Published in final edited form as:
Free Radic Biol Med. 2015 February ; 0: 292–299. doi:10.1016/j.freeradbiomed.2014.11.009.

The dual roles of NRF2 in tumor prevention and progression: possible implications in cancer treatment

Eui Jung Moon and **Amato Giaccia**
Division of Radiation Biology & Oncology, Department of Radiation Oncology, Stanford University, Stanford, CA 94305, USA

Abstract

The Cap'N'Collar (CNC) family serves as cellular sensors of oxidative and electrophilic stresses and shares structural similarities including basic leucine zipper (bZIP) and CNC domains,. They form heterodimers with small MAF proteins to regulate antioxidant and phase II enzymes through antioxidant response element (ARE)-mediated transactivation. Among the CNC family members, NRF2 is required for systemic protection against redox-mediated injury and carcinogenesis. On the other hand, NRF2 is activated by oncogenic pathways, metabolism, and hypoxia. Constitutive NRF2 activation is observed in a variety of human cancers and it is highly correlated with tumor progression and aggressiveness. In this review, we will discuss how NRF2 plays dual roles in cancer prevention and progression depending on the cellular context and environment. Therefore, a better understanding of NRF2 will be necessary to exploit this complex network of balancing antioxidant pathways to inhibit tumor progression.

Keywords

Oxidative stress; NRF2; CNC family; antioxidant response element; ARE; small MAF; cancer

Introduction

In response to intrinsic and extrinsic stimuli, cells activate various adaptive mechanisms to promote reactive oxygen species (ROS) detoxification. The Cap'N'Collar (CNC) family proteins are transcription factors, which contain basic leucine zipper (bZIP) and CNC domains. By regulating various antioxidative genes and phase II detoxifying enzymes, which is required for metabolic detoxification of xenobiotics, they play a pivotal role in the cellular response to oxidative or electrophilic stresses [1–3]. The CNC family consists of Nuclear factor erythroid-derived 2 (NF-E2), NF-E2-related-1 [Nrf1 or NF-E2-like 1 (NFE2L1)], NRF2 (NFE2L2), NRF3 (NFE2L3), and distantly related Broad complex–Tramtrack–Bric-a-brac (BTB) and CNC homology 1 (BACH1) and BACH2. Functionally,

*Corresponding author: giaccia@stanford.edu.

the CNC transcription factors form heterodimers with small MAF proteins [4]. These heterodimers regulate genes containing the antioxidant response element (ARE) or the MAF recognition element (MARE) such as heme oxygenase 1 (*HO-1*), NADP(H):quinone oxidoreductase 1 (*NQO-1*), glutamylcysteine ligase (*GCL*), peroxiredoxin (*PRDX*), and superoxide dismutase (*SOD*), which are involved in detoxification and drug metabolism [5–7]. The ARE core sequence, 5′-RGTGA(C/G)NNNGC-3′, acts as a *cis*-acting enhancer and shows significant homology to the MARE enhancer, 5′-TGCTGAG(C)TCAGCA-3′ [8]. Interestingly, many studies demonstrate that among CNC members, NRF2 is heavily involved in the regulation of antioxidant genes [9–13]. In various cell lines, NRF2 expression increases ARE activity about four to six times higher than NRF1, NRF3, and NF-E2. Knocking out *Nfe2l2* in mouse fibroblast further supports that NRF2 regulates ARE-dependent genes such as GCL and HO-1. On the other hand, BACH1 and BACH2 play a repressive role by competing with NF-E2 and NRF2 [14–19]. Thus, here we review how NRF2 contributes to various physiological and pathological conditions.

Structure of Nrf2 and its stabilization under antioxidant stresses

NRF2 is a soluble protein primarily localized to the cytoplasm. It is highly conserved across species and contains seven functional Nrf2-ECH homology (NEH) domains (Figure 1) [20]. The Neh1 domain possesses the CNC-bZIP domain that is responsible for heterodimerization and ARE binding. Neh2, located in the N-terminus, is the main regulatory domain of NRF2, containing seven lysine residues for ubiquitination, and DLG (Asp-Leu-Gly) and ETGE (Glu-Thr-Gly-Glu) motifs for Kelch-like erythroid cell-derived protein with CNC homology (ECH)-associated protein 1 (KEAP1) binding. Although Neh3, Neh4, and Neh5 act as activation domains by interacting with the transcriptional coactivators [21, 22], Neh6 regulates KEAP1-independent NRF2 stability by recruiting an ubiquitin ligase complex [23]. The Neh7 domain has recently been identified to interact with retinoic X receptor alpha (RXRα), a repressor of NRF2 [24].

Under basal conditions, NRF2 is anchored in the cytoplasm through a direct interaction with the KEAP1 protein that leads to ubiquitination and proteasomal degradation [20]. KEAP1 contains three functional domains, a BTB domain, an intervening region (IVR), and a double glycine repeat (DGR) or a Kelch motif, which contains six copies of the conserved kelch repeat (two adjacent glycine residues and a tyrosine/tryptophan pairs separated by 7 residues) that form β-propeller structure (Figure 1). The BTB domain is required for KEAP1 homodimerization and the interaction with Cul3, which regulates KEAP1 through ubiquitination and proteasomal degradation. The Kelch/DGR domain includes six kelch repeats that mediate binding between KEAP1 and the Neh2 domain of NRF2. Twenty-seven cysteines within the IVR domain residues that act as active stress sensors in human Keap1 [25]. Among these, Cys151, Cys257, Cys273, Cys288, and Cys297 are known to be highly reactive towards oxidative and electrophilic stresses [25, 26]. In response to stresses, these cysteine residues become oxidized and form disulfide bonds or covalent adducts. The cysteine modifications cause a conformational change in KEAP1 to prevent NRF2 ubiquitination [27]. Free NRF2 then translocates into the nucleus, hetermodimerizes with small MAFs, and binds to antioxidant genes containing the ARE domain.

Role of NRF2 in development and systemic defense mechanisms

Nfe2l2 knockout mice are viable and fertile, indicating that *Nfe2l2* is dispensable for mouse development [28]. On the other hand, *Keap1* knockout mice die postnatally due to malnutrition resulting from abnormal hyperkeratosis of the esophagus and forestomach [29]. In these mice, expression of *Nfe2l2* as well as phase II enzymes targeted by *Nfe2l2* such as *Nqo1*, *Gcl*, and *Prdx1* is significantly increased, showing that NRF2 is constitutively activated. The lethality of *Keap1* knockout mice can be rescued with deletion of *Nfe2l2* as well as reduced expression of phase II enzymes. These results indicate that *Keap1* is an upstream repressor of *Nfe2l2*, and *Nfe2l2-Keap1* homeostasis is essential for cellular defense against oxidative stress.

Although there are no significant developmental deficits, dysregulation of ARE-dependent genes causes *Nfe2l2* knockout mice to be highly susceptible to oxidative stress and xenobiotic toxicity (Table 1). *Nfe2l2* knockout mice are more susceptible to hyperoxia due to increased oxidative lung injury and inflammation. Significantly lower expression of antioxidant and phase II enzymes in these mice suggests that *Nfe2l2* contributes to the protection against oxidative lung injury [30, 31]. Likewise, although phase II enzymes are highly induced by phenolic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT)), and N-acetyl-4-aminophenol (APAP, acetaminophen), their induction is largely inhibited in *Nfe2l2* knockout mice, resulting in increased tissue toxicity and high mortality [32–34]. NRF2 also plays a radioprotective role since thoracic irradiation decreases survival of *Nfe2l2* knockout mice when compared with *Nfe2l2* WT mice [35]. These results reflect the critical role of NRF2 in coordinating the antioxidant response.

Tumor suppressive roles of NRF2

Enhanced susceptibility to chemical carcinogens is also observed in *Nfe2l2* knockout mice [36, 37]. After benzo[a]pyrene (B[a]P) or N-titrosobutyl (4-hydroxybutyl) amine (BBN) exposure, *Nfe2l2* knockout mice develop gastric neoplasia or urinary bladder carcinoma at significantly higher rates [36, 37]. In these studies cancer chemopreventive actions of 4-methyl-5-[2-pyrazinyl]-1,2-dithiole-3-thione (oltipraz) are less effective in *Nfe2l2* knockout mice because the induction of phase 2 detoxifying enzymes is significantly reduced. Treatment of 7,12-dimethylbenz(*a*)anthracene (DMBA), 12-O-tetradecanoylphorbol-13-acetate (TPA), or azoxymethane/dextran sulfate sodium (DSS) also enhances incidence of skin, colorectal, or mammary tumors in *Nfe2l2* knockout mice, indicating NRF2 plays a critical role in protecting against carcinogenesis [38–40].

