Nrf2, a multi-organ protector?

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ABSTRACT **NF-E2-related factor 2 (Nrf2) is a basic leucine zipper transcription factor that binds to the promoter sequence "antioxidant responsive element (ARE)" leading to coordinated up-regulation of ARE-driven detoxification and antioxidant genes. Since the expression of a wide array of antioxidant and detoxification genes are positively regulated by the ARE sequence, Nrf2 may serve as a master regulator of the ARE-driven cellular defense system against oxidative stress. In support of this, numerous studies have shown that Nrf2 protects many cell types and organ systems from a broad spectrum of toxic insults and disease pathogenesis. This Nrf2-conferred, multi-organ protection phenomenon raises an interesting question about how a single protein can protect many different organs from various toxic insults. A possible molecular mechanism explaining this phenomenon is that Nrf2 protects many different cell types by coordinately up-regulating classic ARE-driven genes as well as cell type-specific target genes that are required for the defense system of each cell type in its unique environment. This hypothesis is supported by microarray data indicating the protective role of Nrf2 is conveyed through both known ARE-driven genes and novel cell type-specific genes. The widespread nature of Nrf2 may have an important therapeutic potential, allowing prevention of carcinogenesis and neurodegenerative diseases.—Lee, J.-M., Li, J., Johnson, D. A., Stein, T. D., Kraft, A. D., Calkins, M. J., Jakel, R. J., Johnson, J. A. Nrf2, a multi-organ protector? *FASEB J.* 19, 1061–1066 (2005)**

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THE HYPOTHESIS

MANY STUDIES have reported that a basic leucine zipper transcription factor, NF-E2-related factor 2 (Nrf2), plays a critical role in protecting a variety of tissues (lung, liver, kidney, stomach, small intestine, central nervous system, splenocytes, macrophages, erythrocytes, and retinal epithelia) from a wide array of toxic insults (carcinogens, electrophiles, reactive oxygen species, diesel exhaust, inflammation, calcium disturbance, UV light, and cigarette smoke). To explain this "single protein-conferred multi-organ protection phenomenon," we suggest a hypothesis that Nrf2 protects various cell types by coordinately up-regulating not only classic ARE-driven genes but also cell type-specific protective genes essential for the basic defense system of each cell type.

ARE AND Nrf2

Reactive oxygen species (ROS) and electrophiles cause cellular damage leading to many diseases including cancer, autoimmune disease, and neurodegenerative disease; such toxic insults are normally detoxified by phase II detoxification enzymes and antioxidant proteins. Therefore, the *cis*-acting regulatory element "antioxidant responsive element (ARE)" that transcriptionally regulates genes encoding detoxification enzymes and antioxidant proteins plays an important role in cellular defense system. Enhancer sequences originally were described in the rat glutathione *S*-transferase (GST) -P (1), rat GST Ya (2), mouse GST Ya (3), rat NAD(P)H:quinone oxidoreductase-1 (NQO1) (4), and human NQO1 genes (5). Collectively, these studies have identified a common pathway for ARE-driven phase II detoxification gene induction; the underlying ARE activation mechanism was further clarified by investigation of Nrf2. Initially, Venugopal and Jaiswal (6) and Itoh et al (7) implicated Nrf2 function in the ARE-driven gene expression mechanism; subsequent studies have revealed the molecular mechanism by which Nrf2 is activated and participates in ARE-driven gene expression. Briefly, Nrf2 is sequestered in the cytoplasm by Keap1, and ARE activation signals (i.e., protein kinase pathways and electrophiles) disrupt the Nrf2-Keap1 complex leading to nuclear translocation of Nrf2. Upon activation, Nrf2 binds to ARE sites in the promoter regions of many detoxification and antioxidant genes, leading to the coordinate up-regulation of downstream targets that boost cellular detoxification processes and antioxidant potential (**Fig. 1**) (8–16).

