Published in final edited form as:

Biopharm Drug Dispos. 2009 October ; 30(7): 345–355. doi:10.1002/bdd.680.

Nrf2 Plays an Important Role in Coordinated Regulation of Phase II Drug Metabolize Enzymes and Phase III Drug Transporters

Guoxiang Shen^{a,b,c} and Ah-Ng Tony Kong^{a,c}

^aDepartment of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey, 08854

^bDepartment of Metabolism and Pharmacokinetics, Research and Development, Bristol-Myers Squibb Company, Princeton, NJ 08540

Abstract

The nuclear transcription factor E2-related factor 2 (Nrf2) has been shown to play pivotal roles in preventing xenobiotics-induced toxicity and carcinogen-related tumorigenesis. These protective effects are mainly attributed to the induction of Phase II drug metabolizing/detoxification and antioxidant enzymes through the Nrf2-antioxidant response element (ARE) pathways. In this review, we will summarize the current research status on the identification of Nrf2-regulated drug metabolism enzymes (DMEs), especially Phase II DMEs, and Phase III drug transporters. In addition, the molecular mechanisms underlying the coordinated regulation of Phase II DMEs and Phase III transporters are also discussed based on findings published in the literatures.

Keywords

Nrf2; Phase II drug metabolizing enzymes; Phase III transporters; cancer chemoprevention; dietary cancer chemopreventive agents

Introduction

Studies during the past decade have revealed that many drug metabolism enzymes (DME) are coordinately regulated by various nuclear receptors in response to the exposure of both endogenous substances and xenobiotics (reviewed in references [1,2]). Among these nuclear receptors, aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are well characterized for their roles in the induction of cytochrome P450 (CYP450) enzymes [1]. Binding of ligands to the ligand-binding domain (LBD) of a specific nuclear receptor will result in its nuclear translocation and trigger the transcription of its target DME genes. For example, AhR mediates the induction of human CYP1A1 enzyme upon the activation by its ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and its nuclear translocation is facilitated by a chaperone nuclear transporter Ah receptor nuclear translocator (ARNT) [3]. Similarly CAR and PXR are known to be

^oTo whom correspondence should be addressed to either: Guoxiang Shen, Ph.D., Department of Metabolism and Pharmacokinetics, Research and Development, Bristol-Myers Squibb Company, Route 206 and Province Line Road, Princeton, NJ 08540, Phone: 609-252-4089, guoxiang.shen@bms.com Or, A.-N. Tony Kong, Ph.D., Professor II and Glaxo Professor of Pharmaceutics, Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 160 Frelinghuysen Road, Room 228, Piscataway, NJ 08854-8020 USA, Phone: 732-445-3831 ext 228/6, Fax: 732-445-3134, KongT@rci.rutgers.edu. Guest Editor, Dr. Ah-Ng Tony Kong, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ-08854

involved in the coordinated regulation of human CYP2B6, CYP2C9 and CYP3A4 through cross-over mechanisms in response to inducers such as phenobarbital and rifampin [1]. Recently, new findings have shown that all these nuclear receptors are also involved in the regulation of Phase II DMEs as well as phase III drug transporters [1,4]. Given their complementary role in the metabolism and disposition of xenobiotics and endogenous substances, it is not surprising that their regulation also shares similar mechanisms as those for phase I CYP450 system. .

In this review, we will discuss the important but less understood role of another nuclear transcription factor, nuclear transcription factor E2-related factor 2 (Nrf2), in the regulation of DMEs. Nrf2 belongs to the basic lucine zipper nuclear transcription factor family, which share regions of homology with that of *Drosophila* Cap'n'collar (CNC) protein [5]. The Nrf family transcription factors were identified in the search for proteins that regulate the expression of beta-globin genes during differentiation. Human Nrf2 was first isolated in 1994 from a hemin-induced K562 erythroid cell line and showed high sequence homology to the known p45 subunit of NF-E2 (nuclear factor erythroid 2) [5,6]. However, unlike the p45 subunit which is only expressed in erythroid cells and megakaryocytes, expression of Nrf2 has been found in many tissues including liver, intestine, muscle, kidney and lung, suggesting a broad function of Nrf2 in these tissues [5]. Since then, the function of Nrf2 has been extensively investigated using various approaches including the Nrf2-deficient mouse model [7]. Interestingly, although mice having genetic deficient of Nrf2 gene appear to have normal phenotypes, they are more susceptible to butylated hydroxytoluene (BHT) induced lung injury or acetoaminophen induced hepatotoxicity at high doses [7,8]. Therefore, Nrf2 is considered to play an important role in the cellular defense mechanisms against xenobiotic toxicity or carcinogenicity [9]. Further mechanistic studies demonstrated that the major mechanism of protection against carcinogenesis, mutagenesis and other form of toxicity elicited by carcinogen is through Nrf2-mediated induction of DME, particularly Phase II detoxification and antioxidant enzymes, such as glutathione S-transferase (GST), NAD(P)H:quinone oxidoreductase 1(NQO1), heme oxygenase-1 (HO-1) and UDP-glucuronosyltransferase (UGT); and more recently, transporters gene such as multidrug resistance-associated protein (MRP) [10]. Induction of these enzymes in cells is well known to confer resistance against carcinogen, reactive metabolites or reactive oxygen species by enhancing their elimination. Nrf2 regulates the transcription of these target genes through its binding to the consensus antioxidant response element (ARE) within the 5'-flanking promoter/enhancer region of these target genes [11,12]. Because many cancer protective phytochemicals can elicit their cancer chemopreventive effects through Nrf2-dependent up-regulation of Phase II detoxification and antioxidant enzymes in many rodent cancer prevention studies [13], Nrf2 has been considered as an important molecular target to prevent cancer in human [14].

