Review

BRJ
Biomedical Research Journal

Diverging Role of Nrf2 in Cancer Progression and Prevention

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The role of transcription factor, nuclear factor [erythroid-derived 2]-like 2 (Nrf2), is detoxification of xenobiotics, overcoming oxidative stress and offering resistance to ionizing radiation induced cell death. However, the role of Nrf2 in cancer progression remains debatable. Activation of Nrf2 dependent proteins is crucial in maintaining cellular redox homeostasis and combating toxicity of carcinogens. Thus, employing natural or synthetic activators of Nrf2 pathway is a promising approach for development of chemopreventive modalities. Intriguingly, recent reports have highlighted the dark side of Nrf2 suggesting that multiple cancer cells demonstrate constitutive activation of Nrf2 caused by mutations in Nrf2 or Keap-1 proteins, offering survival advantage. Additionally, Nrf2 pathway is also up-regulated in chemoresistant cells and may be a major contributor in acquired chemoresistance. Thus, targeting Nrf2 pathway has emerged as a novel strategy to improve efficacy of chemotherapeutic drugs. This review discusses the dark and bright sides of this transcription factor in line with the recent literature.

INTRODUCTION

The transcription factor, nuclear factor [erythroid-derived 2]-like 2 (Nrf2) was identified as NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the β-globin locus control regions (Moi *et al*., 1994). The Nrf2 gene was cloned and characterized by using the tandem repeats of nuclear factor like erythroid factor-2 (NF-E2)/activator protein-1 (AP1) of the β-globulin locus as a recognition site probe. Nrf2 contains a basic leucine zipper DNA binding domain at the C-terminus and an N-terminal acidic domain (rich in glutamic and aspartic acid residues), which could potentially function as an acidic transactivation domain (Moi *et al*., 1994). Further characterization demonstrated it as Cap`n`Collar (CNC) protein involved in the control of Drosophila head segment development by basic leucine zipper DNA binding domain (bZip) homeotic gene. The CNC family comprises four members, namely Nrf1, Nrf2, Nrf3 and p45NF-E2. Nrf1 and Nrf2 are ubiquitously expressed and are essential for normal development in mice. The expression of Nrf3 is restricted to placenta and liver, while p45NF-E2 expression is restricted to erythrocytes (Ikeda *et al*., 2004; Motohashi

Key words: Cancer, Nrf-2 transcription factor, Keap-1 protein, β-TrCP protein.
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et al., 2002). Expression of Nrf1 is essential for embryonic development and its deficiency leads to hepatic abnormality. The Nrf2 knockout mice are viable and exhibit no phenotypic defects, but are sensitive to oxidative stress (Chan and Kwong, 2000; Chan *et al*., 1998; Leung *et al*., 2003; Ohtsuji *et al*., 2008; Ramos-Gomez *et al*., 2001; Xu *et al*., 2005). Human Nrf2 is homologus to mouse and contains six highly conserved domains called Nrf2-ECH homology domains (Neh). Neh1 domain has a nuclear localisation signal and CNC-type basic leucine zipper necessary for DNA binding and dimerization. The Neh2 domain contains a Keap1 (Kelch-like ECH-associated protein 1a, negative regulator of Nrf2) binding pocket and has seven lysine residues that direct ubiquitin mediated proteasomal degradation of Nrf2 (Fig. 1) (Itoh *et al*., 1999; Zhang *et al*., 2004). Neh3 is essential for interaction of Nrf2 with CHD6 (a chromo-ATPase/helicase DNA binding protein) suggesting involvement in interaction with co-transcription factors (Nioi *et al*., 2005). Neh4 and Neh5 are transactivation domains that interact with the CREB-binding protein (CBP) (Katoh *et al*., 2001). Neh6 domain interacts with β-transducin repeat-containing protein (β-TrCP) (Jain and Jaiswal, 2007). Binding of Keap1 to Nrf2 brings it close to E3 ligase complex through two major domains: BTB (Bric a Brac, tramtrack, broad complex) domain which interacts with Cul3; and kelch domain

