T, Zhou Q, Ye X, Sun R, Itoh J, Ishiguro A, Kijima H, Mimura J, Itoh K, Fukuda S, Saijo Y. 2012. Methylation of the KEAP1 gene promoter region in human colorectal cancer. BMC Cancer 12:66. https://doi.org/10.1186/1471-2407-12-66.
228. Wang D, Ma Y, Yang X, Xu X, Zhao Y, Zhu Z, Wang X, Deng H, Li C, Gao F, Tong J, Yamanaka K, An Y. 2015. Hypermethylation of the Keap1 gene inactivates its function, promotes Nrf2 nuclear accumulation, and is involved in arsenite-induced human keratinocyte transformation. Free Radic Biol Med 89:209–219. https://doi.org/10.1016/j.freeradbiomed.2015.07.153.
229. Kalo E, Kogan-Sakin I, Solomon H, Bar-Nathan E, Shay M, Shetzer Y, Dekel E, Goldfinger N, Buganim Y, Stambolsky P, Goldstein I, Madar S, Rotter V. 2012. Mutant p53R273H attenuates the expression of phase 2 detoxifying enzymes and promotes the survival of cells with high levels of reactive oxygen species. J Cell Sci 125:5578–5586. https://doi.org/10.1242/jcs.106815.
230. Walerych D, Lisek K, Sommaggio R, Piazza S, Ciani Y, Dalla E, Rajkowska K, Gaweda-Walerych K, Ingallina E, Tonelli C, Morelli MJ, Amato A, Eterno V, Zambelli A, Rosato A, Amati B, Wiśniewski JR, Del Sal G. 2016. Proteasome machinery is instrumental in a common gain-of-function program of the p53 missense mutants in cancer. Nat Cell Biol 18:897–909. https://doi.org/10.1038/ncb3380.
231. Wang Z, Chen Z, Jiang Z, Luo P, Liu L, Huang Y, Wang H, Wang Y, Long L, Tan X, Liu D, Jin T, Wang Y, Wang Y, Liao F, Zhang C, Chen L, Gan Y, Liu Y, Yang F, Huang C, Miao H, Chen J, Cheng T, Fu X, Shi C. 2019. Cordycepin prevents radiation ulcer by inhibiting cell senescence via NRF2 and AMPK in rodents. Nat Commun 10:2538. https://doi.org/10.1038/s41467-019-10386-8.