

## Review

### **Nrf2: A MASTER REGULATOR OF CELLULAR DEFENSE MECHANISM AND A NOVEL THERAPEUTIC FACTOR**

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#### **ABSTRACT:**

Nrf2 is a potent regulator for the cellular defense system and belongs to a member of the cap 'n' collar (CNC) subfamily of transcription. Nrf2 senses the chemical, electrophilic and radiation-induced oxidative stress under basal conditions. Nrf2 is repressed and retained in cytoplasm by its negative regulator Keap1 and is polyubiquitinated by cullin E3 ubiquitin ligase complex for its proteasomal degradation, but stress induces the modification in cullin E3 ubiquitin ligase complex and leads towards its reduced ability to polyubiquitinate Nrf2 and results in Nrf2 escape from Keap1 and mediates the transcription of antioxidant electrophile response element (ARE) to encode antioxidant enzymes that neutralize the effect of reactive oxygen species (ROS). Here we highlighted the activity of Nrf2 as a therapeutic agent in oxidatively stressed diseases such as heart failure, cancer, neurological, bone and joint disorders and it can be induced by numerous natural and synthetic compounds.

**Key words:** Nrf2 (nuclear factor- erythroid 2-related factor 2), antioxidant /electrophile response element (ARE/EpRE), cytoprotective genes, Keap1, therapeutic factor

#### **INTRODUCTION**

Nuclear factor-erythroid 2 (Nrf2) is a pleiotropic protein having a molecular weight of 66-kDa, belongs to cap 'n' collar (CNC) subfamily of transcription factors. It contains a leucine zipper DNA binding domain (bZip) on its C-Terminus. (Mitsuishi et al., 2012). Nrf2 (Nrf2, with gene called *NFE2L2*) regulates the cellular redox balance and in case of imbalance it gets activated (O'Connell and Hayes, 2015). Excessive generation of reactive oxygen species (ROS) results in cellular stress and it is a chemical wake up call for cells that leads to upregulation of antioxidant /electrophile response element (ARE/EpRE) (Zhang et al., 2016). Six highly conserved domains named as Neh1, Neh2, Neh3, Neh4, Neh5 and Neh6 are critical for certain important functions. Neh1 domain provides binding site for DNA and forms dimers with other factors. Neh2 domain (rich in lysine residues) is responsible for proteasomal degradation of Nrf2 by binding to its negative regulator Keap1 (Kelch-like ECH-associated protein 1), a 69kDa cytoplasmic protein (Itoh et al., 1999). Neh3 domain binds with other co activators such as CHD6, Neh4 and 5 are trans activating domains (Nioi et al., 2005) and Neh6 domain is highly rich in serine residues (Zhang et al., 2004).

Nrf2 regulates the cellular redox balance and in case of imbalance it is activated. Its activation is a chemical wake up call for cells and leads to upregulation of antioxidant /electrophile response element (ARE/EpRE) (Itoh et al., 2010). It responds by binding to antioxidant response elements (AREs) also known as cis-acting enhancer sequences and

expresses the cascades of cytoprotective and antioxidant proteins, drug efflux pump proteins, xenobiotic detoxification enzymes such as glutathione S-transferases (GST), quinone oxidoreductase 1 (NQO1) and antioxidant enzymes such as catalase (CAT) and sulfiredoxin (SRX) (Zipper and Mulcahy, 2002). Nrf2 is involved in many physiological processes and thereby protecting our body cells from external harms by neutralizing the free radicals which are highly reactive oxygen species (ROS) such as superoxide, nitric oxides and oxygen radicals produced in oxidative stress. Nrf2 upregulates the expression of genes encoding for phase-I, II and III enzymes/transporters which are responsible for reducing the effects of carcinogens. Phase-I enzymes are responsible for metabolizing these carcinogens whereas phase-II are responsible for the formation of water-soluble products that neutralize the effect of carcinogens (Hiller et al., 2016). During carcinogenesis, multiple steps take place that cause alteration in cell environment and lead to their rapid and uncontrolled proliferation. The main stages of carcinogenesis involve Initiation (first stage that is responsible for the transport of carcinogenic agent to its targeted site and causing genotoxic damage), promotion (second stage in which accumulation of actively proliferating pre-neuroblastic cells takes place) and progression (final stage that includes the rapid growth of neuroblastic cells either in invasive and metastatic manner) (Moolgavkar, 1978). NRF2 has significant contribution to control carcinogenesis via p21 gene. Under high oxidative stress p21 gene and competes with the keap1 for binding with Nrf2. p21 directly interacts with Nrf2 via DLG

and ETGE motifs in Nrf2 and 154KRR in p21 which leads to upregulation of the Nrf2-Mediated Anti-oxidant Response (Chen et al., 2009; Gartel and Tyner, 2002).

