Review

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Kelch-like ECH-associated Protein 1/Nuclear Factor Erythroid 2-related Factor 2 Pathway and Its Interplay with Oncogenes in Lung Tumorigenesis

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Nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor regulating cellular redox homeostasis, exhibits a complex role in cancer biology. Genetic mutations in the Kelch-like ECH-associated protein 1 (KEAP1)/NRF2 system, which lead to NRF2 hyperactivation, are found in 20% to 30% of lung cancer cases. This review explores the intricate interplay between NRF2 and key oncogenic pathways in lung cancer, focusing on the interaction of KEAP1/NRF2 system with Kirsten rat sarcoma virus (KRAS), tumor protein P53 (TP53), epidermal growth factor receptor (EGFR), and phosphatidylinositol 3-kinases (PI3K)/AKT signaling. While NRF2 activation alone is insufficient to initiate tumorigenesis, it can significantly impact tumor initiation and progression when combined with oncogenic drivers such as KRAS. The review highlights the context-dependent effects of NRF2, from its protective role against chemical carcinogen-induced tumor initiation to its potential promotion of tumor growth in established cancers. These findings suggest the need for nuanced, stage-specific approaches to targeting the NRF2 pathway in cancer therapy.

Key Words Nuclear factor erythroid 2-related factor 2, Kelch-like ECH-associated protein 1, Lung cancer, Oncogenes

INTRODUCTION

Nuclear factor erythroid 2-related factor 2 (NRF2), encoded by the *NFE2L2* gene, is a transcription factor that regulates cellular redox homeostasis and protects against oxidative and electrophilic stress [1-3]. In response to stress conditions, NRF2 activates the expression of approximately 200 genes associated with xenobiotic metabolism, antioxidant defense, cell proliferation, and cellular metabolic processes [2]. As the main negative regulator of NRF2, Kelch-like ECH-associated protein 1 (KEAP1) controls the turnover of NRF2 protein by binding to it and recruiting Cullin3 (CUL3)-based E3 ligase complex, leading to the degradation of NRF2. Through the regulation by KEAP1, constitutively low levels of NRF2 are maintained in cells [3].

Interestingly, NRF2 exhibits two apparently contradictory roles depending on the cellular context [4-7]. In non-cancer cells, well-balanced NRF2 activation promotes chemopreventive pathways that defend cells against chemical carcinogens [5]. However, in cancer cells, imbalanced and sustained NRF2 activation contributes to oncogenic characteristics, facilitating tumor growth, cancer progression, and chemoresistance to anticancer therapy [6,7].

One of the critical reasons for persistent NRF2 activation in cancer is genetic mutations in *NRF2* and *KEAP1*. Mutations in these genes are detected in various cancer types, such as head and neck squamous cell carcinoma (3%-10%), esophageal carcinoma (3%-8%), and hepatocellular carcinoma (2%-6%) [8-12]. However, the highest frequency of mutations in *NRF2* and *KEAP1* gene occurs in lung cancer [13,14].

Lung cancer is the third most common cancer worldwide. Approximately 85% of lung cancers are non-small-cell lung cancer (NSCLC), composed of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) [15,16]. The remaining 15% are identified as SCLC. Within NSCLC, *KEAP1/NRF2* mutations are mainly found in LUSC (~31%) and LUAD (~22%) [13,14]. These genetic mutations are associated with enhanced tumorigenesis and poor prognosis in lung cancer patients [17,18].

In this review, we will examine the NRF2-KEAP1 signaling

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in cancers, particularly focusing on the relationship between mutations in *NRF2* and *KEAP1* genes and oncogenes in lung cancer tumorigenesis.

STRUCTURE AND REGULATION OF KEAP1/NRF2 SYSTEM

Human NRF2 protein consists of 605 amino acids and includes NRF2-ECH homology (Neh) 1-7 domains [19,20]. The Neh 1 domain, also known as the CNC/bZIP domain, forms a heterodimerization with small musculoaponeurotic fibrosarcoma (sMAF) proteins (MAFG, MAFK, or MAFF). These heterodimers then bind to genomic sites containing the antioxidant response elements (ARE; 5'-A/GTGACnnnGC-3') sequences located in the promoter regions of NRF2 target genes [1,2]. The Neh 2 domain contains DLG (29th-31st amino acids within NRF2) and ETGE (79th-82nd amino acids within NRF2) motifs, which enable NRF2 to bind to the Kelch domain of KEAP1, subsequently interacting with the CUL3-based E3 ligase complex for ubiquitination and degradation of NRF2 [1,2]. Neh 3-5 domains are involved in transactivation of NRF2 target genes through interaction with co-activators, including cAMP response element-binding protein (CREB) binding protein, and p300. The Neh 6 domain binds to β-transducing repeat-containing protein (β-TrCP), another negative regulator of NRF2, while the Neh 7 domain interacts with retinoic X receptor alpha, inhibiting the expression of NRF2 target genes [19,20].