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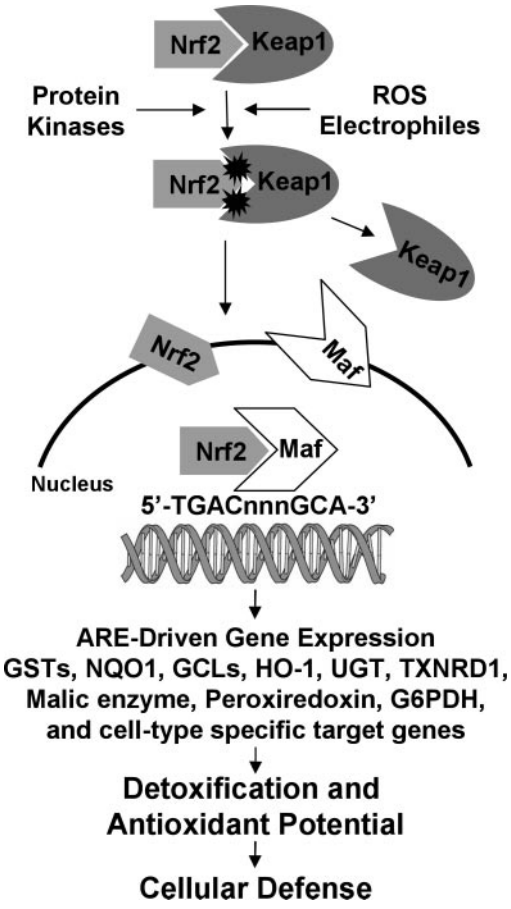

Figure 1. Cellular protection mechanism conferred by Nrf2-ARE pathway.

MULTI-ORGAN PROTECTION BY THE Nrf2 PATHWAY

The function of Nrf2 and its downstream target genes suggests that Nrf2 plays a central role in protecting cells from ROS and electrophiles. In agreement with this idea, numerous studies have shown that Nrf2 protects multiple organs from various toxic insults.

Lung and liver

Nrf2 protects lung from butylated hydroxytoluene-induced acute respiratory distress syndrome (17), hyperoxic injury (18), and bleomycin-mediated pulmonary fibrosis (19) by increasing detoxification pathways and antioxidant potentials. Recent study further demonstrated that genetic ablation of Nrf2 enhanced sensitivity to cigarette smoke-induced emphysema and identified Nrf2-dependent antioxidant and cytoprotective genes using microarray technology (20). Nrf2 plays a role in protecting liver, as evidenced by increased sensitivity to acetaminophen-induced centrilobular hepatocellular necrosis and hepatotoxicity (21, 22) as well as increased levels of lipid peroxidation and DNA damage in $Nrf2^{-/-}$ livers (23).

GI tract

Some studies have shown that Nrf2 protects GI tract from carcinogenesis, implying a role for Nrf2 in cell cycle regulation and cancer prevention. For example, $Nrf2^{-/-}$ mice showed an increased burden of gastric neoplasia after benzo[a]pyrene treatment compared with $Nrf2^{+/+}$ mice; oltipraz significantly decreased gastric tumors only in $Nrf2^{+/+}$ mice, implying that the chemopreventive activity of oltipraz is dependent on the Nrf2-ARE pathway (24). Further support for this anti-carcinogenic effect is evidenced by an increased DNA adduct formation in forestomach after benzo-[a]pyrene administration in $Nrf2^{-/-}$ mice (25). Finally, an ARE-inducer, sulforaphane, is a potent bacteriostatic agent against *Helicobacter pylori* and blocks benzo[a]pyrene-evoked forestomach tumors (26). This chemopreventive effect of sulforaphane is not observed in $Nrf2^{-/-}$ mice, suggesting Nrf2 dependency. Thus, constitutive and inducible expression of phase II enzymes through the Nrf2-ARE pathway can modify the susceptibility of GI tract to carcinogenesis.

Nervous system

The Nrf2-ARE pathway appears to contribute to neuroprotection. First, activation of the Nrf2-ARE pathway by *tert*-butylhydroquinone (tBHQ) protected neuroblastoma cells from oxidative glutamate toxicity (27) and H_2O_2-induced apoptosis (28). Primary astrocytes and neurons derived from $Nrf2^{-/-}$ mice were more sensitive to oxidative damage, calcium disturbance, and mitochondrial toxins than were wild-type cells (29, 30). Overexpression of Nrf2 and small molecule-mediated activation of the Nrf2-ARE pathway in astrocytes increased resistance of neurons to non-excitotoxic glutamate toxicity (31). Similarly, dominant negative-Nrf2 stable cells were more sensitive to NO-induced apoptosis, and siRNA-mediated knockdown of Nrf2-sensitized neuroblastoma cells to NO-induced apoptosis (32). $Nrf2^{-/-}$ mice are more susceptible to lesions produced by the mitochondrial complex II inhibitors, and transplantation of Nrf2-overexpressing astrocytes into the striatum protects from malonate-induced lesions (33). These data reinforce the pivotal role of the Nrf2-ARE pathway in protecting nervous system.