**Regulatory pathway of Nrf2–Keap1:** The balance between the control and formation of the transcription factors is one of the important elements in regulation of body functions. Nrf2 is one of the potent transcriptional activators for the

formation of antitoxic enzymes which are encoded by target genes of Nrf2 responsible for the drug transport, metabolism of xenobiotic compounds, glutathione synthesis and elimination of ROS species by neutralizing their effects (Okawa et al., 2006). Hinge and latch Model presenting the Nrf2 Dependent Antioxidant Response upregulated by p21 is shown in Figure1 and Structure of Keap1 is shown in Figure 2 and3.

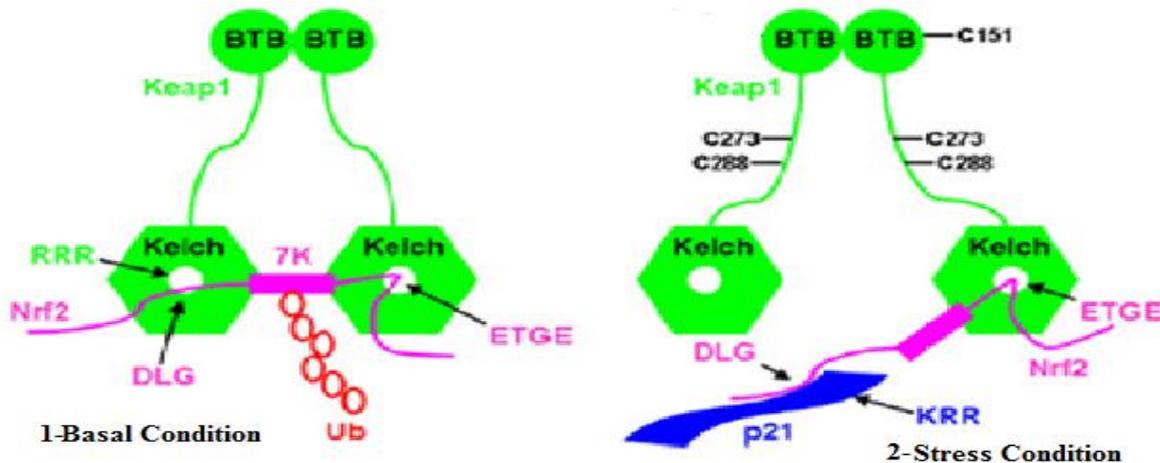


Figure-1: Hinge and latch Model presenting Nrf2- Dependent Antioxidant Response upregulated by p21 (Chen et al., 2009)

This structure is presented in basal and stress condition. **1-**Under basal conditions Nrf2 is constantly ubiquitinated and degraded upon binding with its negative regulator Keap1 and ubiquitin E3 ligase complex via weak binding DLG motif (latch) or a strong binding ETGE motif (hinge) of Nrf2 and Kelch domains of Keap1homodimee. **2-**

Under stressed conditions-the confirmation of Keap1 altered (cysteine residues labelled as C273, C288 and C151 are modified) in such a manner that latch loosen and then p21 competes with Keap1 and binds with DLG motif of Nrf2 through its 154KRR motif and leads to upregulation of cytoprotective genes.

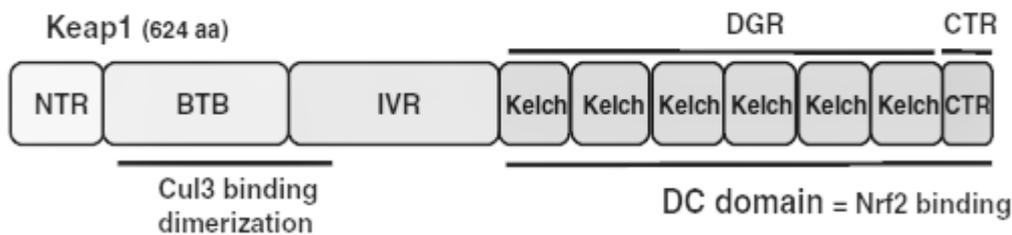


Figure -2: Structure of Keap1 (Taguchi et al., 2011)

**The structure of Keap1 consist 624 amino acids.** BTB domain that is the binding site of the Cul3, IVR domain: linker that consists of several

cysteine residues which sense the insult by oxidative and electrophilic stress, Kelch domain: binding site of the N-terminal Neh2 domain of Nrf2.

