Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Chemical Biology

Small molecule modulators of antioxidant response pathway

Wooyoung Hur[1,2] and Nathanael S Gray[1,2]

*N*uclear factor E2-*r*elated *f*actor *2* (Nrf2) is a transcription factor that regulates *A*ntioxidant *R*esponse *E*lement (ARE)-mediated transcription of a plethora of antioxidant and protective genes to counteract the harmful effects of reactive oxygen species or environmental carcinogens. Studies have demonstrated that pre-emptive activation of the Nrf2–ARE pathway reinforces the cellular defense mechanism against oxidative stress and leads to protection in a variety of disease models. Non-carcinogenic ARE inducers have been identified from a variety of chemical classes that enhance the transcriptional activity of Nrf2 through S-alkylation of reactive cysteines within the cellular redox sensor protein Keap1 (*K*elch-like *E*CH *a*ssociated *p*rotein *1*). Here we review the currently known small molecule ARE inducers and their reported biological activities in various models.

Addresses
[1] Department of Cancer Biology, Dana-Farber Cancer Institute, 250 Longwood Ave, Boston, MA 02115, USA
[2] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 250 Longwood Ave, Boston, MA 02115, USA

Corresponding author: Gray, Nathanael S (Nathanael_Gray@dfci.harvard.edu)

Current Opinion in Chemical Biology 2011, **15**:162–173

This review comes from a themed issue on
Omics
Edited by Kate Carroll and Pieter Dorrestein

Available online 30th December 2010

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2010.12.009

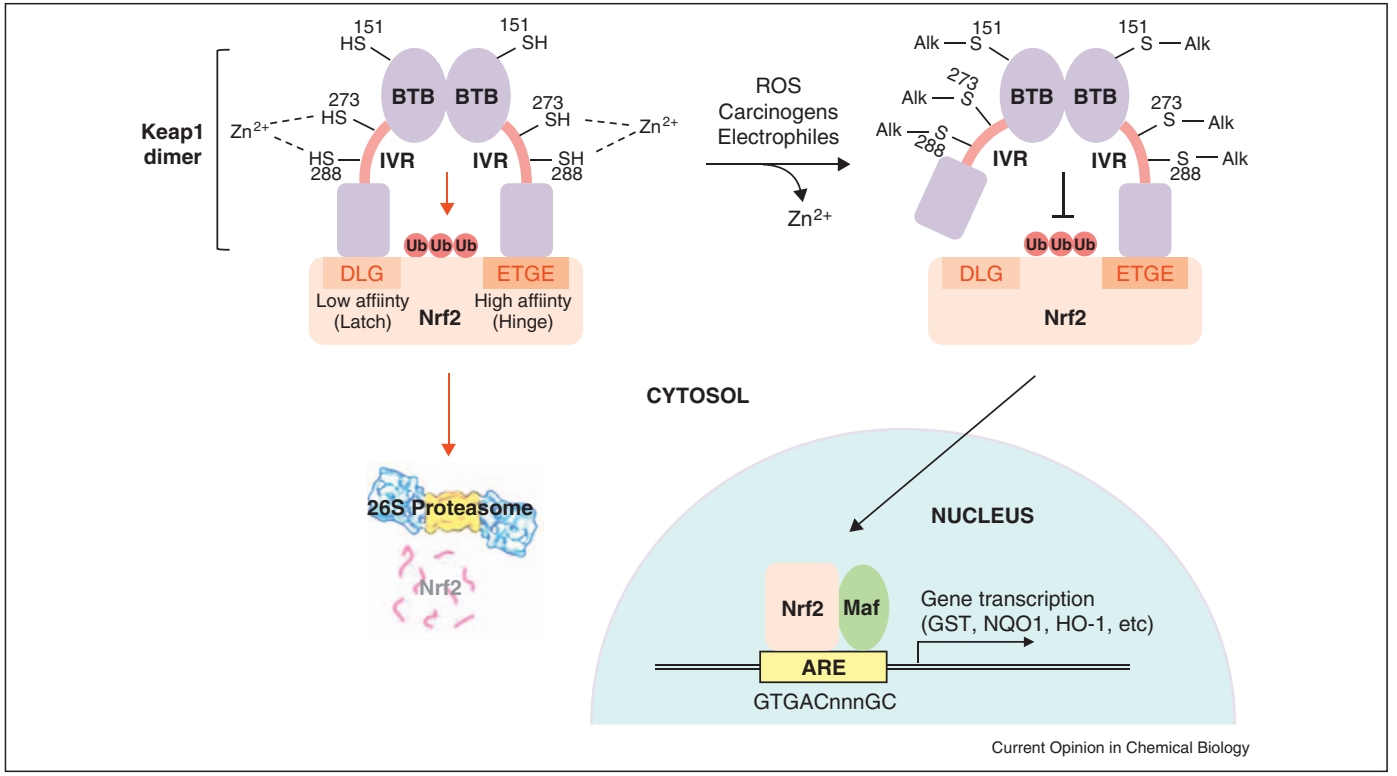
Introduction

When cells encounter oxidative stress or environmental carcinogens, the Nrf2–ARE-mediated defensive cellular response is activated to remove and counteract the harmful effects of the oxidative and xenobiotic agents. Nrf2 (*N*uclear factor E2-*r*elated *f*actor *2*) is a Cap'n'Collar family basic region-leucine zipper transcription factor that binds to the enhancer sequence ARE (*A*ntioxidant *R*esponse *E*lement, 5′-GTGACnnnGC-3′) of a broad range of protective genes, leading to an ARE-directed transcriptional response. The ARE-directed genes are involved in a variety of protective actions including production of direct antioxidants (e.g. heme oxygenase-1 (HO-1)), direct inactivation of reactive oxygen species (e.g. catalase, superoxide dismutase (SOD)), detoxification of toxic xenobiotics (phase II enzymes: e.g. glutathione *S*-transferase (GST), NAD(P)H:quinone oxidoreductase 1 (NQO1)), and glutathione generation (e.g. γ-glutamatecysteine ligase (GCL)) [1,2•]. The cooperative action of this large group of enzymes provides protection from the toxic effects induced by cellular stress.

Nrf2 is tightly regulated at a protein level by its negative regulator Keap1 (*K*elch-like *E*CH *a*ssociated *p*rotein *1*), which acts as an adaptor protein for Cul3-based ubiquitin E3 ligase complex. Keap1 is a cysteine-rich protein (27 cysteines in 626 amino acids of human Keap1), and as a result, it can serve as an excellent redox sensor in cells. Under normal physiological conditions, Nrf2 is sequestered in cytosol and maintained at a low level through Keap1-dependent ubiquitination and proteasomal degradation. Upon cellular stress such as reactive oxygen species (ROS) and environmental chemicals, a group of Keap1 cysteines are oxidatively modified and the resulting conformational change relieves Nrf2 from Keap1-directed degradation. The 'hinge and latch' model proposes that a single Nrf2 molecule binds two molecules of Keap1 with different affinity and that Keap1 modification leads to detachment of the weak interaction (latch) while the high-affinity interaction (hinge) remains intact [3]. Thus, in the presence of ARE inducers, Nrf2 is bound to Keap1 in a manner that prevents ubiquitination and subsequent degradation of Nrf2. When Nrf2 accumulates in cytosol to an extent that surpasses the sequestration capacity of Keap1, the excess Nrf2 moves to the nucleus, where it elicits transcription of the aforementioned downstream protective genes in association with a small Maf protein [2•,4] (Figure 1).

A variety of compounds that stimulate ARE-driven transcription have been identified from natural sources, dietary inputs, metabolites, and synthetic agents. All known ARE inducers are electrophilic species or metabolically activated to become electrophilic, and subsequently react with Keap1 cysteine residues. Classes of the ARE-inducing electrophiles include flavonoids, phenolic compounds, Michael acceptors, isothiocyanates, 1,2-dithiol-3-thiones, dimercaptans, heavy metals, peroxides, and polyenes [1]. ARE inducers inactivate the function of Keap1 by modifying the three important cysteines – C151 in the BTB (*B*road complex, *T*ramtrack, and *B*ric-a-brac) domain, and C273 and C288 in the central intervening region (IVR) [5–7]. The two residues C273 and C288 in IVR coordinate a Zn^{2+} ion and are crucial for maintaining the structural integrity required for Keap1 to associate with Nrf2 [8]. It is believed that oxidative modification of these two cysteine residues releases the zinc ion, resulting in a conformational rearrangement of the Keap1 protein.