The focus of the current review will be on the Phase II DMEs and phase III drug transporters wherein their basal expression or induction are considered to be Nrf2-dependent. Additional focus will be on how these genes were coordinately regulated by Nrf2-dependent signaling pathway. Although a few recent studies also showed that the regulation of Cytochrome P450 genes such as mouse CYP2A5 and CYP3A11 also appears to be Nrf2-dependent [15,16], the regulation of Phase I oxidative DME by Nrf2 will not be discussed in this review due to limited research in this field to date.

Mechanism of gene regulation by Nrf2

Unlike the general mechanism by which other nuclear receptors regulate majority of DMEs that introduced above, Nrf2-mediated gene transcription is not activated through ligand-receptor binding. Compare to nuclear receptors such as PXR, although Nrf2 also has a

DNA-binding domain (DBD), it does not contain a ligand binding domain (LBD) that would bind to structurally specific ligands. This is consistent with previous findings that Nrf2 activators have very diverse chemical structures and at least nine structurally dissimilar classes of inducers have been found [17]. Interestingly, many Nrf2 chemical activators are electrophilic compounds.

Activation of Nrf2-mediated gene transcription involves very complex process and has been extensively investigated (reviewed in reference [9]). Under homeostatic condition, Nrf2 is mainly retained in the cytosol by the Keap1 protein, which is a homologue of the *Drosophila* actin binding protein Kelch that binds to the actin cytoskeleton. Keap1 can suppress the Nrf2 function by binding to the N-terminal Neh2 domain of Nrf2 and retains Nrf2 in the cytoplasm [18,19]. Therefore, the Neh domain on the N-terminus of Nrf2 is distinct from the activation function 1 (AF-1) domain on other nuclear receptor such as PXR and plays a critical role in the regulation of basal activity of Nrf2 through the binding to Keap1. However, upon the challenge of oxidative or chemical stress, the Keap1's sequestration can be reversed so that Nrf2 can be released from Keap1 and translocates into the nucleus to trigger the transcription of its target genes. Since many prototypical Nrf2 chemical activators or phase II inducers are electrophiles, it has been demonstrated that some of these electrophiles can directly modify the four most reactive cysteine residues on Keap1 [20]. Since Keap1 is a high cysteine-rich protein (mouse Keap1 contains 25 cysteines) that are very sensitive to oxidative/electrophile stress, conjugative modification of these cysteines on the Keap1 protein by Nrf2 activators could disrupt the binding between Keap1 and Nrf2, thereby to release the Nrf2 from the complex. Interestingly, studies have also shown that binding to the Keap1 protein also appears to accelerate the degradation of Nrf2 as compared to that of the unbound Nrf2 in the cytoplasm, which further explain the suppression effect of Keap1 binding under homeostatic condition [21,22]. Once Nrf2 is released from Keap1 protein, it will translocate into the nucleus and form heterodimers with small Maf proteins and other Bzip transcription factors [23,24]. Binding of the heterodimers, along with other nuclear transcriptional co-activators such as CBP [25], to the *cis*-acting element of ARE-containing target genes will result in the transcription of a battery of Nrf2 mediated genes.

It is noteworthy that in addition to the direct modification on the Keap1 protein to disrupt the Keap1/Nrf2 binding and activate Nrf2, several kinase signaling cascades including phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC) and mitogen-activated protein kinases (MAPKs) have also been proposed to control the release of Nrf2 from Keap1 binding and its nuclear translocation, especially when these kinases are activated by Phase II enzyme inducers or reactive electrophiles (reviewed in reference [26]). For example, PKC can directly phosphorylate Nrf2 N-terminus to facilitate its nuclear translocation [27]; while MAPK ERK, JNK and p38 pathways could play differential roles in the regulation of Nrf2 transactivation activity [25], probably through the regulation of its binding to AREs and other nuclear transcription co-activators such as CBP.

Interestingly, several findings also indicated that Nrf2 activity can be regulated at transcriptional level. Kwak et al. identified two ARE-like (AREL) elements in the 1 kb 5'-flanking promoter region of the mouse Nrf2 gene (Table 1) [28]. Based on deletion and mutagenesis studies, both AREL elements were found to be necessary to fully activate Nrf2 expression in response to a potent Nrf2 activator 3H-1,2-dithiole-3-thione (D3T). The binding of these two AREL elements to Nrf2 were also demonstrated by both electrophoretic mobility-shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay. Therefore, results from this study suggest that Nrf2 can auto-regulate its expression through the AREL elements in the proximal region of its promoter in response to its activators. The elevated nuclear level of Nrf2 from this mechanism will further enhance the expression of its target genes. In addition to this auto-regulation mechanism, Nrf2

expression was also demonstrated to be under the control of AhR [29]. Three AhR specific xenobiotic response element (XRE)-like elements (XREL) were identified after examining the DNA sequence of the promoter region of the mouse Nrf2 gene. Functionality of these XRELs in the induction of Nrf2 expression by AhR ligand TCDD was also confirmed using various approaches including site mutagenesis, EMSA, ChIP and siRNA of AhR. This new finding suggests that Nrf2-mediated gene transcription could be a downstream event of the AhR/ARNT pathway, and also suggests the existence of an integrated system to coordinately regulate both Phase I oxidation and Phase II DME to detoxify xenobiotics and carcinogens.

