OPEN BIOLOGY

royalsocietypublishing.org/journal/rsob

Review

Cite this article: Dayalan Naidu S, Dinkova-Kostova AT. 2020 KEAP1, a cysteine-based sensor and a drug target for the prevention and treatment of chronic disease. *Open Biol.* **10**: 200105.
http://dx.doi.org/10.1098/rsob.200105

Received: 20 April 2020
Accepted: 22 May 2020

Subject Area:
biochemistry

Keywords:
KEAP1, NRF2, cysteine, anti-inflammatory, antioxidant, redox

Author for correspondence:
Albena T. Dinkova-Kostova
e-mail: a.dinkovakostova@dundee.ac.uk

THE ROYAL SOCIETY PUBLISHING

KEAP1, a cysteine-based sensor and a drug target for the prevention and treatment of chronic disease

Sharadha Dayalan Naidu[1] and Albena T. Dinkova-Kostova[1,2]

[1]Jacqui Wood Cancer Centre, Division of Cellular Medicine, School of Medicine, University of Dundee, Dundee, UK
[2]Department of Pharmacology and Molecular Sciences and Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

ATD-K, 0000-0003-0316-9859

Redox imbalance and persistent inflammation are the underlying causes of most chronic diseases. Mammalian cells have evolved elaborate mechanisms for restoring redox homeostasis and resolving acute inflammatory responses. One prominent mechanism is that of inducing the expression of antioxidant, anti-inflammatory and other cytoprotective proteins, while also suppressing the production of pro-inflammatory mediators, through the activation of transcription factor nuclear factor-erythroid 2 p45-related factor 2 (NRF2). At homeostatic conditions, NRF2 is a short-lived protein, which avidly binds to Kelch-like ECH-associated protein 1 (KEAP1). KEAP1 functions as (i) a substrate adaptor for a Cullin 3 (CUL3)-based E3 ubiquitin ligase that targets NRF2 for ubiquitination and proteasomal degradation, and (ii) a cysteine-based sensor for a myriad of physiological and pharmacological NRF2 activators. Here, we review the intricate molecular mechanisms by which KEAP1 senses electrophiles and oxidants. Chemical modification of specific cysteine sensors of KEAP1 results in loss of NRF2-repressor function and alterations in the expression of NRF2-target genes that encode large networks of diverse proteins, which collectively restore redox balance and resolve inflammation, thus ensuring a comprehensive cytoprotection. We focus on the cyclic cyanoenones, the most potent NRF2 activators, some of which are currently in clinical trials for various pathologies characterized by redox imbalance and inflammation.

1. Introduction

All living organisms are vulnerable to various chemical stressors derived from endogenous and exogenous sources, such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive lipid species (RLS), which play important roles in cell signalling, but when produced in excess lead to oxidative stress. Oxidation–reduction (redox) reactions are common in biology, and the maintenance of redox homeostasis is vital for the correct functioning of most biological processes [1]. Oxidative stress occurs when there is an excess of oxidants, and the antioxidants are insufficient for restoring the intracellular redox balance [2]. Some examples of sources of exogenous oxidative stressors are environmental pollutants, ultraviolet (UV) and ionizing radiation, and genotoxic agents. Endogenous stressors, usually produced intracellularly, are derived from metabolic processes such as mitochondrial respiration and inflammation. Exposure to these chemically reactive species promotes cellular macromolecular damage. Chronic oxidative stress has been implicated in the development and exacerbation of neurodegenerative diseases [3,4], cancer [5,6], diabetes [7,8], autoimmune [9], cutaneous [10–12], pulmonary [13,14] and cardiovascular [14,15] diseases, infection [16], inflammation [17], as well as aging [18–20]. Cells have evolved several mechanisms to combat these

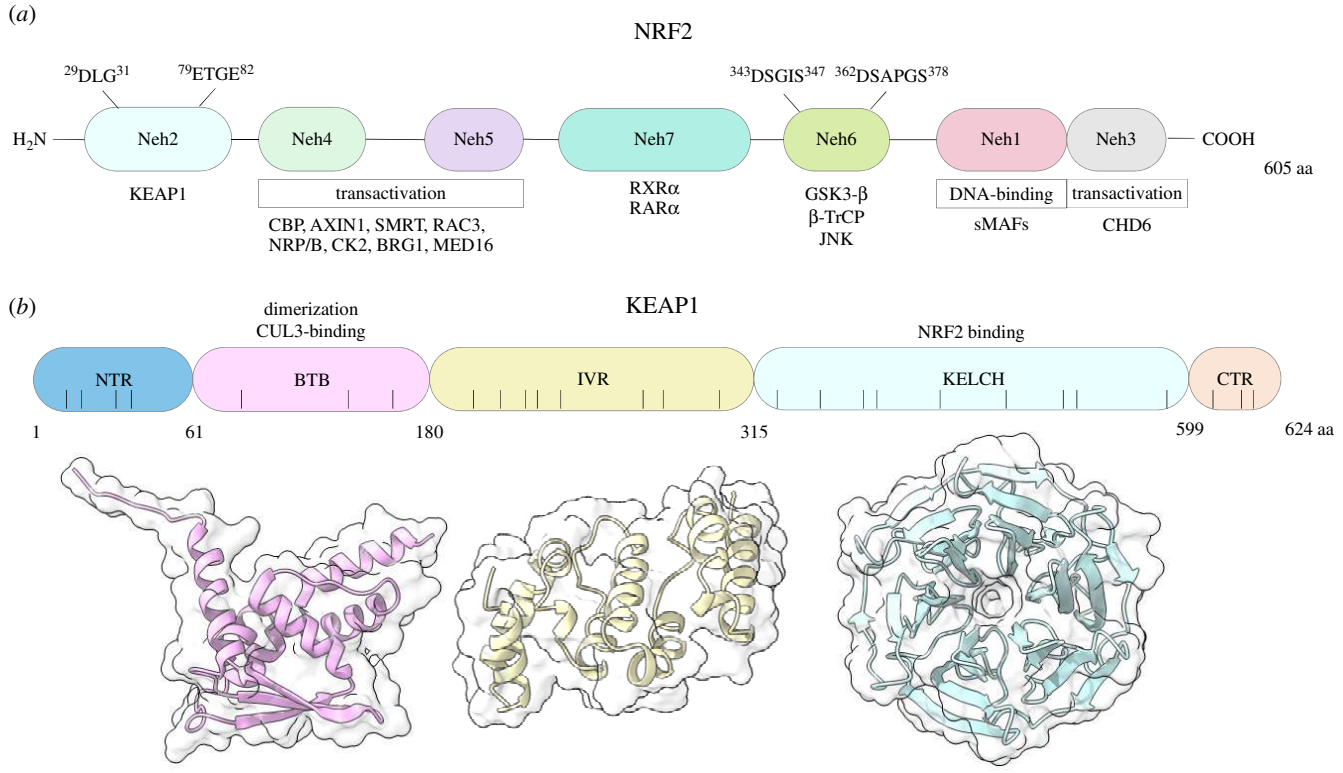

Figure 1. (*a*) Domain structure of human NRF2. There are seven NRF2-ECH (Neh) domains found within NRF2. The N-terminal Neh2 domain contains the KEAP1 binding motifs DLG and ETGE. The Neh4 and 5 domains within the transcription factor are required for its transactivation and the proteins that have exhibited binding to this region are CREB (cAMP responsive element binding protein) binding protein (CBP), AXIN1, silencing mediator of retinoic acid and thyroid hormone receptor (SMRT1), Receptor-associated coactivator 3 (RAC3), nuclear matrix protein (NRP/B), casein kinase 2 (CK2), Brahma-related gene 1 (BRG1) and mediator complex subunit 16 (MED16). The Neh7 domain found in the middle of the NRF2 protein has been shown to interact with retinoid X receptor alpha (RXRα) as well as retinoic acid receptor alpha (RARα). Two motifs, DSGIS and DSAPGS are found within the Neh6 domain, and are important for the binding of β-TrCP to facilitate NRF2-degradation, where the binding is promoted upon glycogen synthase kinase β (GSK3-β)-mediated phosphorylation of the DSGIS motif. C-Jun N-Terminal Kinase (JNK) binds to the Neh6 domain and phosphorylates S335. The Neh1 domain comprises of the DNA-binding motif and the binding region for the sMAF proteins. The carboxy-terminal Neh3 domain is also important for transactivation of NRF2 and chromodomain helicase DNA binding protein 6 (CHD6) interacts with this domain. (*b*) Domain structure of human KEAP1. The KEAP1 protein is a substrate adaptor for the CUL3-based E3 ligase, and is sectioned into five domains: (1) N-terminal region (NTR); (2) Broad complex, tramtrack, and Bric à Brac (BTB) domain allows for the homodimerization of KEAP1 monomers as well as CUL3 binding; (3) Intervening region (IVR); (4) Kelch domain (KELCH) is a structure consisting of a six-bladed β-propeller, where one KELCH subunit within the KEAP1 homodimer binds to the DLG motif and the other binds to the ETGE motifs found within the Neh2 domain of NRF2; and (5) C-terminal region (CTR). The black vertical lines represent the positions of the 27 cysteine residues present within the protein. Cartoon and surface representations of the BTB (pale pink) (PDB ID: 4CXI), IVR (pale yellow) (modelled) and KELCH (pale blue) (PDB ID: 5WFV) were drawn with UCSF ChimeraX software using X-Crystallographic images deposited into the Protein Data Bank (rscb.org) or modelled using the web-based I-TASSER platform.

relentless chemical insults in order to reinstate the redox homeostasis. The activation of the KEAP1/NRF2/ARE pathway is one such mechanism, which orchestrates the upregulation of antioxidant, anti-inflammatory and other cytoprotective proteins.