Figure 1



Activation mechanism of Nrf2–ARE pathway – hinge and latch model: Keap1 homodimer recognizes ETGE and DLG motifs of Nrf2. ARE inducers modify three key reactive cysteines (C151, C273, and C288) within Keap1, and the resulting conformational change leads to detachment of the weak-binding DLG motif (latch) from Keap1. Consequently, ubiquitination of Nrf2 is abolished, while the strong binding through ETGF motif (hinge) remains intact. The stabilized Nrf2 then translocates to the nucleus and activates ARE-directed transcription of protective genes in association with Maf.

Studies have revealed that modification of C151 by ARE inducers including *t*-butylhydroquinone (tBHQ) and sulforaphane induces a conformational change of Keap1 accompanied by dissociation of Keap1 from Cul3, thereby inhibiting Keap1-directed Nrf2 ubiquitination and degradation [6,7,9]. It has been proposed that conformational change of Keap1 induced by C151 alkylation might expose C273 and C288 for further alkylation leading to inactivation of Keap1 [6,9], but more study is warranted. Interestingly, however, Nrf2 can also be activated in a C151-independent manner by heavy metals such as $CdCl_2$ and some arsenic species [10•,11], suggesting that there may be many distinct modifications that result in Keap1 inactivation. In addition, the activity of several kinases including PKC, PI3K, ERK, p38 MAPK, and PERK are implicated in regulating Nrf2 function [2•,12,13]. The activity of these kinases and possibly other kinases regulate stability and localization of Nrf2 through protein phosphorylation in a cell type-dependent fashion [14].

Pre-emptive activation of Nrf2 pathway potentiates the levels of a wide range of protective enzymes that counteract oxidative and environmental stress, and confers resistance to subsequent challenge of the cellular stress. The pharmacological priming of Nrf2–ARE pathway therefore can lead to protection from the stress-related deterioration and disease development. Pharmacological manipulation of the Nrf2–ARE pathway is actively being explored as a 'chemoprotection' or 'chemoprevention' strategy against a variety of disease models [2•,15]. For example, chemoprotection using a variety of ARE inducers has been demonstrated to prevent the initiation and development of carcinogenesis by limiting the exposure of cells to carcinogenic substances or ROS. ARE inducers have been also shown to prevent oxidative stress-induced neuronal death and neurodegeneration accompanying the expression of aggregate-prone proteins (e.g. α-synuclein, β amyloid) [16•]. Moreover, a number of ARE inducers also exhibit anti-inflammatory activity through the actions of Nrf2-directed enzymes [17]. HO-1 generates carbon monoxide as a by-product, which mediates potent anti-inflammatory effects, and peroxiredoxin I directly represses the anti-inflammatory activity of macrophage migration inhibitory factor (MIF). Thus, ARE inducers may possess significant therapeutic potential for a variety of conditions. Below we classify ARE inducers based upon their electrophilic functional groups and describe the biological activities of representative compounds within each category.

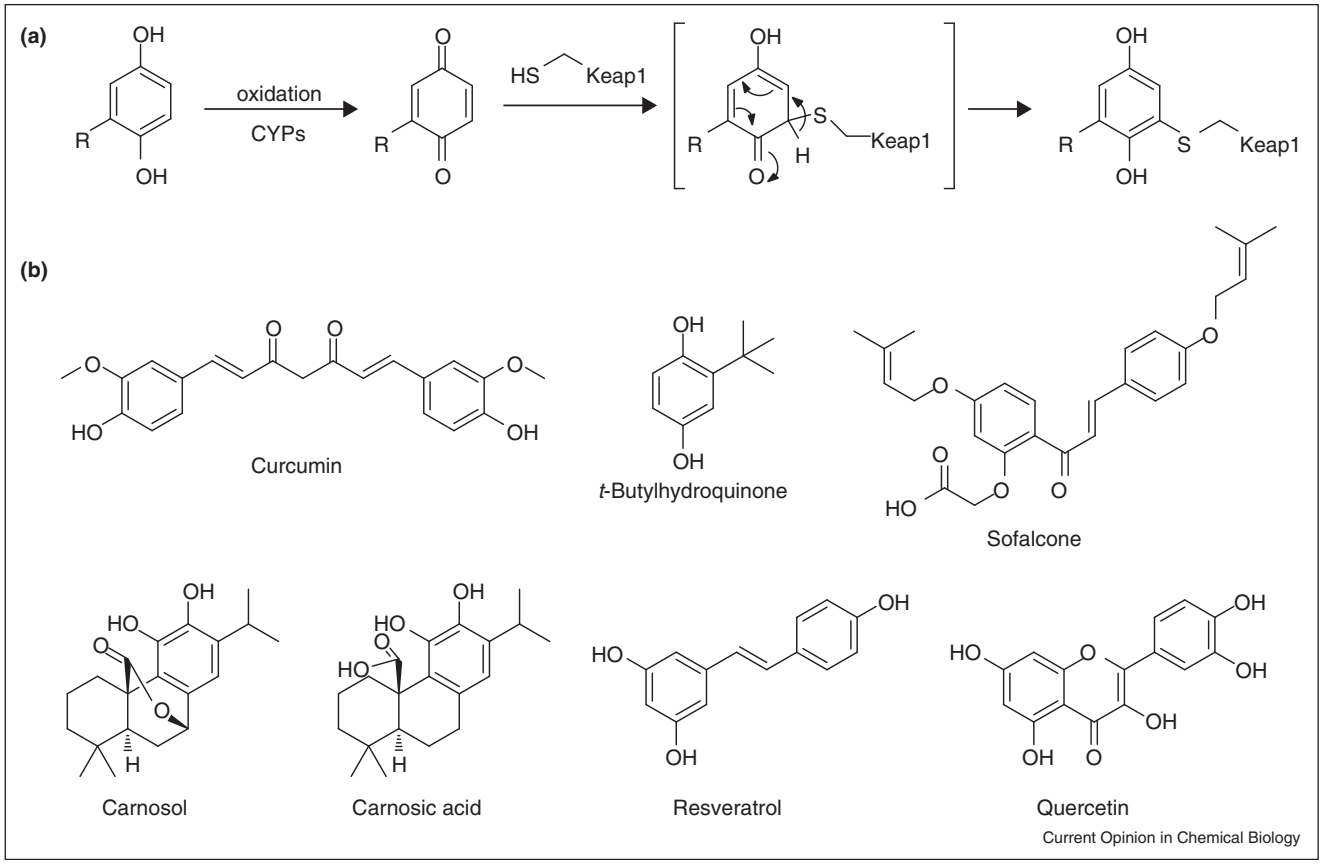
Phenolic or flavonoid antioxidants

Chemoprotection against carcinogen-induced cancers has been observed from phenolic antioxidants since the early 1970s [18]. Later, the regulatory enhancer element responsive to the antioxidants was identified and named 'antioxidant response element (ARE)' [19]. Since then, it has been found that ARE is responsive to a broad range of electrophiles. The phenolic antioxidants have an intrinsic antioxidant activity due to their ability to scavenge reactive radical species. However, their chemoprotective effects are mediated primarily by their quinone metabolites, formed as a result of oxidation by cytochrome P450 enzymes (CYPs), which rapidly react with thiols in Keap1 and ultimately elicits the ARE-dependent defensive response [20] (Figure 2a).