Human KEAP1 protein is composed of 624 amino acids and contains broad-complex, tramtrack and bric a brac (BTB), intervening region (IVR), and Kelch domains [19,20]. The BTB domains facilitate homodimerization of KEAP1, allowing KEAP1 proteins to interact with NRF2 proteins in a 2:1 ratio, stoichiometrically. Along with BTB domains, IVR domains are involved in CUL3 interaction, leading to ubiquitination of NRF2 [1]. Several Kelch domains are positioned in the C-terminal region of KEAP1, mediating NRF2 binding. As mentioned above, these Kelch domains of the KEAP1 dimer bind to DLG and ETGE motifs located in the Neh 2 domain of NRF2 [19,20]. KEAP1 is unique in having abundant cysteine residues. Human KEAP1 protein retains 27 cysteines, which initially raised the potential for a redox-sensing role of KEAP1 via these cysteine residues [21].

In the basal state, the balance of NRF2 protein is optimized by regulation of KEAP1 [22,23]. Specifically, KEAP1 binds to NRF2, forms a complex with CUL3, and recruits an E3 ubiquitin ligase complex, promoting ubiquitination and degradation of NRF2. However, under stressed conditions, electrophiles or reactive oxygen species (ROS) weaken the NRF2-KEAP1 interaction, resulting prevention of KEAP1-mediated ubiquitination of NRF2 [22]. Eventually, NRF2 translocates into the nucleus and transactivates target gene expression.

Regarding the mechanism of NRF2-KEAP1 interaction, the hinge-latch model has been suggested [24,25]. The DLG and ETGE motifs in NRF2 bind to KEAP1 with different binding affinities. The DLG motif has a lower binding affinity than the ETGE motif, so the DLG motif is called the latch and the ETGE motif is called the hinge in this model. Conformational changes in KEAP1 protein, caused by different types of NRF2 inducers, lead to the dissociation from the DLG motif of NRF2. Eventually, newly synthesized NRF2 bypasses KEAP1 binding, moves into the nucleus, resulting in transcriptional activation [23].

Many types of NRF2 inducers are cysteine sensor-dependent stimulants, causing conformational change of KEAP1 via cysteine residue modification [23,26]. These inducers have been subdivided into several classes depending on their reacting cysteine residues. For instance, class I inducers react with Cys151 in the BTB domain, and class II & III inducers interact with Cys273 and Cys288 in the IVR domain [23]. All inducers contribute to conformational changes in KEAP1 and inhibit degradation of NRF2, resulting in stabilization and activation of NRF2.

The other type of NRF2 inducers can act in a cysteine sensor-independent manner. It has been reported that protein-protein interaction inhibitors, including PRL295 and NG262, disrupt the interaction between the DLG motif in NRF2 and the Kelch domain in KEAP1, and induce NRF2 activation [25,27,28].

KEAP1/NRF2 MUTATION IN CANCER

In cancers, the regulatory balance of NRF2 is often disrupted, leading to its abnormal hyperactivation [29-31]. This aberrant activation of NRF2 triggers a cellular system that promotes cancer cell proliferation and survival while also conferring resistance to anticancer therapies [6,32]. Among the multiple lines of evidence supporting NRF2 overactivation in cancers, genetic mutations within *NRF2* and *KEAP1* play a crucial role [33].

NRF2 mutation in cancer

Most *NRF2* mutations are located near the DLG and ETGE motifs in the Neh 2 domain, which are responsible for KEAP1 binding. According to the hinge and latch model, mutations in the DLG motif weaken the NRF2-KEAP1 interaction but maintain some attachment, while mutations in the ETGE motif completely dissociate the NRF2-KEAP1 complex, releasing NRF2 [25]. Consequently, mutated NRF2 evades negative regulation by KEAP1 and continuously activates its target genes [25].

These types of *NRF2* mutations are observed in diverse cancer types. The Cancer Genome Atlas (TCGA) database has identified *NRF2* mutations in 21 out of 33 different tumor types [8]. The highest frequencies of *NRF2* mutations are found in LUSC, followed by esophageal carcinoma, uterine corpus endometrial carcinoma, and head and neck squamous cell carcinoma. Eleven hotspots of *NRF2* mutations

have been identified in or near the DLG and ETGE motifs, including W24, Q26, R34, and D77 [8]. These mutated forms of NRF2 cannot tightly interact with KEAP1, resulting in escape from KEAP1-CUL3 mediated degradation.