2. The NRF2/KEAP1/ARE cytoprotective pathway

Under homeostatic conditions, the transcription factor nuclear factor erythroid-2 p45-related factor 2 (NRF2) (figure 1*a*), is continuously ubiquitinated and targeted for 26S proteasomal degradation by its negative regulator Kelch-like (ECH)-associated protein 1 (KEAP1) (figure 1*b*), which is a substrate adaptor for the Cullin 3 (CUL3)-ring box 1 (RBX1) E3-ubiquitin ligase system [21]. Electrophiles from endogenous and exogenous sources or other small molecules (termed inducers) which activate NRF2 are able to do so via inactivating KEAP1 by reacting with its cysteine(s) residues or by disrupting the KEAP1:NRF2 protein–protein interaction (PPI) interface [22,23]. Consequently, KEAP1 is unable to target the transcription factor for degradation. The interactions between KEAP1 and NRF2 and the effect of inducers on NRF2 stabilization can be visualized by the imaging of live cells expressing KEAP1 and NRF2, each fused to a fluorescent protein [24,25]. Following KEAP1 inactivation, the newly synthesized or free NRF2 is able to accumulate and translocate into the nucleus where it heterodimerizes with a small musculoaponeurotic fibrosarcoma (sMAF) protein and binds to the antioxidant response elements (ARE) with the consensus sequence 5′-TGACxxxGC-3′ found in the promoters of its target genes [21]. The 605-amino acid long NRF2 protein belongs to the family of the Cap'n'Collar (CnC) basic leucine zipper (bZIP) transcription factors, and is composed of seven NRF2-ECH (Neh) domains which are highly conserved (figure 1*a*). The N-terminally lying Neh2 domain of NRF2 contains two KEAP1 (low- and high-affinity) binding motifs, which are the sequences DLG and ETGE, respectively [26].

2.1. NRF2-mediated antioxidant effects

Together, the NRF2 target genes (over 250) are involved in mounting a cellular defence response by encoding a large network of proteins, some of which catalyse phase I, II and III cytoprotective detoxification reactions, while others have antioxidant and anti-inflammatory properties [27]. NRF2 controls the cellular redox homeostasis by regulating key enzymes and proteins involved in processes such as the synthesis, utilization and regeneration of glutathione (GSH), thioredoxin (TXN), peroxiredoxin and NADPH production [28]. The activity of NRF2 is a major determining factor of the cellular redox state.

GSH is an essential thiol-based intracellular tripeptide that plays a vital role in the defence against cellular oxidative stress through its ability to neutralize ROS/RNS as well as electrophilic species [29]. Perturbed glutathione homeostasis has been implicated in numerous pathological conditions [30,31]. NRF2 regulates the gene expression of the catalytic subunit GCLC and the modifier subunit GCLM of γ-glutamate-cysteine ligase (GCL), the enzyme catalysing the rate-limiting step in the GSH biosynthesis [32], as well as the gene expression of the cystine/glutamate antiporter (SLC7A11, system xc−) [33] that is responsible for the import of cystine, which in turn is converted to cysteine, a GSH precursor. The flux of glutamine into anabolic pathways is enhanced under conditions of NRF2 activation [34] thus providing glutamate, the second GSH precursor; of note, glutamate is also necessary for the import of cystine by system xc−. The transporter SLC6A9, another NRF2-regulated gene, provides the third GSH precursor, glycine [35]. In addition to the biosynthesis of GSH, NRF2 also regulates the regeneration of GSH. The transcription factor controls the expression of glutathione peroxidase (GPX), which detoxifies peroxides to produce oxidized glutathione (GSSG). In turn, GSSG is a substrate for the NRF2-target glutathione reductase (GSR), which regenerates GSH from GSSG using NADPH as a hydride donor. Importantly, NRF2 is also involved in the regulation of cellular NADPH levels by controlling the gene expression of the four main enzymes involved in the generation of NADPH: isocitrate dehydrogenase 1 (IDH1), 6-phosphogluconate dehydrogenase (PGD), glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme 1 (ME1) [34,36].

The role of NRF2 in the biosynthesis and maintenance of GSH is particularly important in the brain [37,38], and may also affect the metabolic glutamate–glutamine cycle that allows the inter-cellular exchange of these amino acids between neurons and astrocytes [39]. During neuronal development, expression of *NFE2L2* (the gene encoding NRF2) is repressed by promoter methylation [40], and NRF2 activity in astrocytes is critical for neuronal protection against oxidative stress [41]. In rapidly proliferating cells, such as cancer cells, NRF2 activation channels glucose through the pentose phosphate pathway [34], a major source of reducing equivalents for GSH regeneration, but also increases consumption of glutamate for GSH biosynthesis and glutamate secretion by system xc− [42].

2.2. NRF2-mediated anti-inflammatory effects

In addition to antioxidant, the activation of NRF2 has anti-inflammatory effects, which have been consistently observed in cellular and animal models, as well as in human intervention trials with pharmacological NRF2 activators. Thus, a recent analysis of peripheral blood mononuclear cells (PBMCs) isolated from human subjects following intervention with sulforaphane, a classical NRF2 activator, reported an increase in the expression of NRF2-target genes (i.e. NQO1, HO1, AKR1C1), which was accompanied by a decrease in inflammatory markers (i.e. IL-6, TNFα, IL-1β, COX2) [43]. NRF2 is critical for the resolution of inflammation. The endogenous mildly electrophilic anti-inflammatory mitochondrial immunometabolite itaconate, which accumulates to millimolar concentrations during the metabolic reprogramming in activated macrophages [44,45], is an NRF2 activator. In turn, NRF2 represses the expression of pro-inflammatory cytokines and the type I interferon (IFN) response, promoting the resolution of inflammation [45–49]. Interestingly, NRF2 is also important for the execution of inflammation. Itaconate is downregulated in dysfunctional macrophages from hypercholesterolemic mice, and the levels of NRF2 and the expression of its target genes are lower in lipopolysaccharide (LPS)-stimulated macrophages isolated from mice fed high-fat diet (HFD) compared to standard fat diet (SFD) [50]. These findings illustrate that systemic metabolic changes can suppress NRF2 and consequently interfere with metabolic reprogramming in immune cells, which is necessary for their effector functions.

Thus, the activation of NRF2 is an attractive therapeutic strategy to combat diseases characterized by chronic oxidative stress and inflammation as it provides a multi-targeted approach [51,52]. Indeed, in recent years the pharmaceutical industry has invested heavily in the development of pharmacological modulators of the KEAP1/NRF2/ARE pathway, and there are currently more than 15 ongoing clinical trials as well as a number of compounds undergoing preclinical testing for various disease indications [23,53].

3. KEAP1

KEAP1 is a highly conserved cysteine-rich 624-amino acid protein sharing approximately 92% sequence homology among the mammalian species (figure 2). The existence of KEAP1 was predicted in the 1980s, more than a decade before its discovery. Following a series of extensive structure-activity studies using quantitative chemical biology approaches, Paul Talalay and his associates observed that numerous structurally diverse inducers of the cytoprotective enzymes NAD(P)H:quinone oxidoreductase (NQO1) and glutathione *S*-transferases (GSTs) have a common chemical property, namely sulfhydryl reactivity. This seminal discovery rationalized the perplexing lack of structural similarity among inducers, and led to the explicit suggestion that the primary cellular sensor with which inducers react is a protein endowed with highly reactive cysteines [54,55]. The identification of KEAP1 as a negative regulator of NRF2 by Masayuki Yamamoto and his colleagues in 1999 [56] immediately turned attention to the cysteine residues of KEAP1. Human KEAP1 has 27 cysteines, whereas mouse KEAP1 has 25, nine of which (red boxes in figure 2) are flanked by basic amino acids. Cysteines are unique amino acids due to their sulfhydryl (thiol) functional group that performs various functions, including: (1) forming intra- and intermolecular covalent bonds with other cysteine thiols, (2) binding to metals and metalloids, and (3) undergoing reversible or irreversible oxidation upon reacting with oxidants [57]. The *pKa* value of cysteine (represented by the balance between the thiol and the thiolate anion) indicates its reactivity [58]. The lower the *pKa* value, where the formation of the thiolate anion

Figure 2. Amino acid sequence alignment of various mammalian KEAP1. Alignment performed using the web-based Clustal Omega program found within uniprot.org. The Uniprot IDs are listed. Q14145 (human), Q9Z2X8 (mouse), P57790 (rat), Q684M4 (pig), Q5R774 (orangutan), H2QFB9 (chimpanzee). The cysteine residues are highlighted in yellow and the cysteine residues which have neighbouring basic amino acids are boxed in red. The amino acid sequence identity among these species is 92%.

is favoured, the cysteine reactivity increases. The presence of basic amino acids in close proximity to a cysteine decreases the *pKa*, hence increasing its reactivity [59,60]. The identification of highly reactive cysteines within KEAP1 that serve as inducer sensors [61] solved the puzzling fact that many inducers are active at sub-micromolar concentrations despite the millimolar intracellular concentrations of glutathione.