Curcumin (Figure 2b) is polyphenol yellow pigment in dietary spice turmeric from *Curcuma longa*, and can induce Nrf2 in HUH7 human hepatoma cells in a p38 MAPK-dependent manner [21]. Curcumin induces ROS in HUH7 cells, which appears to be important for Nrf2 activation because curcumin-induced HO-1 expression was diminished by pretreatment with a radical scavenger *N*-acetylcysteine [21]. The α,β-unsaturated carbonyl groups are the important pharmacophore for Nrf2 activation because tetrahydrocurcumin which lacks the α,β-unsaturated carbonyl moiety failed to induce Nrf2 [22]. Dietary curcumin (0.05% in diet, 15 days) in mice induced nuclear translocation of Nrf2 and the following association with ARE sequence, increased the levels of GST and NQO1, and prevented DNA modification challenged by benzo[*a*]pyrene (B[*a*]P) [23]. The curcumin-fed mice also revealed that curcumin inhibits B[*a*]P-induced phase I enzymes such as CYP1A1 and CYP1A2 at mRNA level by downregulating the transcriptional activity of aryl hydrocarbon receptor (AhR) through an unidentified mechanism [23]. This indicates that curcumin blocks the initiation of cancer not only by upregulating phase II enzymes but also by downregulating phase I enzymes. In another murine study, dietary curcumin (1.0% diet, 4 weeks) attenuated the levels of oxidized proteins, DNA, and lipids in kidney tissue that were induced by a renal carcinogen, ferric nitrilotriacetate (Fe-NTA) [24]. In fact, curcumin also has an iron-chelating property and thus prevents the formation of the iron-induced ROS [25], which might also contribute to the antioxidant activity of curcumin in the Fe-NTA induced cancer models.

Curcumin exhibits neuroprotective effects in murine models of Alzheimer's disease (AD). In the AD brain, β amyloid (Aβ) fibrils cause neuroinflammatory response, and the resulting ROS and cytokines promote further deposition of Aβ and increase neuronal death and tissue damage. *In vitro* studies demonstrated that curcumin enhanced Nrf2-mediated expression of HO-1 and GST, and protected PC12 cells and rat prefrontal cortical neurons from Aβ-induced apoptosis [26]. Curcumin exhibited a strong anti-inflammatory effect through inhibition of IκB phosphorylation and subsequent activation of NF-κB [27]. In a study using AD transgenic mouse models that carry a human amyloid precursor protein (APP) with the Swedish double mutation, a relatively low dose of curcumin (24 mg/kg/day) significantly suppressed inflammatory cytokine IL-1β production and decreased the levels of insoluble

Figure 2



ARE inducers – flavonoid and phenolic antioxidants: **(a)** formation of active quinone metabolite and its reaction with Keap1 cysteines and **(b)** structures of flavonoid and phenolic ARE inducers.

Aβ and oxidized proteins in the brain [28]. Curcumin is currently assessed in clinical trials for various diseases including pancreatic cancer, multiple myeloma, and Alzheimer's disease [27].

Carnosol (Figure 2b) is a catechol-type antioxidant from *Rosmarinus officinalis* (rosemary). Carnosol can increase Nrf2 level and HO-1 expression, and reduce lipid peroxidation and ROS generation in PC12 cells at a micromolar concentration [12]. Carnosol exhibited induction of an ARE response with an EC_{50} of an approximately 5 μM as measured in both HepG2 cells transfected with ARE-luc reporter and fibroblasts from ARE-luc transgenic mice [29]. The transgenic mice that received carnosol through oral gavage administration exhibited robust ARE activation in the kidney and liver compared with other organs [29]. A study also demonstrated that carnosol inhibited adipocyte differentiation in mouse 3T3-L1 pre-adipocytes at a micromolar concentration and elevated Nrf2 activity as well at a similar concentration [30]. The increase of cellular reduced glutathione (GSH) level by action of Nrf2 downstream genes might attenuate the ROS-mediated adipocyte differentiation. Carnosic acid (CA, Figure 2b), a carboxylic acid analogue of carnosol, showed Nrf2-mediated neuroprotection in PC12 cells against glutamate or rotenone-induced toxicity [31]. A pulldown experiment using a biotin-tethered CA revealed that CA directly modifies intracellular Keap1 [31]. The strongest covalent modification was seen with the BTB domain, implying that C151 of Keap1 might be preferentially targeted by CA. CA upregulated phase II enzymes including HO-1, GST, and NQO1 in mouse hippocampal HT22 cells and elicited protection against glutamate-induced cell death [32]. Interestingly, CA displayed a superior protection than carnosol in HT22 cells, which might be because CA is less toxic or because the hydrophilic nature of CA is favorable for binding to cellular Keap1. CA (i.p., 1 mg/kg) increased the level of GSH in brain and exhibited a significant protection from brain injury induced by middle cerebral artery occlusion in mice [31].

Quercetin (Figure 2b) is a flavonoid polyphenolic antioxidant ubiquitously found in fruits and vegetables. Quercetin is capable of activating Nrf2 and upregulating phase II enzymes such as NQO1 in HepG2 cells with an

EC_{50} of 15 μM as measured using an ARE-luciferase reporter gene assay [33]. Interestingly, quercetin increased cellular Nrf2 level not only by inhibiting ubiquitination of Nrf2 but also by increasing the level of Nrf2 mRNA in hepatocytes. Quercetin (50 μM) exhibited a marked protection of RAW264.7 macrophages from H_2O_2-induced apoptosis through upregulation of Nrf2-directed enzymes including HO-1 [13]. Quercetin increased phosphorylation of ERK in RAW264.7 cells through an unidentified mechanism, and the ERK activity appeared to be required for quercetin-induced ARE activation, because Nrf2 induction was inhibited by treatment of MEK inhibitor PD098059. But intriguingly, a recent study revealed that quercetin is a potent inhibitor of ERK phosphorylation (IC_{50} = 1.5 μM, immunoprecipitation-kinase assay) presumably via direct binding to MEK1 or Raf [34]. Thus, more study is required to dissect the role of ERK pathway with regard to quercetin-induced ARE activation. Dietary quercetin (1.0% in diet, 2 weeks) suppressed azoxymethane-induced colorectal carcinogenesis in F344 rats, demonstrating that quercetin provokes prevention from the carcinogenesis *in vivo* [35].

