Minireview

The Multi-Faceted Consequences of NRF2 Activation throughout Carcinogenesis

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The oxidative balance of a cell is maintained by the Kelch-like ECH-associated protein 1 (KEAP1)/nuclear factor erythroid 2-related factor 2 (NRF2) pathway. This cytoprotective pathway detoxifies reactive oxygen species and xenobiotics. The role of the KEAP1/NRF2 pathway as pro-tumorigenic or anti-tumorigenic throughout stages of carcinogenesis (including initiation, promotion, progression, and metastasis) is complex. This mini review focuses on key studies describing how the KEAP1/NRF2 pathway affects cancer at different phases. The data compiled suggest that the roles of KEAP1/NRF2 in cancer are highly dependent on context; specifically, the model used (carcinogen-induced vs genetic), the tumor type, and the stage of cancer. Moreover, emerging data suggests that KEAP1/NRF2 is also important for regulating the tumor microenvironment and how its effects are amplified either by epigenetics or in response to co-occurring mutations. Further elucidation of the complexity of this pathway is needed in order to develop novel pharmacological tools and drugs to improve patient outcomes.

Keywords: cancer, initiation, metastasis, NRF2, promotion, transformation

INTRODUCTION

Carcinogenesis is an intricate and heterogeneous process that depends on cooperation among key oncogenic proteins. The mechanisms orchestrating initiation, promotion, and progression vary by the tumor type. Tumor initiation has been heavily investigated (Evans et al., 2019; Grizzi et al., 2006), as has the molecular biology of carcinogenesis (Bouvard et al., 2009; el Ghissassi et al., 2009; Grosse et al., 2009; Straif et al., 2009) and pathways of chemotherapy resistance (Alfarouk et al., 2015; Gupta et al., 2019) that contribute to cancer fitness (McCreery and Balmain, 2017). Extensive tumor heterogeneity permits the selection of distinct phylogenetic clones and consequent treatment failure (Aktipis et al., 2011; Greaves and Maley, 2012; Nowell, 1976; Worsley et al., 2016). Predictably, the heterogeneity of cancer can explain the context-dependent roles for molecules like NFE2-related factor 2 (NRF2) and Kelch-like ECH-associated protein 1 (KEAP1). As a master regulator of antioxidant responses and cellular metabolism, tumors faced with high oxidative stress and metabolic disorders benefit from constitutive NRF2 pathway activation (Wu et al., 2019). Nonetheless, the role of NRF2 throughout multi-stage carcinogenesis is complex.

Despite significant advances in the NRF2 field over the past decade (Pillai et al., 2022; Robledinos-Antón et al., 2019; Rojo de la Vega et al., 2018; Sporn and Liby, 2012; Wu et al., 2019; Zimta et al., 2019), a consensus on the precise role for NRF2 throughout carcinogenesis remains elusive. Abundant evidence confirms that NRF2 activation protects healthy cells from damaging electrophilic and oxidative stress, thus

limiting genomic mutations (Gacesa et al., 2016; Loboda et al., 2016; Mukaigasa et al., 2012) and other cellular damage. This beneficial cytoprotection in normal cells supports the use of pharmacological activators of the NRF2 pathway for cancer prevention. However, these same cytoprotective mechanisms can also enhance survival of transformed cells. Indeed, a tumor-promoting role for NRF2 in cancer initiation has been reported, attributed to protection against redox stress in cells that have acquired mutations in *KRAS* and/or *STK11* (Galan-Cobo et al., 2019). NRF2 activation can also promote chemoresistance (Purohit et al., 2021; Srivastava et al., 2022) and radiation resistance (Feng et al., 2021; Koppula et al., 2022; Matsuoka et al., 2022), as well as metastasis. These disparate findings raise questions as to whether NRF2 is an oncogene, a tumor suppressor gene, or possibly both. These apparent discordant functions may be partly explained by the diverse models used (Best et al., 2019; DeNicola et al., 2011; Ramos-Gomez et al., 2001; Satoh et al., 2013). For example, carcinogen-induced spontaneous tumor models often yield outcomes distinct from xenograft models in which NRF2 is constitutively activated, with further biological complexity found in dual *KEAP1/KRAS*-mutant tumors. Furthermore, there is a paucity of studies on NRF2-activated tumor–immune cell interactions, which are likely important for anti-tumor effects. Without an understanding of the tumor microenvironment, immunodeficient models may yield confounding results. This review will synthesize our current understanding of NRF2 activation throughout the stages of carcinogenesis and address its time- and context-dependent impact on tumor progression.