Others

The protective effects of Nrf2 have proved indispensable in many other cell types as well. Phase II gene induction by sulforaphane protected retinal pigment epithelial cells from photooxidative damage (34), and Nrf2 was important in protecting macrophages and epithelial cells from diesel exhaust chemicals (35). In addition, Nrf2 has been identified as a putative Lith 1 gallstone gene contributing to gallstone resistance (36) and plays a role in wound healing/keratinocyte differentiation (37, 38). Finally, $Nrf2^{-/-}$ mice develop lupus-like autoimmune nephritis (39), systemic autoimmune

disease (23), hemolytic anemia (40), and splenomegaly (23, 40).

Taken together, the studies outlined indicate that Nrf2 protects many different types of organs and cells, and begs an interesting question as to how a single protein can protect multiple organs from an array of different toxic insults.

PROTECTION MECHANISMS

Our hypothesis for Nrf2-conferred multi-organ protection phenomenon is that Nrf2 protects multiple tissues by coordinately up-regulating classic ARE-driven detoxification and antioxidant genes as well as cell type-specific targets that are required for basic defense in each unique environment. Although the observed protective effects are primarily mediated by classic ARE-driven genes, Nrf2-mediated, cell type-specific pathways (other than classic detoxification and antioxidant genes) may substantially contribute to protection of each cell type. This hypothesis is supported by a series of gene expression profiling experiments using oligonucleotide microarrays for various tissues/cell types (**Table 1**).

First, Thimmullappa et al. showed that many xenobiotic metabolizing enzymes, detoxification, and antioxidant proteins were increased by Nrf2 in the small intestine and suggested a pivotal role for Nrf2 in modulating cellular defense against carcinogens and toxins (41). Small intestine-selective Nrf2 target genes identified by microarray experiments (epoxide hydrolase, aflatoxin aldehyde reductase, UGT, GSTs, and multidrug resistance protein) indicate that Nrf2 protects the small intestine by coordinately regulating drug metabolism enzymes in the GI tract, where xenobiotics are absorbed primarily and processed. Considering the basic function of the small intestine, these small intestine-selective genes play a role in maintaining cellular integrity of GI tract against xenobiotics.

Kwak et al. identified Nrf2-dependent, D3T (3*H*-1,2-dithiol-3-thione) -inducible genes in liver (42). D3T induced many chaperones and ubiquitin-proteasome pathway genes in a Keap1-Nrf2-dependent manner. Based on liver-selective Nrf2 target genes, the authors expanded the role of the Nrf2-ARE pathway beyond its primary functions into secondary protective effects such as recognition and repair/removal of damaged proteins (42). This finding indicates that Nrf2 plays an important role in protecting hepatocytes and maintaining hepatocyte function by up-regulating specific target genes involved in recognition, detoxification, repair, and removal of damaged proteins. Since these processes might constantly occur during the xenobiotic metabolism in liver, the Nrf2-ARE pathway is important in supporting specialized hepatocyte functions.

DNA chip experiments have revealed that Nrf2 basally up-regulates many ARE-driven genes in primary astrocytes and neurons. In addition to classical ARE-driven genes (Table 1), these studies revealed that Nrf2

TABLE 1. *Identification of classic Nrf2 target genes by microarray analysis*[a]