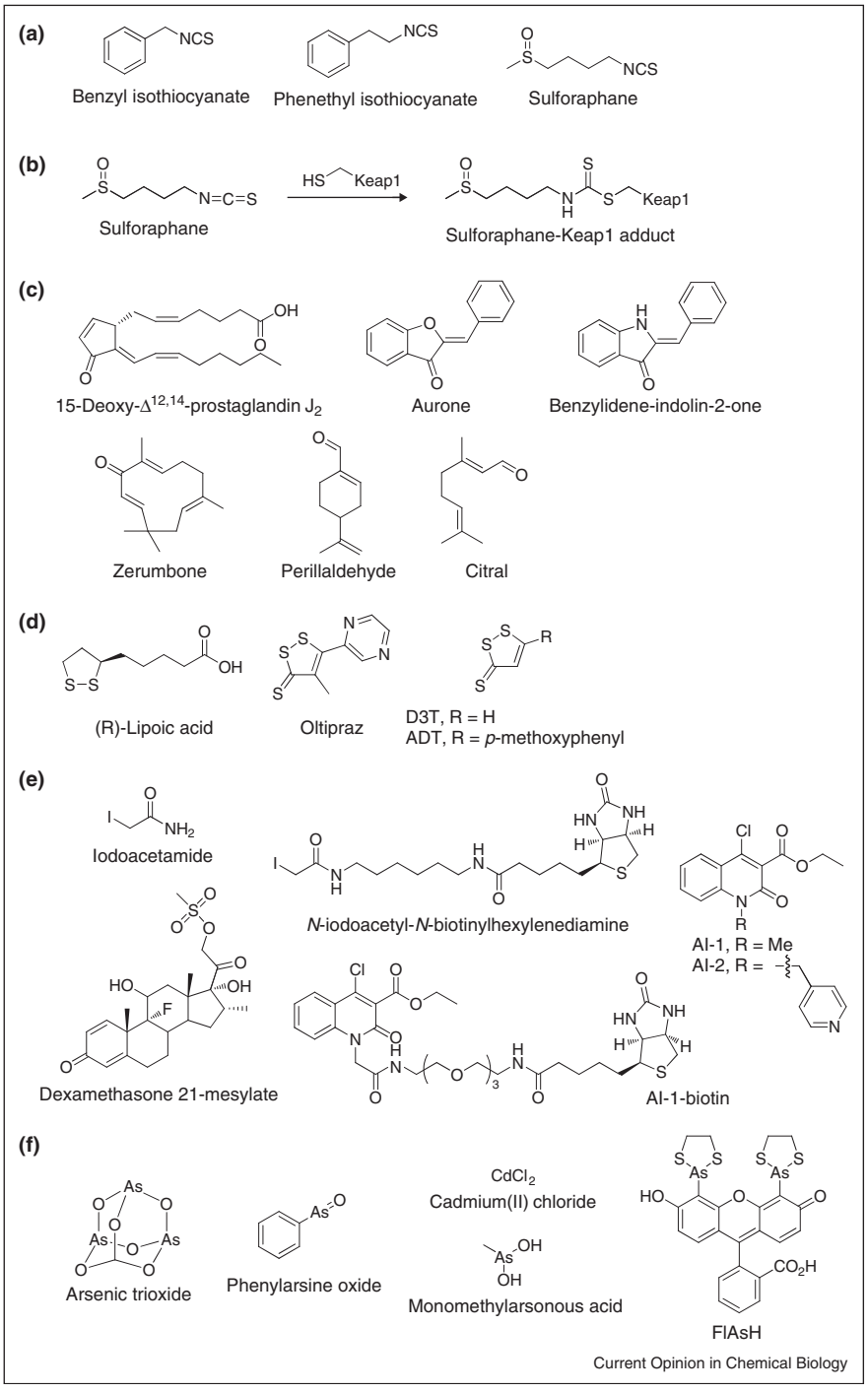
Resveratrol (Figure 2b), a nonflavonoid polyphenolic compound found in grapes, has attracted enormous attention because of its ability to delay age-related conditions including cardiovascular disease, cancer, diabetes, and neurodegeneration. It is believed that resveratrol mimics the anti-aging benefits of caloric restriction through activation of SIRT1 pathway. But recent studies suggest that Nrf2–ARE pathway is also implicated in the beneficial effects of resveratrol. Resveratrol activates Nrf2-dependent antioxidant enzymes in hepatocytes, primary cardiocytes, endothelial cells, and epithelial cells, and is capable of inducing protection against oxidative stress-induced or carcinogen-induced cell death [36]. Similar to quercetin, resveratrol augmented Nrf2 level not only by stabilizing the protein but also by increasing mRNA of Nrf2 in hepatocytes. Resveratrol (10 μM) attenuated cigarette smoke extracts (CSE)-induced ROS and restored CSE-depleted GSH level by upregulating GCL via Nrf2 activation in human alveolar epithelial A549 cells [37]. The antioxidant activity of resveratrol has been demonstrated in various *in vivo* models. Dietary resveratrol (0.24% in diet) markedly attenuated oxidative stress in aortas that were induced by high-fat diet (HFD) in obese mice [38]. A very recent report supported the importance of Nrf2 in the mechanism of resveratrol because it was unable to elicit vasoprotective effects against the HFD-induced oxidative stress in Nrf2 knockout mice [39•]. In addition, a mouse model of Type-2 diabetes ($Lepr^{db}$) exhibited high levels of serum H_2O_2 and aortic nitrotyrosine proteins, but those levels were significantly reduced following oral administration of resveratrol (20 mg/kg/day, 4 weeks) [40]. While two Nrf2-directed antioxidant genes, superoxide dismutase-1 (SOD-1) and glutathione peroxidase (GPX), were downregulated in $Lepr^{db}$ mice, resveratrol upregulated the expression of SOD-1 and GPX in the diabetic mice. Thus, upregulation of Nrf2–ARE pathway induced by resveratrol may be a central mechanism behind this compound's pleotropic activity in numerous disease models.

Sofalcone (Figure 2b) is a synthetic derivative of the chalcone-type natural product sophoradin and is used as an anti-ulcer agent in Asia for the protection of gastric mucosa. The pharmacological efficacy of sofalcone may be due to inhibition of inflammatory cytokines that are associated with *H. pylori*-infection, but further investigations are required. A recent study demonstrated that sofalcone (10 μM) induces Nrf2 nuclear translocation and HO-1 expression in rat gastric mucosal RGM-1 cells [41]. Interestingly, sofalcone dramatically increased the expression of VEGF, and the depletion of HO-1 via either siRNA treatment or pharmacological inhibition suppressed the production of VEGF. The authors proposed that Nrf2-directed HO-1 expression upregulated VEGF-mediated angiogenetic actions, which might promote ulcer healing [41]. In addition, sofalcone (5–10 μM) significantly inhibited the LPS-induced production of pro-inflammatory molecules such as NO, TNF-α, MCP-1 in RAW264.7 macrophages/3T3-F442A pre-adipocytes co-culture system, and pharmacological inhibition of HO-1 attenuated the anti-inflammatory effect [42]. Thus, Nrf2-mediated angiogenetic and anti-inflammatory actions may be associated with the anti-ulcer activity of sofalcone.

Isothiocyanates

Isothiocyanates (ITCs, Figure 3a) such as benzyl isothiocyanate and phenethyl isothiocyanate are found in cruciferous vegetables such as broccoli and cabbage, and cumulative evidence clearly indicates that dietary consumption of ITCs or ITC-containing foods reduce the risk of developing lung, breast, and colon cancers [43]. Sulforaphane (Figure 3a) is abundant in broccoli and is the most well-studied ARE inducer in the ITC class. Mass spectrometric analysis indicated that sulforaphane directly modifies a number of Keap1 cysteine residues including C151, C273, and C288 *in vitro* through formation of carbamodithioate (Figure 3b) [44]. Pretreatment of HepG2 cells with sulforaphane upregulated transcription of phase II enzymes including GST and prevented the modification of DNA induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [45]. Topical application of sulforaphane (100 nmol/day, 14 days) before application of two carcinogens 7,12-dimethylbenz[*a*]anthracene and 12-*O*-tetradecanoylphorbol-13-acetate increased Nrf2 level and dramatically decreased the incidence of skin tumors in the $Nrf2^{+/+}$ mice, whereas no such effects were shown from $Nrf2^{-/-}$ mice, indicating that chemoprotection of sulforaphane was mediated through Nrf2 [46]. The pharmacology of sulforaphane is more complicated as the sulforaphane–cysteine adduct,

Figure 3



Classes of ARE inducers: **(a)** isothiocyanates, **(b)** reaction between sulforaphane and Keap1 cysteines, **(c)** Michael acceptors, **(d)** organosulfur compounds, **(e)** electrophiles containing a leaving group, and **(f)** heavy metal species.

which is formed by metabolic process *in vivo*, inhibits histone deacetylase enzymes (HDACs) [47], an observation that has been corroborated in human clinical studies [48]. Interaction with HDACs was observed from a non-ITC class ARE inducer as well [49•], suggesting that the ability to inhibit HDACs *in vivo* might be shared with many other ARE inducers.

The neuroprotective activity of sulforaphane has been assessed in models of Parkinson's disease (PD). Oxidation

of dopamine (DA) to DA-quinone and the resulting oxidative stress is believed to be a major factor leading to a selective death of dopaminergic neurons in PD. Sulforaphane (1 μM) significantly increased the level of NQO1, an enzyme that catalyzes reduction of DA-quinone and resulted in protection of dopaminergic cell lines and primary mesencephalic dopaminergic neurons against cell death induced by 6-hydroxydopamine (6-OHDA) and tetrahydrobiopterin [50]. But sulforaphane did not protect against 1-methyl-4-phenylpyridinium (MPP^+)-induced dopaminergic cell death where the toxicity does not involve formation of DA-quinone, indicating that sulforaphane protects dopaminergic neurons particularly against the mechanism that involves DA-quinone generation. The protective activity in dopaminergic neuroblastoma SH-SY5Y cells against 6-OHDA was abolished by pharmacological inhibition of GCL, a rate-determining enzyme in GSH synthesis, demonstrating an important role for GSH level in neuroprotection [51]. The neuroprotective effect of sulforaphane has also been demonstrated in Drosophila models of PD. Dietary sulforphane (25 μg/mL, 4 days) increased GSH level and suppressed the neuronal loss of the flies that express either α-synuclein or Drosophila parkin mutants [52].

