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Review article

REVIEW OF NRF2-REGULATED GENES INDUCED IN RESPONSE TO ANTIOXIDANTS

*Ahmed Atia, Azman Abdullah

Department of Pharmacology, Faculty of Medicine, University Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

*Corresponding author email: elbadri83@yahoo.com

ABSTRACT

Nuclear factor (erythroid-derived 2) -like 2 (Nrf2) is a transcription factor that plays an important role in the cellular protection against free radical damage and reduce the incidence of radical derived degenerative diseases such as cancer. Nrf2 is referred to as the "master regulator" of the antioxidant response due to the fact that it modulates the expression of several genes including phase-2 and antioxidant enzymes playing a crucial role in detoxification of electrophiles and reactive oxygen species (ROS), including glutathione-S-transferase (GST), gamma-glutamyl cysteine ligase (γ-GCL), glutathione-S-reductase (GSR), NAD(P)H:quinoneoxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), etc. Following dissociation from it obligatory partner Kelch like ECH-associated protein 1 (Keap1), Nrf2 translocates to the nucleus and transactivates the antioxidant response element (ARE) in the promoter region of several antioxidant genes. In this review, we discuss the role of the Nrf2 system, with particular focus on Nrf2-controlled target genes.

Keyword: Nrf2- keap1- ROS- Cytoprotective genes.

INTRODUCTION

Oxidative and electrophilic stresses provoke physiological responses that induce the expression of severalcytoprotective genes.[1] The transcription factor Nrf2 was identified as the main regulator of the cytoprotective genes encoding phase 2 detoxification and antioxidant enzymes.[2] Nrf2 originates from studies of β-globin gene expression with the description of locus control region as possessing substantial regulatory activities, and relates to transcription factor activating protein-1(AP-1).[3] During the last few years,the role of Nrf2 has been increasingly studied to show that Nrf2 activation can protect against various human diseases such as cancer. Using dietary or synthetic compounds to rise Nrf2-mediated cellular defence responses, Nrf2 has been progressively studied in diseases prevention.[4] Numerous Nrf2 activators have been recognized and their efficacy in cancer prevention has been established both in animal and human experiments.[5,6] Nrf2 functions to promptly alter the sensitivity of cells to oxidative and electrophilic compounds by stimulating the transcriptional activation of various cytoprotective and detoxification genes, including the antioxidants ferritin[7], glutathione-S-reductase (GSR), gamma-glutamyl cysteine ligase (γ-GCL)[8], NAD(P)H:quinoneoxidoreductase-1 (NQO1), and heme oxygenase-1 (HO-1).[9] In homeostatic status, Nrf2 is constantly ubiquitinated through its inhibitory partner Keap1. In response to electrophiles or ROS stress, cytosolic Nrf2 liberates itself from sequestration or negative regulation of Keap1, thereby releasing Nrf2 from proteasomal degradation and translocates into the nucleus. Once in the nucleus, Nrf2forms a heterodimer complex with various

transcriptional regulatory proteins such as small Maf(sMaf) protein. This protein complex then binds to the ARE, which is located in the upstream promoter regions, leading to the induction of diverse target cytoprotective genes.[10]

THE NRF2:INRF2 (OR KEAP1) SYSTEM

The transcription factor Nrf2 is the master of redox homeostasis because it regulates basal and inducible expression of several antioxidants and cytoprotective genes, providing protection against several diseases, such as renal, pulmonary, cardiovascular, neurodegenerative diseases and cancer.[11] Nrf2 is a member of the cap 'n' collar (CNC) family basic region-leucine zipper transcription factor that also includes NF-E2, Nrf1, Nrf3, Bach1, and Bach2.[12] Nrf2 protein has six NRF2-ECH homology domains designated as Neh1-Neh6 respectively.[13] These proteins serve as heterodimeric transcription factors by dimerizing with other bZIP proteins such as the sMaf.[14] As shown in fig. 1, the first conserved domain, Neh1, is located in the half C-terminal of the molecule,constitutes the basic DNA binding domain and the leucine zipper for dimerization with other b-Zip proteins.[15] Neh2 consists of the amino-terminal region of the Nrf2 and serve as a negative regulator of Nrf2.[16] Neh3, in turn,located at the C-terminal end of the protein and required for transcriptional activation of Nrf2.[12] Both Neh4 and Neh5 are considered as transactivation domains, act cooperatively to bind another transcriptional co-activator, the cAMP response element-binding protein (CREB)-binding protein (CBP), in orderto organize the start of transcription.[17] Neh6 is a redox-insensitive degron which is essential for negative regulation of Nrf2.[16]

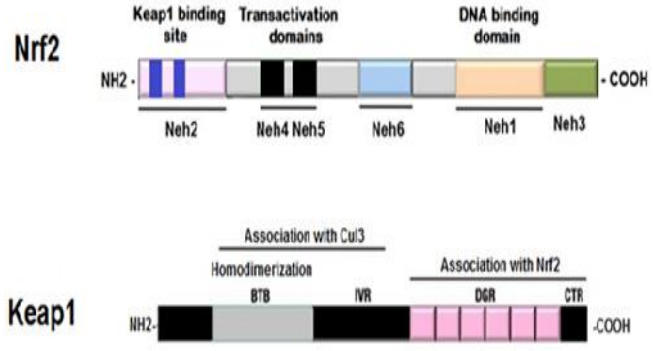

Fig 1: Nrf2 and Keap1 domains.