3.1. KEAP1 structure and its cysteine sensors

KEAP1 is a homodimeric protein belonging to the BTB (Broad complex, Tramtrack, Bric-á-brac)-Kelch family of proteins, which are named Kelch-like 1 to 42 (KLHL1–42). All members of this family are able to bind to CUL3 through their BTB domain (figure 1*b*). The BTB domain is necessary for KEAP1

homodimerization, and it has been reported that mutation of S104 to an alanine residue prevents its homodimerization and causes NRF2 accumulation in the nucleus [62]. There are three cysteines present in the KEAP1 BTB domain, C77, C151 and C171. Single mutants of each of the cysteines in the BTB domain to a serine behave like the wild-type (WT) KEAP1 in terms of their ability to repress NRF2-mediated gene expression [63]. C151 is the most well characterized in the literature. Under basal conditions, the KEAP1 C151S mutant is able to mediate the degradation of NRF2, similarly to the WT protein [63–65]. Zhang *et al*. found that the BTB domain of KEAP1 protected KEAP1 from ubiquitin-mediated degradation [66]. Interestingly, it has been shown by several groups that under basal conditions, when subjected to SDS-polyacrylamide gel electrophoresis (PAGE), WT KEAP1 migrates as two distinct species, one at approximately 65 kDa and the other at 130 kDa and that the slower migrating species does not appear within the C151S mutant [63,67]. Zhang and colleagues suggested that the slow migrating species of KEAP1 is due to posttranslational modifications occurring on the protein, as the mutation of C151S prevents its occurrence. We have observed a similar effect following treatment of cells with the double Michael acceptor dibenzylidene acetone (DBA) (S.D.N. 2016, unpublished observations). Fourquet *et al*. have shown that the intensity of this slower migrating KEAP1 species, which is resistant to reducing agents, is increased upon exposure to oxidants and nitrosative agents. These authors subsequently exposed lysates from induced cells to the reducing agent β-mercaptoethanol and observed a complete reduction in the intensified slower migrating species of KEAP1 hence suggesting that this species mainly consists of the oxidized form of KEAP1 [67]. Most recently, C151 from one subunit of the KEAP1 dimer was shown to form a methylimidazole crosslink with R135 from the second subunit upon accumulation of the reactive metabolite methylglyoxal, the concentration of which is increased in the plasma of diabetic patients [68]. In all cases, these post-translational modifications of C151 result in dimerization of KEAP1, accumulation of NRF2 and activation of the NRF2-driven cytoprotective transcriptional program. Of note, methylglyoxal is a precursor of highly damaging advanced glycation end-products, and NRF2 regulates the expression of glyoxalase 1, the enzyme that detoxifies methylglyoxal, thus protecting against glycation [69].

The cysteine residues of KEAP1, for which chemical modifications by various electrophiles and oxidants have been either demonstrated directly or implicated based on mutagenesis analyses, are summarized in table 1. Some of the well-known NRF2 inducers that modify C151 are the isothiocyanate sulforaphane (SFN), the alkylating agent iodoacetamide (IAA), *tert*-butyl hydroquinone (tBHQ) and diethylmaleate (DEM) [64,65,73,92]. In 2010, using molecular modelling, McMahon *et al*. postulated that the reactivity of C151 in KEAP1 was due to the presence of five basic amino acid residues (H129, K131, R135, K150 and H154) located in close spatial proximity to C151 [64]. These five basic amino acids possess the ability to deprotonate the thiol group within C151, thereby, lowering its *pKa*. This results in the thiol group of C151 to exist as an anion under physiological pH conditions. Indeed, the authors showed that KEAP1 bearing the triple mutations K131M, R135M and K150M, lost the ability to sense electrophiles that specifically targeted C151 [64]. The crystal structure of the BTB domain of KEAP1 with the triterpenoid CDDO was solved in 2014 and deposited in the Protein Data Bank (rscb.org) with the accession number 4CXI [72]. We measured the distances of the 5 positively charged amino acids mentioned that were adjacent to C151 and found that R135 had the closest proximity to C151 with a distance of 3.6 angstroms (Å) (figure 3), which further supports the findings reported by McMahon and colleagues [64].

To date, there is no crystal structure of the intervening (IVR) domain of KEAP1 (aa 180 to 315) available. The KEAP1 IVR domain, flanked by the N-terminal BTB domain and the Kelch domain at the C-terminus, contains 8 cysteine residues (C196, C226, C241, C249, C257, C273, C288 and C297), of which, C273 and C288 are best characterized. Exposure to electrophiles or alkylating agents targeting the cysteines within the IVR domain, predominantly C273 and C288, leads to the inactivation of KEAP1 and subsequent activation of NRF2 [61,93,94]. Single or double mutations of C273 or C288 to serine or alanine render KEAP1 inactive with respect to its ability to repress and target for degradation NRF2 [63,64,93,95,96]. Since these C273S/A and C288S/A mutants inactivated KEAP1, they presented a difficulty to study the electrophiles that could potentially target these cysteines. By systematically mutating these cysteines, Saito and colleagues showed that single or double mutation of C273 and C288 to tryptophan or glutamic acid did not impede the KEAP1-mediated repression and degradation of NRF2 hence allowing to precisely identify electrophiles that are sensed by either or both of these cysteines [65]. In the report published by Saito *et al*. the authors showed that 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) is sensed specifically by C288, extending the earlier observations by Levonen *et al*. who recognized the importance of cysteine thiols within KEAP1 for sensing electrophilic lipids [95]. We performed molecular modelling of the amino acid residues comprising the IVR domain using the web-based I-TASSER platform service [97–99], and found that it is comprised of nine α-helices (figure 4). The basic amino acids adjacent to C273 (i.e. R272 and H274) and C288 (i.e. K287) are expected to cause the deprotonation of the cysteine thiol groups, hence increasing their reactivity.

The Kelch domain of KEAP1 is evolutionarily conserved and contains nine cysteine residues at amino acid positions 319, 368, 395, 406, 434, 489, 513, 518 and 583. The first crystal structure of the KEAP1 Kelch domain was solved at a resolution of 1.85Å in 2004 by Li and colleagues [100]. Several crystal structures of both the human and murine KEAP1 Kelch domains with different resolutions and in combination with compounds or short peptide sequences of the Neh2 domain of NRF2 have since been reported [101–107]. The Kelch domain in KEAP1 contains six Kelch repeats that assemble into a six-bladed β-propeller structure (blades I-VI), where the C-terminal residues form the first strand in the first blade (figure 1*b*). Four-stranded antiparallel β-sheets form one blade, where the shortest β-sheet is found at the central core [100,108]. The Kelch domain also contains double glycine repeats (DGR), which are located at the terminal end of the β-sheets.

First discovered in the laboratory of Masayuki Yamamoto in 1999 by Itoh and colleagues, the N-terminal Neh2 domain within NRF2 has been since reported by various groups to bind to the KEAP1 Kelch domain [56]. Using nuclear magnetic resonance spectroscopy, Tong *et al*. discovered that the Neh2 domain was intrinsically disordered [26]. It was subsequently found that the evolutionarily conserved DLG [109] and ETGE [56] motifs within the NRF2-Neh2 domain

Table 1. Cysteine residues of KEAP1, for which chemical modifications by the indicated electrophiles and oxidants, have been implicated.

	MCE-1	MCE-23	TBE-31	RTA-408	CDDO-Im	SF	PEITC	tBHQ	DEM	DMF	MEF	H_2O_2	H_2S	NO	OA-NO_2	SNAP	4-HNE	15d-PGJ_2	PGA_2	8-NO cGMP	As^{3+}	Cd^{2+}	Se^{4+}	Zn^{2+}	MeHg	Dex-Mes	1,2-NQ	MIND4-17	(Z)-LIG	Ox-LIG	DATS	NAPQI	Acrolein	XH	10-Shogaol	IAA	IAB	BMCC	ISO	Ebselen	4-OI	GSSG
C23																																				◆						
C38						◆									◆																						◆						
C77																															◆						◆					◆
C151	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆		◆	◆	◆	◆				◆				◆		◆	◆	◆	◆		◆	◆	◆	◆	◆			◆	◆	◆	
C171																											◆																
C196																																				◆	◆						
C226												◆	◆		◆						◆	◆	◆	◆								◆				◆			◆				◆
C241																																				◆							
C249																																					◆						
C257										◆					◆										◆						◆				◆	◆					◆		
C273										◆					◆		◆		◆		◆				◆					◆	◆					◆					◆		
C288															◆		◆	◆			◆				◆					◆	◆	◆				◆	◆				◆		
C297																			◆						◆																◆	◆	
C319																														◆				◆		◆						◆	
C368						◆																			◆									◆			◆					◆	
C434																				◆											◆										◆	
C489						◆									◆				◆						◆						◆							◆				
C583																															◆											
C613											◆	◆	◆								◆	◆	◆	◆							◆		◆									
C622												◆																													◆	
C624												◆																													◆	

3-ethynyl-3-methyl-6-oxocydohexa-1,4-dienecarbonitrile (MCE-1) [70], 9a-ethynyl-3-oxo-9,9a-dihydro-3H-fluorene-2-carbonitrile (MCE-23) [70], (±)-(4bS,8aR,10aS)-10a-ethynyl-4b,8,8-trimethyl-3,7-dioxo-3,4b,7,8,8a,9,10,10a, octahydrophenanthrene-2,6-dicarbonitrile (TBE-31) [70], omaveloxolone (RTA-408) [71], 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) [65,72], 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO-Im) [65,72], sulforaphane (SF) [63–65,73,74], phenethyl isothiocyanate (PEITC) [75], *tert*-butylhydroquinone (tBHQ) [63–65,73], diethylmaleate (DEM) [65,73], dimethyl fumarate (DMF) [73,76], monoethyl fumarate (MEF) [76], hydrogen peroxide (H_2O_2) [67,77,78], hydrogen sulfide (H_2S) [78], nitric oxide (NO) [64,67], nitro-oleic acid (OA-NO_2) [63,65], (±)-S-nitroso-N-acetylpenicillamine (SNAP) [65], 4-hydroxy-2-nonenal (4-HNE) [65], 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) [65], prostaglandin A_2 (PGA_2) [79], 8-nitroguanosine 3′,5′-cyclic monophosphate (8-NO cGMP) [80], As^{3+} [64,65], Cd^{2+} [64], Zn^{2+} [64,81], methylmercury (MeHg) [82], dexamethasone 21-mesylate (Dex-Mes) [61], 1,2-naphthoquinone (1,2-NQ) [79,83], 5-nitro-2-{[5-(phenoxymethyl)-4-phenyl-4H-1,2,4-triazol-3-yl]thio}pyridine (MIND4-17) [84], (Z)-ligustilide (Z-LIG) [85], oxidised-ligustilide (Ox-LIG) [85], diallyl trisulfide (DATS) [86], N-acetyl-p-benzoquinoneimine (NAPQI) [87], Se^{4+} [64], acrolein [64], xanthohumol (XH) [88], 1-(4-hydroxy-3-methoxyphenyl)-4-tetradecen-3-one (10-shogaol) [88], iodoacetamide (IAA) [87], N-biotinylhexylenediamine (IAB) [89], 1-biotinamido-4-(4′-[maleimidoethyl-cyclohexane]-carboxamido) butane (BMCC) [89], isoliquiritigenin (ISO) [88], 2-phenyl-1,2-benzisoselenazol-3(2H)-one (Ebselen) [79,90], 4-octyl itaconate (4-OI) [45] and glutathione disulfide (GSSG) [91].