Michael acceptors

15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2, Figure 3c) is a cyclopentenone prostaglandin metabolite produced by the action of COX-2 that acts as a ligand of the nuclear receptor PPARγ. It possesses an α,β-unsaturated carbonyl group in the cyclopentenone ring, and some important activities of 15d-PGJ_2 including its anti-inflammatory activity are mediated through its covalent reaction with the cysteine residues in target proteins [53]. It directly inhibits NF-κB pathway through covalent modification of cysteines in the activation loop of IKKβ and the DNA-binding domains of NF-κB subunits. 15d-PGJ_2 at 2 μM markedly increased the levels of HO-1 and GSH in bovine aortic endothelial cells, and covalent modification of intracellular Keap1 was detected using a biotin-tethered 15d-PGJ_2 conjugate [54]. Among the three electrophilic centers, C9 within the cyclopentenone ring is the reaction site for Keap1 because the analogue that lacks the endocyclic double bond in the cyclopentenone showed no Nrf2-mediated cytoprotective effects [55]. 15d-PGJ_2 exerted protection from H_2O_2-induced apoptosis in primary rat cortical neurons. Intraventricularly injected 15d-PGJ_2 reduced brain infarct volume induced by ischemia-reperfusion injury and abrogated caspase-3 activation in the ischemic cortex [56]. 15d-PGJ_2 also exhibited Nrf2-dependent efficacy in a murine model of acute inflammation [57].

Zumberone (Figure 3c), a sesquiterpene compound, enhances the expression of phase II enzymes and inhibits LPS-induced inflammation in RAW264.7 mouse macrophages [58]. Comparative analysis with a reduced version of zumberone (α-humulene) indicates that the α,β-unsaturated carbonyl functionality is essential for both the antioxidant and anti-inflammatory activities [59]. A pull-down study using a biotin-conjugated zumberone revealed covalent association with Keap1 [60]. Aurone (Figure 3c) is a representative of a diverse group of natural products that confer bright yellow color on ornamental flowers. Recently, synthetic efforts focused on aurone analogues have revealed the structure–activity relationship (SAR) with respect to NQO1 upregulation [61]. A series of benzylidene-indolin-2-ones (Figure 3c), where the oxygen of the benzofuran ring within aurone is replaced with an NH group, exhibited the ability to activate Nrf2 in murine Hepa1c1c7 cells and possessed antiproliferative activity in human cancer cell lines (MCF-7, HCT116). SAR analysis suggested that substitutions at the core structure affected NQO1 induction and antiproliferative activities in dissimilar ways, demonstrating that the two activities probably relate to different biological targets [62]. Perillaldehyde and citral (Figure 3b), terpenoid unsaturated aldehydes isolated from herbs, were shown to upregulate thioredoxin expression via Nrf2 activation and protect human leukemic K562 cells and rat epithelial RGM-1 cells against H_2O_2-induced stress [63].

Organosulfur compounds

Lipoic acid (LA, Figure 3d) contains an intramolecular disulfide and is produced in the mitochondria where it serves as a cofactor of α-ketoglutarate dehydrogenase [64]. Unlike endogenously produced LA, orally administered LA elicits multiple biological activities. LA acts as a scavenger of various ROS *in vitro*, but its cellular antioxidant activity appears to be due to its ability to induce a variety of antioxidant enzymes through Nrf2 activation because LA accumulates only at micromolar concentrations, which are considerably lower than the millimolar concentration of intracellular GSH [65]. In addition to covalent interaction with Keap1 cysteines, the ability to activate various kinases may be involved in LA-induced Nrf2 activation [64]. LA activates Erk1/2, p38 MAPK, PI3K/Akt pathways, possibly through direct inhibition of phosphatases such as PTP1B, PP2A, and PTEN via cysteine modification, that promote accumulation of Nrf2 in the nucleus. LA at low millimolar concentrations increased the expression of phase II enzymes including NQO1 and glutathione reductase, and enhanced Nrf2 level while it decreased Keap1 level in HL-60 cells [66]. A study demonstrated that Nrf2 activation and GSH levels are severely impaired in aged rats but that administration of LA (40 mg/kg, i.p.) to old rats increased the levels of nuclear Nrf2 and GCL, and attenuated the age-related loss of GSH [67•].

Besides an ability to activate Nrf2, LA has a variety of properties that can interfere with the pathogenesis or progression of AD [64]. LA chelates redox active transition

metals such as Cu, Fe, and Zn ions, and thus prevents the formation of Aβ-metal-induced neurotoxic hydroxyl radicals and potentially inhibits the formation of Aβ plaques. LA inhibits NF-κB pathway, which could lead to suppression of chronic inflammatory processes associated with amyloid plaques. LA also increases glucose uptake via multiple mechanisms, which could alleviate insulin resistance and abnormalities identified in AD brain. Chronic administration of LA (0.01%, 4 weeks) reversed oxidative stress in brain and memory impairment that were induced by Aβ in 12-month-old SAMP8 mice [68]. AD transgenic Tg2576 mice fed with dietary LA (0.1% in diet, 6 months) significantly improved learning and memory retention compared to untreated Tg2576 mice. However, there was no difference in levels of brain soluble, insoluble Aβ, and nitrotyrosine between LA-treated and untreated Tg2576 mice [69]. These results suggest that chronic dietary LA reduces memory deficits and Aβ-induced oxidative stress in AD mice, but does not affect Aβ levels or plaque deposition *in vivo*.

Oltipraz (Figure 3d) is a synthetic ARE inducer and the most extensively studied compound within a class of 1,2-dithiol-3-thiones [70]. Other popular compounds in this class are 3*H*-1,2-dithiol-3-thione (D3T) and 5-(4-methoxyphenyl)-3*H*-1,2-dithiol-3-thione (ADT). D3T showed a marked increase of NQO1 activity in murine Hepa1c1c7 cells at a micromolar concentration, whereas its isomer, 1,3-dithiol-2-thione, was ineffective even at a high concentration, indicating that the 1,2-dithiol substructure is crucial for ARE activation [71]. Oltipraz showed induction of an ARE response in ARE-GFP transfected HepG2 cells (EC_{50} = ca. 50 μM). Oral administration of a single dose of oltipraz (500 mg/kg) significantly increased the activity of hepatic GST and NQO1 in $Nrf2^{+/-}$ mice, whereas the effect was abrogated in $Nrf2^{-/-}$ mice [72]. Oltipraz prevents carcinogenesis in a variety of organs including bladder, colon, liver, lung, stomach, and pancreas induced by various carcinogens such as aflatoxin B_1 (AFB_1), B[*a*]P, and diethylnitrosamine [70]. However, oltipraz is also able to induce phase I enzymes such as CYP1A, CYP1B1, and CYP3A2 *in vivo* through activation of AhR–XRE pathway, which may antagonize the protective effects [73]. Despite efficient anti-carcinogenic effects shown in rodent models, oltipraz exhibited no significant effects in preventing oxidative DNA damage in two recent placebo-controlled human clinical trials with chronic smokers and patients who were exposed to a high level of AFB_1, which raises questions as to whether oltipraz will find use as a therapeutic in humans [74,75].

Electrophiles bearing a leaving group

Electrophiles that have a leaving group, such as dexamethasone 21-mesylate and *N*-iodoacetyl-*N*-biotinylhexylenediamine (IAB, Figure 3e), have been used for *in vitro* analysis to identify the reactive cysteines of Keap1. IAB contains an iodoacetyl reactive group and is the most extensively used activity probe for Keap1. IAB covalently modified a number of cysteines *in vitro* including C151, C273, and C288 [9,76]. However, despite its ability to efficiently modify Keap1, it only exhibits moderate activity as an activator of Nrf2, which may be related to the high cytotoxicity or the promiscuous targeting of intracellular proteins [76,77]. The weak activity of IAB as an activator of cellular Nrf2 limits its use to *in vitro* analysis.

AI-1 (Figure 3e), a quinolinone-scaffold compound, contains a chloro group at β-position of the two α,β-unsaturated carbonyl systems that can readily be displaced by Keap1 cysteines. We describe AI-1 in this group rather than classify it as a Michael acceptor because replacement of the chloro group of AI-1 with hydrogen eliminates the ARE-inducing activity, although it maintains the Michael acceptor functionality. AI-1 is the first ARE inducer identified using high-throughput screening of unbiased small molecule library [49•]. It exhibited a single digit micromolar EC_{50} for inducing ARE-luciferase reporter gene activity and Nrf2 stabilization in IMR-32 neuroblastoma cells. AI-1 has attractive features such as a potent Nrf2 activation, low cytotoxicity, and a versatile chemistry for derivatization, and a clear SAR. A biotin-modified AI-1, AI-1-biotin (Figure 3d), was used to reveal that AI-1 alkylates C151 of Keap1 both *in vitro* and in cells [49•]. AI-2 was found as a more potent analogue, and as seen from carnosic acid, the hydrophilicity of the pyridine group within AI-2 might assist in localizing to cellular Keap1. Like tBHQ and sulforaphane, AI-1 weakens the interaction between Cul3 and Keap1 in a C151-dependent manner, thereby inhibiting Nrf2 ubiquitination and activating ARE-mediated transcription [49•].