In addition to preventing primary tumor growth, *Nfe2l2* has a protective function against tumor metastasis [41]. After intravenous injection of Lewis lung carcinoma (3LL) cell lines, *Nfe2l2* knockout mice show a significantly increased number of metastatic nodules in the lung. On the other hand, *Keap1* conditional knockout mice using the Cre-Lox system exhibit a significantly decreased number of lung metastasis after injection of 3LL cells. These results indicate that although *Nfe2l2* is dispensable during embryonic development, it is required for systemic protection against carcinogenesis and metastasis.

Oncogenic roles of NRF2

Although knockout mouse studies indicate that loss of *Nfe2l2* reduces cellular protection against oxidative stress and carcinogenesis, prolonged activation of the NRF2 protein is correlated with cancer progression in several cases. Increased NRF2 expression has been extensively studied in patients with head and neck cancer [42–44], lung cancer [45–50], gall bladder cancer [51, 52], epithelial ovarian cancer [53], osteosarcoma [54], breast cancer [55], bladder cancer [56], colorectal cancer [57], gastric cancer [58], glioblastoma [59, 60], and pancreatic cancer [61]. In these patients, Nrf2 expression is significantly correlated with increased proliferation and treatment resistance to radiation, cisplatin, and 5-fluorouracil (5-FU), seemingly through the induction of antioxidant genes [44, 45, 48, 53, 55, 58]. Patient survival and multivariate analysis further demonstrate that NRF2 is a poor prognostic factor in cancer patients (Table 2).

Although loss-of-function mutations in *KEAP1* contribute to constitutive activation of NRF2 in tumors [45, 51, 53], oncogenes and oncogenic signaling pathways are also involved in increased NRF2 expression (Figure 2). Mouse embryonic fibroblasts (MEF) and NIH3T3 fibroblasts transduced with oncogenic alleles of *K-Ras*, *B-Raf*, and *Myc* (K-Ras^{G12D}, B-Raf^{V619E} (corresponding to human B-Raf^{V600E}), and Myc^{ERT2}) increase *Nfe2l2* transcription and simultaneously, decrease ROS production [62]. In the K-Ras mutant mouse model of pancreatic cancer and B-Raf^{V619E} expressing mouse lung adenoma, NQO1 expression is increased, whereas DNA oxidation, stained by 7,8-dihydro-8-oxo-29-deoxyguanosine (8-oxo-dGuo) is decreased. *Nfe2l2* deletion in these mice results in reduced cell proliferation and tumor burden as well as elevated DNA oxidation. Therefore, Nrf2 activation, which is mediated by oncogenic signaling, contributes to tumor growth and progression through ROS detoxification.

In addition, a number of other oncogenic protein kinases also contribute to enhanced NRF2 stability. Protein kinase C (PKC) phosphorylates NRF2 on Ser 40 and induces its dissociation from KEAP1 [63, 64]. Similarly, PKR-like ER kinase (PERK), which is essential for ER stress, also phosphorylates NRF2 and inhibits its binding to Keap1 [65]. PERK-mediated NRF2 activation promotes cell survival in response to ER stress. Inhibition of MAP kinases and PI3K blocks NRF2 nuclear translocation and ARE-mediated gene transcription [66–69]. Since oncogenic *K-RAS* signaling activates PI3K and PKC regulates the MEK-ERK pathway in cancer, there may be crosstalk between a variety of signaling pathways to regulate NRF2 [70, 71]. However, the direct roles of these oncogenic pathways in regulating NRF2-induced redox responses and tumor progression remain unclear.

The role of NRF2 in metabolism

Metabolic switches are one hallmark of cancer [72]. In addition to its role in antioxidative responses for cancer cell protection, microarray and ChIP-sequencing analysis further show that NRF2 regulates cell proliferation and survival by activating metabolic enzymes involved in pentose phosphatase pathways (PPPs) [73–75]. PPP prevents oxidative stress by generating NADPH. NRF2 mediates upregulation of metabolic genes such as glucose-6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase (PGD), transketolase

(TKT), transaldolase 1 (TALDO1), malic enzyme 1 (ME1), and isocitrate dehydrogenase 1 (IDH1), suggesting that Nrf2 regulates the PPP and NADPH production pathways. Mechanistically Nrf2-mediated changes in these metabolic genes alter glucose and glutamine metabolism to promote cancer cell proliferation. Fumarate accumulation due to fumarate hydratase (FH)-deficiency results in hereditary leiomyomatosis renal cell carcinoma (HLRCC) and type-2 papillary renal carcinoma (pRCC) [76]. A recent study also shows that fumarate, a metabolite of the Krebs cycle, activates NRF2 through the succination of cysteine residues of KEAP1 [77]. Elevated NRF2 expression in FH deficient pRCC further indicates that NRF2-mediated metabolic alteration plays a role in the aggressive growth of cancer.

The role of NRF2 in treatment resistance

NRF2 is also involved in resistance to cancer treatment. Inhibiting NRF2 expression in tumors enhances sensitivity to ionizing radiation and chemotherapeutic drugs such as doxorubicin, cisplatin, etoposide, and 5-FU [51, 78–83]. Doxorubicin, cisplatin, and ionizing radiation increase glutathione synthesis (GSH) levels and NRF2 activation [84, 85]. Levels of cellular GSH, which is catalyzed by GCS, are critical for detoxification of anticancer drugs [86]. Inhibition of NRF2 reverses GSH induction and radiation resistance, linking NRF2-mediated GSH induction and treatment resistance. Radiotherapy and chemotherapy induce multidrug resistance proteins (MRPs) [87, 88]. MRPs are ATP-dependent transporters that can efflux chemicals and metabolites. Thus, increased expression of MRP proteins is correlated with treatment resistance. Knockout mouse studies show that NRF2 is required for the induction of MRP1, MRP2, MRP3, and MRP4 [89–91]. In conclusion, NRF2 induces chemo- and radio-resistance while maintaining cellular homeostasis. Therefore, targeting NRF2-regulated pathways may be a good strategy to overcome resistance to cancer treatment.

Hypoxia-mediated regulation of Nrf2

Tumor hypoxia is caused by an imbalance between oxygen consumption and supply. HIF is a major regulator of hypoxic gene regulation, and HIF transactivates many downstream genes involved in crucial steps of tumor progression, including angiogenesis, glycolytic metabolism, and treatment resistance to both radiotherapy and chemotherapy [92–94]. HIF is a dimeric transcription factor comprising α and β subunits. Unlike HIF-1β, which is constitutively expressed, HIFα is regulated by various factors including oxygen, free radicals, and oncogenic signaling pathways [95–99]. Under normal conditions, HIFα is hydroxylated by prolyl hydroxylases (PHD) [100]. Prolyl hydroxylation leads to von Hippel-Lindau (VHL)-mediated proteasomal degradation of HIFα. Under hypoxia, PHD activity is inhibited, which results in HIFα stabilization and activation. Meanwhile, hypoxia is known to increase mitochondrial ROS production, suggesting a linkage between hypoxia, HIF, and the NRF2 pathway [101]. Indeed, several studies have shown that NRF2 expression or its activation is induced by chronic or intermittent hypoxia [102, 103]. Microarray data also reveals that gain-of-function mutants of NRF2 are significantly correlated with hypoxia signatures in patients with head and neck cancer [47]. In terms of target gene regulation, NRF2 and HIF can act together or independently in regulating gene

expression such as HO-1, a common target of both NRF2 and HIF [104]. Interestingly, HO-1 can be induced in Chinese Hamster Ovary (CHO) cells even in the absence of HIF-1 activity, indicating that Nrf2 regulates HO-1 in a HIF-independent manner. On the other hand, HIF-dependent NRF2 regulation is also reported in human colorectal cancer cell lines, HCT116 and HT29 [105]. Knocking down *NFE2L2* inhibits the hypoxic induction of HIF-1 as well as a pro-angiogenic factor, vascular endothelial growth factor (VEGF). As a result, *in vivo* tumor growth and tumor angiogenesis are significantly curtailed. In this study, it is suggested that competition between NRF2 and PHD for oxygen availability activates the HIF-VEGF pathway. In patients with glioblastoma, expression of NRF2 and HIF-1 is correlated, further indicating these two proteins may cooperate or interrelate for cancer progression [59]. Thus, NRF2 may be a valuable target for repression of HIF-mediated tumor growth and angiogenesis (Figure 3).