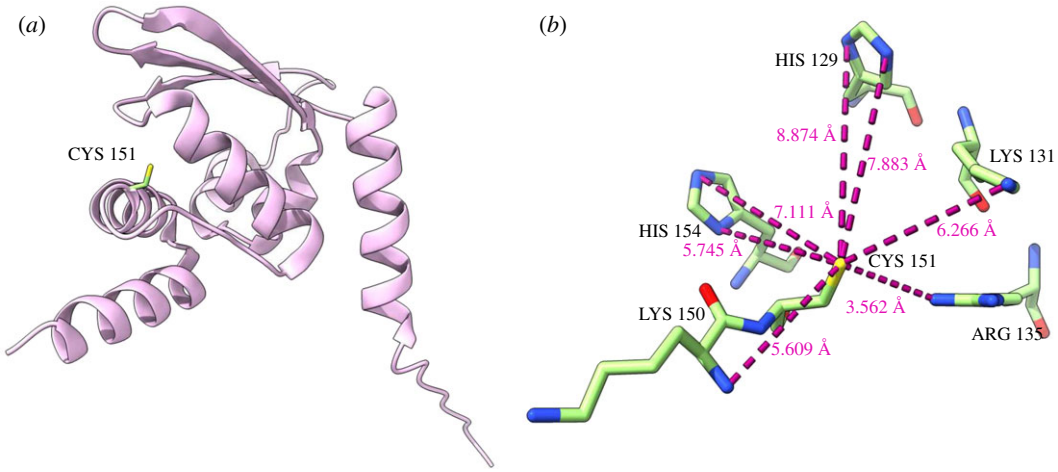

Figure 3. Structure of the human KEAP1 BTB domain. (*a*) Cartoon representation of the human KEAP1 BTB domain (pale pink) showing the side chain of CYS 151 in green. (*b*) The side chains of the basic amino acids (HIS 129, LYS 131, ARG 135, LYS 150 and HIS 154) adjacent to and surrounding the CYS 151 residues are represented with green stick drawings coloured by their elements. Structure drawn using UCSF ChimeraX software using the PDB accession 4CXI. The distances have been calculated in angstroms (Å) between these basic residues and CYS 151.

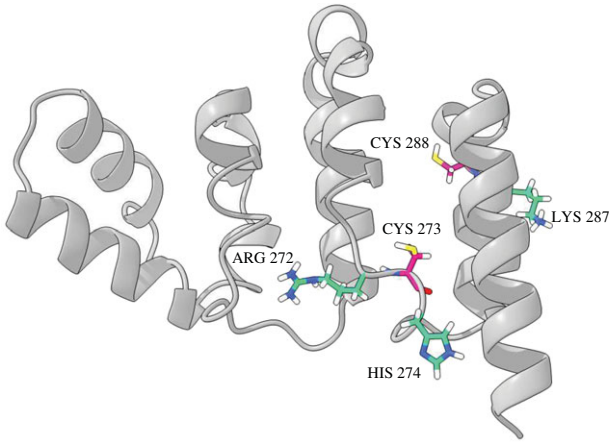

Figure 4. Modelled structure of the human KEAP1 IVR domain. Cartoon representation of the I-TASSER program modelled human KEAP1 IVR domain displaying 9 α-helices (grey). The basic amino acids (ARG 272 and HIS 274, green) found adjacent to the CYS 273 residue (pink) as well as the hydrophobic LYS 287 (green) residue found adjacent to CYS 288 (pink) where their side chains are represented with stick drawings coloured by their respective elements. The structure was drawn with UCSF ChimeraX software.

(figure 1*a*) were responsible for binding to the KEAP1 Kelch domains [26,110]. Compared with the ETGE motif, the DLG motif has a 200-fold lower affinity for the Kelch domain of KEAP1 [102]. The DLG and ETGE motifs flank a lysine-rich α-helix to allow for the conjugation of ubiquitin molecules by the activated ubiquitin conjugating E2 enzyme. Attachment of NRF2 to KEAP1 via both of these motifs is required for ubiquitination of the transcription factor, and a 'fixed-ends' or a 'hinge-and-latch' model for NRF2 ubiquitylation was proposed, where each binding motif of one molecule of NRF2 is tethered to a separate subunit of the KEAP1 homodimer [110,111]. Based on this knowledge, the Kelch domain of KEAP1 has become the target for the development of non-electrophilic NRF2 activators, which function as PPI inhibitors [112]. One example includes a series of 1,4-diphenyl-1,2,3-triazole compounds, which have been shown to disrupt the KEAP1:NRF2 PPIs *in vitro* using a fluorescence polarization assay, as well as in live cells expressing EGFP-NRF2 and KEAP1-mCherry fusion proteins using a Förster resonance energy transfer-based system and multiphoton fluorescence lifetime imaging microscopy [113].

The C-terminal domain of KEAP1 is the home of three of the four cysteine sensors of KEAP1, which are used redundantly to mediate NRF2 activation in response to hydrogen peroxide (H_2O_2), the major ROS in redox regulation of biological processes. Very recently, Suzuki *et al*. [77] generated a construct for mammalian cell expression of a KEAP1 mutant, which lacks 11 out of the 25 cysteine residues of the murine protein. This mutant KEAP1 was still able to target NRF2 for ubiquitination and proteasomal degradation, but was unable to respond to most cysteine-reactive NRF2 activators, including H_2O_2. A series of elegant experiments involving mouse embryonic fibroblast cells expressing various KEAP1 cysteine mutants as well as five distinct KEAP1 mutant mouse lines revealed that KEAP1 uses C226, C613, C622 and C624 redundantly to sense H_2O_2 [77].

Although no crystal structure of full-length KEAP1 is available to date, a 24 Å resolution reconstituted electron microscopy (EM) structure has been described [114]. It shows that the KEAP1 dimer resembles a cherry-bob, where two large spheres, corresponding to the Kelch domains, are attached by short linker arms. Interestingly, each IVR domain surrounds the core of the Kelch domain, suggesting that chemical modifications of cysteines within the IVR domains may affect the KEAP1–NRF2 interactions through the Kelch domains.

3.2. KEAP1-CUL3 interaction

The primary system for protein degradation in the cell is the ubiquitin-proteasome system, where E3 ubiquitin ligases are essential components. Cullins are a family of hydrophobic proteins that confer substrate specificity by acting as scaffolds for E3 ubiquitin ligase complexes [115,116]. Thus far, in mammals, there have been seven Cullins (1, 2, 3, 4A, 4B, 5 and 7) identified. CUL3 is the only member of its family that is able to recognize BTB domains-containing proteins [116]. Since KEAP1 contains BTB domains, in 2004, Kobayashi and colleagues hypothesized that under basal conditions, CUL3 could be mediating the degradation of NRF2 through binding of the substrate adaptor KEAP1 [93]. Indeed, three independent groups simultaneously

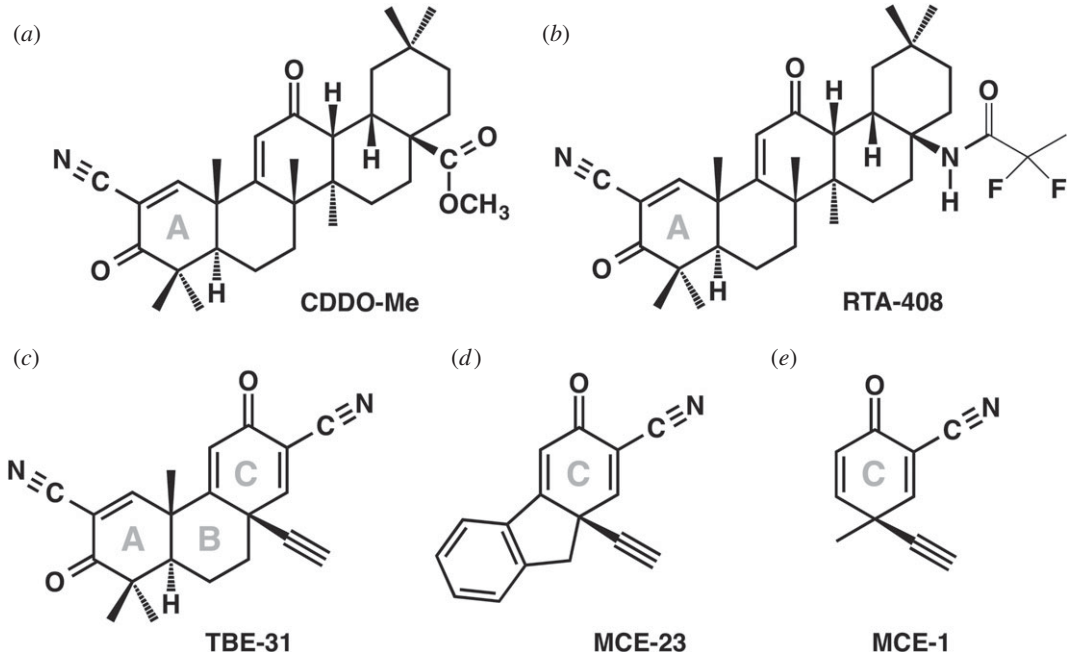

Figure 5. Chemical structures of selected cyclic cyanoenone NRF2 activators.

discovered that KEAP1 forms a functional E3 ubiquitin ligase complex with CUL3/RBX1 [93,117,118]. Shortly after, Furukawa & Xiong reported that KEAP1 and CUL3 binding occurs between the BTB-domain of the former and the N-terminal domain of the latter [119]. Subsequently, it was discovered that CUL3 homodimerization requires the presence of its N-terminal domain and is dependent on its interaction with BTB domain-containing substrates (e.g. KEAP1) which also are able to homodimerize at their BTB domains [120].