Heavy metal species

Arsenic containing compounds are environmental carcinogens, but they also possess therapeutic benefits in certain human diseases including leukemia and psoriasis. Arsenic trioxide (ATO, Figure 3f) is an FDA-approved drug for patients with relapsed or refractory acute promyelocytic leukemia (APL). ATO induces the caspase-mediated and proteasome-mediated degradation of the PML–PARα fusion protein through interactions with cysteines in PML, resulting in terminal differentiation or apoptosis of APL cells [78]. Recently, it was reported that ATO (2 μM) induces nuclear translocation of Nrf2 and activates a number of Nrf2 downstream genes including NQO1 and HO-1 in human myeloma cell lines such as KMS11, MM.1s, and U266 cells [79]. Exposure of KMS11 cells to ATO generates ROS independently of its ability to activate Nrf2. ATO exhibits potent cytotoxicity that overwhelms the protective effects of Nrf2 activation, but it could be beneficial to antitumor action.

A recent report demonstrated that the well-known arsenic-based fluorophore (FlAsH, Figure 3f) stabilizes

Nrf2 and increases HO-1 level at micromolar concentrations in Hepa1c1c7 cells [80]. It binds to Keap1 without the tetracysteine tag (CCPGCC) in the protein that is usually required for interaction with the fluorophore. Interestingly, when FlAsH is associated with Keap1, it emits a strong fluorescence signal at 508 nm. By using FlAsH as a probe, it was found that other arsenic reagents such as phenylarsine oxide (PAO) compete with FlAsH for binding to Keap1, demonstrating its potential as a pharmacological probe for many biological assays such as high-throughput drug binding assays. PAO (Figure 3f) exhibits a relatively high potency for Nrf2 stabilization (submicromolar EC_{50}) in Hepa1c1c7 cells, and its direct binding to Keap1 was demonstrated using pulldown using PAO-coupled affinity resins [81]. Interestingly, while PAO showed a Keap1 C151-dependent Nrf2 activation, other trivalent arsenic species such as arsenite ($NaAsO_2$) and monomethylarsonous acid activated Nrf2 in a C151-independent manner [11].

Conclusion

The Nrf2–ARE pathway elicits transcriptional activation of antioxidant genes and detoxifying genes that protect cells and organisms from oxidative and chemical stress. Pharmacological 'priming' of the ARE-mediated cellular defense response has been thoroughly demonstrated as a chemoprevention strategy using a variety of *in vivo* disease models. The identification of potent, non-toxic ARE inducers therefore will be of great value as pharmacological probes of mechanism and as potential new therapeutics.

The currently known ARE inducers are electrophilic molecules that are capable of covalently modifying the reactive cysteines of the cellular redox sensor Keap1. Because Keap1 accommodates a broad spectrum of ARE inducers, presumably due to its ability to react with a diverse assortment of electrophiles, various naturally occurring compounds and synthetic drugs have been identified as ARE inducers. Because of the promiscuity of Keap1, it is reasonable to speculate that most compounds bearing an electrophilic group could function as potential ARE inducers. However, such compounds also have an ability to react with other thiol-containing enzymes, which gives them the ability to exhibit polypharmacology. It will be worthwhile to identify a non-electrophilic ARE inducer, as it could potentially present fewer off-target effects and would significantly increase our knowledge of Keap1 at a mechanistic level.

In most cases, cancer chemopreventive activities of ARE inducers have been demonstrated in animal models where tumor formation is forced by acute treatment of high-dose carcinogens. However these cancer models are unlikely to accurately reflect low-dose chronic carcinogen exposure as it occurs in a modern environment. These challenges to preclinical and clinical validation of chemoprevention strategy will require further investigation before a therapeutic impact in the human population is likely to be achieved.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Kensler TW, Wakabayashi N, Biswal S: **Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway**. *Annu Rev Pharmacol Toxicol* 2007, **47**:89-116.

2. • Kensler TW, Wakabayashi N: **Nrf2: friend or foe for chemoprevention?** *Carcinogenesis* 2010, **31**:90-99.
An excellent review article about Nrf2–ARE pathway and provides its implication in physiology in great detail.

3. Tong KI, Kobayashi A, Katsuoka F, Yamamoto M: **Two-site substrate recognition model for the Keap1-Nrf2 system: a hinge and latch mechanism**. *Biol Chem* 2006, **387**: 1311-1320.

4. Kobayashi M, Yamamoto M: **Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species**. *Adv Enzyme Regul* 2006, **46**:113-140.

5. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P: **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* 2002, **99**:11908-11913.

6. Zhang DD, Hannink M: **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* 2003, **23**:8137-8151.

7. Yamamoto T, Suzuki T, Kobayashi A, Wakabayashi J, Maher J, Motohashi H, Yamamoto M: **Physiological significance of reactive cysteine residues of Keap1 in determining Nrf2 activity**. *Mol Cell Biol* 2008, **28**:2758-2770.

8. Dinkova-Kostova AT, Holtzclaw WD, Wakabayashi N: **Keap1, the sensor for electrophiles and oxidants that regulates the phase 2 response, is a zinc metalloprotein**. *Biochemistry* 2005, **44**:6889-6899.

9. Rachakonda G, Xiong Y, Sekhar KR, Stamer SL, Liebler DC, Freeman ML: **Covalent modification at Cys151 dissociates the electrophile sensor Keap1 from the ubiquitin ligase CUL3**. *Chem Res Toxicol* 2008, **21**:705-710.

10. • McMahon M, Lamont DJ, Beattie KA, Hayes JD: **Keap1 perceives stress via three sensors for the endogenous signaling molecules nitric oxide, zinc, and alkenals**. *Proc Natl Acad Sci U S A* 2010, **107**:18838-18843.
This paper reveals three different modules of Keap1 for sensing three different endogenous electrophiles, NO, Zn^{2+}, and alkenals. It also shows that Zn^{2+} and other metal ions are sensed not by C151 but by C226/C613 of Keap1.

11. Wang XJ, Sun Z, Chen W, Li Y, Villeneuve NF, Zhang DD: **Activation of Nrf2 by arsenite and monomethylarsonous acid is independent of Keap1-C151: enhanced Keap1-Cul3 interaction**. *Toxicol Appl Pharmacol* 2008, **230**:383-389.

12. Martin D, Rojo AI, Salinas M, Diaz R, Gallardo G, Alam J, De Galarreta CM, Cuadrado A: **Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol**. *J Biol Chem* 2004, **279**:8919-8929.

13. Chow JM, Shen SC, Huan SK, Lin HY, Chen YC: **Quercetin, but not rutin and quercitrin, prevention of H2O2-induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages**. *Biochem Pharmacol* 2005, **69**:1839-1851.

14. Lee JM, Hanson JM, Chu WA, Johnson JA: **Phosphatidylinositol 3-kinase, not extracellular signal-regulated kinase, regulates activation of the antioxidant-responsive element in IMR-32 human neuroblastoma cells**. *J Biol Chem* 2001, **276**:20011-20016.

15. Kwak MK, Kensler TW: **Targeting NRF2 signaling for cancer chemoprevention**. *Toxicol Appl Pharmacol* 2010, **244**:66-76.

16. • Barnham KJ, Masters CL, Bush AI: **Neurodegenerative diseases and oxidative stress**. *Nat Rev Drug Discov* 2004, **3**:205-214.
An excellent review article about the implication of oxidative stress in various neurodegenerative diseases.

17. Kundu JK, Surh YJ: **Nrf2-Keap1 signaling as a potential target for chemoprevention of inflammation-associated carcinogenesis**. *Pharm Res* 2010, **27**:999-1013.