Gene	Fold change							
Species	Mouse	Mouse	Mouse	Rat	Rat	Mouse	Mouse	Mouse
Cell type/gender	Small intestine	Primary astrocyte	Primary neuronal	Primary glial	Primary neuronal	Liver/ male	Liver/ female	Spleen/ female
Reference	(41)	(30)	(29)	(43)	(43)	(23)	(23)	(23)
Nrf2	–	↑ 35.6	↑ 22.7	–	–	↑ 40.6	↑ 18.8	↑ 21.0
NQO1	↑ 3.7	↑ 1.9	↑ 4.9	↑ 4.3	↑ 5.4	↑ 4.3	↑ 7.5	–
GST A4 (Ya)	–	↑ 1.5	↑ 4.7	↑ 21.7	↑ 4.7	↑ 2.0	↑ 1.4	–
GST A2 (Yc2)	–	–	–	↑ 17.6	↑ 8.8	↑ 2.6	–	–
GST P1	–	↑ 1.3	–	–	–	–	–	–
GST P2	–	–	–	↑ 3.4	↑ 2.4	–	–	–
GST mu 1 (8.7)	↑ 2.7	↑ 1.9	↑ 2.6	–	–	↑ 2.7	↑ 1.7	–
GST mu 2	↑ 1.9	↑ 1.3	–	–	–	↑ 2.2	–	–
GST mu 3 (9.3)	↑ 6.1	↑ 1.6	↑ 1.7	–	–	↑ 3.6	↑ 2.4	↑ 1.2
GCLM	–	↑ 1.3	–	↑ 13.0	↑ 5.1	–	–	–
GCLC	–	↑ 1.9	↑ 1.3	–	–	↑ 2.0	–	–
HO-1	–	↑ 1.5	–	↑ 2.8	↑ 21.3	–	–	–
Malic enzyme	↑ 3.4	↑ 1.5	↑ 1.6	↑ 8.3	↑ 10.1	↑ 2.1	↑ 2.1	–
TXNRD1	–	↑ 1.7	–	↑ 2.6	↑ 3.4	↑ 1.4	–	–
G6PDH	↑ 1.7	↑ 1.3	–	–	–	–	↑ 2.0	–

[a] Primarily, RNA from $Nrf2^{-/-}$ and wild-type control mice (small intestine, primary astrocytes, primary neuronal, liver, and spleen) was used for gene expression profiling. Rat glial and neuronal cells were used for overexpression of Nrf2 and identification of Nrf2 target genes. Many classic Nrf2 target genes were identified by gene expression profiling experiments using oligonucleotide microarrays. Fold changes of Nrf2 target genes were obtained from the comparisons of *1)* $Nrf2^{+/+}$ cells vs. $Nrf2^{-/-}$ cells (23, 29, 30, 41) and *2)* Nrf2-overexpressed cell vs. vector control (43). GCLM, glutamate-cysteine ligase modulatory subunit; GCLC, glutamate-cysteine ligase catalytic subunit; HO-1, heme oxygenase-1; TXNRD1, thioredoxin reductase 1; and G6PD, glucose-6-phosphate dehydrogenase. –, Either not available or no change.

TABLE 2. *Examples of nervous system-selective Nrf2-dependent genes*[a]

Cell type	Category	Gene
Primary Astrocytes	Reducing potential	Glucose-6-phosphate dehydrogenase
		Transaldolase
		Transketolase
	Immune/inflammation	PAF acetylhydrolase
		Prostaglandin-endoperoxide synthase 2
		Dithiolethione-inducible gene
		Tachykinin 2
Primary Neuronal	Calcium homeostasis	Calbindin-28K
		Synaptotagmin-1
		Hippocalcin
		S100 calcium binding protein A1
	Growth factor	Nerve growth factor-γ
		Fibroblast growth factor-13
		Fibroblast growth factor-14
		Brain-derived neurotrophic factor
	Signaling	Neuronal GEF
		Protein kinase C-β
		G-protein-γ3
		Adrenomedullin
		Corticotropin-releasing hormone
	Receptor/channel	Chloride channel
		GABA-A receptor-1
		GABA-A receptor, gamma 3
		GABA-B receptor-1

[a] In addition to known classic Nrf2-target genes, oligonucleotide microarray experiments further identified novel cell type-specific Nrf2-dependent genes from many cell types. Some examples of novel Nrf2-dependent genes identified from primary astrocytes and neurons by microarray analysis (29, 30, 31, 43) are listed.

regulates genes involved in the reducing potential and immune/inflammation in astrocytes as well as calcium homeostasis, growth factors, signaling molecules, and receptors/channels in neuronal cultures (**Table 2**) (29, 30). Nrf2-regulated, astrocyte-specific genes may explain the observed antioxidant/reducing potential and anti-inflammatory effects of Nrf2 (18, 37), insinuating important functional roles for Nrf2-activated astrocytes in supporting neurons. Furthermore, neuronal functions (i.e., signaling and receptors) and defense activities of neurons (calcium buffering capacity) are greatly enhanced in the presence of Nrf2, suggesting toxic by-products occurring during normal neuronal activities is mitigated by Nrf2-ARE pathway.