Modulation of NRF2 signaling: NRF2 activators

Given the fact that NRF2 plays a role in disease prevention, NRF2 is an attractive target for activation. Dimethyl fumarate (DMF or Tecfidera) is a methyl ester of fumaric acid (FA), which alkylates Cys151 of KEAP1, inhibits NRF2-KEAP1 binding, and hence, stabilizes NRF2 [106]. DMF treatment reduces inflammatory responses and promotes neuroprotection. Currently DMF is approved by the FDA to treat patients with relapsing multiple sclerosis (MS). Other NRF2 activators are also being investigated in clinical trials for a variety of diseases [107, 108]. Sulforaphane (SFN) is an isothiocyanate that is naturally present in cruciferous vegetables such as broccoli and cabbage. SFN forms a covalent adduct with cysteine residues with KEAP1 at cysteine residues including Cys151 and enhances NRF2 activation [109]. Promising preclinical studies demonstrate that SFN prevents mice from forming carcinogen-induced mammary tumors, colonic crypt foci, gastric cancer, and lung cancer [110–113]. Similarly, SFN inhibited prostate cancer progression and pulmonary metastasis from TRAMP transgenic mice harboring an adenocarcinoma of the mouse prostate [114]. *In vitro*, SFN mediates cell cycle arrest, apoptosis, and inhibition of endothelial cell proliferation and migration, suggesting its potential as an anti-cancer molecule [115–117]. SFN treatment is currently being investigated in clinical trials as an NRF2 activator in patients with breast, prostate, pancreatic, and colon cancers [118–121].

The synthetic triterpenoids, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) and its derivative, bardoxolone methyl (CDDO-me) are known for their anti-inflammatory and anti-tumor effects [122]. The interaction between CDDO and the BTB domain of KEAP1 inhibits binding to NRF2 and leads to NRF2 activation [123]. However, phase III clinical trials in patients with chronic kidney disease were terminated prematurely due to patients having severe chronic renal disease and adverse cardiovascular effects, indicating that a detailed understanding of NRF2 activation in tissues needs to be gained before the clinical use of the NRF2 activator [124].

Modulation of NRF2 signaling: NRF2 inhibitors

In contrast to NRF2 activators, NRF2 inhibitors block tumor progression. Brusatol, a plant extract from *Brucea javanica*, reduces ARE-dependent gene expression by enhancing

degradation of NRF2 [125]. Brusatol inhibits *in vivo* tumor growth while enhancing chemosensitivity, indicating its beneficial effect as a combinatorial therapy. Luteolin, a flavonoid that also exists in plants and vegetables, blocks subcutaneous lung tumor cell growth and proliferation in mice while increasing cytotoxicity of cisplatin treatment [126, 127]. Ascorbic acid and *all-trans*-retinoic acid (ATRA), other natural compounds, suppress NRF2 activation by interfering with nuclear localization of Nrf2 or its binding to ARE [128, 129]. Specifically, ATRA, a metabolite of vitamin A, inhibits NRF2 by activating RA receptor α (RARα). RARα directly interacts with NRF2 and prevents its binding to ARE [128]. However, these inhibitors are electrophiles that share non-specific off-target effects and can target cysteine residues in other proteins or enzymes [108]. Therefore, development of NRF2-targeting agents with enhanced specificity is required to improve the effectiveness of cancer treatment. Additionally, conflicting roles of NRF2 in cancer prevention and cancer progression indicate that more questions need to be addressed to determine the optimal use of NRF2 activators or inhibitors in the clinic.

The relationship between NRF2 and small MAF family proteins

Consisting of MAFF, MAFK, and MAFG, the small MAF proteins are essential binding partners of the CNC family [4, 6, 130]. Both bZiP protein arrays and ChIP-sequencing analysis indicate the fact that Nrf2 forms heterodimers mainly with small MAFs [74, 131]. Specifically, ChIP-sequencing data indicates significant overlap between NRF2 and MAFG binding sites. The JUN family (JUN, JUND, and JUNB) [11, 132], FOSB [133, 134], and ATF4 [135] are also known to bind to Nrf2. However, specific roles of these complexes are still under investigation.

The original MAF protein was first described as a viral oncogene, *v*-Maf from **m**usculo**a**poneurotic **f**ibrosarcoma, in chickens [136]. The MAF family contains the basic DNA binding domain and the leucine zipper dimerization domain [6]. The extended homology region (EHR), which is highly conserved in MAF family members, is also required for DNA binding (Figure 1). MAF proteins can be divided into two groups: large MAFs and small MAFs. Large MAF proteins, including MAFA (L-MAF), MAFB [137], c-MAF (MAF) [138], and Neura Retinal (NRL), comprise an acidic TAD in their N-terminus. Unlike large MAFs, small Maf proteins, which are 18 kDa in size, lack a TAD. Small MAF family proteins form homodimers or heterodimers with CNC family proteins, FOS, and FOSB and binds to MARE or ARE [4].

Although small MAF proteins interact with NRF2 to activate antioxidant response genes through ARE pathways, increased expression of small MAFs in response to oxidative and electrophilic stresses indicates that they are also regulated by redox pathways [139, 140]. Interestingly, the repressive role of small MAF family proteins has also been identified when they are overexpressed. Overexpression of MAFG and MAFK inhibits the NRF2-mediated induction of NQO1, Glutatione S-transferase (GST), and GCS [86, 141]. Excessive expression of small MAF proteins may lead to formation of small MAF homodimers, which inhibit antioxidant gene transcription. Therefore, functional consequences of small MAFs are highly dependent on the quantitative balance between small MAF and its CNC partners [142–144].

Similarly to NRF2, small MAF proteins are also associated with carcinogenesis. Comparative genomic hybridization arrays reveal that DNA copy number of MAFG is amplified in lung adenocarcinoma [145]. A study of lung epithelial cells suggests that negative regulation of mir-218 increases MAFG after smoking and possibly leads to lung carcinogenesis [146]. In hepatocellular carcinoma patients with *CTNNB1* (β-catenin) mutations, MAFG is also highly expressed, indicating that MAFG might be a proto-oncogene [147]. In addition, genetic disruption of small MAF proteins has also been reported in familial pancreatic cancer and CML patients, indicating a broader oncogenic role [148, 149]. In contrast, high expression of MAFF in patients with ovarian and prostate cancer is correlated with prolonged survival, suggesting a role for MAFF in tumor suppression [150].

Small MAFs also respond to microenvironmental changes. The interaction of HIF-1α with MAFG or MAFK has been shown by yeast two-hybrid and surface plasmon resonance [151]. In this study, MAFG and MAFK bind to the PAS-A domain of HIF-1α. Knocking down MAFG inhibits nuclear localization of HIF-1α and, as a result, decreases expression of erythropoietin (EPO), one of HIF-1α's target genes under hypoxia. On the other hand, MAFG does not affect protein levels of HIF-1α, indicating its specific role in HIF-1α translocation. Interestingly, overexpression of MAFG also decreases the accumulation of HIF-1α in nuclei as well as EPO expression, suggesting that it acts as an activator and a repressor depending on its abundance. In addition to hypoxia, small MAF proteins are regulated by extracellular pH [152]. For example, acidic pH (pH 6.6) significantly increases small MAF expression as well as FOSB. Co-localization studies show that both MAFG and FOSB are expressed and interact with each other in the nucleus, and that this heterodimer can activate MMP1 by binding to an AP-1 consensus site in the MMP1 promoter.

Taken together, small MAF proteins play either repressive or activating roles in antioxidant responses and carcinogenesis and these might be due to competition between small MAF homodimers and NRF2–small MAF heterodimers for binding to ARE embedded in MARE. However, other than quantity based regulation, the factors that determine the formation of different binding complexes still need to be investigated. Overall, these results indicate that small MAF expression must be finely tuned in order to activating or repressive effects on Nrf2 and other transcription factors.

Conclusion

In response to oxidative and carcinogenic stimuli, NRF2 transactivates various genes involved in defensive and adaptive pathways to prevent normal tissue damage. As a result, NRF2 deficiency causes mutant mice to be more susceptible to oxidative challenge and carcinogen exposure. Ironically, its roles in tumor progression indicate that NRF2 should be considered as a potential therapeutic target as well. NRF2 activity is also controlled by oncogenic signaling pathways, metabolism, and cellular microenvironment. Additional studies reveal NRF2 is also involved in pathogenic pathways and physiological changes, such as angiogenesis, and metastasis. As binding partners of NRF2, small MAF proteins fulfill both positive and negative functions depending on their formation of homo- or heterodimers. Thus, whether NRF2 inhibits or activates carcinogenesis is highly context

dependent. Therefore, it is still important to determine whether NRF2 is activated or suppressed in various environments and under the influence of diverse stimuli.