18. Wattenberg LW: **Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin**. *J Natl Cancer Inst* 1972, **48**:1425-1430.

19. Rushmore TH, Pickett CB: **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* 1990, **265**:14648-14653.

20. Wang XJ, Hayes JD, Higgins LG, Wolf CR, Dinkova-Kostova AT: **Activation of the NRF2 signaling pathway by copper-mediated redox cycling of para- and ortho-hydroquinones**. *Chem Biol* 2010, **17**:75-85.

21. McNally SJ, Harrison EM, Ross JA, Garden OJ, Wigmore SJ: **Curcumin induces heme oxygenase 1 through generation of reactive oxygen species, p38 activation and phosphatase inhibition**. *Int J Mol Med* 2007, **19**:165-172.

22. Farombi EO, Shrotriya S, Na HK, Kim SH, Surh YJ: **Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1**. *Food Chem Toxicol* 2008, **46**:1279-1287.

23. Garg R, Gupta S, Maru GB: **Dietary curcumin modulates transcriptional regulators of phase I and phase II enzymes in benzo[a]pyrene-treated mice: mechanism of its anti-initiating action**. *Carcinogenesis* 2008, **29**:1022-1032.

24. Iqbal M, Okazaki Y, Okada S: **Curcumin attenuates oxidative damage in animals treated with a renal carcinogen, ferric nitrilotriacetate (Fe-NTA): implications for cancer prevention**. *Mol Cell Biochem* 2009, **324**:157-164.

25. Jiao Y, Wilkinson Jt, Di X, Wang W, Hatcher H, Kock ND, D'Agostino R Jr, Knovich MA, Torti FM, Torti SV: **Curcumin, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator**. *Blood* 2009, **113**:462-469.

26. Qin XY, Cheng Y, Cui J, Zhang Y, Yu LC: **Potential protection of curcumin against amyloid beta-induced toxicity on cultured rat prefrontal cortical neurons**. *Neurosci Lett* 2009, **463**:158-161.

27. Hatcher H, Planalp R, Cho J, Torti FM, Torti SV: **Curcumin: from ancient medicine to current clinical trials**. *Cell Mol Life Sci* 2008, **65**:1631-1652.

28. Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM: **The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse**. *J Neurosci* 2001, **21**:8370-8377.

29. Balstad TR, Carlsen H, Myhrstad MC, Kolberg M, Reiersen H, Gilen L, Ebihara K, Paur I, Blomhoff R: **Coffee, broccoli and spices are strong inducers of electrophile response element-dependent transcription in vitro and in vivo—studies in electrophile response element transgenic mice**. *Mol Nutr Food Res* 2010, **54**:1-13.

30. Takahashi T, Tabuchi T, Tamaki Y, Kosaka K, Takikawa Y, Satoh T: **Carnosic acid and carnosol inhibit adipocyte differentiation in mouse 3T3-L1 cells through induction of phase2 enzymes and activation of glutathione metabolism**. *Biochem Biophys Res Commun* 2009, **382**:549-554.

31. Satoh T, Kosaka K, Itoh K, Kobayashi A, Yamamoto M, Shimojo Y, Kitajima C, Cui J, Kamins J, Okamoto S *et al.*: **Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1**. *J Neurochem* 2008, **104**:1116-1131.

32. Satoh T, Izumi M, Inukai Y, Tsutsumi Y, Nakayama N, Kosaka K, Shimojo Y, Kitajima C, Itoh K, Yokoi T *et al.*: **Carnosic acid protects neuronal HT22 Cells through activation of the antioxidant-responsive element in free carboxylic acid- and catechol hydroxyl moieties-dependent manners**. *Neurosci Lett* 2008, **434**:260-265.

33. Tanigawa S, Fujii M, Hou DX: **Action of Nrf2 and Keap1 in ARE-mediated NQO1 expression by quercetin**. *Free Radic Biol Med* 2007, **42**:1690-1703.

34. Lee KW, Kang NJ, Heo YS, Rogozin EA, Pugliese A, Hwang MK, Bowden GT, Bode AM, Lee HJ, Dong Z: **Raf and MEK protein kinases are direct molecular targets for the chemopreventive effect of quercetin, a major flavonol in red wine**. *Cancer Res* 2008, **68**:946-955.

35. Dihal AA, de Boer VC, van der Woude H, Tilburgs C, Bruijntjes JP, Alink GM, Rietjens IM, Woutersen RA, Stierum RH: **Quercetin, but not its glycosidated conjugate rutin, inhibits azoxymethane-induced colorectal carcinogenesis in F344 rats**. *J Nutr* 2006, **136**:2862-2867.

36. Rubiolo JA, Mithieux G, Vega FV: **Resveratrol protects primary rat hepatocytes against oxidative stress damage: activation of the Nrf2 transcription factor and augmented activities of antioxidant enzymes**. *Eur J Pharmacol* 2008, **591**:66-72.

37. Kode A, Rajendrasozhan S, Caito S, Yang SR, Megson IL, Rahman I: **Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells**. *Am J Physiol Lung Cell Mol Physiol* 2008, **294**:L478-L488.

38. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E *et al.*: **Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span**. *Cell Metab* 2008, **8**:157-168.

39. • Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K, de Cabo R, Csiszar A: **Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2**. *Am J Physiol Heart Circ Physiol* 2010, **299**:H18-24.
This paper strongly supports that protective activity of resveratrol is mediated by Nrf2. It reveals that resveratrol protects endothelial cells from HFD-induced oxidative stress in wild-type mice, but not in Nrf2 knockout mice.

40. Zhang H, Zhang J, Ungvari Z, Zhang C: **Resveratrol improves endothelial function: role of TNF2010 and vascular oxidative stress**. *Arterioscler Thromb Vasc Biol* 2009, **29**:1164-1171.

41. Shibuya A, Onda K, Kawahara H, Uchiyama Y, Nakayama H, Omi T, Nagaoka M, Matsui H, Hirano T: **Sofalcone, a gastric mucosa protective agent, increases vascular endothelial growth factor via the Nrf2-heme-oxygenase-1 dependent pathway in gastric epithelial cells**. *Biochem Biophys Res Commun* 2010, **398**:581-584.

42. Tanaka H, Nakamura S, Onda K, Tazaki T, Hirano T: **Sofalcone, an anti-ulcer chalcone derivative, suppresses inflammatory crosstalk between macrophages and adipocytes and adipocyte differentiation: implication of heme-oxygenase-1 induction**. *Biochem Biophys Res Commun* 2009, **381**:566-571.

43. Seow A, Yuan JM, Sun CL, Van Den Berg D, Lee HP, Yu MC: **Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study**. *Carcinogenesis* 2002, **23**:2055-2061.

44. Hong F, Freeman ML, Liebler DC: **Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane**. *Chem Res Toxicol* 2005, **18**:1917-1926.

45. Bacon JR, Williamson G, Garner RC, Lappin G, Langouet S, Bao Y: **Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes**. *Carcinogenesis* 2003, **24**:1903-1911.

46. Xu C, Huang MT, Shen G, Yuan X, Lin W, Khor TO, Conney AH, Kong AN: **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* 2006, **66**:8293-8296.

47. Myzak MC, Karplus PA, Chung FL, Dashwood RH: **A novel mechanism of chemoprotection by sulforaphane: inhibition of histone deacetylase**. *Cancer Res* 2004, **64**:5767-5774.

48. Myzak MC, Tong P, Dashwood WM, Dashwood RH, Ho E: **Sulforaphane retards the growth of human PC-3 xenografts and inhibits HDAC activity in human subjects**. *Exp Biol Med (Maywood)* 2007, **232**:227-234.

49. • Hur W, Sun Z, Jiang T, Mason DE, Peters EC, Zhang DD, Luesch H, Schultz PG, Gray NS: **A small-molecule inducer of the antioxidant response element**. *Chem Biol* 2010, **17**:537-547.
The first identification of ARE inducers from high-throughput small molecule screening. It provides a systematic strategy for screening and the final lead compounds. Broad SAR information and mechanistic study of AI-1 are also described.

50. Han JM, Lee YJ, Lee SY, Kim EM, Moon Y, Kim HW, Hwang O: **Protective effect of sulforaphane against dopaminergic cell death**. *J Pharmacol Exp Ther* 2007, **321**:249-256.