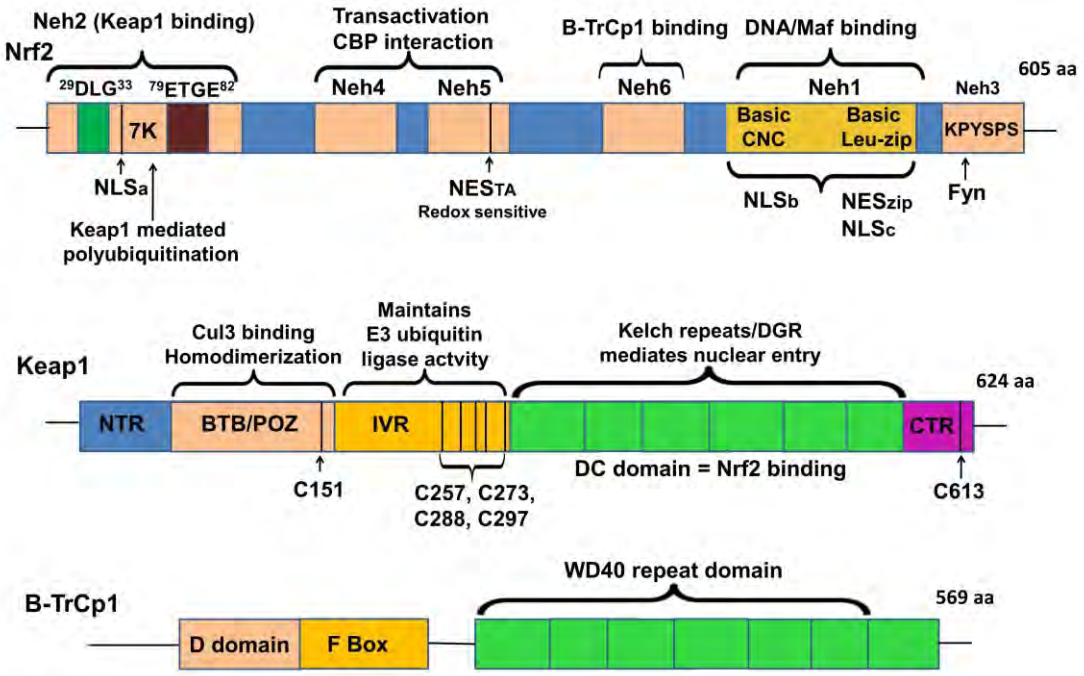

Figure 1: Structures and functions of Nrf2 and its repressors Keap1 and β-TrCP1. The relative position of the Neh domains is shown. The DLG and ETGE motifs present in Neh2 domain that bind to Keap1 are represented above with the numbering of amino acids based on the human cap'n'collar (CNC)-basic-region leucine zipper (bZIP) protein.

which binds to Nrf2. Interaction of Neh2 domain with Keap1 depends on low-affinity binding via DLG motif and high-affinity binding of an ETGE motif which results in a hinge and latch mechanism of binding. The N-terminal BTB/POZ (Pox virus Zinc finger) domain forms homodimers enabling Keap1–Nrf2 interaction (Adams *et al*., 2000; Kensler *et al*., 2007, Li *et al*., 2004; Lo *et al*., 2006; Padmanabhan *et al*., 2005).

Activation of Nrf2 dependent genes

Exposure of cells to low levels of oxidative stress, electrophiles or chemopreventive compounds leads to activation of Nrf2. Upon activation, Nrf2 dissociates from inhibitory protein Keap1 and translocates to the nucleus. In the nucleus it forms a heterodimer with co-transcription factor Maf and binds to the anti-oxidant response element (ARE) sequence to induce transcription of several different genes (Zhang, 2006). ARE sequence is the 'core' sequence of 5′-RTGACnnnGCR-3′ identified using murine GST-Ya ARE. The sequence was used to identify genes present in the promoter region (Rushmore *et al*., 1991). The Nrf2 downstream genes include phase II detoxifying enzymes like glutathione S-transferase (GST), NAD(P)H quinone oxidoreductase-1 (NQO1), and UDP-glucuronosyltransferase (UGT), intracellular cytoprotective proteins like glutamate cysteine ligase (GCL), glutathione peroxidase (GPx), thioredoxin (Trx), thioredoxin reductase (TrxR), peroxiredoxin (Prx), heme oxygenase-1 (HO-1) and transporters like multidrug resistance-associated protein (MRP) (Banning *et al*., 2005; Ishii *et al*., 2000; Ishii and Yanagawa, 2007; Kim *et al*., 2001; Maher *et al*., 2005; Moinova and Mulcahy 1999; Sakurai *et al*., 2005). Phase II enzymes reduce the toxicity of xenobiotics by making them water soluble, thereby facilitating their elimination. Efflux of endogenous molecules and xenobiotics is also governed by Nrf2 mediated expression of transporters. Constitutive expression of Nrf2 by tumor cells may offer an advantage for ambient growth and detoxification of xenobiotics, the phenomena coined as “dark side of Nrf2” (Lau *et al*., 2008; Wang *et al*., 2008c). The present review emphasizes the putative dual role of Nrf2 pathway during cancer progression and highlights its potential as a target for chemoprevention.

Mechanism of Nrf2 Activation

Nrf2 is sequestered in the cytoplasm by Keap1 which regulates Nrf2 stabilization and levels inside the cell. The interaction between the two proteins is a dynamic process regulated in such a manner that enables Nrf2 to control both the basal and inducible expression of dependent genes. Under homeostasis conditions, Nrf2 is maintained at low basal levels for expression of cytoprotective genes (Fig. 2) (Itoh *et al*., 1999). Nrf2 is at low levels when bound to Keap1 homodimer through its