KEAP1 mutation in cancer

Mutations within the *KEAP1* gene are distributed throughout all domains but are primarily enriched in the Kelch domains, which are responsible for NRF2 binding [33]. These mutations impair the NRF2-KEAP1 interaction, leading to disruption of the NRF2-KEAP1-CUL3 complex. Consequently, mutated KEAP1 cannot interact with NRF2, allowing NRF2 to become constitutively activated [33].

KEAP1 mutations are most frequently found in LUAD, followed by LUSC, hepatocellular carcinoma, and head and neck squamous cell carcinoma [8]. The mutations are mainly located in the Kelch domain, including V271, G333, G417, R470, and G480 amino acids. Most mutation patterns are missense mutations leading to a loss-of-function of KEAP1 for NRF2 interaction [8,10].

KEAP1/NRF2 mutation in lung cancer

A genomic characterization study of 178 LUSC patients revealed that genetic mutations within tumor protein P53 (*TP53*) are most highly prevalent, occurring in approximately 80% of cases [13]. This study also found that the rate of mutations in KEAP1/NRF2 pathway is over 30% in LUSC, accounting for 15% in *NRF2* and 12% in *KEAP1*, respectively. Adherently, the oxidative stress response pathway associated with KEAP1/NRF2 has been identified as one of the altered signaling pathways in LUSC patients, along with the phosphatidylinositol 3-kinases (PI3K)/receptor tyrosine kinases (RTK)/rat sarcoma virus (RAS) signaling pathway (~69%) and the squamous differentiation pathway [13].

In LUAD, a study of 230 patients showed that *TP53* mutations had the highest frequency at about 46%. Kirsten RAS (*KRAS*) and epidermal growth factor receptor (*EGFR*) mutations were also common, occurring in approximately 33% and 14% of cases, respectively [14]. Among the altered signaling pathways in LUAD patients, the oxidative stress response pathway involving KEAP1/NRF2 was notable. Mutations in this pathway was found in around 23% of LUAD cases [14]. These findings indicate that *KEAP1/NRF2* mutations are common in both LUSC and LUAD, underscoring the significant role of KEAP1/NRF2 signaling in lung cancer. In line with this, clinical observations indicate that these mutations are correlated with poor prognosis of lung cancer patients [34,35].

ROLE OF NRF2 IN TUMOR PROMOTION

Current substantial body of evidence suggests that NRF2 hyperactivation by *KEAP1/NRF2* mutation endows cancer cells with enhanced survival and growth through the upregulation of genes involved in ROS/electrophile removal, NADPH production, metabolic reprogramming, cell proliferation, and anti-apoptotic response [2,20,30]. This notion has been consistently evidenced by studies observing the role of NRF2 in cancer cells after the initiation process. The potential contribution of NRF2 to cancer initiation remains an intriguing question. Until now, there were no reports that NRF2 activation alone is sufficient to initiate tumorigenesis [36]. Several studies have explored the relationship between tumorigenesis and NRF2 activation, particularly in carcinogen-induced cancer models.

A study using a pharmacological NRF2 activator revealed that the role of NRF2 varies depending on the cell state during tumorigenesis [37]. In a vinyl carbamate (VC)-exposed mouse model, treatment with NRF2 activating sulforaphane prior to VC exposure decreased the number of lung tumors, while post-treatment increased tumor formation. Subsequently, in a genetic mutation model of KRAS activation (G12D substitution), pre-treatment with NRF2 activator had no effect on tumor formation or numbers. However, after tumor initiation by *Kras* mutation, post-treatment with sulforaphane increased tumor number and size [37].

The association of NRF2 with oncogene has been suggested. In a urethane-induced lung cancer model, *Nrf2*-deficient mice showed a higher number of lung tumors in short-term observations (8 weeks), but the tumor number decreased in middle- and long-term observations (16 and 24 weeks) [38]. Conversely, wild-type mice showed a lower incidence of lung tumors than *Nrf2*-deficient mice in short-term observations; however, they showed 100% incidence in middle-term observations, indicating the positive role of NRF2 in tumorigenesis over a long-term period. Interestingly, cancer cells from *Nrf2*-deficent tumors failed to grow in nude mice, while *Nrf2*-wild-type tumors gradually grew in nude mice [38]. This difference was mediated by oncogene KRAS activation. Sequencing analysis revealed that *Nrf2*-deficient tumors had a low frequency of *Kras* mutations (1 out of 13), while all wild-type tumors harbored *Kras* mutations in all tumors (15 out of 15). These results suggest that NRF2 promotes tumor growth in the long-term period via KRAS oncogene activation. Taken together, current evidence indicates that NRF2 can accelerate tumor growth after cancer initiation, whether through chemical carcinogens or oncogenic mutations.