Nrf2 regulated Phase II drug metabolize enzymes and drug transporters

Phase II DMEs and antioxidant enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione S-transferases (GST) play critical roles in protecting cells from xenobiotic- or carcinogen-induced oxidative stress, cytotoxicity, mutagenicity and carcinogenicity. Because many of these enzymes were found to be induced by planar aromatic compounds and phenolic antioxidants in early studies [11], both AhR-dependent and -independent regulation mechanisms were proposed [30,31]. Efforts to address the AhR-independent regulation mechanism lead to the identification of consensus ARE *cis*-element [11] in the 5'-flanking promoter region of these genes such as Rat GST Ya subunit and NQO1. However, the ARE-mediated regulation mechanism was not completely understood until the isolation of nuclear transcription factor Nrf2 that specifically binds to the *cis*-acting ARE component. Since then, many Nrf2 target genes were identified based on the existence and functional characterization of ARE or ARE-like elements in their 5'-flanking promoter region. Interestingly, although the core ARE sequence has been identified as 5'-GTGACnnnGC-3' where n represents any nucleotide [11], but this sequence alone is not sufficient to mediate the induction [12]. In stead, the ARE sequence has been defined as 5'-TMAnnnRTGAYnnnGCRWW-3' (M = A or C, R = A or G, Y = C or T, W = A or T, S = G or C), in which Nrf2 is proposed to bind to the RTGAYnnn portion of the core sequence (underlined). In addition, the presence a tandem core sequence (underlined) at upstream 5'-flanking region of the consensus sequence will confer the maximum induction capability by inducers. Importantly, the establishment of genetic Nrf2-deficient mouse strain provided the critical link to identify Nrf2 target genes. With the aid of genome-wide microarray techniques, many Nrf2-dependent genes are characterized based on their differential basal/constitutive or inducible expression pattern between Nrf2 wild type and Nrf2 knockout (KO) mice.

Phase II drug metabolize enzymes

NQO1

NQO1 detoxifies quinones and its derivatives to protect cells against redox cycling and oxidative stress. Human NQO1 [32] gene contains a twenty-four base pairs of ARE in its promoter region that is essential for its high basal transcription as well as the induction by prototypical Nrf2 activator such as *tert*-butyl-hydroquinone (t-BHQ) and β -naphthoflavone (β -NF) (Table 1). Interestingly, the consensus ARE in human NQO1 contains one perfect and one imperfect AP1 element that may suppress the basal expression of NQO1 by the binding of c-Fos and Fra1 [33]. Nevertheless, both Nrf1 and Nrf2 were found to be involved in the ARE-mediated expression and induction of human NQO1 by these xenobiotics. Similarly, in addition to the Xenobiotic Response Element (XRE), a consensus ARE sequence was also characterized in rat NQO1 gene (Table 1) and was involved in the induction of rat NQO1 by t-BHQ and β -NF, but not by TCDD [34]. Although ARE hasn't been reported in mouse NQO1 gene, but the induction of NQO1 by Phase II enzyme inducer including D3T and butylated hydroxyanisole (BHA) was largely abolished in both the liver

and small intestine (SIT) of Nrf2-deficient mouse as compared to that in the Nrf2 wild type mouse [23,35]. Interestingly, the basal level expression of NQO1 was only significantly decreased in the SIT of the Nrf2 knockout mouse, but not affected in the liver [24,35,36]. These findings from the Nrf2-deficient mouse studies suggest that the basal transcriptional regulation of NQO1 gene in the liver probably involves multiple pathways other than Nrf2-ARE pathway [24]. This hypothesis is very likely to be true considering the presence of numerous nuclear receptors controlling the expression of DMEs in the liver than in the SIT, which may result in the overall lower contribution from Nrf2-dependent mechanism in the liver than in SIT on the basal transcription of mouse NQO1.

GST

The GSTs are Phase II enzymes that catalyze the conjugation of reduced glutathione to electrophilic xenobiotics or reactive metabolites, products from Phase I oxidation. Mouse and rat alpha class GST Ya subunit gene is the first GST gene its expression and induction by Phase II inducers are found to be dependent on the ARE sequence in its 5'-flanking region (Table 1) [11,17,37,38]. The promoter regions of several GSTs Pi class of genes in both mouse (GSTpiA and GSTpiB) and rat (GSTpi) have also been investigated [23,39,40]. Their ARE sequences containing the ARE core sequence differ slightly from the ARE consensus (Table 1).

In studies utilizing the homozygous Nrf2-deficient mouse model, researchers have found that the induction of GST alpha, Mu and pi isoforms was largely impaired in both the liver and the SIT of Nrf2-deficient mouse after BHA treatment [23]. In another similarly designed study [24], although the basal level of class alpha and Mu isoforms were 3–6 folds lower in the SIT of Nrf2-deficient mouse, the induction of some of these GSTs (GSTA1/2,4 and M1) by ethoxyquin (EQ) and BHA seems to be independent of Nrf2 status based on western blotting analysis. Although the promoter of GSTA1 contains a perfect ARE located 4 bp downstream from a closely related imperfect ARE (Table 1) [23], the region encompasses both elements can not form an effective palindromic structure but a head-to-tail tandem direct repeat which may not be able to bind Nrf2 complex tightly [12,24]. In the same study, constitutional expression level of GSTpi class was not affected in SIT. However, study using microarray technique to compare the global gene expression profiles between Nrf2 wild-type and Nrf2-deficient mouse showed that basal level of few GSTs (GSTM1/2) were decreased by 3~6 folds in the Nrf2-deficient mouse [36]. When treated with a potent Phase II inducer sulforaphane (SFN), the inducibility of many GSTs (GSTA1/2/3 and GSTM1) was diminished due to the deficiency of Nrf2 in SIT. Based on studies described above, although incongruent results have been observed regarding the inducibility of individual GST isoforms (such as GSTPi), it is likely that the regulation of constitutive and inducible expression of GSTs by Nrf2 is dependent on multiple factors, including chemical inducers, tissues and species.