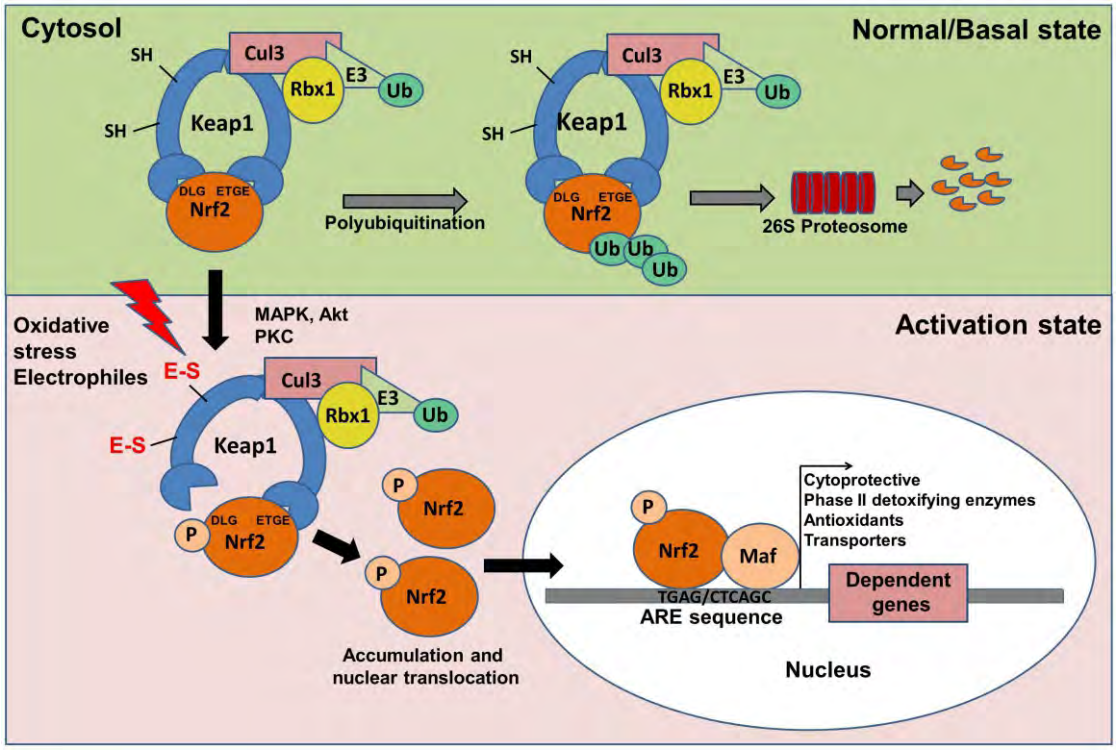

Figure 2: Schematic model of Nrf2 activation under normal and oxidative stress conditions.

kelch repeats domains at C terminal, leading to Cullin3/Rbx1-mediated polyubiquitination and subsequent proteasomal degradation. Keap1 protein contains numerous cysteine (cys) residues with potential to act as a redox sensor (Hong *et al*., 2005b).

Role of cys residues in Nrf2 activation

The significance of Keap1 as a central regulator of Nrf2 activation was revealed while addressing the negative regulation of antioxidant machinery by Keap1 dependent proteasomal degradation of Nrf2 (McMahon *et al*., 2003). The half life of Nrf2 increases from 15 min to 30 min in cells expressing mutated ETGE motif containing Nrf2 and Keap1 (Du *et al*., 2008). Using *in vitro* alkylation and *in vivo* site-directed mutagenesis, cys151 was identified as the major site directly alkylated by Nrf2 inducers along with critical residues cys273 and cys288 (Dinkova-Kostova *et al*., 2002; Eggler *et al*., 2005; Hong *et al*., 2005a; Levonen *et al*., 2004). Mutation at cys151 abolished induction of Nrf2 by activators like sulforaphane and *tert*-butylhydroquinone but had no impact on Keap1:Nrf2 binding. Keap1-cys151 restores phenotypes like over-expression of Nrf2 and post-natal lethality as observed in Keap1 null mice (Wakabayashi *et al*., 2004). However, activation of Nrf2 by arsenite in cys151 Keap1 mutant MDA-MB231 cells, indicated a possible redox independent mode of Nrf2 induction (Wand *et al*., 2008b). Further

cys273ser and cys288ser mutations showed abrogated repression of Nrf2 by Keap1 (Levonen *et al*., 2004; Wakabayashi *et al*., 2004). These observations demonstrated that cys151 is required for the activation of Nrf2, whereas cys273 and cys288 are needed for Nrf2 inhibition. Besides, a significant contribution of the critical cysteine residues during Nrf2 activation and regulation under oxidative stress was indicated. Several cellular redox modifiers modulate activation of Nrf2 via modification of the critical cysteine residues in Keap1. Further, the afore-mentioned three critical cysteine residues undergo thiol modifications leading to conformational change in the Cul3–E3 ligase complex leading to loss of E3 ligase ubiquitin activity. The cysteine residues act as redox sensors to further perturb the efficiency of nuclear export signal on Keap1, and mutant form of Keap1 at leu308 and leu310 was unable to locate in the cytoplasm (Kobayashi *et al*., 2009; Nguyen *et al*., 2005; Velichkova and Hasson, 2005). These studies suggested that under normal conditions, the signals from nuclear export sequence (NES) of Keap1 maintained the Keap1 dimer in association with Nrf2 in the cytoplasm.

Exposure of cells to oxidative, xenobiotic or electrophilic stress abrogates Keap1 induced degradation of Nrf2. Perturbation in the cellular redox status results in modifications of critical cysteine residues in Keap1. The conformational change renders release of Nrf2 from the low affinity binding motif (Cullinan *et al*., 2004; Kobayashi *et al*., 2006). The change confers stabilisation and accumulation of Nrf2 in the cytosol followed by nuclear translocation. According to hinge and latch model, ETGE motif remains bound to the Keap1 following activation. This results in saturation of Keap1 which is no longer able to compete with free Nrf2 inducing translocation to the nucleus and binding to ARE to induce expression of cytoprotective machinery of the host cell (Jain and Jaiswal, 2006). An alternate model of induction is attributed to the polyubiquitination of Keap1 at lys63, leading to subverted Cullin3 interaction and dissociation of Nrf2 from Keap1 (Zhang *et al*., 2005). The ubiquitin-specific protease-15 deubiquitinase restored Keap1 activity (Villeneuve *et al*., 2013).

Apart from Keap1 and Cul3/Rbx1, other mediators also contribute in regulating the low basal levels of Nrf2. Phosphorylation status of tyr568 on Nrf2 is governed by Src subfamily kinases Fyn, Src and Fgr, which influence the nuclear export of Nrf2. Under oxidative stress conditions, glycogen synthase kinase-3 beta (GSK-3β), a serine/threonine protein kinase, plays an important role in the nuclear export of Nrf2 by phosphorylating Fyn. Another Src member Bach1 has been shown to govern export of Nrf2 from the nucleus, thereby negatively regulating expression of its dependent genes. Bach1 competes with Nrf2 for binding to ARE sequence, resulting in

suppression of ARE mediated expression of Nrf2 dependent genes (Jain and Jaiswal, 2006; Niture *et al*., 2011).