COLLABORATIVE ROLE OF NRF2 AND ONCOGENES IN LUNG CANCER

In human cancers, mutations in *NRF2* and *KEAP1* frequently co-occur with alterations in other genes, notably *TP53* (a tumor suppressor) and *KRAS* (a tumor-driver) [39]. Clinical data analysis of NSCLC patients demonstrated significant concurrent mutations in these genes. In *NRF2*-mutated NSCLC, *TP53* mutations were most common (40.8%), followed by *KRAS* (22.5%) and *EGFR* (6%) mutations. Similarly, in

KEAP1-mutated NSCLC, *TP53* mutations were most prevalent (44.9%), with *KRAS* mutations occurring in 40.5% of cases [40]. These findings suggest the potential interplay between NRF2/KEAP1 and other key cancer-related genes.

Researchers have extensively studied the interaction between activated NRF2 and oncogenes during tumorigenesis using mouse models that mimic mutations in *Nrf2*, *Keap1*, and various oncogenes. A consistent finding across multiple studies is that loss-of-function mutations in *Keap1*, which lead to NRF2 activation, can accelerate tumorigenesis in *Kras*-mutant LUAD [41-45]. For instance, a study by Romero et al. [41] provided compelling evidence for this interaction using a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system. They demonstrated that *Keap1* deletion, resulting in NRF2 overactivation, significantly enhanced LUAD tumorigenesis in mice with *Kras* mutation. Further supporting these findings, Best et al. [42] showed that *Keap1* loss accelerated tumor initiation in *Kras*-mutant LUAD, an effect that occurred independently of *Tp53* or *Liver kinase B1 (Lkb1)* status. The impact of *Keap1* loss extends beyond tumor initiation. Lignitto et al. [43] revealed that *Keap1* deficiency also promotes migration and metastasis of lung cancer in *Kras*-mutant and *Tp53*-deleted mice through a *Bach1*-dependent mechanism. Hayashi et al. [44] further demonstrated that NRF2 hyperactivation via *Keap1* knockdown enhanced tumor growth in *Kras*-mutant mice.

However, the role of NRF2 activation in tumorigenesis is not straightforward, as some studies have reported conflicting results. Rogers et al. [46] found that *Keap1* knockout was insufficient to induce tumorigenesis in mouse models of *Kras*-mutant LUAD, even when combined with *Tp53* or *Lkb1* loss. Similarly, Cai et al. [47] showed that *Keap1* loss was not associated with tumor initiation in *Kras*-mutant lung cancer models, although they observed that *Keap1* loss enhanced tumor growth in long-term observations (26 weeks). In a study by Foggetti et al. [48], *Keap1* deletion did not promote tumorigenesis in a LUAD model with *Egfr* mutation and *Tp53* loss. Kang et al. [49] observed that in a mouse model of *Kras* mutation combined with *Tp53* loss and *Keap1* knockout, lung tumors were smaller compared to those in mice with wild-type *Keap1* and the same genetic alterations.

These conflicting findings highlight the complexity of KEAP1/NRF2 signaling in lung cancer and suggest that its effects may be context-dependent, varying with specific genetic backgrounds and experimental conditions. In particular, a recent comprehensive study on NRF2 function during tumorigenesis reported that NRF2 activation showed pro- or anti-tumorigenic effects depending on the stage of tumorigenesis. DeBlasi et al. [50] conducted a study in which they introduced mutations frequently found in human NSCLC into mouse models. Specifically, they incorporated *Keap1* (R554Q substitution) or *Nrf2* (D29H substitution) mutations into mice with either wild-type genetics or existing alterations in *Kras*, *Tp53*, or *Lkb1* genes.