γGCS

In accordance with the induction of GSTs, expression of heavy chain of γGCS, an enzyme which catalyze the rate-limiting step in the *de novo* synthesis pathway of glutathione, was also found to be dependent on Nrf2 in the mouse. In the liver of Nrf2-deficient mouse, decreased basal level of γGCS heavy chain mRNA was observed [8]; furthermore, its induction was found to be largely abolished in SIT of Nrf2-deficient mouse in response to ethoxyquin (EQ) and BHA induction [24]. Two AREs (ARE3 and ARE4) separated by 34 bp were identified in the -3802 to -2752 bp promoter region of human γGCS heavy chain gene (Table 1) [41]. Interestingly, although ARE3 seems to be not involved to the β-NA induction, the tandem structure between ARE3 and ARE4 dramatically enhance the basal expression level of γGCS promoter-linked luciferase reporter activity. This result is

consistent with the previous functional analysis of ARE sequences [12]. The identification γ GCS and various GSTs as Nrf2 target genes is another good example of coordinated regulated mechanism on the glutathione-related detoxification process.

UGT

UDP-glucuronosyltransferases (UGTs) catalyze one of the major Phase II conjugation and detoxification process, the glucuronidation. Decreased basal expression level of UGT1A6 was observed in the liver, SIT and lung of Nrf2-deficient mouse [7,8,36,42,43]. In addition to the binding sites of AhR and PXR receptors, human UGT1A6 gene promoter region also has three ARE-like sequences (Table 1) [44]. However, the involvement of these three AREs in t-BHQ induction was not fully characterized, since the t-BHQ effects were not abolished by site-directed mutagenesis in both ARE3 and XRE (15bp upstream of ARE3), while the function of the other two AREs containing consensus ARE sequence were not investigated in that study. In contrast, rat UGT1A6 gene promoter has no ARE-like sequence but an AhR binding sequence [45], but significant induction of UGT1A6 and UGT1A7 were observed in rat liver after treatments of Nrf2 activators EQ and oltiparaz (OTZ) [46]. Several other UGTs were also been implicated as Nrf2 target genes. For example, expression of UGT1A8 and UGT1A10 were inhibited by siRNA of Nrf2 in human caco-2 cells [47]. UGT2 family members such as UGT2B1, UGT2B3, UGT2B12 were all induced by prototypical Nrf2 activators EQ and OTZ in rat liver or duodenum [46]. UGT2B5 induction was also found to be largely diminished in Nrf2-deficient mouse after curcumin treatment [48]. Overall, some of the UGTs have been shown to be Nrf2 target genes, however, the contribution of Nrf2-ARE pathway on the regulation of specific UGTs may be species-dependent and may also not be as prominent due to involvement of other nuclear receptors such as PXR and AhR.

Sulfotransferases (SULT)

Most recently, the expression of several sulfotransferases (SULTs) and 3'-phosphoadenosine 5'-phosphosulfate synthase (PAPSs) isozymes has been demonstrated to be regulated by several prototypical Nrf2 activators such as OTZ, EQ and BHA (Table 1) [49]. The induction of SULTs and PAPS2 by these chemical activators is more prominent than AhR, CAR, PXR and PPAR activators, and the induction was only observed in the liver of male mice (not in females). This result is in agreement with the findings that many of these inducible SULTs contains ARE sequences in their promoter regions based on the authors' *in silico* DNA analysis (unpublished observations). Interestingly, most of the AhR activators suppress the expression of both SULTs and PAPSs, which may explain the observation that OTZ induced much less SULTs than BHA and EQ since OTZ can also activate the AhR pathway. Since Nrf2-ARE pathway has been proposed to be downstream of the AhR pathway, the differential regulation of SULTs and PAPSs by AhR activators (mainly suppression) and Nrf2 activators (mainly induction) indicated a more complex regulatory mechanism. One possible explanation is that these chemical inducers may also affect many other pathways at high concentrations *in vivo*.

Drug Transporters

Multidrug Resistance-Associated Protein Transporters (MRPs)

Drug transporters can function synergistically with Phase II conjugating enzymes to facilitate the cellular excretion of conjugated metabolites, which is believed to be an important detoxification process to remove carcinogens, reactivate metabolites and xenobiotics. Since Nrf2 has been shown to play a pivotal role in the induction of many Phase II enzymes, it is not surprising recently to find that Nrf2-ARE pathway also regulates the expression of many drug transporters, such as multidrug resistance-associated protein