Keap1 independent activation of Nrf2

Multiple studies have highlighted Keap1 independent activation of Nrf2. Along with Keap1 dependent degradation of Nrf2, an alternate mechanism controls activation and stabilisation of Nrf2 mediated by β-transducin repeat-containing protein (β-TrCP) (Rada *et al*., 2012). Mouse Nrf2 contains two binding sites for β-TrCP which acts as an adapter for the Skp1-Cul1-Rbx1 ubiquitin ligase complex. GSK-3β phosphorylates serine residue in SCF/β-TrCP destruction motif "DSGIS" in Neh6 domain leading to Keap1 independent degradation (Jain and Jaiswal, 2007). Post translational modification also governs Nrf2 activation. Nrf2 contains multiple serine, threonine and tyrosine residues which serve as potential sites for phosphorylation. Different pathways for activation of Nrf2 are identified including protein kinase C (PKC), mitogen-activated protein kinases (MAPK), phosphatidyl inositol 3-kinase (PI3K), and RNA-dependant protein kinase-like endoplasmic reticulum kinase (PERK) (Cullinan and Diehl, 2004; Lee *et al*., 2001; Yu *et al*., 2000). PKC has multiple isoforms which play essential roles in growth, differentiation, cytoprotection, apoptosis, survival and carcinogenesis and PKC can be activated by oxidative stimuli. PKC phosphorylated ser40 residue in the Neh2 domain leading to disruption of Keap1/Nrf2 interaction in response to oxidative stress induced by tBHQ and β-naphthoflavone. Mutation in the serine residue results in abrogation of PKC induced activation of Nrf2 (Huang *et al*., 2002). Interestingly, phosphorylation of ser40 was required for release of Nrf2 from Keap1, but does not play a role in nuclear translocation (Bloom and Jaiswal, 2003). Nuclear localisation sequence (NLS) and nuclear export sequence in Nrf2 regulates localization in the cell. The NLS motifs are identified by adapter proteins like importins that facilitate transfer inside to nucleus (Theodore *et al*., 2008). Another conserved protein kinase that influences Nrf2 activation is casein kinase II (CK2). CK2 possesses an array of potential targets and plays a role in complex cellular processes including cytoprotection. Nrf2 contains 13 potential phosphorylation targets for CK2 abundant in Neh4/Neh5 transcriptional domains. Phosphorylation dependent nuclear translocation of Nrf2 is sensitive to Ck2 inhibitor (Apopa *et al*., 2008; Pi *et al*., 2007).

Role of MAPK in activation of Nrf2

PI3K and extracellular signal-regulated protein kinase (ERK) are proposed to regulate Nrf2 pathway (Cullinan *et al*., 2003; Kang *et al*., 2001). tBHQ enhances NQO1 protein expression and activity in a PI3K dependent manner in human neuroblastoma cells. tBHQ

elicited ARE mediated induction of GST in hepatoma cells in a PI3K dependent manner. PI3K inhibitor (Ly294002) abrogated tBHQ mediated NQO1 induction, indicating a role of PI3K in Nrf2 activation (Lee *et al*., 2001). PERK, a transmembrane kinase, phosphorylates Nrf2 *in vitro* leading to dissociation from Keap1. A pivotal role of PERK mediated activation of Nrf2 was proposed as a mechanism for maintenance of glutathione levels that act as a cytoprotective buffer against oxidative insult (Cullinan *et al*., 2003). An important role of MAPK in the activation of Nrf2 via phosphorylation has been reported by several investigators. Yu *et al.* (2000) studied MAPK mediated activation of phase II detoxification enzymes using multiple inducers (Jeong *et al*., 2006). In hepatoma cells, sulforaphane and tBHQ induced activation of ERK, MAPK kinase and Raf-1, to mediate induction of phase II detoxification enzymes via Nrf2/ARE pathway (Yuan *et al*., 2006). MAPK/ERK upon activation initiates phosphorylation cascade that modulates activity of multiple downstream transcription factors (Shen *et al*., 2004; Zipper and Mulcahy, 2000). Dithiolcarbamate was shown to activate ERK and p38 resulting in transcriptional up-regulation of Nrf2 dependent γ-glutamylcysteine synthetase (Wild *et al*., 1999). Shen *et al.* (2004) investigated the transactivation potential of different Nrf2 domains and observed differential effects of multiple MAPKs in activating Nrf2. The authors further demonstrated Raf-1 mediated activation of Nrf2 attributing it to up-regulation of the co-activator CREB binding protein.

Pro-oncogenic Effects of Nrf2: The Dark Side

It is well documented that oxidative stress plays a pivotal role in the initiation and progression of cancer, with magnitude of oxidative stress a key determinant of the response of a cell towards the oncogenic stimuli. Chronic exposure of cells to oxidative insult causes cytotoxicity due to irreversible damage to vital macromolecules; whereas transient increase leads to the activation of redox sensitive pro-survival transcription factors. Therefore, in order to survive and proliferate, tumor cells maintain a moderate oxidative intracellular niche achieved by taking advantage of the antioxidant defense machinery of the cell like Nrf2 pathway. Constitutive activation of Nrf2 and expression of dependent cytoprotective genes, permits tumor cells to nurture and expand in an ambient redox niche. High levels of Nrf2 expression is reported in multiple cancers including cancers of lung, breast, gall bladder, pancreatic, colorectal and head and neck (Jaramillo and Zhang, 2013; Lau *et al*., 2008; Shelton and Jaiswal, 2013; Sporn and Liby, 2012). Ikeda *et al*. (2004) demonstrated constitutive up-regulation of Nrf2 and GSTP1 in hepatocellular carcinoma indicating role of

Nrf2 in cancer promotion. Nrf2 regulates expression of an exclusive neoplastic lesion marker GSTP1 in an ARE dependent mechanism. Higher levels of Nrf2 have been associated with poor clinical outcome and poor responsiveness in pancreatic, cervical and lung cancer (Geismann *et al*., 2014; Sporn and Liby, 2012).