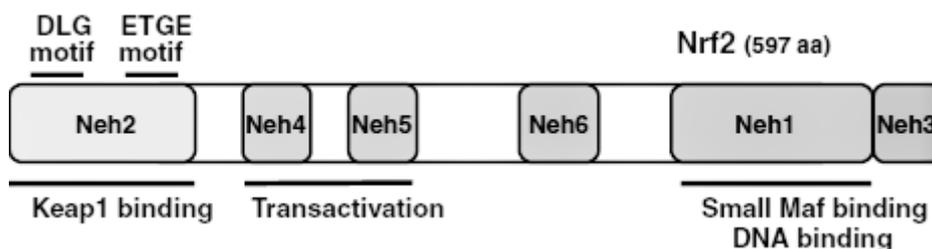


Figure-3: Structure of Nrf2 (Taguchi et al., 2011)

**The Structure of Nrf2 contains 597 amino acids:** Neh2 domain consist of weak binding DLG motif (latch) or a strong binding ETGE motif (hinge). The binding affinity of Kelch to the ETGE motif is approximately 100-fold higher than that of Kelch to the DLG motif. Neh4, 5 are transactivating domains, Neh6 domain rich in serine residues, Neh1 domain provides a binding site for DNA and dimer formation with other factors and Neh3 domain for binding with other coactivators.

**Normal Condition:** Under normal condition, Nrf2 has a half-life of < 20 min. (Kato et al., 2005). Nrf2 and Keap1 bind with each other via Neh2 domain of Nrf2 and DC domain of Keap1 (DC domain is responsible for the formation of barrel structure) (Tong et al., 2006). Then ubiquitin E3 ligase complex/cullinE3 ubiquitin ligase complex is formed by binding of Cullin3(Cul3) and Keap1 which polyubiquitinates the Nrf2 and degrades it via proteasomal degradation pathway.

**Stressed Condition:** Under electrophilic or oxidative stress, certain pathways become activated such as PKC (Protein kinase C) phosphorylate the Nrf2 at Ser40 and this phosphorylation helps in destabilizing the association of Keap1 and Nrf2 (Numazawa et al., 2003) and along with it electrophile directly attack and modify the thiol group (present in the neighbouring side of cysteine residues such as C151, C273, or C288 in the BTB or linker domain) of Keap1 (Furukawa and Xiong, 2005; McMahon et al., 2006), this modification leads to reduced activity of cullinE3 ubiquitin ligase complex and preventing the proteasomal degradation of Nrf2 thus leading to accumulation of NRF2 and inducible activation of many cytoprotective genes (Sekhar et al., 2010). This Nrf2 binds with the MAP kinase and induces the transcription of antioxidant elements, which in turns neutralize the toxic effects (Holland et al., 2008; Sekhar et al., 2010). Many natural and synthetic compounds are involved in the induction of Nrf2-ARE defensive pathway known as natural and synthetic inducers. But epigenetic alteration leads to destabilization of Keap1 and stabilization of Nrf2 (Wang et al., 1999).

**Natural inducers:** Carsonic acid derived from the plant extracts of *Rosmarinus officinalis* provides protection to neurons against oxidative stress by induction of Nrf2-ARE mediated pathway. It binds to Keap1 cysteine residues and acts as neuroprotectant (Sato et al., 2008). Eckol is a naturally occurring compound present in brown algae such as *E. cava*. Treatment of Chinese Hamster Lung fibroblasts V79-4 cell lines with eckol resulted in enhanced expression of a Nrf2 factor that regu-

lates Heme Oxygenase enzyme (HO-1) via Erk and P13K/Akt signaling. This enzyme is a key player in releasing oxidative stress (Kim et al., 2010). Resveratrol is naturally produced by several plants which confer endothelial protection by activating antioxidative transcription factor Nrf2. Resveratrol cultured cells of coronary artery endothelial layer showed significant Nrf2 activity. It upregulates different Nrf2 targeting genes such as gamma-glutamylcysteine synthetase, NADPH: quinone oxi reductase 1 and heme oxygenases 1 (Ungvari et al., 2010).

**Synthetic inducers:** Dimethyl fumarate is a synthetic compound which is a potent neuroprotective agent. Application of dimethyl fumarate on human and rodent astrocytes stabilized the Nrf2 factor. Furthermore, it modifies the Nrf2 inhibitors at cysteine 151 and in this way acts as cytoprotective compound (Linker et al., 2011). Similarly, many other chemical compounds e.g., SF, TBHQ, PGA2, H<sub>2</sub>O<sub>2</sub>, D3T and DEM etc induces the expression of glutathione S-transferase which is regulated by a Nrf2 factor. Zebrafish cells were cultured with these synthetic compounds and enhanced expression of *gtsp* was observed. While Nrf2-knockout cells showed the least expression of *gtsp* gene (Kobayashi et al., 2009).