4. The cyclic cyanoenones, the most potent class of NRF2 activators

To date, the cyanoenone triterpenoids are the most potent NRF2 activators known. They were designed and developed starting from the natural product oleanolic acid [121–123], and new generations of analogues have been synthesized [124]. These semi-synthetic compounds are highly electrophilic, bind covalently and reversibly to sulfhydryl groups [125], and have favourable pharmacokinetic and pharmacodynamic profiles *in vivo*, including in humans [126–128]. Currently, two cyanoenone triterpenoids are in clinical trials led by Reata Pharmaceuticals (USA) and Kyowa Hakko Kirin (Japan) for the treatment of diseases, which have been linked with chronic inflammation and abnormal redox homeostasis. One is CDDO-Me (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid methyl ester; bardoxolone methyl, figure 5*a*) for the treatment of connective tissue disease–pulmonary arterial hypertension, pulmonary hypertension, Alport's syndrome, polycystic kidney disease, renal insufficiency and liver disease. The other is RTA-408 (omaveloxolone, figure 5*b*) for the treatment of Friedreich's ataxia, mitochondrial myopathy, ocular inflammation, ocular pain, corneal endothelial cell loss, cataract surgery, melanoma and radiation dermatitis in breast cancer patients [23]. Recently, Reata Pharmaceuticals reported the evaluation of the pharmacokinetics and tissue distribution of orally administered RTA-408 to cynomolgus monkeys after single and multiple oral doses, and the initial results from a clinical trial in Friedreich's ataxia patients [126]. Dose-dependent plasma levels of RTA-408 and induction of NRF2 target genes were detected in peripheral blood mononuclear cells, liver, lung, and brain of the animals. In patients, improvements in neurological functions were observed at doses of 80 mg or greater; these doses resulted in plasma drug concentrations consistent with those inducing NRF2 target genes in animals.

To improve their potencies as anti-inflammatory agents and understand the details of their mechanism of action, numerous pentacyclic, tricyclic and monocyclic compounds containing cyanoenone functionalities were designed, synthesized and tested for their anti-inflammatory and NRF2-inducing activities in a programme of work led by Michael Sporn, Gordon Gribble, Tadashi Honda and Karen Liby at Dartmouth College [121,129–131]. In collaboration with the laboratory of Paul Talalay, these researchers found a linear correlation ranging over six orders of magnitude of concentrations between the potencies of 18 pentacyclic derivatives to inhibit inducible nitric oxide synthase (iNOS) and to activate the prototypic NRF2 target enzyme NQO1 [132]. Subsequently, this correlation was confirmed more broadly, for all main classes of NRF2 activators [133]. The high potency of the triterpenoid analogues in inducing NRF2 and inhibiting inflammation requires the presence of activated Michael reaction (enone) functions at critical positions in rings A and/or C. Among the cyclic cyanoenone derivatives, the acetylenic tricyclic bis(cyanoenone) TBE-31 is an exceptionally potent inducer (figure 5*c*). TBE-31 is active at sub- to low-nanomolar concentrations, with Concentration that Doubles the specific enzyme activity of NQO1 in murine Hepa1c1c7 cells (CD value) of 0.9 nM [134–137]. This compound is highly bioavailable and suitable for chronic oral administration [138,139]. The presence of two cyanoenone functionalities (in rings A and C) integrated in a three-ring structure confers particularly high inducer potency [138]. Another tricyclic cyanoenone, MCE-23 contains an identical ring C present in TBE-31, however, it does not have the cyanoenone moiety in its ring A (figure 5*d*), and is comparatively less potent, with a CD value of 41 nM [140]. MCE-1, a monocyclic cyanoenone (figure 5*e*), contains the

ring C of MCE-23 and TBE-31. Similar to MCE-23 and TBE-31, MCE-1 also induces NQO1 (CD = 22 nM) [131,138]. All of these cyanoenones exhibit anti-inflammatory activity in RAW 264.7 cells as well as primary macrophage (PMΦ) cells derived from mice [131,138]. Furthermore, in a murine inflammation-mediated depression model, MCE-1 and TBE-31 exhibit anti-depressant effects [141].

CDDO-Me, RTA-408, MCE-1, MCE-23 and TBE-31 possess electrophilic Michael acceptor(s) within their chemical structures, and are therefore extremely reactive with sulfhydryl groups. Early studies employing ultraviolet–visible (UV-VIS) spectroscopy had shown that compounds of this class react with cysteines in KEAP1, but the identity of the specific cysteine sensor(s) within the protein was not known [132,136]. In collaboration with Takafumi Suzuki and Masayuki Yamamoto (Tohoku University), we generated KEAP1-knockout mouse embryonic fibroblast (MEF) cells that were reinstated with wild-type or various cysteine mutants of KEAP1, and monitored the stabilization of NRF2 upon exposure to cyanoenones [70]. In addition, we isolated PMΦ cells from wild-type KEAP1 ($KEAP^{+/+}$) or the knock-in mutant KEAP-C151S ($KEAP^{C151S/C151S}$) mice, which were generated by use of the CRISPR/Cas9 technology. This study revealed that C151 is the primary sensor for the cyanoenone class of NRF2 inducers, irrespective of their molecular shape or size. Furthermore, C151S mutation in KEAP1 (i.e. in the $KEAP1^{C151S/C151S}$ PMΦ cells) not only abolished the inducer activity of low concentrations of TBE-31, but it also diminished its anti-inflammatory activity. This effect was confirmed using transgenic mice expressing human interleukin 6 (IL-6)-luciferase reporter that were either KEAP1 wild-type or $KEAP^{C151S/C151S}$ mutant. Taken together, these experiments highlight the anti-inflammatory effect of NRF2 activation.

It is noteworthy that, although C151 is the primary sensor for the cyanoenone class of NRF2 inducers, the concentration of the inducer is critical for on-target selectivity. Thus, at low cyanoenone concentrations, C151 is essential for NRF2 stabilization, however, at higher cyanoenone concentrations, NRF2 stabilization proceeds in the absence of C151 [70]. These findings underscore the importance of the inherent flexibility of the sensor cysteines in KEAP1, explain the apparent discrepancies between the results from some of the published studies, and highlight the immense importance of determining the accurate dose of even the most selective electrophilic NRF2 inducer for achieving on-target selectivity.

Because RTA-408 can cross the blood–brain barrier and is currently in clinical development [23], its disease-modifying efficacy was tested in a rat model of status epilepticus, a disease, where cytotoxicity and inflammation constitute major pathogenic drivers. In a study led by Matthew Walker, Andrey Abramov and their colleagues at University College London, it was found that 3 daily doses of RTA-408 given in the first week of established disease potently inhibited epileptogenesis during the subsequent 12 weeks, and preserved both neurons and astrocytes in the hippocampus of the animals [71]. This remarkable effect indicates that breaking the vicious circle of redox imbalance causing macromolecular damage and cell death triggering inflammation leading to neuronal death and seizures, which in turn cause more neuronal death and greater inflammation, and more seizures, can be highly effective in managing this disease. Most importantly, the unprecedented high efficacy of RTA-408 in this model, and the fact that it is currently in clinical trials, suggests the potential for this drug as a disease-modifying treatment in epilepsy and perhaps other neurological conditions.

5. Concluding remarks

The extraordinary ability of KEAP1 to sense a multitude of inducers that vary in shape, size and reactivity, and are able to 'read' an intricately complex 'cysteine code', coupled with its intrinsic flexibility, guarantees a finely tuned, and tightly regulated antioxidant response. More than two decades of research conducted by numerous independent groups of investigators has convincingly demonstrated that the ability to mount this cytoprotective response is critical for adaptation and survival, and can be exploited to protect against or delay the onset of pathological processes, particularly those that involve oxidative stress and inflammation. Indeed, KEAP1 is the target of several small-molecule NRF2 activators, which are currently in clinical trials and hold promise for the prevention and treatment of chronic disease.

Data accessibility. This article has no additional data.

Competing interests. A.T.D-K. is a member of the Scientific Advisory Board of Evgen Pharma and a consultant for Aclipse Therapeutics and Vividion Therapeutics.

Funding. We thank Cancer Research UK (C20953/A18644) and Reata Pharmaceuticals for financial support.

Acknowledgements. We are immensely grateful to Michael Sporn, Gordon Gribble, Tadashi Honda and Karen Liby (Dartmouth College, USA) for introducing us to the cyanoenone class of NRF2 activators and most enjoyable collaborative interactions.