Dysregulation of Nrf2 pathway in cancer

Persistent Nrf2 activation is responsible for the pro-tumorogenic effect due to genetic and epigenetic alterations in Nrf2/Keap1 (frequencies of up to 30% in lung or ovarian cancer). Copy number loss in a member of E3 ubiquitin ligase complex or oncogenic pathways or persistent exposure to oxidative stress leads to persistent activation (Barbano *et al*., 2013; Martinez *et al*., 2014; Zhang *et al*., 2010). A mutation in the Keap1 protein or loss of heterozygosity has been reported to result in persistent Nrf2 activation in multiple cancers (Padmanabhan *et al*., 2006; Singh *et al*., 2006). DNA methylation of CpG sites in the promoter region of Keap1 was observed in 51% of breast, 20% of colorectal, and 12% of lung cancers, accounting for decreased levels of Keap1 and consequent enhanced Nrf2 activation (Bryan *et al*., 2013; Wang *et al*., 2008a). Approximately 15% patients with lung cancer posses somatic mutations in Keap1, resulting in impaired and inefficient Nrf2 repression (Hayes and McMahon, 2009). The prevailing Keap1 mutations were classified based on their functional impact into passenger mutations, null mutations and hypomorphic mutations. Passenger mutations do not have any effect on Keap1/Nrf2 interaction, whereas null mutations diminished the ability of Keap1 to repress Nrf2. Most of the mutations do not affect the Nrf2 levels, but impact the activity as Keap1 is unable to act as a negative regulator (Hast *et al*., 2014; Hayes and McMahon, 2009; Shibata *et al*., 2008). Japanese patients with lung adenocarcinoma demonstrated Keap1 mutations (Ohta *et al*., 2008). Dysregulated suppression of Nrf2 by Keap1 in breast cancer resulted due to mutation in cys23 residue (Nioi and Nguyen, 2007). Under hypoxic/ reoxygenation conditions Nrf2 was upregulated and protected cancer cells from deleterious effects of oxidative stress (Kim *et al*., 2007).

Mutations in the DLG motifs of the DC domain in Keap1 show highest frequency in lung cancers (Ganan-Gomez *et al*., 2013). Interestingly frame shift mutations in Keap1 are frequent in DGR domain (65%) essential for interaction with Nrf2 (Taguchi *et al*., 2011). Other mutations in the intervening region and BTB domain of Keap1 occur in prostate, lung and ovarian cancers. Mutation in these domains influence the critical cysteine residues that inhibit its interaction with Cullin3, leading to inhibition of poly-ubiquitination of Nrf2. Mutation in other amino acids like ser104, gly186,423 and

arg320 within the DC, BTB and IVR domains are cancer derived mutations that results in impaired homo-dimerization of Keap1 needed for repression of Nrf2 (Hast *et al*., 2014). A single nucleotide deletion in Keap1 gene was associated with marked drug resistance against BRAF and cisplatin in melanoma cells (Miura *et al*., 2014). Although mutations in Keap1 play a central role in constitutive Nrf2 activation, deficiency of Keap1 *per se* does not result in cancer. Interestingly, Keap1 knockdown mice (floxed *Keap1* allele) did not develop spontaneous cancer and survived for 2 years. Keap1 knockdown mice showed constitutive activation of Nrf2 in multiple tissues including lung and liver. These studies indicated that impaired Nrf2/Keap1 pathway may result in cancer cell proliferation or resistance to anti-cancer modalities, but it does not set off cancer initiation (Taguchi *et al*., 2010). In addition, mutations in Nrf2 gene are focussed in Keap1 binding domain near ETGE and DLG motifs termed as hot spot regions. Mutations in Nrf2 were observed in lung, head and neck, oesophagus and skin cancers, but are less abundant (Kim *et al*., 2010; Shibata *et al*., 2008). Nrf2 deficient mice were more susceptible to urethane induced lung cancer compared to Nrf2 wild type (Bauer *et al*., 2013). The Nrf2 mutations are clustered within ETGE (57%) and DLG (43%) moti, which were indispensable for Keap1 binding. Mutations in ETGE motif disrupt the high affinity binding with Keap1 and thus prevent Nrf2 from ubiquitination, whereas mutations in the DLG motif disrupt low affinity binding but Nrf2 remains bound to Keap1. Both these mutations result in Nrf2 stabilisation and accumulation in nucleus (Taguchi *et al*., 2011). Along with the somatic mutations in Keap1/Nrf2, an alternate mechanism for activation of Nrf2 in tumorigenesis is mediated by oncogenic signalling. Expression of oncogenes like Kras, Braf and Myc activate Nrf2, elevating antioxidant machinery resulting in depletion of the intracellular ROS levels, thus providing a conducive reduced environment for tumor growth (DeNicola *et al*., 2011).