Mice with mutant in either *Keap1* or *Nrf2* alone failed to initiate lung tumorigenesis, with overall survival of 650 to 750 days, showing no difference compared to wild type *Keap1* or *Nrf2* mice. Even when combined with loss of *Tp53* or *Lkb1* tumor suppressor genes, these animal models could not initiate tumorigenesis [50]. In a subsequent approach with *Kras*-mutant mouse models (G12D substitution), mutations in *Keap1* or *Nrf2* showed similar overall survival between cohorts, approximately 200 days. However, *Kras*-mutant mice with *Keap1* homozygous and *Nrf2* heterozygous mutations showed a higher number of lung tumors compared to mice with *Kras*-mutant alone, indicating the effect of concurrent mutations in *Keap1*/*Nrf2* and *Kras*. Particularly, these mutant mice had significantly increased grade 1 tumors, which suggests that *Keap1* or *Nrf2* mutations enhance lung tumor initiation and early progression in *Kras*-mutant mouse models. Of note, grade 3 tumors are rarely observed in *Keap1* or *Nrf2* mutant tumors. This observation suggests that a certain threshold for genetic NRF2 activation exists, which determines its role in either promoting the initiation of tumorigenesis and early progression or inhibiting late progression to high-grade tumors [50].

The complexity of KEAP1/NRF2 signaling is further illustrated by studies on concurrent mutations. Galan-Cobo et al. [51] demonstrated that mutations in *KEAP1/NRF2* enabled LUAD cells with concurrent *KRAS* mutations and *LKB1* loss to promote survival by achieving metabolic homeostasis in a glutaminolysis-dependent manner. Building on this, Lee et al. [52] found that *KRAS*-mutant lung cancer cells with concurrent mutations in *KEAP1* and *LKB1* increased serine-glycine-one-carbon metabolism to meet their increased demand for one-carbon units.

These results collectively indicate that NRF2 activation can play diverse roles in lung cancer initiation and progression, depending on the genetic context and the level of NRF2 activation. While NRF2 activation alone may not be sufficient to initiate tumorigenesis, it can significantly impact tumor initiation and progression when combined with other oncogenic drivers, particularly *KRAS* mutations. The exact mechanisms underlying these differential effects of NRF2 activation and the precise thresholds and required oncogene combination that determine its impact on various stages of tumorigenesis remain to be fully elucidated. Future research should focus on defining these thresholds and understanding how varying levels of NRF2 activation interact with other genetic alterations to influence lung cancer initiation, progression, and potential therapeutic responses.

CROSSTALK BETWEEN NRF2 AND ONCOGENES

NRF2 and KRAS

Current substantial evidence suggests the collaborative role of KEAP1/NRF2 and KRAS in lung cancer tumor initiation

and progression. This positive collaboration represents a significant example of the crosstalk between NRF2 signaling and oncogenic pathways.

In a study by DeNicola et al. [53] demonstrated the direct link between NRF2 and oncogenes. In mouse embryonic fibroblasts (MEFs) harboring mutations in *Kras*, *Braf*, and *Myc*, enhanced transcription of *Nrf2* and decreased intracellular ROS levels were observed. In vivo experiments using *Kras*-mutant-induced LUAD, *Nrf2*-deficiency suppressed *Kras*-driven adenomatous alveolar hyperplasia, bronchiolar hyperplasia, and adenoma, resulting in increased median survival rates.

The crosstalk between NRF2 and KRAS has been further elucidated in subsequent studies. Tao et al. [54] proposed that KRAS mediates upregulation of NRF2 through binding to the TPA response element (TRE) regulatory region located in exon 1 of the *NRF2* gene (Fig. 1A). In NSCLC cell lines, overexpression of KRAS G12D mutant enhanced the transcription of *NRF2* and its target genes, contributing to increased cell viability and resistance to cisplatin treatment. Moreover, in a mouse model, *Kras*-mutant lung tumor tissues exhibited higher expression of NRF2 and its target genes compared to normal tissues.

Another study by Yang et al. [55] expanded on this interaction, demonstrating that the crosstalk between NRF2 and KRAS oncogene is also associated with p53 (Fig. 1B). When *KRAS* was silenced in human lung cancer cell lines, the expression of NRF2 and its target genes decreased, leading to increased ROS levels. This elevation in ROS promoted the phosphorylation of serine 15 within p53 by ataxia telangiectasia mutated kinase, resulting in p53 stabilization. In essence, this study revealed that the interplay between NRF2 and KRAS contributes to KRAS-mediated suppression of p53.

Taken together, the upregulation of NRF2 by oncogenic KRAS not only promotes tumorigenesis and drug resistance but also indirectly suppresses p53 function through ROS modulation.