References

1. Hayes JD, McMahon M. Molecular basis for the contribution of the antioxidant responsive element to cancer chemoprevention. Cancer letters. 2001; 174:103–113. [PubMed: 11689285]
2. Jaiswal AK. Nrf2 signaling in coordinated activation of antioxidant gene expression. Free radical biology & medicine. 2004; 36:1199–1207. [PubMed: 15110384]
3. Motohashi H, Yamamoto M. Carcinogenesis and transcriptional regulation through Maf recognition elements. Cancer science. 2007; 98:135–139. [PubMed: 17129360]
4. Kannan MB, Solovieva V, Blank V. The small MAF transcription factors MAFF, MAFG and MAFK: current knowledge and perspectives. Biochimica et biophysica acta. 2012; 1823:1841–1846. [PubMed: 22721719]
5. Itoh K, Ishii T, Wakabayashi N, Yamamoto M. Regulatory mechanisms of cellular response to oxidative stress. Free radical research. 1999; 31:319–324. [PubMed: 10517536]
6. Blank V. Small Maf proteins in mammalian gene control: mere dimerization partners or dynamic transcriptional regulators? Journal of molecular biology. 2008; 376:913–925. [PubMed: 18201722]
7. Eychene A, Rocques N, Pouponnot C. A new MAFia in cancer. Nature reviews Cancer. 2008; 8:683–693.
8. Nerland DE. The antioxidant/electrophile response element motif. Drug metabolism reviews. 2007; 39:235–248. [PubMed: 17364885]
9. Alam J, Stewart D, Touchard C, et al. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. The Journal of biological chemistry. 1999; 274:26071–26078. [PubMed: 10473555]
10. Kobayashi A, Ito E, Toki T, et al. Molecular cloning and functional characterization of a new Cap'n' collar family transcription factor Nrf3. The Journal of biological chemistry. 1999; 274:6443–6452. [PubMed: 10037736]
11. Jeyapaul J, Jaiswal AK. Nrf2 and c-Jun regulation of antioxidant response element (ARE)-mediated expression and induction of gamma-glutamylcysteine synthetase heavy subunit gene. Biochemical pharmacology. 2000; 59:1433–1439. [PubMed: 10751553]
12. Leung L, Kwong M, Hou S, et al. Deficiency of the Nrf1 and Nrf2 transcription factors results in early embryonic lethality and severe oxidative stress. The Journal of biological chemistry. 2003; 278:48021–48029. [PubMed: 12968018]
13. Zhang Y, Kobayashi A, Yamamoto M, Hayes JD. The Nrf3 transcription factor is a membrane-bound glycoprotein targeted to the endoplasmic reticulum through its N-terminal homology box 1 sequence. The Journal of biological chemistry. 2009; 284:3195–3210. [PubMed: 19047052]
14. Yoshida C, Tokumasu F, Hohmura KI, et al. Long range interaction of cis-DNA elements mediated by architectural transcription factor Bach1. Genes to cells : devoted to molecular & cellular mechanisms. 1999; 4:643–655. [PubMed: 10620011]
15. Oyake T, Itoh K, Motohashi H, et al. Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. Molecular and cellular biology. 1996; 16:6083–6095. [PubMed: 8887638]
16. Muto A, Hoshino H, Madisen L, et al. Identification of Bach2 as a B-cell-specific partner for small maf proteins that negatively regulate the immunoglobulin heavy chain gene 3′ enhancer. The EMBO journal. 1998; 17:5734–5743. [PubMed: 9755173]
17. Sun J, Hoshino H, Takaku K, et al. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. The EMBO journal. 2002; 21:5216–5224. [PubMed: 12356737]
18. Dhakshinamoorthy S, Jain AK, Bloom DA, Jaiswal AK. Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. The Journal of biological chemistry. 2005; 280:16891–16900. [PubMed: 15734732]

19. Tahara T, Sun J, Igarashi K, Taketani S. Heme-dependent up-regulation of the alpha-globin gene expression by transcriptional repressor Bach1 in erythroid cells. Biochemical and biophysical research communications. 2004; 324:77–85. [PubMed: 15464985]
20. Itoh K, Wakabayashi N, Katoh Y, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes & development. 1999; 13:76–86. [PubMed: 9887101]
21. Katoh Y, Itoh K, Yoshida E, et al. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. Genes to cells : devoted to molecular & cellular mechanisms. 2001; 6:857–868. [PubMed: 11683914]
22. Nioi P, Nguyen T, Sherratt PJ, Pickett CB. The carboxy-terminal Neh3 domain of Nrf2 is required for transcriptional activation. Molecular and cellular biology. 2005; 25:10895–10906. [PubMed: 16314513]
23. McMahon M, Thomas N, Itoh K, et al. Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. The Journal of biological chemistry. 2004; 279:31556–31567. [PubMed: 15143058]
24. Wang H, Liu K, Geng M, et al. RXRalpha inhibits the NRF2-ARE signaling pathway through a direct interaction with the Neh7 domain of NRF2. Cancer research. 2013; 73:3097–3108. [PubMed: 23612120]
25. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, et al. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:11908–11913. [PubMed: 12193649]
26. Sekhar KR, Rachakonda G, Freeman ML. Cysteine-based regulation of the CUL3 adaptor protein Keap1. Toxicology and applied pharmacology. 2010; 244:21–26. [PubMed: 19560482]
27. Zhang DD, Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Molecular and cellular biology. 2003; 23:8137–8151. [PubMed: 14585973]
28. Chan K, Lu R, Chang JC, Kan YW. NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:13943–13948. [PubMed: 8943040]
29. Wakabayashi N, Itoh K, Wakabayashi J, et al. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. Nature genetics. 2003; 35:238–245. [PubMed: 14517554]
30. Cho HY, Jedlicka AE, Reddy SP, et al. Role of NRF2 in protection against hyperoxic lung injury in mice. American journal of respiratory cell and molecular biology. 2002; 26:175–182. [PubMed: 11804867]
31. Reddy NM, Kleeberger SR, Kensler TW, et al. Disruption of Nrf2 impairs the resolution of hyperoxia-induced acute lung injury and inflammation in mice. Journal of immunology. 2009; 182:7264–7271.
32. Itoh K, Chiba T, Takahashi S, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochemical and biophysical research communications. 1997; 236:313–322. [PubMed: 9240432]
33. Chan K, Kan YW. Nrf2 is essential for protection against acute pulmonary injury in mice. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96:12731–12736. [PubMed: 10535991]
34. Enomoto A, Itoh K, Nagayoshi E, et al. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicological sciences : an official journal of the Society of Toxicology. 2001; 59:169–177. [PubMed: 11134556]
35. Travis EL, Rachakonda G, Zhou X, et al. NRF2 deficiency reduces life span of mice administered thoracic irradiation. Free radical biology & medicine. 2011; 51:1175–1183. [PubMed: 21712086]
36. Ramos-Gomez M, Kwak MK, Dolan PM, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice.

Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:3410–3415. [PubMed: 11248092]