Upper panel: in Nrf2, Neh1 is the basic DNA binding domain and the leucine zipper for dimerization. Neh2 is the Keap1 binding domain. Neh3 is required for transcriptional activation of the protein. Neh4 and Neh5 domains are important for binding to ARE. Neh6 is required for the negative control of Nrf2.

Lower panel: in Keap1, BTB domain serves as a substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. IVR domain is a domain of intervention which is distinguished for its high number of cysteine residues. DGR domain is associated with actin filaments, giving stability to Keap1 (Adapted from Yoichiro et al).[18]

It has been demonstrated that Nrf2 through its Neh2 domain interacts with Keap1 protein, also known as inhibitor of Nrf2 (INrf2), and negatively controls Nrf2 function.[13] Keap1, as shown in fig.1, is composed of five major domains: an N-terminal region (NTR), broad complex, tramtrack, and bric-a-brac domain (BTB), a cysteine-rich intervening region (IVR), the double glycine repeat region (DGR) or Kelch domain, and a C terminal Kelch region (CTR). Keap1 forms a homodimer and each dimer binds one molecule of Nrf2 by its two Kelch domains, with one high affinity binding site (ETGF motif) and one weak affinity binding site (DLG motif). Both motifs are located in Neh2 domain of Nrf2.[19] The ETGF motif has a higher affinity for Keap1 than the DLG motif, and this is the so-called ''hinge-and-latch'' model.[20] Keap1 contains several reactive cysteine residues that serve as sensors of intracellular redox state, among which C273 and C288, connected to IVR, are critical for the dissociation of Nrf2 from Keap1 under basal conditions and their modulation by inducers may diminish the rate of ubiquitination and degradation of Nrf2. Cysteine residues C151, in the BTB domain, is essential for repression of Nrf2 ubiquitination by electrophiles or oxidants.[21] Oxidative or covalent modification of thiols in some of these cysteine residues cause Nrf2 to be released from keap1 sequestration with consequent translocation to the nucleus.[22] Once in the nucleus, Nrf2 heterodimerizes with the sMaf protein in the upstream promoter regions of the ARE, leading to the induction of genes encoding antioxidant and phase 2 detoxifying enzyme.[15,23] These cytoprotective enzymes are crucial for cell defence by improving the removal of ROS and, thus, plays a protective role against oxidative stress.[24] Furthermore, Nrf2 is a key transcription factor involved in cytoprotection against

inflammation due to its ability to antagonize the transcription factor nuclear factor-kB (NF-kB), which coordinates the expression of inflammatory genes.[25] Additionally, studies have suggested BTB and CNC homology-1 (Bach1) as another control mechanism for Nrf2 activation. Bach1 is known to bind to the Nrf2 site as a heterodimer with sMaf.[26] Upon induction, Bach1 is substituted by Nrf2, leading to activation and suggesting competition between Bach1 and Nrf2 for the same DNA binding site in various cellular conditions.[27]

NRF2 TARGET GENES

NRF2, as mentioned, is a transcription factor that controls the expression of phase 2 detoxification genes. It heterodimerizes with members of the Maf family of transcription factors and binds to the ARE in the promoter regions of various phase 2 genes. The impacts and functions of some of the main Nrf2-target genes will be discussed below. However, it is not easy to distinguish which particular genes activated by Nrf2 are most significant for its cytoprotectiverole;nevertheless it is clear that the coordinated induction of those genes has a dramatic effect on cellular homeostasis.