51. Tarozzi A, Morroni F, Merlicco A, Hrelia S, Angeloni C, Cantelli-Forti G, Hrelia P: **Sulforaphane as an inducer of glutathione prevents oxidative stress-induced cell death in a dopaminergic-like neuroblastoma cell line**. *J Neurochem* 2009, **111**:1161-1171.

52. Trinh K, Moore K, Wes PD, Muchowski PJ, Dey J, Andrews L, Pallanck LJ: **Induction of the phase II detoxification pathway suppresses neuron loss in Drosophila models of Parkinson's disease**. *J Neurosci* 2008, **28**:465-472.

53. Uchida K, Shibata T: **15-Deoxy-Delta(12,14)-prostaglandin J2: an electrophilic trigger of cellular responses**. *Chem Res Toxicol* 2008, **21**:138-144.

54. Oh JY, Giles N, Landar A, Darley-Usmar V: **Accumulation of 15-deoxy-delta(12,14)-prostaglandin J2 adduct formation with Keap1 over time: effects on potency for intracellular antioxidant defence induction**. *Biochem J* 2008, **411**:297-306.

55. Song NY, Kim DH, Kim EH, Na HK, Surh YJ: **15-Deoxy-delta 12, 14-prostaglandin J2 induces upregulation of multidrug resistance-associated protein 1 via Nrf2 activation in human breast cancer cells**. *Ann N Y Acad Sci* 2009, **1171**:210-216.

56. Lin TN, Cheung WM, Wu JS, Chen JJ, Lin H, Chen JJ, Liou JY, Shyue SK, Wu KK: **15d-prostaglandin J2 protects brain from ischemia-reperfusion injury**. *Arterioscler Thromb Vasc Biol* 2006, **26**:481-487.

57. Itoh K, Mochizuki M, Ishii Y, Ishii T, Shibata T, Kawamoto Y, Kelly V, Sekizawa K, Uchida K, Yamamoto M: **Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-Delta(12,14)-prostaglandin j(2)**. *Mol Cell Biol* 2004, **24**:36-45.

58. Nakamura Y, Yoshida C, Murakami A, Ohigashi H, Osawa T, Uchida K: **Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes**. *FEBS Lett* 2004, **572**:245-250.

59. Murakami A, Takahashi D, Kinoshita T, Koshimizu K, Kim HW, Yoshihiro A, Nakamura Y, Jiwajinda S, Terao J, Ohigashi H: **Zerumbone, a Southeast Asian ginger sesquiterpene, markedly suppresses free radical generation, proinflammatory protein production, and cancer cell proliferation accompanied by apoptosis: the alpha,beta-unsaturated carbonyl group is a prerequisite**. *Carcinogenesis* 2002, **23**:795-802.

60. Ohnishi K, Irie K, Murakami A: **In vitro covalent binding proteins of zerumbone, a chemopreventive food factor**. *Biosci Biotechnol Biochem* 2009, **73**:1905-1907.

61. Lee CY, Chew EH, Go ML: **Functionalized aurones as inducers of NAD(P)H:quinone oxidoreductase 1 that activate AhR/XRE and Nrf2/ARE signaling pathways: synthesis, evaluation and SAR**. *Eur J Med Chem* 2010, **45**:2957-2971.

62. Zhang W, Go ML: **Functionalized 3-benzylidene-indolin-2-ones: inducers of NAD(P)H-quinone oxidoreductase 1 (NQO1) with antiproliferative activity**. *Bioorg Med Chem* 2009, **17**:2077-2090.

63. Masutani H, Otsuki R, Yamaguchi Y, Takenaka M, Kanoh N, Takatera K, Kunimoto Y, Yodoi J: **Fragrant unsaturated aldehydes elicit activation of the Keap1/Nrf2 system leading to the upregulation of thioredoxin expression and protection against oxidative stress**. *Antioxid Redox Signal* 2009, **11**:949-962.

64. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM: **Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential**. *Biochim Biophys Acta* 2009, **1790**:1149-1160.

65. Teichert J, Kern J, Tritschler HJ, Ulrich H, Preiss R: **Investigations on the pharmacokinetics of alpha-lipoic acid in healthy volunteers**. *Int J Clin Pharmacol Ther* 1998, **36**:625-628.

66. Elangovan S, Hsieh TC: **Control of cellular redox status and upregulation of quinone reductase NQO1 via Nrf2 activation by alpha-lipoic acid in human leukemia HL-60 cells**. *Int J Oncol* 2008, **33**:833-838.

67. • Suh JH, Shenvi SV, Dixon BM, Liu H, Jaiswal AK, Liu RM, Hagen TM: **Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid**. *Proc Natl Acad Sci U S A* 2004, **101**:3381-3386.
A good *in vivo* study demonstrating that lipoic acid is able to attenuate aging-related oxidative stress by increasing Nrf2-mediated glutathione production.

68. Farr SA, Poon HF, Dogrukol-Ak D, Drake J, Banks WA, Eyerman E, Butterfield DA, Morley JE: **The antioxidants alpha-lipoic acid and N-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice**. *J Neurochem* 2003, **84**:1173-1183.

69. Quinn JF, Bussiere JR, Hammond RS, Montine TJ, Henson E, Jones RE, Stackman RW Jr: **Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice**. *Neurobiol Aging* 2007, **28**:213-225.

70. Zhang Y, Munday R: **Dithiolethiones for cancer chemoprevention: where do we stand?** *Mol Cancer Ther* 2008, **7**:3470-3479.

71. Egner PA, Kensler TW, Prestera T, Talalay P, Libby AH, Joyner HH, Curphey TJ: **Regulation of phase 2 enzyme induction by oltipraz and other dithiolethiones**. *Carcinogenesis* 1994, **15**:177-181.

72. Prince M, Li Y, Childers A, Itoh K, Yamamoto M, Kleiner HE: **Comparison of citrus coumarins on carcinogen-detoxifying enzymes in Nrf2 knockout mice**. *Toxicol Lett* 2009, **185**: 180-186.

73. Miao W, Hu L, Kandouz M, Batist G: **Oltipraz is a bifunctional inducer activating both phase I and phase II drug-metabolizing enzymes via the xenobiotic responsive element**. *Mol Pharmacol* 2003, **64**:346-354.

74. Kelley MJ, Glaser EM, Herndon JE 2nd, Becker F, Bhagat R, Zhang YJ, Santella RM, Carmella SG, Hecht SS, Gallot L *et al.*: **Safety and efficacy of weekly oral oltipraz in chronic smokers**. *Cancer Epidemiol Biomarkers Prev* 2005, **14**:892-899.

75. Glintborg B, Weimann A, Kensler TW, Poulsen HE: **Oltipraz chemoprevention trial in Qidong, People's Republic of China: unaltered oxidative biomarkers**. *Free Radic Biol Med* 2006, **41**:1010-1014.

76. Hong F, Sekhar KR, Freeman ML, Liebler DC: **Specific patterns of electrophile adduction trigger Keap1 ubiquitination and Nrf2 activation**. *J Biol Chem* 2005, **280**:31768-31775.

77. Christodoulides N, Durante W, Kroll MH, Schafer AI: **Vascular smooth muscle cell heme oxygenases generate guanylyl cyclase-stimulatory carbon monoxide**. *Circulation* 1995, **91**:2306-2309.

78. Platanias LC: **Biological responses to arsenic compounds**. *J Biol Chem* 2009, **284**:18583-18587.

79. Morales AA, Gutman D, Cejas PJ, Lee KP, Boise LH: **Reactive oxygen species are not required for an arsenic trioxide-induced antioxidant response or apoptosis**. *J Biol Chem* 2009, **284**:12886-12895.

80. He X, Ma Q: **NRF2 cysteine residues are critical for oxidant/electrophile-sensing, Kelch-like ECH-associated protein-1-dependent ubiquitination-proteasomal degradation, and transcription activation**. *Mol Pharmacol* 2009, **76**:1265-1278.

81. He X, Ma Q: **Critical cysteine residues of Kelch-like ECH-associated protein 1 in arsenic sensing and suppression of nuclear factor erythroid 2-related factor 2**. *J Pharmacol Exp Ther* 2010, **332**:66-75.