37. Iida K, Itoh K, Kumagai Y, et al. Nrf2 is essential for the chemopreventive efficacy of oltipraz against urinary bladder carcinogenesis. Cancer research. 2004; 64:6424–6431. [PubMed: 15374950]
38. Xu C, Huang MT, Shen G, et al. Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. Cancer research. 2006; 66:8293–8296. [PubMed: 16912211]
39. Khor TO, Huang MT, Prawan A, et al. Increased susceptibility of Nrf2 knockout mice to colitis-associated colorectal cancer. Cancer prevention research. 2008; 1:187–191. [PubMed: 19138955]
40. Becks L, Prince M, Burson H, et al. Aggressive mammary carcinoma progression in Nrf2 knockout mice treated with 7,12-dimethylbenz[a]anthracene. BMC cancer. 2010; 10:540. [PubMed: 20932318]
41. Satoh H, Moriguchi T, Taguchi K, et al. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. Carcinogenesis. 2010; 31:1833–1843. [PubMed: 20513672]
42. Stacy DR, Ely K, Massion PP, et al. Increased expression of nuclear factor E2 p45-related factor 2 (NRF2) in head and neck squamous cell carcinomas. Head & neck. 2006; 28:813–818. [PubMed: 16637057]
43. Huang CF, Zhang L, Ma SR, et al. Clinical significance of Keap1 and Nrf2 in oral squamous cell carcinoma. PloS one. 2013; 8:e83479. [PubMed: 24386210]
44. Kawasaki Y, Okumura H, Uchikado Y, et al. Nrf2 is Useful for Predicting the Effect of Chemoradiation Therapy on Esophageal Squamous Cell Carcinoma. Annals of surgical oncology. 2014
45. Singh A, Misra V, Thimmulappa RK, et al. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS medicine. 2006; 3:e420. [PubMed: 17020408]
46. Solis LM, Behrens C, Dong W, et al. Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinicopathologic features. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010; 16:3743–3753. [PubMed: 20534738]
47. Shibata T, Saito S, Kokubu A, et al. Global downstream pathway analysis reveals a dependence of oncogenic NF-E2-related factor 2 mutation on the mTOR growth signaling pathway. Cancer research. 2010; 70:9095–9105. [PubMed: 21062981]
48. Yang H, Wang W, Zhang Y, et al. The role of NF-E2-related factor 2 in predicting chemoresistance and prognosis in advanced non-small-cell lung cancer. Clinical lung cancer. 2011; 12:166–171. [PubMed: 21663859]
49. Inoue D, Suzuki T, Mitsuishi Y, et al. Accumulation of p62/SQSTM1 is associated with poor prognosis in patients with lung adenocarcinoma. Cancer science. 2012; 103:760–766. [PubMed: 22320446]
50. Merikallio H, Paakko P, Kinnula VL, et al. Nuclear factor erythroid-derived 2-like 2 (Nrf2) and DJ1 are prognostic factors in lung cancer. Human pathology. 2012; 43:577–584. [PubMed: 21943684]
51. Shibata T, Kokubu A, Gotoh M, et al. Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. Gastroenterology. 2008; 135:1358–1368. 1368 e1351–1354. [PubMed: 18692501]
52. Wang J, Zhang M, Zhang L, et al. Correlation of Nrf2, HO-1, and MRP3 in gallbladder cancer and their relationships to clinicopathologic features and survival. The Journal of surgical research. 2010; 164:e99–105. [PubMed: 20828733]
53. Konstantinopoulos PA, Spentzos D, Fountzilas E, et al. Keap1 mutations and Nrf2 pathway activation in epithelial ovarian cancer. Cancer research. 2011; 71:5081–5089. [PubMed: 21676886]
54. Park JY, Kim YW, Park YK. Nrf2 expression is associated with poor outcome in osteosarcoma. Pathology. 2012; 44:617–621. [PubMed: 23172081]

55. Hartikainen JM, Tengstrom M, Kosma VM, et al. Genetic polymorphisms and protein expression of NRF2 and Sulfiredoxin predict survival outcomes in breast cancer. Cancer research. 2012; 72:5537–5546. [PubMed: 22964583]
56. Hayden A, Douglas J, Sommerlad M, et al. The Nrf2 transcription factor contributes to resistance to cisplatin in bladder cancer. Urologic oncology. 2014
57. Ji L, Wei Y, Jiang T, Wang S. Correlation of Nrf2, NQO1, MRP1, cmyc and p53 in colorectal cancer and their relationships to clinicopathologic features and survival. International journal of clinical and experimental pathology. 2014; 7:1124–1131. [PubMed: 24695690]
58. Hu XF, Yao J, Gao SG, et al. Nrf2 overexpression predicts prognosis and 5-fu resistance in gastric cancer. Asian Pacific journal of cancer prevention : APJCP. 2013; 14:5231–5235. [PubMed: 24175806]
59. Ji X, Wang H, Zhu J, et al. Correlation of Nrf2 and HIF-1alpha in glioblastoma and their relationships to clinicopathologic features and survival. Neurological research. 2013; 35:1044–1050. [PubMed: 24070025]
60. Ji XJ, Chen SH, Zhu L, et al. Knockdown of NF-E2-related factor 2 inhibits the proliferation and growth of U251MG human glioma cells in a mouse xenograft model. Oncology reports. 2013; 30:157–164. [PubMed: 23673813]
61. Soini Y, Eskelinen M, Juvonen P, et al. Nuclear Nrf2 expression is related to a poor survival in pancreatic adenocarcinoma. Pathology, research and practice. 2014; 210:35–39.
62. DeNicola GM, Karreth FA, Humpton TJ, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature. 2011; 475:106–109. [PubMed: 21734707]
63. Huang HC, Nguyen T, Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. The Journal of biological chemistry. 2002; 277:42769–42774. [PubMed: 12198130]
64. Bloom DA, Jaiswal AK. Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated NAD(P)H:quinone oxidoreductase-1 gene expression. The Journal of biological chemistry. 2003; 278:44675–44682. [PubMed: 12947090]
65. Cullinan SB, Zhang D, Hannink M, et al. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Molecular and cellular biology. 2003; 23:7198–7209. [PubMed: 14517290]
66. Yu R, Lei W, Mandlekar S, et al. Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals. The Journal of biological chemistry. 1999; 274:27545–27552. [PubMed: 10488090]
67. Kang KW, Ryu JH, Kim SG. The essential role of phosphatidylinositol 3-kinase and of p38 mitogen-activated protein kinase activation in the antioxidant response element-mediated rGSTA2 induction by decreased glutathione in H4IIE hepatoma cells. Molecular pharmacology. 2000; 58:1017–1025. [PubMed: 11040049]
68. Zipper LM, Mulcahy RT. Inhibition of ERK and p38 MAP kinases inhibits binding of Nrf2 and induction of GCS genes. Biochemical and biophysical research communications. 2000; 278:484–492. [PubMed: 11097862]
69. Nakaso K, Yano H, Fukuhara Y, et al. PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells. FEBS letters. 2003; 546:181–184. [PubMed: 12832036]
70. Wu WS, Wu JR, Hu CT. Signal cross talks for sustained MAPK activation and cell migration: the potential role of reactive oxygen species. Cancer metastasis reviews. 2008; 27:303–314. [PubMed: 18299806]
71. Eser S, Schnieke A, Schneider G, Saur D. Oncogenic KRAS signalling in pancreatic cancer. British journal of cancer. 2014; 111:817–822. [PubMed: 24755884]
72. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–674. [PubMed: 21376230]
73. Mitsuishi Y, Taguchi K, Kawatani Y, et al. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer cell. 2012; 22:66–79. [PubMed: 22789539]

74. Hirotsu Y, Katsuoka F, Funayama R, et al. Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. Nucleic acids research. 2012; 40:10228–10239. [PubMed: 22965115]

75. Malhotra D, Portales-Casamar E, Singh A, et al. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. Nucleic acids research. 2010; 38:5718–5734. [PubMed: 20460467]

76. Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nature genetics. 2002; 30:406–410. [PubMed: 11865300]

77. Adam J, Hatipoglu E, O'Flaherty L, et al. Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. Cancer cell. 2011; 20:524–537. [PubMed: 22014577]

78. Homma S, Ishii Y, Morishima Y, et al. Nrf2 enhances cell proliferation and resistance to anticancer drugs in human lung cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009; 15:3423–3432. [PubMed: 19417020]

79. Shim GS, Manandhar S, Shin DH, et al. Acquisition of doxorubicin resistance in ovarian carcinoma cells accompanies activation of the NRF2 pathway. Free radical biology & medicine. 2009; 47:1619–1631. [PubMed: 19751820]

80. Wang XJ, Sun Z, Villeneuve NF, et al. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. Carcinogenesis. 2008; 29:1235–1243. [PubMed: 18413364]

81. Jiang T, Chen N, Zhao F, et al. High levels of Nrf2 determine chemoresistance in type II endometrial cancer. Cancer research. 2010; 70:5486–5496. [PubMed: 20530669]

82. McDonald JT, Kim K, Norris AJ, et al. Ionizing radiation activates the Nrf2 antioxidant response. Cancer research. 2010; 70:8886–8895. [PubMed: 20940400]

83. Singh A, Bodas M, Wakabayashi N, et al. Gain of Nrf2 function in non-small-cell lung cancer cells confers radioresistance. Antioxidants & redox signaling. 2010; 13:1627–1637. [PubMed: 20446773]

84. Iida T, Mori E, Mori K, et al. Co-expression of gamma-glutamylcysteine synthetase sub-units in response to cisplatin and doxorubicin in human cancer cells. International journal of cancer Journal international du cancer. 1999; 82:405–411. [PubMed: 10399958]

85. Jayakumar S, Kunwar A, Sandur SK, et al. Differential response of DU145 and PC3 prostate cancer cells to ionizing radiation: role of reactive oxygen species, GSH and Nrf2 in radiosensitivity. Biochimica et biophysica acta. 2014; 1840:485–494. [PubMed: 24121106]

86. Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. The Journal of biological chemistry. 1999; 274:33627–33636. [PubMed: 10559251]

87. Januchowski R, Wojtowicz K, Sujka-Kordowska P, et al. MDR gene expression analysis of six drug-resistant ovarian cancer cell lines. BioMed research international. 2013; 2013:241763. [PubMed: 23484165]

88. Henness S, Davey MW, Harvie RM, Davey RA. Fractionated irradiation of H69 small-cell lung cancer cells causes stable radiation and drug resistance with increased MRP1, MRP2, and topoisomerase IIalpha expression. International journal of radiation oncology, biology, physics. 2002; 54:895–902.