Glutamate-cysteine ligase (GCL). GCL, also known as γ-glutamylcysteinesynthetase, is an enzyme composed of two subunits: a modifier subunit (GCLM) and a catalytic subunit (GCLC), both of which contain ARE sequences in their promoters.[4,5]GCLcatalyzes the first step in glutathione synthesis (GSH), the most significant non-protein thiol in the cell. GSH maintains intracellular redox balance and plays an important role in detoxifying of xenobiotics, electrophiles and protection of the cell from reactive molecules and oxidative insults. GSH can also detoxify chemicals through enzymatic conjugation by glutathione-S-transferases.[28,29] Since GCL is a master determinant of the total capacity of GSH synthesis, regulation of GCL subunits has been subjected to extensive research.[30] Studies have identified that GCL has different levels of regulation, at the kinetic, post translational and transcriptional levels, which ultimately affect either the catalytic or modifier subunits or both.[31] The regulation of GCL at the transcriptional level provides morepersistent effect and thus is essential for the maintenance of GSH homeostasis in response tooxidative stress.[32]The Nrf2/ARE signalling is one of the major regulatory pathways. Both of GCLC and GCLM contain AREs in their promoters.[33] Further, manytranscription factors have been reported to bind ARE, such as Nrf2 family members(Nrf1/2/3), sMaf (maf G/K/F), AP-1, and Fos family members (c-Fos, FosB, Fra1, Fra2).[34] Among them, ARE-dependent GCLC gene expression is highly dependent upon Nrf2.[35]In addition, researchers have identified that levels of GCLC and GCLM are decreased in Nrf2 knockout mice, this leads to lack in GSH synthesis which is lethal during embryogenesis.[36]

Glutathione-S-transferases (GST). GSTs are multi-gene family of phase II detoxification enzymes that catalyse the conjugation of several endogenous and exogenous electrophilic compounds with GSH.[37] GSTs are largely distributed in nature, being presented in all eukaryotes and in various prokaryotes.[38] In mammals, GSTs are divided into numerous cytosolic, mitochondrial and microsomal GST isoenzymes, also known as MAPEG (membrane associated proteins in eicosanoid and glutathione) proteins, according to their homologies and properties.[39] The cytosolic enzymes are encoded by five related gene families (known as; alpha, mu, pi, sigma, and theta GST), while the membrane-bound enzymes and microsomal GST are encoded by single genes and both have originated separately from the soluble GST.[37] It has been suggested that phase II conjugation enzymes, particularly GSTs,are Nrf2 target genes with an extremely transactivational ARE-like motifs demonstrated on the gene promoter.[24] Studies have revealed that Nrf2 knockout mice exhibited reduced constitutive and inducible expressions of many GST isoforms in livers.[40] Moreover, induction of hepatic and intestinal GST isoforms by Butylatedhydroxytoluene (BHA) and ethoxyquin is also reduced in the absence of Nrf2.[41]

NAD(P)H quinoneoxidoreductase 1 (NQO1). NQO1 is a cytosolic homodimericflavoprotein that is widely expressed in many tissues, and its expression is regulated by the ARE both in basal and during oxidative stress conditions.[42] NQO1catalyzes two electron-reduction and detoxification of a wide range of substrates, most prominentlyquinones and its derivatives, protecting cells from reactive forms of oxygen, oxidative stress, and neoplasia.[43] However, in many cases, the reduction of quinones by NQO1 leads to the formation cytotoxic hydroquinones which

play a key role in targeting NQO1-rich cancer cells.[44]NQO1 is expressed predominantly in all the tissues, and its level of expression differs among human tissues. NQO1 is expressed at relatively high levels in several tumor tissues including lung, liver, colon, breast and pancreatic tissues, and its expression induced in response to a variety of xenobiotics, antioxidants, oxidants, and heavy metals.[45] Mutations and deletions in the NQO1 gene promoter aided in recognizing the core ARE sequence.[32] ARE is basically needed for expression and coordinated induction of NQO1 and other detoxifying enzyme genes. Nrf2 bind ARE and regulate expression and induction of NQO1 gene. Meanwhile, Nrf2 knockout exhibited reduction in the constitutive expression of NQO1 and impairs its induction.[42]In addition, treatment with BHA, known Nrf2 inducer, increases hepatic and intestinal NQO1 levels in wild-type, but not in Nrf2 knockout mice.[45]All of these data confirm a role for Nrf2 in the expression of NQO1 in various tissues.

Superoxide dismutases (SODs). SODsare the first and most significant line of antioxidant enzyme defence systems against several ROS,particularly superoxide anion radicals. SOD is an extremely efficient enzyme, catalyses the dismutation of two superoxide radicals to form hydrogen peroxide and oxygen. Product of this gene is suggested to defend the lungs and other tissues from oxidative stress.[46] In mammalians, three distinct isoforms of SOD have been identified;cytoplasmic (SOD1), mitochondrial (SOD2), and extracellular (SOD3).[47] SOD1 is a soluble cytoplasmic protein that binds zinc and copper ions, and works as a homodimer. SOD2, a protein found in mitochondria in a homotetramer form, binds the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen. SOD3, in turn, is secreted into the extracellular space and forms a glycosylated homotetramer that is linked to the extracellular matrix and cell surfaces via an interaction with heparin sulfate proteoglycan and collagen.[46] Researchers have identified that SOD3 but not SOD2 and SOD1 is induced by antioxidants, and is regulated through Nrf2. Therefore, SOD3 suggested as an important gene in defence against oxidant stress and in the prevention of estrogen-mediated breast cancer.[48]