References

1. Sies H, Jones DP. In press. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* (doi:10.1038/s41580-020-0230-3)
2. Sies H. 2015 Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* **4**, 180–183. (doi:10.1016/j.redox.2015.01.002)
3. Andersen JK. 2004 Oxidative stress in neurodegeneration: cause or consequence? *Nat. Med.* **10**, S18-S25. (doi:10.1038/nrn1434)
4. Fukui H, Moraes CT. 2008 The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? *Trends Neurosci.* **31**, 251–256. (doi:10.1016/j.tins.2008.02.008)
5. Mahalingaiah PK, Ponnusamy L, Singh KP. 2015 Chronic oxidative stress causes estrogen-independent aggressive phenotype, and epigenetic inactivation of estrogen receptor alpha in MCF-7 breast cancer cells. *Breast Cancer Res. Treat.* **153**, 41–56. (doi:10.1007/s10549-015-3514-0)
6. Mehlman MA. 1992 Dangerous and cancer-causing properties of products and chemicals in the oil refining and petrochemical industry. VIII. Health effects of motor fuels: carcinogenicity of gasoline–scientific update. *Environ. Res.* **59**, 238–249. (doi:10.1016/S0013-9351(05)80243-9)
7. Ceriello A. 2006 Oxidative stress and diabetes-associated complications. *Endocr*

Pract. **12**(Suppl. 1), 60–62. (doi:10.4158/EP.12.S1.60)

8. Bravi MC, Armiento A, Laurenti O, Cassone-Faldetta M, De Luca O, Moretti A, De Mattia G. 2006 Insulin decreases intracellular oxidative stress in patients with type 2 diabetes mellitus. *Metabolism* **55**, 691–695. (doi:10.1016/j.metabol.2006.01.003)
9. Ramani S, Pathak A, Dalal V, Paul A, Biswas S. In press. Oxidative stress in autoimmune diseases: an under dealt malice. *Curr. Protein Pept Sci.* (doi:10.2174/1389203721666200214111816)
10. Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K. 2015 Oxidative stress in aging human skin. *Biomolecules* **5**, 545–589. (doi:10.3390/biom5020545)
11. Kruk J, Duchnik E. 2014 Oxidative stress and skin diseases: possible role of physical activity. *Asian Pac. J. Cancer Prev.* **15**, 561–568. (doi:10.7314/apjcp.2014.15.2.561)
12. Okayama Y. 2005 Oxidative stress in allergic and inflammatory skin diseases. *Curr. Drug Targets Inflamm. Allergy* **4**, 517–519. (doi:10.2174/1568010054526386)
13. Hector A, Griese M, Hartl D. 2014 Oxidative stress in cystic fibrosis lung disease: an early event, but worth targeting? *Eur. Respir. J.* **44**, 17–19. (doi:10.1183/09031936.00038114)
14. Bast A, Weseler AR, Haenen GR, den Hartog GJ. 2010 Oxidative stress and antioxidants in interstitial lung disease. *Curr. Opin Pulm. Med.* **16**, 516–520. (doi:10.1097/MCP.0b013e32833c645d)
15. Costa S, Reina-Couto M, Albino-Teixeira A, Sousa T. 2016 Statins and oxidative stress in chronic heart failure. *Rev. Port. Cardiol.* **35**, 41–57. (doi:10.1016/j.repc.2015.09.006)
16. Ivanov AV, Bartosch B, Isaguliants MG. 2017 Oxidative stress in infection and consequent disease. *Oxid. Med. Cell Longev.* **2017**, 3496043. (doi:10.1155/2017/3496043)
17. Muntane J, Puig-Parellada P, Mitjavila MT. 1995 Iron metabolism and oxidative stress during acute and chronic phases of experimental inflammation: effect of iron-dextran and deferoxamine. *J. Lab. Clin. Med.* **126**, 435–443.
18. Liguori I *et al.* 2018 Oxidative stress, aging, and diseases. *Clin. Interv. Aging.* **13**, 757–772. (doi:10.2147/CIA.S158513)
19. Fukagawa NK. 1999 Aging: is oxidative stress a marker or is it causal? *Proc. Soc. Exp. Biol. Med.* **222**, 293–298. (doi:10.1046/j.1525-1373.1999.d01-146.x)
20. Joseph JA, Roth GS. 1992 Cholinergic systems in aging: the role of oxidative stress. *Clin. Neuropharmacol.* **15(**Suppl 1 Pt A), 508A-509A. (doi:10.1097/00002826-199201001-00264)
21. Hayes JD, Dinkova-Kostova AT. 2014 The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem. Sci.* **39**, 199–218. (doi:10.1016/j.tibs.2014.02.002)
22. Dinkova-Kostova AT, Kostov RV, Canning P. 2017 Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. *Arch. Biochem. Biophys.* **617**, 84–93. (doi:10.1016/j.abb.2016.08.005)
23. Cuadrado A *et al.* 2019 Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat. Rev. Drug Discov.* **18**, 295–317. (doi:10.1038/s41573-018-0008-x)
24. Baird L, Lleres 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. USA* **110**, 15 259–15 264. (doi:10.1073/pnas.1305687110)
25. Baird L, Dinkova-Kostova AT. 2013 Diffusion dynamics of the Keap1-Cullin3 interaction in single live cells. *Biochem. Biophys. Res. Commun.* **433**, 58–65. (doi:10.1016/j.bbrc.2013.02.065)
26. 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. (doi:10.1128/MCB.26.8.2887-2900.2006)
27. Dodson M, de la Vega MR, Cholanians AB, Schmidlin CJ, Chapman E, Zhang DD. 2019 Modulating NRF2 in disease: timing is everything. *Annu. Rev. Pharmacol. Toxicol.* **59**, 555–575. (doi:10.1146/annurev-pharmtox-010818-021856)
28. Tonelli C, Chio II. C., Tuveson DA. 2018 Transcriptional regulation by Nrf2. *Antioxid Redox Signal.* **29**, 1727–1745. (doi:10.1089/ars.2017.7342)
29. Lu SC. 2009 Regulation of glutathione synthesis. *Mol. Aspects Med.* **30**, 42–59. (doi:10.1016/j.mam.2008.05.005)
30. Teskey G *et al.* 2018 Glutathione as a marker for human disease. *Adv. Clin. Chem.* **87**, 141–159. (doi:10.1016/bs.acc.2018.07.004)
31. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. 2004 Glutathione metabolism and its implications for health. *J. Nutr.* **134**, 489–492. (doi:10.1093/jn/134.3.489)
32. Wild AC, Mulcahy RT. 2000 Regulation of gamma-glutamylcysteine synthetase subunit gene expression: insights into transcriptional control of antioxidant defenses. *Free Radic. Res.* **32**, 281–301. (doi:10.1080/10715760000300291)
33. Sasaki H *et al.* 2002 Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. *J. Biol. Chem.* **277**, 44 765–44 771. (doi:10.1074/jbc.M208704200)
34. 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. (doi:10.1016/j.ccr.2012.05.016)
35. 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**, 10 228–10 239. (doi:10.1093/nar/gks827)
36. Singh A *et al.* 2013 Transcription factor NRF2 regulates miR-1 and miR-206 to drive tumorigenesis. *J. Clin. Invest.* **123**, 2921–2934. (doi:10.1172/JCI66353)
37. Vargas MR, Johnson DA, Sirkis DW, Messing A, Johnson JA. 2008 Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *J. Neurosci.* **28**, 13 574–13 581. (doi:10.1523/JNEUROSCI.4099-08.2008)
38. Cuadrado A, Moreno-Murciano P, Pedraza-Chaverri J. 2009 The transcription factor Nrf2 as a new therapeutic target in Parkinson's disease. *Expert Opin. Ther. Targets.* **13**, 319–329. (doi:10.1517/13543780802716501)
39. Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, McKenna MC. 2014 Glutamate metabolism in the brain focusing on astrocytes. *Adv. Neurobiol.* **11**, 13–30. (doi:10.1007/978-3-319-08894-5_2)
40. Bell KF *et al.* 2015 Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. *Nat. Commun.* **6**, 7066. (doi:10.1038/ncomms8066)
41. Chen PC, Vargas MR, Pani AK, Smeyne RJ, Johnson DA, Kan YW, Johnson JA. 2009 Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: critical role for the astrocyte. *Proc. Natl Acad. Sci. USA* **106**, 2933–2938. (doi:10.1073/pnas.0813361106)
42. Sayin VI *et al.* 2017 Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. *Elife* **6**, e28083. (doi:10.7554/eLife.28083)
43. Liu H, Zimmerman AW, Singh K, Connors SL, Diggins E, Stephenson KK, Dinkova-Kostova AT, Fahey JW. 2020 Biomarker exploration in human peripheral blood mononuclear cells for monitoring sulforaphane treatment responses in autism spectrum disorder. *Sci. Rep.* **10**, 5822. (doi:10.1038/s41598-020-62714-4)
44. Bambouskova M *et al.* 2018 Electrophilic properties of itaconate and derivatives regulate the IkappaBzeta-ATF3 inflammatory axis. *Nature* **556**, 501–504. (doi:10.1038/s41586-018-0052-z)
45. Mills EL *et al.* 2018 Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* **556**, 113–117. (doi:10.1038/nature25986)
46. Kobayashi EH *et al.* 2016 Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nat. Commun.* **7**, 11624. (doi:10.1038/ncomms11624)
47. Kong X, Thimmulappa R, Craciun F, Harvey C, Singh A, Kombairaju P, Reddy SP, Remick D, Biswal S. 2011 Enhancing Nrf2 pathway by disruption of Keap1 in myeloid leukocytes protects against sepsis. *Am. J. Respir. Crit. Care Med.* **184**, 928–938. (doi:10.1164/rccm.201102-0271OC)
48. Olagnier D *et al.* 2017 Activation of Nrf2 signaling augments vesicular stomatitis virus oncolysis via autophagy-driven suppression of antiviral immunity. *Mol. Ther.* **25**, 1900–1916. (doi:10.1016/j.ymthe.2017.04.022)
49. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, Biswal S. 2006 Nrf2 is a

critical regulator of the innate immune response and survival during experimental sepsis. *J. Clin. Invest.* **116**, 984–995. (doi:10.1172/JCI25790)