89. Maher JM, Dieter MZ, Aleksunes LM, et al. Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters via the nuclear factor-E2-related factor-2 transcriptional pathway. Hepatology. 2007; 46:1597–1610. [PubMed: 17668877]

90. Vollrath V, Wielandt AM, Iruretagoyena M, Chianale J. Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. The Biochemical journal. 2006; 395:599–609. [PubMed: 16426233]

91. Hayashi A, Suzuki H, Itoh K, et al. Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. Biochemical and biophysical research communications. 2003; 310:824–829. [PubMed: 14550278]

92. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. Cancer research. 1998; 58:1408–1416. [PubMed: 9537241]

93. Rankin EB, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. Cell death and differentiation. 2008; 15:678–685. [PubMed: 18259193]

94. Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nature reviews Cancer. 2008; 8:425–437.

95. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A. 1995; 92:5510–5514. [PubMed: 7539918]

96. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem. 1995; 270:1230–1237. [PubMed: 7836384]

97. Laughner E, Taghavi P, Chiles K, et al. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol. 2001; 21:3995–4004. [PubMed: 11359907]

98. Chandel NS, Maltepe E, Goldwasser E, et al. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci U S A. 1998; 95:11715–11720. [PubMed: 9751731]

99. Goyal P, Weissmann N, Grimminger F, et al. Upregulation of NAD(P)H oxidase 1 in hypoxia activates hypoxia-inducible factor 1 via increase in reactive oxygen species. Free Radic Biol Med. 2004; 36:1279–1288. [PubMed: 15110393]

100. Berra E, Benizri E, Ginouves A, et al. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 2003; 22:4082–4090. [PubMed: 12912907]

101. Mansfield KD, Guzy RD, Pan Y, et al. Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. Cell metabolism. 2005; 1:393–399. [PubMed: 16054088]

102. Kolamunne RT, Dias IH, Vernallis AB, et al. Nrf2 activation supports cell survival during hypoxia and hypoxia/reoxygenation in cardiomyoblasts; the roles of reactive oxygen and nitrogen species. Redox biology. 2013; 1:418–426. [PubMed: 24191235]

103. Malec V, Gottschald OR, Li S, et al. HIF-1 alpha signaling is augmented during intermittent hypoxia by induction of the Nrf2 pathway in NOX1-expressing adenocarcinoma A549 cells. Free radical biology & medicine. 2010; 48:1626–1635. [PubMed: 20347035]

104. Gong P, Hu B, Stewart D, et al. Cobalt induces heme oxygenase-1 expression by a hypoxia-inducible factor-independent mechanism in Chinese hamster ovary cells: regulation by Nrf2 and MafG transcription factors. The Journal of biological chemistry. 2001; 276:27018–27025. [PubMed: 11356853]

105. Kim TH, Hur EG, Kang SJ, et al. NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1alpha. Cancer research. 2011; 71:2260–2275. [PubMed: 21278237]

106. Phillips JT, Fox RJ. BG-12 in multiple sclerosis. Seminars in neurology. 2013; 33:56–65. [PubMed: 23709213]

107. Venci JV, Gandhi MA. Dimethyl fumarate (Tecfidera): a new oral agent for multiple sclerosis. The Annals of pharmacotherapy. 2013; 47:1697–1702. [PubMed: 24259625]

108. Magesh S, Chen Y, Hu L. Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. Medicinal research reviews. 2012; 32:687–726. [PubMed: 22549716]

109. Hu C, Eggler AL, Mesecar AD, van Breemen RB. Modification of keap1 cysteine residues by sulforaphane. Chemical research in toxicology. 2011; 24:515–521. [PubMed: 21391649]

110. Zhang Y, Kensler TW, Cho CG, et al. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91:3147–3150. [PubMed: 8159717]

111. Chung FL, Conaway CC, Rao CV, Reddy BS. Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. Carcinogenesis. 2000; 21:2287–2291. [PubMed: 11133820]

112. Fahey JW, Haristoy X, Dolan PM, et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach

tumors. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:7610–7615. [PubMed: 12032331]

113. Conaway CC, Wang CX, Pittman B, et al. Phenethyl isothiocyanate and sulforaphane and their N-acetylcysteine conjugates inhibit malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice. Cancer research. 2005; 65:8548–8557. [PubMed: 16166336]
114. Singh SV, Warin R, Xiao D, et al. Sulforaphane inhibits prostate carcinogenesis and pulmonary metastasis in TRAMP mice in association with increased cytotoxicity of natural killer cells. Cancer research. 2009; 69:2117–2125. [PubMed: 19223537]
115. Bertl E, Bartsch H, Gerhauser C. Inhibition of angiogenesis and endothelial cell functions are novel sulforaphane-mediated mechanisms in chemoprevention. Molecular cancer therapeutics. 2006; 5:575–585. [PubMed: 16546971]
116. Davis R, Singh KP, Kurzrock R, Shankar S. Sulforaphane inhibits angiogenesis through activation of FOXO transcription factors. Oncology reports. 2009; 22:1473–1478. [PubMed: 19885601]
117. Lenzi M, Fimognari C, Hrelia P. Sulforaphane as a promising molecule for fighting cancer. Cancer treatment and research. 2014; 159:207–223. [PubMed: 24114482]
118. Cente SKCC. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2009. Study to Evaluate the Effect of Sulforaphane in Broccoli Sprout Extract on Breast Tissue. [cited 2014 June 24]. Available from: http://clinicaltrials.gov/ct2/show/NCT00982319. NLM identifier: NCT00982319
119. Institute OKC. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2010. Sulforaphane in Treating Patients With Recurrent Prostate Cancer. [cited 2014 June 24]. Available from: http://clinicaltrials.gov/ct2/show/NCT01228084. NLM identifier: NCT01228084
120. University H. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2013. Pilot Study Evaluating Broccoli Sprouts in Advanced Pancreatic Cancer [POUDER Trial]. [cited 2014 June 24]. Available from: http://clinicaltrials.gov/ct2/show/NCT01879878. NLM identifier: NCT01879878
121. University TAM. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US); 2011. Cruciferous Vegetable Intake and Histone Status in Screening Colonoscopy Patients. [cited 2014 June 24]. Available from: http://clinicaltrials.gov/ct2/show/NCT01344330. NLM identifier: NCT01879878
122. Patlolla JM, Rao CV. Triterpenoids for cancer prevention and treatment: current status and future prospects. Current pharmaceutical biotechnology. 2012; 13:147–155. [PubMed: 21466427]
123. Cleasby A, Yon J, Day PJ, et al. Structure of the BTB domain of Keap1 and its interaction with the triterpenoid antagonist CDDO. PloS one. 2014; 9:e98896. [PubMed: 24896564]
124. de Zeeuw D, Akizawa T, Agarwal R, et al. Rationale and trial design of Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes: the Occurrence of Renal Events (BEACON). American journal of nephrology. 2013; 37:212–222. [PubMed: 23467003]
125. Ren D, Villeneuve NF, Jiang T, et al. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:1433–1438. [PubMed: 21205897]
126. Tang X, Wang H, Fan L, et al. Luteolin inhibits Nrf2 leading to negative regulation of the Nrf2/ARE pathway and sensitization of human lung carcinoma A549 cells to therapeutic drugs. Free radical biology & medicine. 2011; 50:1599–1609. [PubMed: 21402146]
127. Chian S, Thapa R, Chi Z, et al. Luteolin inhibits the Nrf2 signaling pathway and tumor growth in vivo. Biochemical and biophysical research communications. 2014; 447:602–608. [PubMed: 24747074]
128. Wang XJ, Hayes JD, Henderson CJ, Wolf CR. Identification of retinoic acid as an inhibitor of transcription factor Nrf2 through activation of retinoic acid receptor alpha. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:19589–19594. [PubMed: 18048326]