transporters (MRPs), especially to the response of oxidative and electrophilic stress. MRP transporters are adenosine triphosphate-dependent drug transporters that efflux endogenous substances (such as bilirubin), xenobiotics and their metabolite (especially phase II conjugated metabolites). Using prototypical chemical activators for different nuclear receptors that regulate DME genes, Klaassen's group has identified many MRPs that were induced by Nrf2 activators [10]. Maher *et al.* first found that mouse liver MRP2-6 were all induced by Nrf2 activators including OTZ, BHA and EQ [50]. In a more mechanistic follow-up study [51], the same group demonstrated that basal expression as well as the induction of mouse liver Mrp2, 3, 4 genes by Nrf2 activators BHA and OTZ are Nrf2-dependent. *In vitro* mechanistic studies in mouse Hepa1c1c7 cells demonstrated the specific binding of Nrf2 to the multiple putative ARE sequences in the promoter region of all three MRPs (Table 1). These results are consistent with the identification of ARE or ARE-like sequences in both mouse and human MRP1/2 genes (Table 1) [52–54]. The binding of Nrf2 to the mouse MRP2 AREs were also demonstrated in the previous study [53]. Regulation of rat MRP gene by Nrf2 has not been extensively investigated, however, induction of rat liver MRP3 gene by phenolic acid GA (gallic acid) and p-CA (p-coumaric acid) was observed and this induction was associated with the accumulation of Nrf2 level in liver cells [55]. Taken together, activation of Nrf2-ARE pathway by chemical inducers appears to be able to stimulate the coordinated induction of multiple hepatic MRPs.

OATPs

The regulation of organic anion-transporting polypeptides (OATPs) in the mouse liver was also investigated using similar approaches by Klaassen's group [15] as MRP's as described above. However, unlike MRPs, only mouse OATP2B1 genes was induced by Nrf2 activators BHA and EQ, suggesting the potential limited involvement of Nrf2 in the regulation of this class of transporters.

Summary

Research over the past decade utilizing mouse genetic knockout models, promoters/enhancers mapping technologies and prototypical chemical inducers have revealed that DMEs are coordinately regulated by a wide variety of nuclear receptors including AhR, CAR, PXR, FXR, LXR and PPAR [1]. However, the function of Nrf2 in the regulation of DMEs, especially Phase II DMEs and drug transporters, has not been fully investigated and gained much attention. Although some Phase II conjugation and transporter genes are characterized as Nrf2-dependent genes based on their inducibility by prototypical Nrf2 chemical activators or decreased basal expression levels in Nrf2-deficient mice, evidence of direct binding between Nrf2 and AREs in their promoter region is still necessary to support that conclusion. In addition, the contribution of Nrf2 in the regulation of these DMEs compared to other nuclear receptors (such as CAR and PXR) may highly dependent on the type of chemical inducers/activators, since numerous nuclear receptor binding elements were reported in the promoter regions of these genes such as UGT1A6 and MRP2. In addition, cross-talk between Nrf2-ARE pathway and other pathways such as AhR and CAR has also been proposed [29,56], which makes the interpretation of induction studies using chemical inducers more complicated because of the lack of specificity of some of these chemical activators. Nevertheless, the coordinated regulation of phase II antioxidant and conjugation enzymes, as well as phase III drug transporters, and the potential cross-talk with other nuclear receptors, clearly indicates the pivotal role of Nrf2 in xenobiotics metabolism and detoxification process at these in these rodent models. Translating these observations to human situations are clearly warranted.

Acknowledgments

We thank all the members of Ah-Ng Tony Kong's laboratory for their helpful discussions. This study was supported in part by Institutional Funds and by RO1-CA094828, RO1-CA073674 and RO1-CA118947 to Dr Ah-Ng Tony Kong from the National Institutes of Health (NIH).

References

1. Nakata K, Tanaka Y, Nakano T, Adachi T, Tanaka H, Kaminuma T, et al. Nuclear receptor-mediated transcriptional regulation in Phase I, II, and III xenobiotic metabolizing systems. *Drug Metab Pharmacokinet.* 2006; 21:437–457. [PubMed: 17220560]
2. Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch Pharm Res.* 2005; 28:249–268. [PubMed: 15832810]
3. Lusska A, Shen E, Whitlock JP Jr. Protein-DNA interactions at a dioxin-responsive enhancer. Analysis of six bona fide DNA-binding sites for the liganded Ah receptor. *J Biol Chem.* 1993; 268:6575–6580. [PubMed: 8384216]
4. Urquhart BL, Tirona RG, Kim RB. Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. *J Clin Pharmacol.* 2007; 47:566–578. [PubMed: 17442683]
5. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A.* 1994; 91:9926–9930. [PubMed: 7937919]
6. Chan JY, Han XL, Kan YW. Isolation of cDNA encoding the human NF-E2 protein. *Proc Natl Acad Sci U S A.* 1993; 90:11366–11370. [PubMed: 8248255]
7. Chan K, Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. *Proc Natl Acad Sci U S A.* 1999; 96:12731–12736. [PubMed: 10535991]
8. Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, et al. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. *Toxicol Sci.* 2001; 59:169–177. [PubMed: 11134556]
9. Shen G, Jeong WS, Hu R, Kong AN. Regulation of Nrf2, NF-kappaB, and AP-1 signaling pathways by chemopreventive agents. *Antioxid Redox Signal.* 2005; 7:1648–1663. [PubMed: 16356127]
10. Klaassen CD, Slitt AL. Regulation of hepatic transporters by xenobiotic receptors. *Curr Drug Metab.* 2005; 6:309–328. [PubMed: 16101571]
11. Rushmore TH, Morton MR, Pickett CB. The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J Biol Chem.* 1991; 266:11632–11639. [PubMed: 1646813]
12. Wasserman WW, Fahl WE. Functional antioxidant responsive elements. *Proc Natl Acad Sci U S A.* 1997; 94:5361–5366. [PubMed: 9144242]
13. Surh YJ. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer.* 2003; 3:768–780. [PubMed: 14570043]
14. Lee JS, Surh YJ. Nrf2 as a novel molecular target for chemoprevention. *Cancer Lett.* 2005; 224:171–184. [PubMed: 15914268]
15. Cheng X, Maher J, Dieter MZ, Klaassen CD. Regulation of mouse organic anion-transporting polypeptides (Oatps) in liver by prototypical microsomal enzyme inducers that activate distinct transcription factor pathways. *Drug Metab Dispos.* 2005; 33:1276–1282. [PubMed: 15919853]
16. Abu-Bakar A, Lamsa V, Arpiainen S, Moore MR, Lang MA, Hakkola J. Regulation of CYP2A5 gene by the transcription factor nuclear factor (erythroid-derived 2)-like 2. *Drug Metab Dispos.* 2007; 35:787–794. [PubMed: 17303623]
17. Prester T, Holtzclaw WD, Zhang Y, Talalay P. Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci U S A.* 1993; 90:2965–2969. [PubMed: 8385353]
18. Adams J, Kelso R, Cooley L. The kelch repeat superfamily of proteins: propellers of cell function. *Trends Cell Biol.* 2000; 10:17–24. [PubMed: 10603472]

19. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev.* 1999; 13:76–86. [PubMed: 9887101]
20. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, et al. 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. [PubMed: 12193649]
21. Nguyen T, Sherratt PJ, Huang HC, Yang CS, Pickett CB. Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. *J Biol Chem.* 2003; 278:4536–4541. [PubMed: 12446695]
22. 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. [PubMed: 14585973]
23. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun.* 1997; 236:313–322. [PubMed: 9240432]
24. McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LJ, et al. The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res.* 2001; 61:3299–3307. [PubMed: 11309284]
25. Shen G, Hebbar V, Nair S, Xu C, Li W, Lin W, et al. Regulation of Nrf2 transactivation domain activity. The differential effects of mitogen-activated protein kinase cascades and synergistic stimulatory effect of Raf and CREB-binding protein. *J Biol Chem.* 2004; 279:23052–23060. [PubMed: 15020583]
26. Kong AN, Owuor E, Yu R, Hebbar V, Chen C, Hu R, et al. Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). *Drug Metab Rev.* 2001; 33:255–271. [PubMed: 11768769]
27. Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. *J Biol Chem.* 2002; 277:42769–42774. [PubMed: 12198130]
28. Kwak MK, Itoh K, Yamamoto M, Kensler TW. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. *Mol Cell Biol.* 2002; 22:2883–2892. [PubMed: 11940647]
29. Miao W, Hu L, Scrivens PJ, Batist G. Transcriptional regulation of NF-E2 p45-related factor (NRF2) expression by the aryl hydrocarbon receptor-xenobiotic response element signaling pathway: direct cross-talk between phase I and II drug-metabolizing enzymes. *J Biol Chem.* 2005; 280:20340–20348. [PubMed: 15790560]
30. De Long MJ, Santamaria AB, Talalay P. Role of cytochrome P1-450 in the induction of NAD(P)H:quinone reductase in a murine hepatoma cell line and its mutants. *Carcinogenesis.* 1987; 8:1549–1553. [PubMed: 2820604]
31. Talalay P, De Long MJ, Prochaska HJ. Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc Natl Acad Sci U S A.* 1988; 85:8261–8265. [PubMed: 3141925]
32. Li Y, Jaiswal AK. Regulation of human NAD(P)H:quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element. *J Biol Chem.* 1992; 267:15097–15104. [PubMed: 1340765]
33. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc Natl Acad Sci U S A.* 1996; 93:14960–14965. [PubMed: 8962164]
34. Favreau LV, Pickett CB. Transcriptional regulation of the rat NAD(P)H:quinone reductase gene. Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J Biol Chem.* 1991; 266:4556–4561. [PubMed: 1900296]