NRF2 and TP53

TP53 mutation is the most frequent genetic alteration in NSCLC [13,14,56]. Several studies have suggested an association between NRF2 and p53 across various cancer types. In a mouse model of N-nitrosobutyl(4-hydroxybutyl)amine-induced bladder cancer, mice with double knockout of *Nrf2* and *Tp53* showed higher tumorigenesis when compared to *Nrf2* knockout alone, suggesting these factors cooperatively contribute to preventing carcinogen-induced tumorigenesis [57]. A recent study demonstrated that simultaneous expression of NRF2L30F, a gain-of-function *Nrf2* mutation, combined with *Tp53* loss led to the development of lesions resembling esophageal squamous cell carcinoma in mice [58]. Additionally, *Nrf2* deletion suppressed tumor initiation in pancreas-specific double mutant mice with *Kras* mutation and *Tp53* loss, further indicating a potential interaction between NRF2 and p53 [59]. In line with these findings, direct crosstalk between NRF2 and p53 has been demonstrated in several studies.

Clinical data have shown that lung cancer patients with *TP53* mutations exhibit higher expression levels of NRF2 compared to those with wild-type p53 [60]. A comparison of lung cancer cell lines with mutant or wild-type p53 revealed putative binding sites for p53 and Sp1 on *NRF2* promoter regions (Fig. 2A). When wild-type p53 was transfected into cells, it bound to the *NRF2* promoter regions, suppressing its expression and causing Sp1 disassociation in a dose-dependent manner. Conversely, *TP53* knockdown led to enhanced NRF2 expression through increased Sp1 binding to the *NRF2* promoter regions. Further evidence of this regulatory relationship comes from studies showing that p53 binds to the ARE and represses transcription of NRF2 target genes

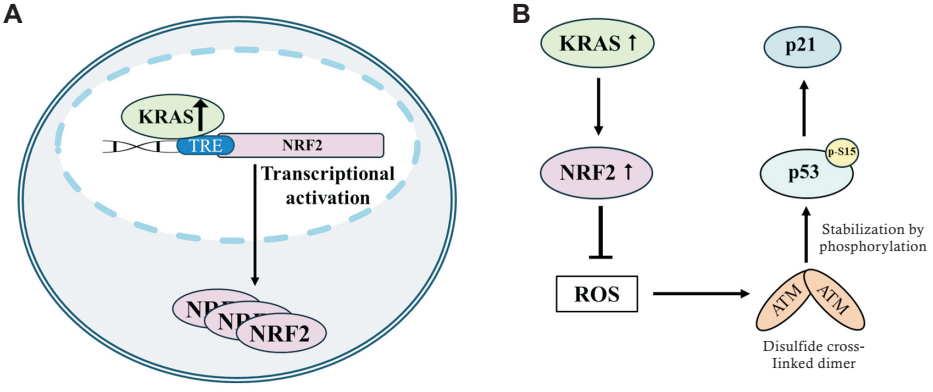

Figure 1. Crosstalk between NRF2 and KRAS. KRAS mediates the upregulation of NRF2, promoting tumorigenesis and indirectly suppressing p53. (A) KRAS induces the transcriptional activation of NRF2 by binding to the TRE regulatory region located in exon 1 of the *NRF2* gene. (B) The interplay between NRF2 and KRAS regulates p53 stability through ROS modulation. These studies demonstrate the positive crosstalk between NRF2 and KRAS. NRF2, nuclear factor erythroid 2-related factor 2; KRAS, Kirsten rat sarcoma virus; ROS, reactive oxygen species; TRE, TPA response element.