129. Tarumoto T, Nagai T, Ohmine K, et al. Ascorbic acid restores sensitivity to imatinib via suppression of Nrf2-dependent gene expression in the imatinib-resistant cell line. Experimental hematology. 2004; 32:375–381. [PubMed: 15050748]
130. Motohashi H, Shavit JA, Igarashi K, et al. The world according to Maf. Nucleic acids research. 1997; 25:2953–2959. [PubMed: 9224592]
131. Newman JR, Keating AE. Comprehensive identification of human bZIP interactions with coiled-coil arrays. Science. 2003; 300:2097–2101. [PubMed: 12805554]
132. Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. Oncogene. 1998; 17:3145–3156. [PubMed: 9872330]
133. Novotny V, Prieschl EE, Csonga R, et al. Nrf1 in a complex with fosB, c-jun, junD and ATF2 forms the AP1 component at the TNF alpha promoter in stimulated mast cells. Nucleic acids research. 1998; 26:5480–5485. [PubMed: 9826775]
134. Ogita K, Kubo M, Nishiyama N, et al. Enhanced binding activity of nuclear antioxidant-response element through possible formation of Nrf2/Fos-B complex after in vivo treatment with kainate in murine hippocampus. Neuropharmacology. 2004; 46:580–589. [PubMed: 14975682]
135. He CH, Gong P, Hu B, et al. Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation The Journal of biological chemistry. 2001; 276:20858–20865.
136. Nishizawa M, Kataoka K, Goto N, et al. v-maf, a viral oncogene that encodes a “leucine zipper” motif. Proceedings of the National Academy of Sciences of the United States of America. 1989; 86:7711–7715. [PubMed: 2554284]
137. Kataoka K, Fujiwara KT, Noda M, Nishizawa M. MafB, a new Maf family transcription activator that can associate with Maf and Fos but not with Jun. Molecular and cellular biology. 1994; 14:7581–7591. [PubMed: 7935473]
138. Sakai M, Imaki J, Yoshida K, et al. Rat maf related genes: specific expression in chondrocytes, lens and spinal cord. Oncogene. 1997; 14:745–750. [PubMed: 9038383]
139. Crawford DR, Leahy KP, Wang Y, et al. Oxidative stress induces the levels of a MafG homolog in hamster HA-1 cells. Free radical biology & medicine. 1996; 21:521–525. [PubMed: 8886803]
140. Moran JA, Dahl EL, Mulcahy RT. Differential induction of mafF, mafG and mafK expression by electrophile-response-element activators. The Biochemical journal. 2002; 361:371–377. [PubMed: 11772409]
141. Nguyen T, Huang HC, Pickett CB. Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK The Journal of biological chemistry. 2000; 275:15466–15473.
142. Marini MG, Chan K, Casula L, et al. hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2. The Journal of biological chemistry. 1997; 272:16490–16497. [PubMed: 9195958]
143. Motohashi H, Katsuoka F, Shavit JA, et al. Positive or negative MARE-dependent transcriptional regulation is determined by the abundance of small Maf proteins. Cell. 2000; 103:865–875. [PubMed: 11136972]
144. Katsuoka F, Motohashi H, Ishii T, et al. Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes. Molecular and cellular biology. 2005; 25:8044–8051. [PubMed: 16135796]
145. Choi JS, Zheng LT, Ha E, et al. Comparative genomic hybridization array analysis and real-time PCR reveals genomic copy number alteration for lung adenocarcinomas. Lung. 2006; 184:355–362. [PubMed: 17086460]
146. Schembri F, Sridhar S, Perdomo C, et al. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:2319–2324. [PubMed: 19168627]
147. Ding X, Yang Y, Han B, et al. Transcriptomic Characterization of Hepatocellular Carcinoma with CTNNB1 Mutation. PloS one. 2014; 9:e95307. [PubMed: 24798046]
148. Lucito R, Suresh S, Walter K, et al. Copy-number variants in patients with a strong family history of pancreatic cancer. Cancer biology & therapy. 2007; 6:1592–1599. [PubMed: 17912030]

149. Martinez-Hernandez A, Gutierrez-Malacatt H, Carrillo-Sanchez K, et al. Small MAF genes variants and chronic myeloid leukemia. European journal of haematology. 2014; 92:35–41. [PubMed: 24118457]

150. Amit I, Citri A, Shay T, et al. A module of negative feedback regulators defines growth factor signaling. Nature genetics. 2007; 39:503–512. [PubMed: 17322878]

151. Ueda K, Xu J, Morimoto H, et al. MafG controls the hypoxic response of cells by accumulating HIF-1alpha in the nuclei. FEBS letters. 2008; 582:2357–2364. [PubMed: 18538669]

152. Shimokawa N, Kumaki I, Qiu CH, et al. Extracellular acidification enhances DNA binding activity of MafG-FosB heterodimer. Journal of cellular physiology. 2005; 205:77–85. [PubMed: 15828020]

- NRF2, a Cap'N'Collar (CNC) transcription factor, plays dual roles in cancer prevention and progression depending on the cellular context and environment.
- Overexpression of NRF2 in many human cancers, is significantly involved in tumor metabolism, angiogenesis, and treatment resistance, which results in poor patient survival.
- Conflicting roles of NRF2 in cancer prevention and cancer progression remain to be challenged to determine the optimal use of NRF2 activators or inhibitors in the clinic.

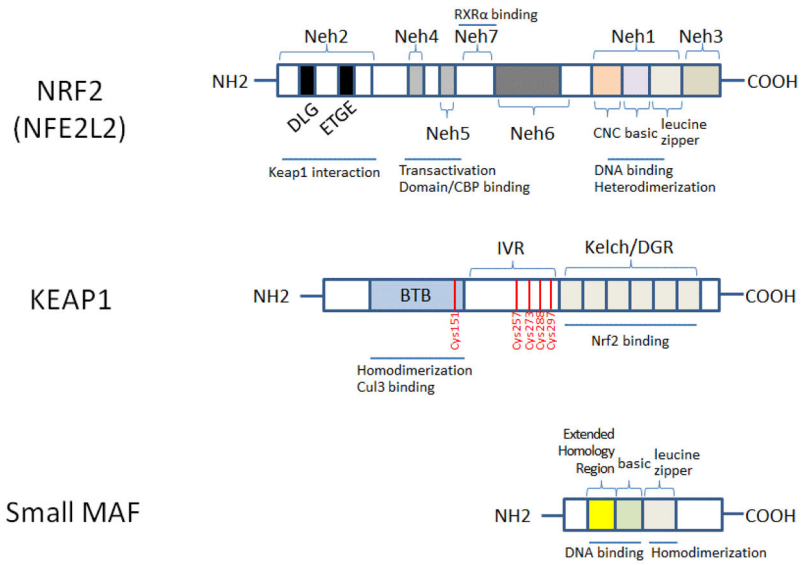

Figure 1. Structural domains of NRF2 and KEAP1

NRF2 includes the CNC-bZIP domain and functional Neh domains. The CNC-bZIP domain including Neh1 is required for DNA binding and interaction with ubiquitin conjugating enzymes. The TAD that consists of Neh5, Neh2 is involved n Keap1 binding [20]. KEAP1 contains the BTB domain for KEAP1 homodimerization and interaction with Cul3. Six kelch repeats in the Kelch/DGR domain interact with the Neh2 domain of NRF2 for binding. The IVR domain links the BTB and Kelch/DGR domains and has several critical cysteine residues for KEAP1 activation and NRF2 repression. Cysteine residues such as Cys151, Cys257, Cys273, Cys288, and Cys297 act as stress sensors to activate Keap1 and to repress NRF2 [25, 26]. Small MAF proteins utilize the highly conserved extended homology region (HER) for DNA binding [6]. The basic DNA binding domain and the leucine zipper dimerization domain are also required for their biological functions.