50. Baardman J *et al*. 2018 A defective pentose phosphate pathway reduces inflammatory macrophage responses during hypercholesterolemia. *Cell Rep.* **25**, 2044–2052. (doi:10.1016/j.celrep.2018.10.092)
51. Cuadrado A *et al*. 2018 Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach. *Pharmacol. Rev.* **70**, 348–383. (doi:10.1124/pr.117.014753)
52. Sporn MB, Liby KT. 2012 NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer.* **12**, 564–571. (doi:10.1038/nrc3278)
53. Robledinos-Anton N, Fernandez-Gines R, Manda G, Cuadrado A. 2019 Activators and inhibitors of NRF2: a review of their potential for clinical development. *Oxid. Med. Cell. Longev.* **2019**, 9372182. (doi:10.1155/2019/9372182)
54. Prochaska HJ, De Long MJ, Talalay P. 1985 On the mechanisms of induction of cancer-protective enzymes: a unifying proposal. *Proc. Natl Acad. Sci. USA* **82**, 8232–8236. (doi:10.1073/pnas.82.23.8232)
55. 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. USA* **85**, 8261–8265. (doi:10.1073/pnas.85.21.8261)
56. 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. (doi:10.1101/gad.13.1.76)
57. Marino SM, Gladyshev VN. 2012 Analysis and functional prediction of reactive cysteine residues. *J. Biol. Chem.* **287**, 4419–4425. (doi:10.1074/jbc.R111.275578)
58. Roos G, Foloppe N, Messens J. 2013 Understanding the pK(a) of redox cysteines: the key role of hydrogen bonding. *Antioxid Redox Signal.* **18**, 94–127. (doi:10.1089/ars.2012.4521)
59. Gould NS, Evans P, Martinez-Acedo P, Marino SM, Gladyshev VN, Carroll KS, Ischiropoulos H. 2015 Site-specific proteomic mapping identifies selectively modified regulatory cysteine residues in functionally distinct protein networks. *Chem. Biol.* **22**, 965–975. (doi:10.1016/j.chembiol.2015.06.010)
60. Snyder GH, Cennerazzo MJ, Karalis AJ, Field D. 1981 Electrostatic influence of local cysteine environments on disulfide exchange kinetics. *Biochemistry* **20**, 6509–6519. (doi:10.1021/bi00526a001)
61. 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. USA* **99**, 11 908–11 913. (doi:10.1073/pnas.172398899)
62. Zipper LM, Mulcahy RT. 2002 The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. *J. Biol. Chem.* **277**, 36 544–36 552. (doi:10.1074/jbc.M206530200)
63. 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. (doi:10.1128/mcb.23.22.8137-8151.2003)
64. 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. USA.* **107**, 18 838–18 843. (doi:10.1073/pnas.1007387107)
65. Saito R *et al*. 2015 Characterizations of three major cysteine sensors of keap1 in stress response. *Mol. Cell Biol.* **36**, 271–284. (doi:10.1128/MCB.00868-15)
66. Zhang DD, Lo SC, Sun Z, Habib GM, Lieberman MW, Hannink M. 2005 Ubiquitination of Keap1, a BTB-Kelch substrate adaptor protein for Cul3, targets Keap1 for degradation by a proteasome-independent pathway. *J. Biol. Chem.* **280**, 30 091–30 099. (doi:10.1074/jbc.M501279200)
67. 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. (doi:10.1074/jbc.M109.051714)
68. Bollong MJ *et al*. 2018 A metabolite-derived protein modification integrates glycolysis with KEAP1-NRF2 signalling. *Nature* **562**, 600–604. (doi:10.1038/s41586-018-0622-0)
69. Xue M *et al*. 2012 Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* **443**, 213–222. (doi:10.1042/BJ20111648)
70. Dayalan Naidu S *et al*. 2018 C151 in KEAP1 is the main cysteine sensor for the cyanoenone class of NRF2 activators, irrespective of molecular size or shape. *Sci. Rep.* **8**, 8037. (doi:10.1038/s41598-018-26269-9)
71. Shekh-Ahmad T, Eckel R, Dayalan Naidu S, Higgins M, Yamamoto M, Dinkova-Kostova AT, Kovac S, Abramov AY, Walker MC. 2018 KEAP1 inhibition is neuroprotective and suppresses the development of epilepsy. *Brain* **141**, 1390–1403. (doi:10.1093/brain/awy071)
72. Cleasby A *et al*. 2014 Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. *PLoS ONE* **9**, e98896. (doi:10.1371/journal.pone.0098896)
73. 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. (doi:10.1016/j.freeradbiomed.2012.06.023)
74. Hu C, Eggler AL, Mesecar AD, van Breemen RB. 2011 Modification of keap1 cysteine residues by sulforaphane. *Chem. Res. Toxicol.* **24**, 515–521. (doi:10.1021/tx100389r)
75. Dayalan Naidu S, Suzuki T, Yamamoto M, Fahey JW, Dinkova-Kostova AT. 2018 Phenethyl isothiocyanate, a dual activator of transcription factors NRF2 and HSF1. *Mol. Nutr. Food Res.* **62**, e1700908. (doi:10.1002/mnfr.201700908)
76. Brennan MS, Matos MF, Li B, Hronowski X, Gao B, Juhasz P, Rhodes KJ, Scannevin RH. 2015 Dimethyl fumarate and monoethyl fumarate exhibit differential effects on KEAP1, NRF2 activation, and glutathione depletion in vitro. *PLoS ONE* **10**, e0120254. (doi:10.1371/journal.pone.0120254)
77. Suzuki T *et al*. 2019 Molecular mechanism of cellular oxidative stress sensing by Keap1. *Cell Rep.* **28**, 746–758. (doi:10.1016/j.celrep.2019.06.047)
78. Hourihan JM, Kenna JG, Hayes JD. 2013 The gasotransmitter hydrogen sulfide induces Nrf2-target genes by inactivating the Keap1 ubiquitin ligase substrate adaptor through formation of a disulfide bond between Cys-226 and Cys-613. *Antioxid Redox Signal.* **19**, 465–481. (doi:10.1089/ars.2012.4944)
79. Kobayashi M *et al*. 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. (doi:10.1128/MCB.01080-08)
80. Fujii S *et al*. 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**, 23 970–23 984. (doi:10.1074/jbc.M110.145441)
81. 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. (doi:10.1016/j.taap.2018.09.033)
82. Kumagai Y, Kanda H, Shinkai Y, Toyama T. 2013 The role of the Keap1/Nrf2 pathway in the cellular response to methylmercury. *Oxid. Med. Cell Longev.* **2013**, 848279. (doi:10.1155/2013/848279)
83. Shinkai Y *et al*. 2015 Reactive sulfur species-mediated activation of the Keap1-Nrf2 pathway by 1,2-Naphthoquinone through sulfenic acids formation under oxidative stress. *Chem. Res. Toxicol.* **28**, 838–847. (doi:10.1021/tx500416y)
84. Quinti L *et al*. 2017 KEAP1-modifying small molecule reveals muted NRF2 signaling responses in neural stem cells from Huntington's disease patients. *Proc. Natl Acad. Sci. USA* **114**, E4676-E4685. (doi:10.1073/pnas.1614943114)
85. Dietz BM *et al*. 2008 Angelica sinensis and its alkylphthalides induce the detoxification enzyme NAD(P)H: quinone oxidoreductase 1 by alkylating Keap1. *Chem. Res. Toxicol.* **21**, 1939–1948. (doi:10.1021/tx8001274)
86. Kim S, Lee HG, Park SA, Kundu JK, Keum YS, Cha YN, Na HK, Surh YJ. 2014 Keap1 cysteine 288 as a potential target for diallyl trisulfide-induced Nrf2 activation. *PLoS ONE* **9**, e85984. (doi:10.1371/journal.pone.0085984)
87. 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. (doi:10.1002/hep.22472)
88. 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. (doi:10.1016/j.jasms.2007.09.015)