Nrf2 in chemoresistance

A distinctive property of constitutive activation of Nrf2 is chemoresistance, protecting cancer cells from anti-cancer drugs used in chemotherapy. Several studies have highlighted the pivotal role of Nrf2 in chemoresistance such as cisplatin in ovarian cancer, cervical cancer or endometrial serous carcinoma; gemcitabine in pancreatic cancer; doxorubicin in liver cancer and 5-fluorouracil in gastric cancer (Chen *et al*., 2012; Duong *et al*., 2014; Jiang *et al*., 2010; Ma *et al*., 2012). Elevated Nrf2 induces autophagy in ovarian carcinoma imparting resistance against cisplatin and tamoxefin (Bao *et al*., 2014). Due to the cytoprotective and detoxifying potential, several Nrf2 dependent genes are implicated in conferring Nrf2 mediated

chemoresistance, e.g., HO-1 is over-expressed in multiple cancers. Due to the cytoprotective nature, over-expression is undesirable in cancer cells. Over-expression of HO-1 was associated with increased cell proliferation and endothelial cell division leading to angiogenesis (Was *et al*., 2006). Other Nrf2 dependent genes including NQO1, GPX, TrxR and Prx1 were shown to be up-regulated in multiple cancer cells. GPx, a selenoprotein that detoxifies H_2O_2, is implicated in the control of malignant growth. Elevated GPx levels were observed in advanced stages of colorectal cancer, Barrett`s esophageal mucosa and gastrointestinal cancers associated with cell proliferation, growth and inhibition of apoptosis (Banning *et al*., 2005; Chu *et al*., 2004; Was *et al*., 2006). Peroxi-redoxins (Prx) are thiol specific antioxidants that detoxify peroxides and are elevated in non small lung cancer (NSLC) and thyroid cancer, a predictive factor for disease and associated with prognosis (Kim *et al*., 2007; Yanagawa *et al*., 1999). Trx and TrxR collectively form a redox couple with a pivotal role in maintaining cellular redox status in cellular functions (Brigelius-Flohe, 2008). Despite its protective role as redox couple, TrxR1 was elevated in gastrointestinal cancer tissues (Arner and Holmgren, 2006; Iida *et al*., 2004). TrxR knockdown lung carcinoma cells showed reversal of tumorigenicity and invasion. Enhanced cellular expression of TrxR has been attributed to cisplatin resistance, and inhibition in TrxR activity abrogates resistance against cisplatin (Sasada *et al*., 1999). NQO1 is another Nrf2 dependent gene over-expressed in adrenal gland, bladder, breast, colon, liver, lung, ovary, and thyroid cancers (Basu *et al*., 2004; Siegel and Ross, 2000). Suppression in NQO1 expression sensitizes A549 cells to etoposide, cisplatin and doxorubicin (Wang *et al*., 2008c).

Chemopreventive Effects of Nrf2: The Bright Side

Several compounds derived from natural or synthetic origin with chemopreventive activity act *via* Nrf2. Administration of methylcholanthrene reduced cancer incidence in rats caused by carcinogenic azo dyes, served as a nucleation point for use of dietary compounds as chemopreventive agents (Richardson and Borsos-Nachtnebel, 1951). Multiple plant derived products possess chemopreventive effect by inducing Nrf2 activation (Kelloff *et al*., 2000; Sporn and Suh, 2000; Talalay and Fahey, 2001; Yang *et al*., 2001). Nrf2 activation results in increased expression of cytoprotective proteins preventing biomolecules from the damaging effects of oxidative and xenobiotic stress. Nrf2 knockout mice studies strengthened the notion of Nrf2 serving as a novel chemopreventive factor controlling sensitivity to carcinogens (Slocum and Kensler, 2011). Ablation in Nrf2 led to enhanced tissues damage caused by cigarette smoke, hyperoxia, ischemic

reperfusion, portal vein embolization, and chemical toxins (Chan *et al*., 1996; Cho and Kleeberger, 2010; Kudoh *et al*., 2014; Shirasaki *et al*., 2014; Zhao *et al*., 2011). Mice with Nrf2 over-expression resulting from *Keap1* knockout shows increased resistance to lung cancer cell metastasis (Satoh *et al*., 2010). Nrf2 ablation was associated with enhanced sensitivity to mutagens and showed increased carcinogenesis in bladder, skin, hepatocytes and colon on exposure to nitrosoamine, ultraviolet, aflatoxin, dextran sulphate sodium and azoxymethane (Iida *et al*., 2004; Khor *et al*., 2006; Osburn *et al*., 2007; Saw *et al*., 2011; Xu *et al*., 2006; Yates *et al*., 2006). Curcumin, sulforaphane, oltipraz and CDDO-imidazole activate Nrf2 while exerting chemopreventive effects and Nrf2 deficiency in mouse models abrogated their chemopreventive effects (McMahon *et al*., 2001; Ramos-Gomez *et al*., 2003; Shen *et al*., 2006; Slocum and Kensler, 2011; Sussan *et al*., 2009). Nrf2 has also been implicated in protecting against ROS dependent genetic lesions that promote metastasis (Satoh *et al*., 2010). Under conditions of increased ROS levels, Nrf2 induced expression of Kruppel-like factor 9 (Klf9), which further enhanced oxidative stress mediated cell death (Zucker *et al*., 2014). The anti tumor potential of Klf9 in different cancer types has been reported with inhibition of glioblastoma stemness through transcriptional repression, and induced apoptosis in prostate cancer cells by Akt inhibition (Huang *et al*., 2015; Ying *et al*., 2014). Nrf2 is also an anti-inflammatory transcription factor and activation of Nrf2 and dependent genes reduce chronic inflammation associated cancers like colorectal or pulmonary cancer. A protective role of Nrf2 is supported by studies in mice with a single-nucleotide polymorphism (SNP) in the promoter region. The polymorphism was associated with increased susceptibility to hyperoxia induced lung damage, due to low expression of Nrf2 (Cho *et al*., 2002; Yamamoto *et al*., 2004).