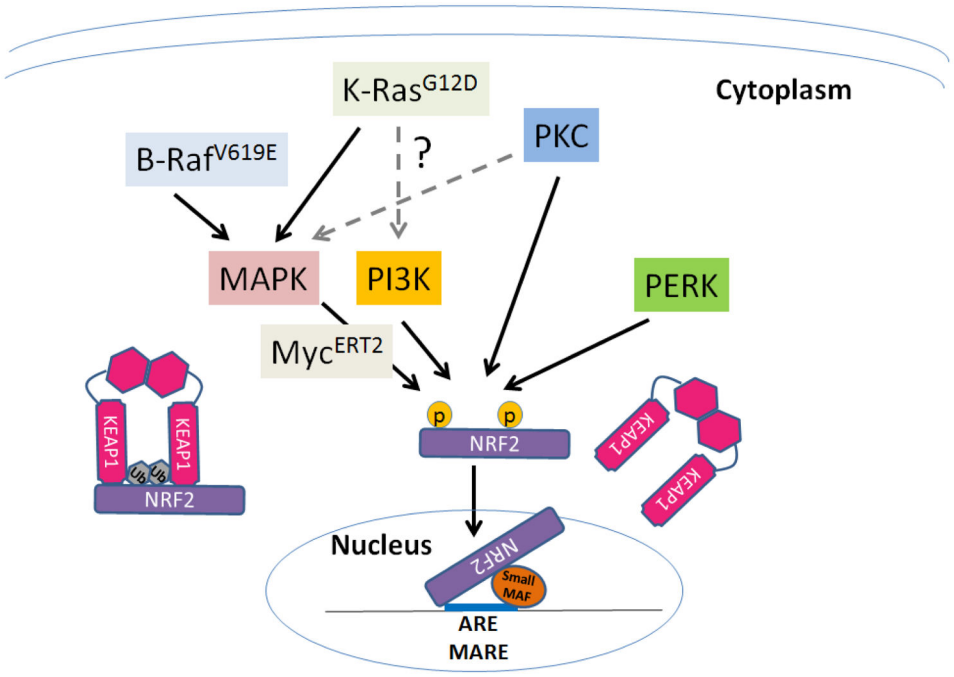

Figure 2. Oncogenic signaling pathways involved in NRF2 expression and activation

Oncogenic mutation of *K-Ras*, *B-Raf*, and *Myc* (K-Ras^{G12D}, B-Raf^{V619E} (corresponding to human B-Raf^{V600E}), and Myc^{ERT2}) increase *Nfe2l2* transcription while reducing production of free radicals and DNA oxidation which results in enhanced cell proliferation and tumor burden [62]. Protein kinase C (PKC) and PKR-like ER kinase (PERK) also phosphorylate and activate NRF2 by inhibiting its binding to KEAP1 [65]. While inhibition of MAP kinases and PI3K reduce NRF2 nuclear translocation and ARE-dependent activation, a crosstalk between various signaling pathways may be involved in regulating NRF2 [66–69].

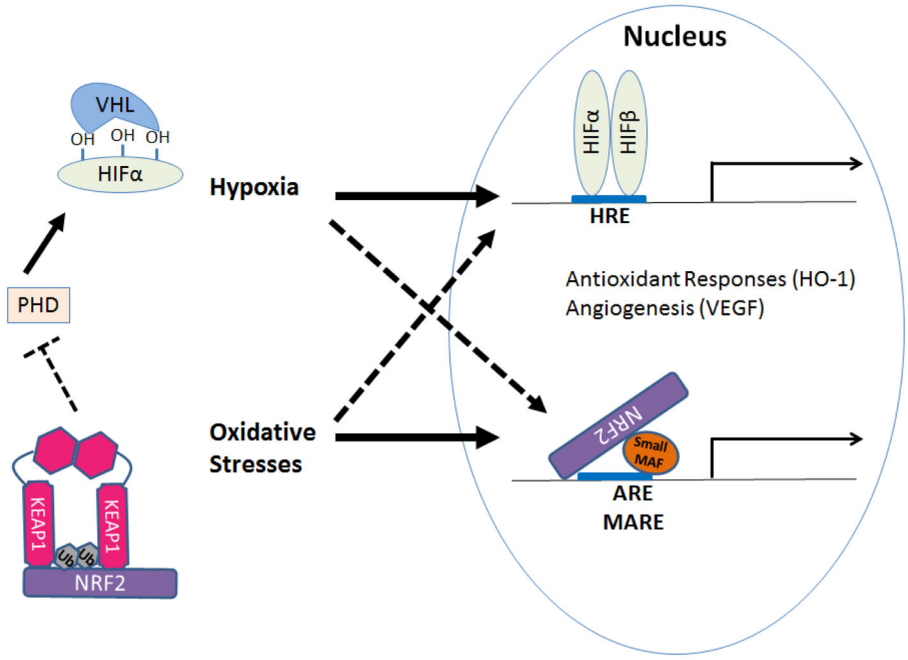

Figure 3. Activation of NRF2 and HIF under various environmental stimuli

Hypoxia inhibits prolyl hydroxylation of HIFα, which leads to its stabilization and activation as a heterodimer transcription factor with HIFβ. Free radicals produced by oxidative stresses are also known to activate the HIF-pathway. NRF2, a main regulator of the antioxidative responses, has been recently recognized as a tumorigenic factor, which plays a role under hypoxia. In human colorectal cancer cells, when NRF2 is deficient, the HIF-VEGF pathway and tumor angiogenesis are inhibited, indicating that these two proteins may cooperate for tumor progression [105].

Table 1

Examples of *Nfe2l2*-mediated protection against redox-mediated injury and carcinogenesis in knockout mouse model.

	Stressors	Symptoms	Reference
Nfe2l2 knockout mice	Hyperoxia	Acute lung injury & Inflammation	[31]
	Butylated hydroxytoluene (BHT)	Increased mortality & pulmonary injury	[33]
	N-acetyl-4-aminophenol (APAP, Acetaminophen)	Increased mortality & hepatic damage	[34]
	Radiation (16Gy)	Increased mortality	[35]
	Benzo[a]pyrene (B[a]P)	Increased gastric neoplasia	[36]
	N-titrosobutyl (4-hydroxbutyl) amine (BBN)	Increased incidence of urinary bladder carcinoma	[37]
	7,12-dimethylbenz(*a*)anthracene (DMBA) &12-O-tetradecanoylphorbol-13-acetate (TPA)	Increased skin tumorigenesis	[38]
	Azoxymethane/dextran sulfate sodium (DSS)	Increased incidence of colorectal tumor & inflammation	[39]
	Medroxyprogesterone acetate & DMBA	Increased progression of mammary carcinoma	[40]
	Tail vein injection of 3LL cells	Enhanced tumor lung metastasis	[41]

Table 2

NRF2 as an independent prognostic factor in cancer patients.

CNC Family	Tumor Type	Observation	Method	Other Variables for Multivariate Analysis	Reference
NRF2/Keap1	NSCLC (patients: 122)	NRF2 high/Keap1 low Poor **OS**, **RFS**	IHC	• Age at surgery • Tumor Stage • Smoking	[46]
NRF2		Increased nuclear expression Poor **OS**, RFS			
	HNSCC (patients: 60)	Increased mutant NRF2 signatures Poor OS	Microarray		[47]
	NSCLC (patients: 60)	Increased expression Poor **OS**, **PFS**	IHC	Not Significant	[48]
	Lung Cancer (patients: 109)	Increased NRF2 expression Poor DSS	IHC		[49]
	Lung Cancer (patients: 289)	Increased NRF2 expression Poor OS	IHC		[50]
	Gallbladder Cancer (patients: 59)	Increased expression Poor **OS**	IHC	• Metastasis	[52]
	Epithelial Ovarian Cancer (patients: 30)	Increased NRF2 pathway Poor OS	Microarray		[53]
	Breast Cancer (patients: 452)	rs2886162 genotype AA Poor **OS**	SNP Genotyping	• Nodal Status • HER2 Status	[55]
	Bladder Cancer (patients: 225)	Increased NRF2 expression Poor OS, DSS, RFS	IHC		[56]
	Gastric Cancer (patients: 186)	Increased NRF2 expression Poor **OS**, **DFS**	IHC	• TNM stage (DFS only)	[58]
	Glioblastoma (patients: 68)	Increased NRF2 expression Poor **OS**	IHC	• HIF1α • Resection Degree	[59]
	Glioblastoma (patients: 49)	Increased NRF2 expression Poor OS	IHC		[60]
	Pancreatic Adenocarcinoma (patients: 103)	Increased nuclear expression Poor OS	IHC		[61]
	SCLC (patients: 262)	*NFE2L2* Mutation Poor **OS**	PCR	• Pathological Stage	[149]
	Lung Adenocarcinoma (patients: 387)	*NFE2L2* SNP Homozygous (c.617>A/A) Poor OS	SNP Genotyping		[150]

OS: Overall Survival, RFS: Recurrence Free Survival, PFS: Progression Free Survival, DSS: Disease Specific Survival; significance from Kaplan-Meier method, Highlighted in Red, OS: Overall Survival, RFS: Recurrence Free Survival, PFS: Progression Free Survival, DFS: Disease Free Survival; Independent prognostic factor using multivariate analysis, HNSCC: Head and Neck Squamous Cell Carcinoma, NSCLC: Non-Small Cell Lung Carcinoma, SCLC: Squamous Cell Lung Cancer, IHC: Immunohistochemistry, SNP: Single Nucleotide Polymorphism, TNM: Tumor Node Metastasis