35. Kwak MK, Egner PA, Dolan PM, Ramos-Gomez M, Groopman JD, Itoh K, et al. Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutat Res.* 2001; 480–481:305–315.
36. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res.* 2002; 62:5196–5203. [PubMed: 12234984]
37. Friling RS, Bensimon A, Tichauer Y, Daniel V. Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element. *Proc Natl Acad Sci U S A.* 1990; 87:6258–6262. [PubMed: 2166952]
38. Rushmore TH, King RG, Paulson KE, Pickett CB. Regulation of glutathione S-transferase Ya subunit gene expression: identification of a unique xenobiotic-responsive element controlling inducible expression by planar aromatic compounds. *Proc Natl Acad Sci U S A.* 1990; 87:3826–3830. [PubMed: 2160079]
39. Xu X, Stambrook PJ. Two murine GSTpi genes are arranged in tandem and are differentially expressed. *J Biol Chem.* 1994; 269:30268–30273. [PubMed: 7982937]
40. Okuda A, Imagawa M, Maeda Y, Sakai M, Muramatsu M. Structural and functional analysis of an enhancer GPEI having a phorbol 12-O-tetradecanoate 13-acetate responsive element-like sequence found in the rat glutathione transferase P gene. *J Biol Chem.* 1989; 264:16919–16926. [PubMed: 2777813]
41. Mulcahy RT, Wartman MA, Bailey HH, Gipp JJ. Constitutive and beta-naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence. *J Biol Chem.* 1997; 272:7445–7454. [PubMed: 9054446]
42. Chan K, Han XD, Kan YW. An important function of Nrf2 in combating oxidative stress: detoxification of acetaminophen. *Proc Natl Acad Sci U S A.* 2001; 98:4611–4616. [PubMed: 11287661]
43. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzymeinducers is lost in nrf2 transcription factor-deficient mice. *Proc Natl Acad Sci U S A.* 2001; 98:3410–3415. [PubMed: 11248092]
44. Munzel PA, Schmohl S, Buckler F, Jaehrling J, Raschko FT, Kohle C, et al. Contribution of the Ah receptor to the phenolic antioxidant-mediated expression of human and rat UDP-glucuronosyltransferase UGT1A6 in Caco-2 and rat hepatoma 5L cells. *Biochem Pharmacol.* 2003; 66:841–847. [PubMed: 12948865]
45. Auyeung DJ, Kessler FK, Ritter JK. An alternative promoter contributes to tissue-and inducer-specific expression of the rat UDP-glucuronosyltransferase 1A6 gene. *Toxicol Appl Pharmacol.* 2001; 174:60–68. [PubMed: 11437649]
46. Shelby MK, Klaassen CD. Induction of rat UDP-glucuronosyltransferases in liver and duodenum by microsomal enzyme inducers that activate various transcriptional pathways. *Drug Metab Dispos.* 2006; 34:1772–1778. [PubMed: 16855052]
47. Yang XY, Zhao WP, Li YQ, Sun ZY, Zhang Y, Guo YT, et al. [The role of NF-E2-related factor 2 in the induction of uridine 5'-diphosphate-glucuronosyltransferase 1A and its isoforms by epigallocatechin gallate in colon cancer cells]. *Zhonghua Yi Xue Za Zhi.* 2006; 86:82–87. [PubMed: 16620709]
48. Shen G, Xu C, Hu R, Jain MR, Gopalkrishnan A, Nair S, et al. Modulation of nuclear factor E2-related factor 2-mediated gene expression in mice liver and small intestine by cancer chemopreventive agent curcumin. *Mol Cancer Ther.* 2006; 5:39–51. [PubMed: 16432161]
49. Alnouti Y, Klaassen CD. Regulation of sulfotransferase enzymes by prototypical microsomal enzyme inducers in mice. *J Pharmacol Exp Ther.* 2008; 324:612–621. [PubMed: 17993606]
50. Maher JM, Cheng X, Slitt AL, Dieter MZ, Klaassen CD. Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metab Dispos.* 2005; 33:956–962. [PubMed: 15833929]
51. Maher JM, Dieter MZ, Aleksunes LM, Slitt AL, Guo G, Tanaka Y, et al. Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters via the nuclear

- factor-E2-related factor-2 transcriptional pathway. *Hepatology*. 2007; 46:1597–1610. [PubMed: 17668877]
52. Hayashi A, Suzuki H, Itoh K, Yamamoto M, Sugiyama Y. Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. *Biochem Biophys Res Commun*. 2003; 310:824–829. [PubMed: 14550278]
53. Vollrath V, Wielandt AM, Iruretagoyena M, Chianale J. Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. *Biochem J*. 2006; 395:599–609. [PubMed: 16426233]
54. Stockel B, Konig J, Nies AT, Cui Y, Brom M, Keppler D. Characterization of the 5'-flanking region of the human multidrug resistance protein 2 (MRP2) gene and its regulation in comparison with the multidrug resistance protein 3 (MRP3) gene. *Eur J Biochem*. 2000; 267:1347–1358. [PubMed: 10691972]
55. Yeh CT, Yen GC. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance-associated protein 3 mRNA expression. *J Nutr*. 2006; 136:11–15. [PubMed: 16365051]
56. Slitt AL, Cherrington NJ, Dieter MZ, Aleksunes LM, Scheffer GL, Huang W, et al. trans-Stilbene oxide induces expression of genes involved in metabolism and transport in mouse liver via CAR and Nrf2 transcription factors. *Mol Pharmacol*. 2006; 69:1554–1563. [PubMed: 16449384]
57. Li Y, Jaiswal AK. Identification of jun-B as third member in human antioxidant response element-nuclear proteins complex. *Biochem Biophys Res Commun*. 1992; 188:992–996. [PubMed: 1445367]
58. Kauffmann HM, Pfannschmidt S, Zoller H, Benz A, Vorderstemann B, Webster JI, et al. Influence of redox-active compounds and PXR-activators on human MRP1 and MRP2 gene expression. *Toxicology*. 2002; 171:137–146. [PubMed: 11836020]