Using a cell sorting technology, Kraft et al. isolated an astrocyte-specific (detoxification and antioxidant) and neuron-specific gene cluster (cell adhesion, synaptic transmission, calcium mobilization) (31), supporting the notion that Nrf2 regulates target gene expression depending on a cell's function and the toxic by-products generated in the cell's microenvironment during routine activities.

Finally, Li et al. showed that Nrf2$^{-/-}$ mice develop an autoimmune disease with multiple organ pathology that closely resembles human systemic lupus erythmatosus (SLE). These data indicate that the lack of Nrf2 can induce pathologies in multiple organs, and SLE might be evoked by oxidative tissue damage. In support of this, the authors identified Nrf2 target genes in liver and spleen. While Nrf2 regulates many classical ARE-driven genes in liver, it also regulates many cytokines as

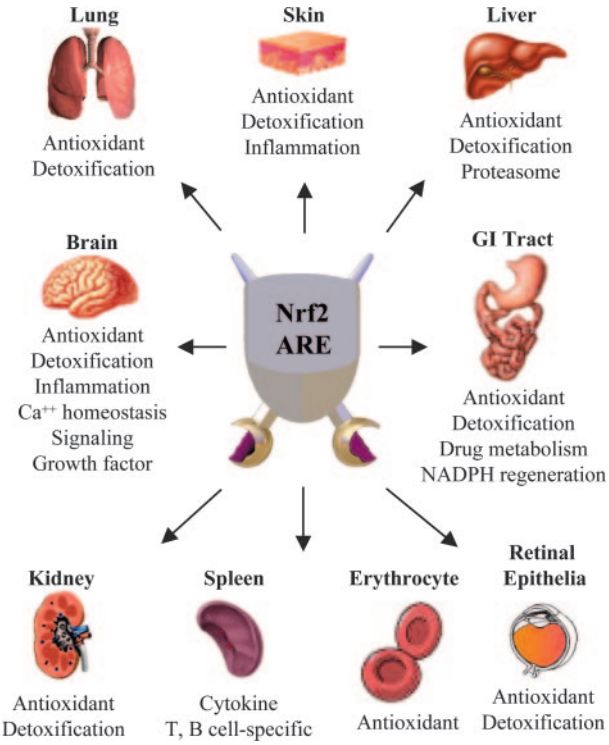

Figure 2. A multi-organ protector, Nrf2. Nrf2 protects various cell types (lung, liver, kidney, stomach, small intestine, central nervous system, splenocytes, macrophages, erythrocytes, and retinal epithelia) by coordinately increasing classic ARE-driven detoxification and antioxidant genes. Nrf2 contributes to cellular protection by enhancing cellular resistance to potential harmful insults that occur during cells' normal activities.

well as B cell and T cell-specific genes, which are important for immune and phagocytosis function of splenocytes (23).

Overall, these observations show that Nrf2-dependent, ARE-driven detoxification and antioxidant genes commonly play an important role in cellular protection (Table 1, Fig. 1). Furthermore, there are cell type-dependent Nrf2 target genes that further contribute to cellular protection. Although some of these genes have been shown to be regulated through the Nrf2-ARE pathway, many have not been verified as being under direct control of Nrf2. It is quite possible that some of the changed genes could be secondary to an Nrf2 controlled gene; such information has yet to be investigated.

The basic defense system of each cell type has evolved to deal with those most likely toxic insults that each cell could encounter in its specific environment (**Fig. 2**). It would also appear that cell utilizes the Nrf2-ARE pathway as a common mechanism to regulate/activate its defense system.

CONCLUSIONS

In summary, we have proposed and presented evidence supporting the hypothesis that Nrf2 has the capability to confer and is central to a multi-organ protection phenomenon. Expansion of these known characteristics of the Nrf2-ARE pathway are important in identifying novel pathways necessary to prevent or cure toxic effects involved in multiple disease states. As numerous studies have demonstrated the great potential for the Nrf2-ARE pathway as a therapeutic target in preventing cancer, autoimmune disease, and neurodegenerative disease, it is important to identify ways to modulate cell-specific Nrf2 activity so as to facilitate the development of novel therapeutic strategies for treatment of these diseases. FJ

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