Though higher levels of Nrf2 are observed in multiple malignancies, the role in initiation, promotion or transformation of normal cells remains contentious. Low levels of Nrf2 were essential for oncogenic transformation of mesenchymal stem cells (Funes *et al*., 2014). Epigenetic reactivation of Nrf2 attenuated skin epidermal cell transformation (Su *et al*., 2014). Over-expression of Nrf2 in cancer cells may enable survival under conditions of oxidative stress, or detoxify xenobiotics leading to better survival. Nrf2 prevented initiation of lung cancer, but accelerated progression through the Kras signalling pathway. Thus Nrf2 activators may pave the way for prevention of lung cancer (Satoh *et al*., 2013).

Nrf2 as Target for Therapeutic Interventions

Nrf2 activation and enhanced expression of its

dependent genes associated with redox regulating proteins, phase II detoxifying enzymes and transporters are exploited by cancer cells to survive and proliferate. Therefore, agents that inhibit Nrf2 expression in cancer cells may provide a novel strategy for therapeutic interventions to enhance efficacy of existing chemotherapeutic drugs. Brusatol, a plant extract from *Bruceajavanica*, selectively inhibits Nrf2 by increasing ubiquitination and degradation. It reduced resistance towards cisplatin in cultured xenografts (Ren *et al*., 2011). 6-Hydroxy-1-methylindole-3-acetonitrile (6-HMA) protected against cisplatin induced oxidative nephrotoxicity by inhibiting Nrf2 activation (Moon *et al*., 2013). Luteolin, a plant derived flavanoid, inhibited proliferation of tumor cells and reduced toxicity of cisplatin in a mice model (Lin *et al*., 2010; Sun *et al*., 2012; Tang *et al*., 2011). All-trans retinoic acid (ATRA) inhibited Nrf2 by activating retinoic acid receptor α, which directly interacts with Nrf2 and restrain binding to ARE (Wand *et al*., 2013a). However, the use of Nrf2 inhibitors in cancer therapy is at a nascent stage and requires development of specific agents to minimize non-specific off-target effects.

Assuming Nrf2 as a target for cancer prevention, several population-based clinical trials were conducted with diverse chemopreventive drugs including phenethyl isothiocyanate, oltipraz, curcumin, resveratrol, fumaric acid esters and synthetic oleanane triterpenoids. Administration of several Nrf2 activators in clinical trials was well tolerated, resulting in elevated levels of cytoprotective enzymes (Kensler *et al*., 2012; Linker *et al*., 2011; Palsamy and Subramanian, 2011; Scannevin *et al*., 2012). In a Chinese study, aflatoxin intoxication as a risk factor was reduced by oltipraz (Kensler *et al*., 2003). Favourable effects of sulforaphane, a potent Nrf2 activator, were observed in the promotion or progression phase of cancer, and sulforaphane inhibited cancers of multiple sites including skin, lung, bladder, breast, colon and stomach (Conaway *et al*., 2005; Dinkova-Kostova *et al*., 2006; Gills *et al*., 2006; Hu *et al*., 2006; Shen *et al*., 2007). Chemoprevention with sulforaphane-rich extracts of broccoli are in clinical trials in China (Egner *et al*., 2011). Similarly, synthetic oleanane triterpenoids reduced progression of lung, breast and pancreatic cancers, and delayed onset of tumor driven by Kras, Trp53, Brca1 and Erbb2 oncogenes (Liby *et al*., 2010).

Salutary health effect of phytochemicals that induce Nrf2 highlights the role of Nrf2-activating foods and spices in human diet. Food products like curcumin from turmeric root, sulforaphane from broccoli, and seaweed-based extracts from green alga *Ulva lactuca* were shown to activate the Nrf2 pathway *in vivo*. Extract with sulforaphane concentration that is achieved by dietary broccoli consumption, offered protection

against particulate pollution in humans (Boddupalli *et al*., 2012; James *et al*., 2012; Wang *et al*., 2013b). Similarly, phytochemical constituents of garlic, tomatoes, grapes, green tea, coffee, and berries show Nrf2 activating properties, indicating beneficial effects by dietary consumption (Kropat *et al*., 2013). Numerous dietary supplement companies have developed mixtures of known Nrf2 activators to increase the antioxidant system in body. Protandim (LifeVantage, Inc, Sandy, UT, USA), reduced oxidative stress in humans (Nelson *et al*., 2006).

Apart from the implication of phytochemicals and dietary intake, life style of an individual also plays an important role in Nrf2 activation. A relationship between physical activity and Nrf2 activation was established in a mouse model and exercise-induced oxidative stress was higher in Nrf2 knockout mice (Miller *et al*., 2012; Muthusamy *et al*., 2012; Zhao *et al*., 2013). Evidence from several studies provide a strong incentive for development of novel Nrf2 activators as putative cancer chemopreventive agents in normal healthy individuals without affecting pro-survival potential. However, caution must be exercised as a pro-tumorogenic role of Nrf2 in various cancers indicates dual nature of Nrf2 activation. Nrf2 activation may provide a survival advantage to pre-existing cancer cells and also participate in resistance to chemotherapy or radiotherapy.