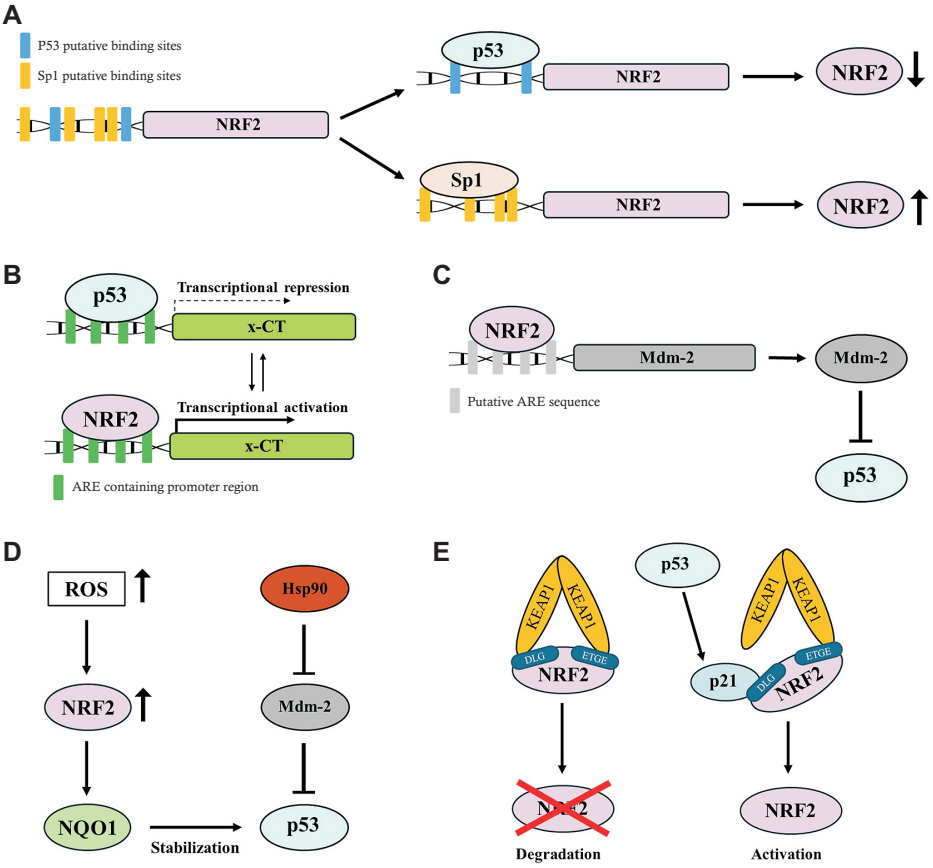

Figure 2. Interplay between NRF2 and TP53. P53 interacts with NRF2 either positively or negatively, depending on biological and cellular context. (A) Wild-type p53 binds to putative binding sites in *NRF2* promoter regions, suppressing NRF2 expression. (B) P53 binds to ARE-containing promoter regions of NRF2 target genes, inducing their transcriptional repression. (C) NRF2 binds to putative ARE sequences in the promoter regions of *Mdm2*, a repressor protein of p53. These reports suggest a negative regulatory relationship between NRF2 and p53. (D) *NQO1*, an NRF2 target gene, stabilizes p53 independently of MDM2 regulation. (E) P21, a target gene of p53, mediates NRF2 activation by binding to the DLG motif within NRF2, which is a competitive binding site for KEAP1. These two studies show a positive crosstalk between NRF2 and p53. NRF2, nuclear factor erythroid 2-related factor 2; TP53, tumor protein P53; ARE, antioxidant response elements; MDM2, mouse double minute 2 homolog; NQO1, NAD(P)H: quinone oxidoreductase-1.

[61], suggesting a negative regulatory role of p53 in NRF2 signaling (Fig. 2B).

Several studies have reported the regulatory role of NRF2 in p53 at the post-translational levels. First, NRF2 has been demonstrated to negatively regulate p53 via transcriptional regulation of mouse double minute 2 homolog (MDM2), a repressor protein of p53 (Fig. 2C). The promoter region of the murine *Mdm2* gene contains putative ARE sequences that can potentially bind NRF2 [62]. In vitro demonstrations have shown that low NRF2 activity inhibited MDM2 expression, leading to increased p53 activity and expression of its target gene, p21. Similarly, an additional study reported that NRF2-mediated induction of MDM2 inhibited p53 activity and promoted cell proliferation [63].

NAD(P)H: quinone oxidoreductase-1 (NQO1), one of the target genes of NRF2, also engages with p53, contributing to its stabilization and accumulation [64]. This positive interaction between NQO1 and p53 is independent of MDM2 pathway (Fig. 2D). Furthermore, p21, a known target gene of p53, promotes NRF2 activation by inhibiting the NRF2-KEAP1 interaction. The KKR motif (154th-156th amino acids) within p21 interacts with the DLG motif within NRF2, competing with KEAP1 binding and preventing NRF2 degradation (Fig. 2E) [65].

The seemingly contradictory findings from these reports suggest that the crosstalk between NRF2 and p53 can be either positive or negative, depending on the specific biological and cellular context.

NRF2, EGFR and PI3K/AKT

Mutations in *EGFR* are additional prevalent mutations in lung cancer [13,14]. Several studies have suggested a positive relationship between NRF2 and EGFR. A study by Huo et al. [66] revealed an intriguing interaction between the intracellular domain of EGFR and the IVR and Kelch domains of KEAP1, leading to the dissociation of KEAP1 from NRF2 and resulting in transcriptional activation of NRF2. In a mouse model with liver-specific NRF2 hyperactivation, NRF2-mediated elevation of EGFR and EGF, and consequent activation of AKT (also known as protein kinase B) were identified as underlying mechanism of hepatomegaly [67]. In melanoma, NRF2 inhibition suppressed EGFR-mediated AKT activation. Reciprocally, EGF stimulated NRF2 nuclear accumulation and transcriptional activation, supporting a positive link between NRF2 and EGFR [68].