Table 1

Summary of Nrf2-regulated drug metabolism enzymes (DME) and transporters

Genes	Species/Tissue	Reported ARE sequence	Inducer	References
Nrf2	mouse	ARE1: (−492) CCTGACTCCGCCAT	D3T	[28]
NQO1	human	(−472)5'-GCAGTCACAGT <u>GACTCAGC</u> AGAATCT-3' (−445)	β-NF, t-BHQ	[57]
	rat	(−434)5'-TCTAGAGTCACAGT <u>GACTTGGC</u> AAAATCTGA-3' (−404)	β-NF, t-BHQ	[34]
	Mouse (liver & SIT)		BHA, D3T, SFN, I3C, OTZ, CMRN, AGLN	[23,24,35,36]
GST				
GSTA1	mouse	(−754)5'-TAGCTTGGAAATGACATTGCTAATGGT <u>GACAAAGCA</u> ACTTT-3' (−714)	β-NF, t-BHQ, SFN; catechol, hydroquinone, 3- MC, EQ	[17,37]
GSTA	rat	(−722)5'-GAGCTTGGAAATGGCATTGCTAATGGT <u>GACAAAGCA</u> ACTTT-3' (−682)		[11]
GSTP1A	mouse	5'- <u>TGACTCAGCAT</u> CCGGGGCGG-3'		[23,39]
GSTP1B	mouse	5'- <u>TGAGTCAGCAT</u> CCGGGGCGG-3'		[23,39]
GSTP1	rat	5'-CAAAATAGTCAGTCACTATGATTCAGCAACAAACCC-3'		[40]
GSTM1/2/5	mouse (SIT)		curcumin	[24,36,48]
γGCS	human	5'-CGCACCGCCTCCCCGTGACTCAGCGCTTTGTGCGGG-3'	β-NA, EQ, BHA	[41][41]
	Mouse (liver & SIT)			[8,24,48]
UGT				
UGT1A6	human	ARE1: 5'-CAGAAGCTCAGGTGAAGCTGACACGGCCATAGT-3' ARE2: 5'-TTACCCACAACTTCTGTCTGACTTGGCAAAAAT-3' ARE3: 5'-AACTCGCGTGCCAGCCAGGTGTGCATGACTAGCTCTGG-3' (XRE)	t-BHQ	[44]
	mouse			[7,8,36]
	rat (liver)		EQ, OTZ	[46]
UGT1A7	rat (liver)		EQ, OTZ	[46]
UGT1A8	human		EGCG	[47]
UGT1A10	human		EGCG	[47]
UGT2B1	rat (liver)		EQ, OTZ	[46]
UGT2B3	rat (liver & SIT)		EQ, OTZ	[46]
UGT2B5	mouse (SIT)		curcumin	[36,48]
UGT2B12	rat (liver & SIT)		EQ, OTZ	[46]
SULT				
SULT1A1	mouse(liver)		BHA	[49]
SULT1B1	mouse(liver)		OTZ, EQ	[49]
SULT1C1	mouse(liver)		BHA	[49]
SULT1C2	mouse(liver)		EQ	[49]
SULT1D1	mouse(liver)		BHA	[49]
SULT1E1	mouse(liver)		BHA	[49]
SULT3A1	mouse(liver)		EQ, BHA	[49]

Genes	Species/Tissue	Reported ARE sequence	Inducer	References
SULT5A1	mouse(liver)		OTZ, EQ	[49]
MRP				
MRP1	human	ARE1: (−499) 5′-GTGACTCAGC-3′ (−490) ARE2: (−1843) 5′-GTGACAAAGCA-3′ (−1833)	t-BHQ, quercetin	[52,58]
	mouse	ARE1: (−1240) 5′-TTGAGTTAGCT-3′ (−1229) ARE2: (−2020) 5′-TTGAGACAGCA-3′ (−2010)	DEM	[52]
MRP2	human	AREL1: (−340) 5′-TGAAAGACTGTGCACTCTTGA-3′ AREL2: (−1090) 5′-AAGCAATTAAAGTGACAGTACAAAAGG-3′ AREL3: (−2865) 5′-TACTGATGCTGCCCTTT-3′ (−2856)	2AAF	[53,54]
	mouse	ARE1: (−95) 5′-ATGACATAGCA-3′ (−85) ARE2: (−1391) 5′-CTGACATGGCA-3′ (−1381)	t-BHQ, BHA, β-NF, 2AAF, OTZ, EQ	[50,51,53]
MRP3	mouse	ARE1: (−9919) 5′-GCAGTTAGTTAATGACTCTGCTTGTGAGTATCA-3′	t-BHQ, BHA, OTZ, EQ	[50,51]
	rat (liver)		GA, p-CA	[55]
MRP4	mouse	ARE1: (−3295) 5′-AGAGGCTCACAGTGACCTGGCAAAAAGCAG-3′ AREL2&3: (−3753) 5′-GAGCTGCTGAGTCACTCCTGACTCCAGCTTCTG-3′ ARE4: (−9104) 5′-TCTTAGACGTACAGTGACGTTGCTGTCTCCTAAAC-3′ ARE5: (−10462) 5′-GTGCCAACCTCCTCTGACAAGGCCCTTGGCTCCTG-3′	t-BHQ, BHA, OTZ, EQ	[50,51]
MRP5	mouse (liver)		BHA, OTZ, EQ	[50,51]
MRP6	mouse (liver)		BHA, OTZ, EQ	[50,51]
OATP2B	mouse (liver)		BHA, EQ	[15]

The consensus ARE sequence has been characterized as (A/G) TGA(C/T)NNNGC(A/G) (N represents any nucleotides) and the core ARE sequence is TGACNNNGC. The core ARE sequences in the promoter region are underlined (imperfect nucleotides were bolded). The position of ARE or ARE like (AREL) sequences is also provided according to the original reports.

Abbreviations: β-naphthoflavone (β-NF); *tert*-butyl-hydroquinone (t-BHQ); 1,2-dithiole-3-thione (D3T); butylated hydroxyanisole (BHA); ethoxyquin (EQ); oltipraz (OTZ); indole-3-carbinol (I3C); sulforaphane (SFN); coumarin (CMRN); α-agelicalactone (AGLN); 3-methylcholanthrene (3-MC); diethyl maleate (DEM); (−)-epigallocatechin-3-gallate (EGCG); 2-acetylaminofluorene (2AAF); gallic acid (GA); p coumaric acid (p-CA)