Future perspective and conclusion:

Given the dual role of Nrf2 in cancer, the prime query is the role of Nrf2 in cancer initiation or cell transformation. Transient activation of Nrf2 by pharmacological activators is safe for the purpose of chemoprevention as the activators do not seem to increase the tumor burden. A major concern of use of Nrf2 activators is their cytotoxicity and non-specific mechanism of action. The activators show a tendency to modulate cellular redox and are reactive towards cysteine residues which may lead to modulation of signalling pathways. Thus, designing specific Nrf2 activators like ETGE and DLG mimetic, based on co-crystal structure of Neh2 domain and Keap1, may reduce the off target effects. Further, demonstration of miRNA mediated regulation Nrf2 pathway provides a new conduit to explore additional targets. Multiple studies highlight the cross talk of Nrf2 with other signalling pathways imperative for cell survival. Results from our laboratory have demonstrated implication of Nrf2 cross talk with NF-κB as a prime target for anti-inflammatory effect (Gambhir *et al*., 2014). Thus, novel agents targeting Nrf2 pathway specifically needs investigation.

SUMMARY

Nrf2 is a redox sensitive transcription factor, maintained at low basal levels under normal conditions. Upon activation, it mediates

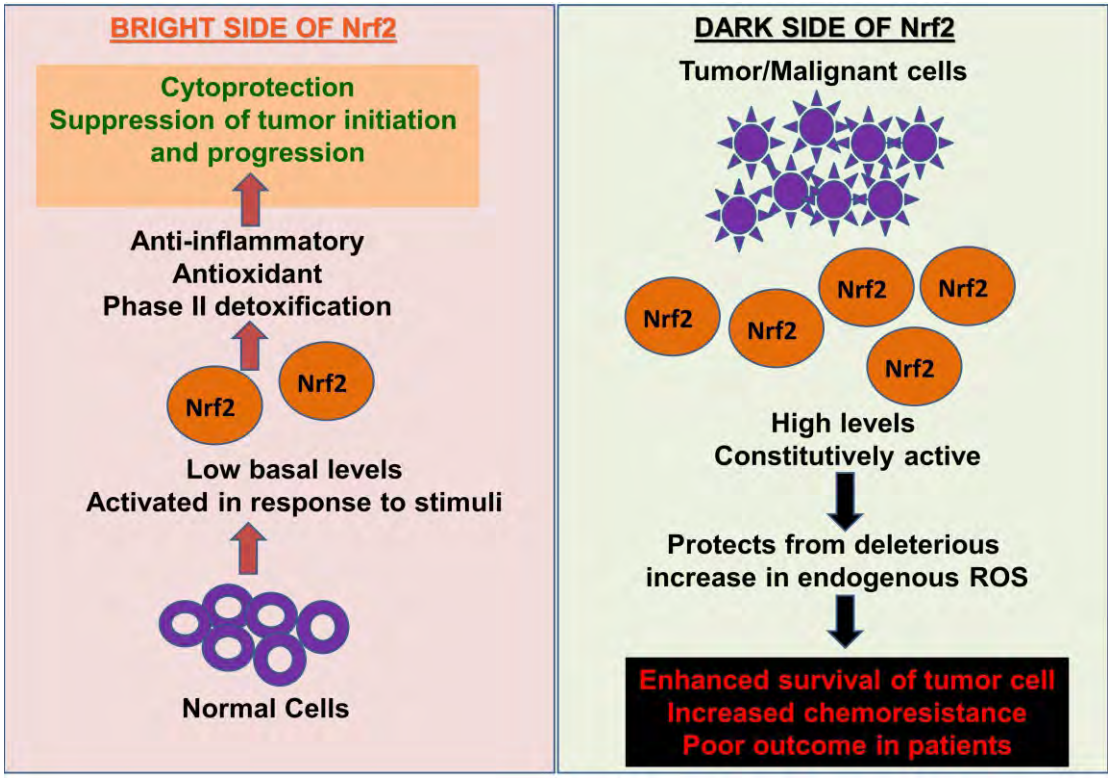

Figure 3: Dual role of Nrf2. The bright side is indicated by Nrf2 functions in normal cells where it acts as cytoprotective transcription factor inducing expression of an array of cytoprotective proteins, antioxidants and detoxifying enzymes leading to increased survival and cancer prevention. In tumor cells, constitutive high levels of Nrf2 provides an ambient niche for cancer cells to grow by reducing toxicity of endogenous ROS and xenobiotics. High levels of Nrf2 may increase cancer cell survival and imparts chemoresistance leading to poor clinical outcome.

expression of dependent cytoprotective genes, phase II detoxifying enzymes and antioxidant machinery. Evidences illustrating a positive role of Nrf2 in cancer prevention has been documented. Thus, efforts are underway to identify novel agents that can activate Nrf2. However, constitutive expression of Nrf2 may prevent death of precancerous lesions and promote survival of cancer cells under oxidative stress suggesting a dual role (Fig. 3). The transient activation of Nrf2 is beneficial, in countering ill effects of xenobiotics, oxidative stressors, carcinogens and mutagens. Whereas, persistent activation of Nrf2 in tumor cells confers survival advantage and makes them refractory to chemotherapy and/or radiotherapy. Evidence to directly implicate Nrf2 in cancer initiation needs confirmation. However, Nrf2 facilitates a reducing environment through up-regulation of the antioxidant and cytoprotective machinery. Thus providing armour for cancer cell to create an ambient growth niche and resist toxicity of xenobiotics. Hence, Nrf2 may serve as an additional target for therapeutic interventions, increasing susceptibility of cancer in conjuction with chemotherapy or radiotherapy treatment modalities.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Anu Ghosh and Dr. S. Jayakumar for proofreading the review. The authors would also like to acknowledge Dr. S. Chattopadhyay, Associate Director, Bioscience Group, Bhabha Atomic Research Centre, Mumbai, for his constant encouragement.

CONFLICT OF INTEREST

The authors claim no conflict of interest.

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