The PI3K/AKT pathway is also deregulated in lung cancer, and resulting AKT overactivation has been associated with increased tumor growth and progression [69]. Aberrant acti-

vation of PI3K/AKT signaling can lead to NRF2 accumulation and activation through the AKT-mediated inhibition of glycogen synthase kinase 3 activity and subsequent β-TrCP-dependent NRF2 degradation [2,70,71]. In this context, forced expression of PI3K/AKT pathway by deletion of the *phosphatase and tensin homolog* gene increased NRF2 activity and subsequently facilitated metabolic shift toward glutamine metabolism, purine nucleotide synthesis, and NADPH production [72].

CONCLUSION

The role of NRF2 activation in cancer development presents a complex and sometimes conflicting picture. A comprehensive review of previous studies reveals that NRF2 activation alone is insufficient to initiate tumorigenesis [36-38]. Conversely, genetic deficiency of *Nrf2* or treatment with NRF2 inhibitors resulted in increased susceptibility to carcinogens, leading to tumorigenesis initiation [37,38]. Interestingly, there is a consensus among these studies that NRF2 activation promotes cancer progression once it is initiated [37]. The effects of NRF2 activation, when coupled with oncogenes, can be either pro-tumorigenic or anti-tumorigenic, depending on the timing and context. To fully understand the collaborative effect of NRF2 and oncogenes on tumorigenesis, from initiation through progression, it is crucial to consider both the stage of tumorigenesis and the threshold of NRF2 expression levels [50].

This review has also summarized the direct and indirect interactions between the KEAP1/NRF2 system and oncogenic signaling pathways such as KRAS, TP53, EGFR, and PI3K/AKT. The majority of studies demonstrate a positive interplay between these elements, associated with aggravated tumorigenesis and cancer progression [53-55,60]. These insights into the relationship between the KEAP1/NRF2 system and oncogenes during tumorigenesis suggest new opportunities for drug development that target this interplay. As reported, NRF2 inhibitors suppress the progression of *Nrf2*-mutated tumors, while NRF2 activators exacerbate tumorigenesis once tumors are initiated [37]. Additionally, NRF2 activation within cells surrounding tumors, such as immune cells, can restrict the progression of *Nrf2*-mutated tumors, which suggesting additional layer of complex interplay between NRF2 and oncogene signaling [44].

Additional interplay with between NRF2 and NOTCH signaling can also influence tumor initiation and progression. NOTCH signaling, which is involved in diverse cellular responses such as differentiation, proliferation, and survival, has been shown to exert oncogenic functions through mechanisms of cell metastasis [73]. Activating mutations of *NOTCH1* have been identified as common genetic alterations in NSCLC [74]. A direct link between NRF2 and NOTCH has been suggested. Wakabayashi et al. [75] demonstrated that, the expression of NOTCH1 and its target genes was significantly reduced in *Nrf2*-null MEFs. Consistently, *Nrf2*-null mice exhibited approximately 40% reduction in *Notch1* transcript levels. Further investigation revealed a functional ARE sequence in the promoter region of *Notch1*, confirming that NRF2 directly regulates *Notch1* as a target gene. A subsequent study discovered that the promoter region of *Nrf2* contains a binding site for Notch intracellular domain (NICD) complex. In this context, NOTCH signaling activation leads to the transcriptional activation of NRF2 via nuclear translocation of NICD and transactivation [76]. This reciprocal regulation creates a positive feedback loop between NRF2 and NOTCH signaling pathways, potentially amplifying the effects of both pathways in cellular processes. The interplay between NRF2 and NOTCH signaling adds another layer of complexity to our understanding of the role of NRF2 in cellular regulation and cancer development.

In conclusion, the multifaceted role of NRF2 in cancer biology, its complex interactions with various oncogenic pathways, and its context-dependent effects on tumor initiation and progression underscore the need for a nuanced approach in targeting the NRF2 pathway for cancer therapy. Future research should focus on elucidating the precise mechanisms and thresholds that determine the effect of NRF2, with the ultimate goal of developing stage-specific and personalized therapeutic strategies that can effectively modulate the NRF2 pathway and its intricate network of interactions in cancer.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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