89. Hong F, Sekhar KR, Freeman ML, Liebler DC. 2005 Specific patterns of electrophile adduction trigger Keap1 ubiquitination and Nrf2 activation. *J. Biol. Chem.* **280**, 31 768–31 775. (doi:10.1074/jbc.M503346200)
90. Sakurai T, Kanayama M, Shibata T, Itoh K, Kobayashi A, Yamamoto M, Uchida K. 2006 Ebselen, a seleno-organic antioxidant, as an electrophile. *Chem. Res. Toxicol.* **19**, 1196–1204. (doi:10.1021/tx0601105)
91. Holland R, Hawkins AE, Eggler AL, Mesecar AD, Fabris D, Fishbein JC. 2008 Prospective type 1 and type 2 disulfides of Keap1 protein. *Chem. Res. Toxicol.* **21**, 2051–2060. (doi:10.1021/tx800226m)
92. Bryan HK, Olayanju A, Goldring CE, Park BK. 2013 The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. *Biochem. Pharmacol.* **85**, 705–717. (doi:10.1016/j.bcp.2012.11.016)
93. 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. (doi:10.1128/MCB.24.16.7130-7139.2004)
94. Wu JH, Miao W, Hu LG, Batist G. 2010 Identification and characterization of novel Nrf2 inducers designed to target the intervening region of Keap1. *Chem. Biol. Drug Des.* **75**, 475–480. (doi:10.1111/j.1747-0285.2010.00955.x)
95. 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. (doi:10.1042/BJ20031049)
96. 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. USA* **101**, 2040–2045. (doi:10.1073/pnas.0307301101)
97. Zhang Y. 2009 I-TASSER: fully automated protein structure prediction in CASP8. *Proteins* **77**(Suppl. 9), 100–113. (doi:10.1002/prot.22588)
98. Roy A, Yang J, Zhang Y. 2012 COFACTOR: an accurate comparative algorithm for structure-based protein function annotation. *Nucleic Acids Res.* **40**, W471–W477. (doi:10.1093/nar/gks372)
99. Yang J, Zhang Y. 2015 I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res.* **43**, W174–W181. (doi:10.1093/nar/gkv342)
100. Li X, Zhang D, Hannink M, Beamer LJ. 2004 Crystal structure of the Kelch domain of human Keap1. *J. Biol. Chem.* **279**, 54 750–54 758. (doi:10.1074/jbc.M410073200)
101. 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. (doi:10.1038/sj.emboj.7601243)
102. 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. (doi:10.1128/MCB.00753-07)
103. Komatsu M *et al.* 2010 The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat. Cell Biol.* **12**, 213–223. (doi:10.1038/ncb2021)
104. 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. (doi:10.1128/MCB.01191-13)
105. Winkel AF *et al.* 2015 Characterization of RA839, a noncovalent small molecule binder to Keap1 and selective activator of Nrf2 signaling. *J. Biol. Chem.* **290**, 28 446–28 455. (doi:10.1074/jbc.M115.678136)
106. Davies TG *et al.* 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. (doi:10.1021/acs.jmedchem.6b00228)
107. Saito T *et al.* 2016 p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. *Nat. Commun.* **7**, 12030. (doi:10.1038/ncomms12030)
108. Canning P, Sorrell FJ, Bullock AN. 2015 Structural basis of Keap1 interactions with Nrf2. *Free Radic. Biol. Med.* **88**, 101–107. (doi:10.1016/j.freeradbiomed.2015.05.034)
109. 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**, 31 556–31 567. (doi:10.1074/jbc.M403061200)
110. 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**, 24 756–24 768. (doi:10.1074/jbc.M601119200)
111. 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. (doi:10.1515/BC.2006.164)
112. Wells G. 2015 Peptide and small molecule inhibitors of the Keap1-Nrf2 protein-protein interaction. *Biochem. Soc. Trans.* **43**, 674–679. (doi:10.1042/BST20150051)
113. 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. (doi:10.1021/acs.jmedchem.5b00602)
114. 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. USA* **107**, 2842–2847. (doi:10.1073/pnas.0914036107)
115. Sarikas A, Hartmann T, Pan ZQ. 2011 The cullin protein family. *Genome Biol.* **12**, 220. (doi:10.1186/gb-2011-12-4-220)
116. Anderica-Romero AC, Gonzalez-Herrera IG, Santamaria A, Pedraza-Chaverri J. 2013 Cullin 3 as a novel target in diverse pathologies. *Redox Biol.* **1**, 366–372. (doi:10.1016/j.redox.2013.07.003)
117. 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. (doi:10.1128/MCB.24.19.8477-8486.2004)
118. Zhang HF, Tomida A, Koshimizu R, Ogiso Y, Lei S, Tsuruo T. 2004 Cullin 3 promotes proteasomal degradation of the topoisomerase I-DNA covalent complex. *Cancer Res.* **64**, 1114–1121. (doi:10.1158/0008-5472.can-03-2858)
119. 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. (doi:10.1128/MCB.25.1.162-171.2005)
120. Choo YY, Hagen T. 2012 Mechanism of cullin3 E3 ubiquitin ligase dimerization. *PLoS ONE* **7**, e41350. (doi:10.1371/journal.pone.0041350)
121. Sporn MB, Liby KT, Yore MM, Fu L, Lopchuk JM, Gribble GW. 2011 New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress. *J. Nat. Prod.* **74**, 537–545. (doi:10.1021/np100826q)
122. Honda T, Rounds BV, Bore L, Finlay HJ, Favaloro Jr FG, Suh N, Wang Y, Sporn MB, Gribble GW. 2000 Synthetic oleanane and ursane triterpenoids with modified rings A and C: a series of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **43**, 4233–4246. (doi:10.1021/jm0002230)
123. Honda T, Honda Y, Favaloro Jr FG, Gribble GW, Suh N, Place AE, Rendi MH, Sporn MB. 2002 A novel dicyanotriterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile, active at picomolar concentrations for inhibition of nitric oxide production. *Bioorg. Med. Chem. Lett.* **12**, 1027–1030. (doi:10.1016/s0960-894x(02)00105-1)
124. Fu L, Lin QX, Onyango EO, Liby KT, Sporn MB, Gribble GW. 2017 Design, synthesis, and biological activity of second-generation synthetic oleanane triterpenoids. *Org. Biomol. Chem.* **15**, 6001–6005. (doi:10.1039/c7ob01420a)
125. Couch RD, Browning RG, Honda T, Gribble GW, Wright DL, Sporn MB, Anderson AC. 2005 Studies

on the reactivity of CDDO, a promising new chemopreventive and chemotherapeutic agent: implications for a molecular mechanism of action. *Bioorg. Med. Chem. Lett.* **15**, 2215–2219. (doi:10.1016/j.bmcl.2005.03.031)

126. Reisman SA, Gahir SS, Lee CI, Proksch JW, Sakamoto M, Ward KW. 2019 Pharmacokinetics and pharmacodynamics of the novel Nrf2 activator omaveloxolone in primates. *Drug Des. Dev. Ther.* **13**, 1259–1270. (doi:10.2147/DDDT.S193889)
127. Lynch DR *et al.* 2019 Safety, pharmacodynamics, and potential benefit of omaveloxolone in Friedreich ataxia. *Ann. Clin. Transl. Neurol.* **6**, 15–26. (doi:10.1002/acn3.660)
128. Creelan BC *et al.* 2017 Safety, pharmacokinetics, and pharmacodynamics of oral omaveloxolone (RTA 408), a synthetic triterpenoid, in a first-in-human trial of patients with advanced solid tumors. *Onco Targets Ther.* **10**, 4239–4250. (doi:10.2147/OTT.S136992)
129. Favaloro Jr FG, Honda T, Honda Y, Gribble GW, Suh N, Risingsong R, Sporn MB. 2002 Design and synthesis of tricyclic compounds with enone functionalities in rings A and C: a novel class of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **45**, 4801–4805. (doi:10.1021/jm025565f)
130. Honda T *et al.* 2011 Tricyclic compounds containing nonenolizable cyano enones. A novel class of highly potent anti-inflammatory and cytoprotective agents. *J. Med. Chem.* **54**, 1762–1778. (doi:10.1021/jm101445p)
131. Zheng S *et al.* 2012 Synthesis, chemical reactivity as Michael acceptors, and biological potency of monocyclic cyanoenones, novel and highly potent anti-inflammatory and cytoprotective agents. *J. Med. Chem.* **55**, 4837–4846. (doi:10.1021/jm3003922)
132. Dinkova-Kostova AT *et al.* 2005 Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc. Natl Acad. Sci. USA* **102**, 4584–4589. (doi:10.1073/pnas.0500815102)
133. Liu H, Dinkova-Kostova AT, Talalay P. 2008 Coordinate regulation of enzyme markers for inflammation and for protection against oxidants and electrophiles. *Proc. Natl Acad. Sci. USA* **105**, 15 926–15 931. (doi:10.1073/pnas.0808346105)
134. Yates MS *et al.* 2007 Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. *Mol. Cancer Ther.* **6**, 154–162. (doi:10.1158/1535-7163.MCT-06-0516)
135. Honda T, Dinkova-Kostova AT, David E, Padegimas EM, Sundararajan C, Visnick M, Bumeister R, Christian Wigley W. 2011 Synthesis and biological evaluation of 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]-4-ethynylimidazole. A novel and highly potent anti-inflammatory and cytoprotective agent. *Bioorg. Med. Chem. Lett.* **21**, 2188–2191. (doi:10.1016/j.bmcl.2011.03.018)
136. Liby K *et al.* 2008 A novel acetylenic tricyclic bis-(cyano enone) potently induces phase 2 cytoprotective pathways and blocks liver carcinogenesis induced by aflatoxin. *Cancer Res.* **68**, 6727–6733. (doi:10.1158/0008-5472.CAN-08-1123)
137. Honda T, Sundararajan C, Yoshizawa H, Su X, Honda Y, Liby KT, Sporn MB, Gribble GW. 2007 Novel tricyclic compounds having acetylene groups at C-8a and cyano enones in rings A and C: highly potent anti-inflammatory and cytoprotective agents. *J. Med. Chem.* **50**, 1731–1734. (doi:10.1021/jm070141c)
138. Dinkova-Kostova AT *et al.* 2010 An exceptionally potent inducer of cytoprotective enzymes: elucidation of the structural features that determine inducer potency and reactivity with Keap1. *J. Biol. Chem.* **285**, 33 747–33 755. (doi:10.1074/jbc.M110.163485)
139. Kostov RV, Knatko EV, McLaughlin LA, Henderson CJ, Zheng S, Huang JT, Honda T, Dinkova-Kostova AT. 2015 Pharmacokinetics and pharmacodynamics of orally administered acetylenic tricyclic bis(cyanoenone), a highly potent Nrf2 activator with a reversible covalent mode of action. *Biochem. Biophys. Res. Commun.* **465**, 402–407. (doi:10.1016/j.bbrc.2015.08.016)
140. Li W *et al.* 2015 New monocyclic, bicyclic, and tricyclic ethynylcyanodienones as activators of the Keap1/Nrf2/ARE pathway and inhibitors of inducible nitric oxide synthase. *J. Med. Chem.* **58**, 4738–4748. (doi:10.1021/acs.jmedchem.5b00393)
141. Yao W *et al.* 2016 Antidepressant effects of TBE-31 and MCE-1, the novel Nrf2 activators, in an inflammation model of depression. *Eur. J. Pharmacol.* **793**, 21–27. (doi:10.1016/j.ejphar.2016.10.037)