Epoxide hydrolases (EHs).EHs are multifunctional enzymes that are essential for both the activation and inactivation of reactive species. EHs catalyse the hydrolysis of epoxides, which formed by the cytochrome P450-dependent monooxygenase (CYP) superfamily, to a diol (known as dihydrodiols) by the addition of water.[49] Two mammalian enzymes, microsomal (mEHor EPHX1) and soluble epoxide hydrolase (sEHor EPHX2), have been characterized to play diverse roles in xenobiotic metabolism. mEH is expressed in two types. Type-I mEHis typically located in the hepatic endoplasmic reticulum to oppose epoxides of polycyclic aromatics and make them into diols. Type-II mEH is positioned in the hepatocyte plasma membrane, where it controls the absorption of bile acids in the liver in association with a taurocholate binding protein. Meanwhile, Soluble epoxide hydrolasealso forms diols from different endogenous and exogenous epoxides, and is expressed in most tissues such as; liver, lung and kidney. sEH regulates many pathways linked to endogenous systems including; fatty acid and leukotriene epoxide metabolism, and is involved in blood pressure regulation and inflammatory responses.[50] There are some evidence indicated that EHs are regulated by the Nrf2 system. The mRNA expression of mEH has shown to be decreased in various tissues of Nrf2 knockout mice. In addition, treatmentof Nrf2 knockdown mice with prototypical Nrf2 inducers, oltipraz, have not been found to induce mEH in compare to the wild type. These results highlights a role for Nrf2-mediated control.[51,52]

Heme oxygenase-1 (HO-1). HO-1 is the extremely inducible rate-controlling enzyme of heme catabolism.[53] It catalyzes the rate-limiting step in the degradation of heme into biliverdin, carbon monoxide (CO), and free iron.[54]HO-1 has antioxidant, cytoprotective, anti-inflammatory, and immune-modulatory activities. These activities are suggested to be due to its ability to reducehigh levels of potentially toxic heme within cells and to alter heme to less reactive iron which can be stored in the nontoxic form of ferritin.[55]HO-1 plays a crucial role in the maintenance of cellular redox homeostasis and subsequentlyhinders transformation of normal cells to malignant cells by abolishing ROS-mediated carcinogenesis.[56] However, increased levels of HO-1 and Nrf2 have been demonstrated in several types of human malignancies. Studies have reported that Nrf2

regulates the induction of HO-1 in response to diverse forms of cellular stress, including oxidative, hemodynamic, and endoplasmic reticulum stress. Additionally, Nrf2-knockout animals have been found to express HO-1 at low levels, further implicating Nrf2 in the induction of HO-1.[57]

Multidrug resistance-associated proteins (MRPs): MRPs are ATP-dependent efflux transporters known to transport a variety of compounds, particularly glutathione, glucuronide, and sulfate conjugates, out of cells. Four MRP transporters (MRP2, 3, 4, and 6) of the eight MRPs are expressed to a significant extent in liver.[58] MRP2 is the only MRP localized to the canalicular membrane and involves in excretion of chemicals into bile. While, MRP3, MRP4 and MRP6 are localized to the basolateral membrane and serve as an efflux pump transporting chemicals from hepatocytes into blood.[59] MRPs play major roles in hepatic removal of metabolites, and modification of MRPs expression in liver can convert drug disposition.[60] MRPs have been demonstrated to be induced by various Nrf2 activators, including BHA, oltipraz, and ethoxyquin.[61] Moreover, other transcription factors, such as NF-κB, have been implicated in MRPs regulation, suggesting that another regulatory mechanisms might control MRPs induction.[62]

Beside the mentioned genes that are regulated by Nrf2, there are many other genes regulated by this process that we cannot possibly include them in this review. But, what has shown in this section is the most known genes that regulated by this signallingmechanism, addressingtheir implication with Nrf2 in cytoprotection against ROS-mediated carcinogenesis.

SUMMARY

Transcription factor Nrf2 regulates basal and inducible expression of phase II detoxification genes that protect animal cells against toxic effects of electrophiles and ROS. Under normal physiological conditions, Nrf2 is sequestered in the cytoplasm by Keap1, a multi-domain, cysteinerich protein that is bound to the actin cytoskeleton. Keap1 acts both as a repressor of the Nrf2 transactivation and as a sensor of phase 2 inducers. Electrophiles and oxidative stress loose the interaction between Nrf2 and the Keap1 protein, allowing Nrf2 to translocate to the nucleus, where it forms a heterodimer bound with its obligatory partner sMaf protein, and eventually activates ARE-dependent gene expression. These genes are essential for detoxification of xenobiotics and endogenous reactive intermediates.

Conflicts of Interest: The authors declare that they have no conflict of interests.

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