**Nrf2 as a therapeutic agent:** NRF2 is a potent regulatory element in antioxidant reactions. It enables antioxidant enzymes to suppress the action of ROS (reactive oxygen species) and other electrophiles that cause numerous genetic alterations and inflammatory reactions. It plays important role in regulation of other antioxidant genes and also degrades the damaged proteins present in the cell by proteasomic action (Sykiotis and Bohmann, 2010). Using these characteristics of this factor, it can be used as a therapeutic agent in various diseases.

**Heart Failure:** Hypertension initiates many cardiac disorders such as the hyperphobic condition of the heart and eventually its failure. Hydrophobic heart contains higher levels of enzymes such as superoxide dismutase, glutathione peroxidase, and NADPH: quinone oxidoreductase 1 that causes heart failure as studied in mice cardiomyocytes (Purdom-Dickinson et al., 2007). The need is to reduce this oxidative stress. Resveratrol, an antioxidant compound derived from a plant, induces the expression of NRF2 and also help in regulation of antioxidant genes such as GCLC, NQO1, and HMOX1 (Ungvari et al., 2010).

**Impaired Bone Healing:** Studies have shown that diabetic patients have impaired bone healing process (Gandhi et al., 2005) and joint destruction

involves oxidative stress. Transcription factor Nrf2 has an important role in the regulation of cartilage injuries. Nrf2-knockout models of mice have shown that they have more joint injuries than wild type (Wruck et al., 2011). So Nrf2 factor can be an alternative therapeutic agent, in diabetic patients with impaired bone healing, other than insulin.

**Carcinogenesis:** Different daily life chemicals have been reported to be carcinogenic in nature such as diesel exhaust and arsenic etc. Arsenic is well known to cause skin cancer by inducing oxidative stress and causes malfunctioning of important enzymes (Pi et al., 2003). Similarly, diesel exhaust chemicals cause dysfunctioning of pulmonary pathways (Li et al., 2004). Under oxidative stress, NQO1 (NADPH: quinone oxidoreductase 1) enzyme and glutathione S-transferase enzymes are a key player in protecting the cell from carcinogenesis. These enzymes expression is regulated by a Nrf2 factor. Ishii *et al.*, (2000) showed that GSH was the potent antioxidant under oxidative stress, using peritoneal macrophages from Nrf2 deficient mice. A number of proteins such as peroxiredoxin MSP 23, heme-oxygenase 1 (HO1), stress protein A70 and cysteine membrane transporter are expressed to aid in the defensive mechanism (Ishii et al., 2000). Similarly some organic agents such as dimethylfumarate and synthetic terpenoids have been reported to cure lung carcinogenesis via activating Nrf2 anti-inflammatory pathway (To et al., 2015).

**Retinopathy:** Retinopathy is the obliteration of retinal blood vessels due to high oxidative stress which causes the apoptosis of endothelial cells. Nrf2 plays important role in protection of retinal blood vessels. (Xu et al., 2014). Mice models have been studied for retinopathy. In Nrf2 knockout mice, under about 75% O<sub>2</sub>, a number of retinal capillaries greatly decreased as compared to Nrf2+ mice models (Uno et al., 2010). Studies show that diabetic patients are at higher risk of retinopathy due to the high glucose level. Statin-an antioxidative compound helps in the treatment of retinopathy by blocking the expression of NADPH oxidase and STAT3 (Al-Shabrawey et al., 2008). Certain agents are known to date that aid in nuclear translocation of NRF2 protein and induce the cytoprotective enzymes. Some of the agents are butylated hydroxyanisole, quinone, flavonoids and ethoxyquin etc. (Landers and Pang, 2011)

**Neuroprotection:** Different neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease have oxidative stress condition. Nrf2 acts as feedback limiting system to relieve this oxidative stress using ARE pathway and antioxi-

dative enzymes (Shih et al., 2005). Furthermore, this defensive mechanism can be induced by nitric oxide. Human neuroblastoma cell lines SH-sy5y were cultured with NO and it was studied that NO upregulated the expression of antioxidative enzymes via Nrf2 factors whereas DN-Nrf2 (Dominant negative-Nrf2) cells were prone to apoptosis as they were lacking the factor Nrf2 (Dhakshinamoorthy and Porter, 2004). This neuroprotection is not limited to Nrf2 factor but tBHQ (tert-butylhydroquinone) also acts as ARE-pathway inducer (Shih et al., 2003). Likewise *in vitro* studies have shown that fumarates exert neuroprotective effects via activation of Nrf2 antioxidant pathway and help in curing neurological disorders such as autoimmune encephalomyelitis and axonal destruction etc. (Linker et al., 2011)

#### CONCLUSION:

Nrf2 is a key player in reducing environmental stresses via inducing Nrf2 mediated-ARE pathway. It is an important novel therapeutic factor for diseases which involve oxidative stress and its expression can be enhanced by various natural or synthetic compounds. Nrf2 has significant applications in clinical areas in near future.

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