NRF2 AND THE STAGES OF CARCINOGENESIS

Because NRF2 activation can prevent or promote cancer depending on the phase of carcinogenesis, we will discuss implications of NRF2 activation during each of the following stages: Transformation and Initiation, Promotion and Progression, and Metastasis.

TRANSFORMATION AND INITIATION

- *Transformation*: "Process of converting a normal cell into a cell having some or many of the attributes of a cancer cell."
- *Initiation*: "Process of changing a cell, usually in a stable fashion, so that it is able to respond subsequently to the growth-stimulatory actions of a tumor-promoting agent"; "Such a process, with the implication that the change involves a mutation."; "The first step in multi-step tumorigenesis."
Taken from Weinberg (2014).

As a master regulator of the oxidative stress response (Gacesa et al., 2016; Loboda et al., 2016; Mukaigasa et al., 2012), the NRF2 pathway is highly conserved in multicellular animals throughout evolution (Fuse and Kobayashi, 2017; Gacesa et al., 2016; Toyokuni et al., 2020) to defend against one of the most potent and prevalent cellular insults: oxygen. Oxidative imbalance leads to the formation of free radicals which can cause DNA damage and disruption of cellular homeostasis,

leading to transformation (Klaunig et al., 1998; Toyokuni et al., 2020; Valko et al., 2006). Reactive oxygen species (ROS) can contribute to carcinogenesis directly by inducing mutations in proto-oncogenes and tumor suppressor genes and indirectly by activating kinases that induce growth-promoting cellular functions (Cerutti, 1985; Son et al., 2013; Waris and Ahsan, 2006). Activation of the NRF2 pathway induces the transcription of genes encoding antioxidant and detoxification enzymes that counteract dangerous accumulation of ROS (Kwak et al., 2003; Lee et al., 2003) and protects cells from transformation (Hao et al., 2020; Schaue et al., 2022; Wang et al., 2022b; Zhang and Gordon, 2004). Several of these protective genes include *GSTP1* (Fang et al., 2020; Zhou et al., 2022), *TXN*, *NQO1*, and *HMOX1* (Tonelli et al., 2018). Chronic exposure of human BEAS-2B lung epithelial cells to the carcinogen hexavalent chromium decreases KEAP1 protein levels, leading to increased basal NRF2 activity and decreased intracellular ROS (Wang et al., 2022a). Additionally, epigenetic activation of NRF2-mediated gene transcription protects mouse skin cells (Yang et al., 2018b), rat mammary cells (Singh et al., 2014), and human colorectal cells (Zuo et al., 2018) from transformation; and pharmacological activation of NRF2 blocks transformation in mouse prostate cells (Yang et al., 2018a). Conversely, inhibition of the NRF2 pathway by glucocorticoids enables the development of breast cancer (Alam et al., 2017; Giudice et al., 2022), and aberrant expression patterns of NRF2 correlate with transformation and progression of colorectal carcinoma (El-Deek et al., 2019).

Although the antioxidant activities that follow NRF2 activation largely protect against transformation, the metabolic consequences of the NRF2-mediated transcription program can have pro-cancer effects. For example, 3-nitrobenzanthrone, a compound in diesel exhaust, is metabolized to a product which forms DNA adducts and promotes mutagenicity (Enya et al., 1997). Phase II metabolic genes under transcriptional control by NRF2, including *AKR1C1*, *AKR1C2*, *AKR1C3*, and *NQO1*, enhance this bioactivation (Murray et al., 2019). On balance, however, most phase II enzymes under transcriptional control by NRF2 metabolize and inactivate a wide variety of carcinogens and toxicants (Lee and Surh, 2005).

Chronic inflammation is known to promote transformation and tumor initiation (Hanahan, 2022) and is driven by multiple signaling pathways. Uncontrolled activation of the NF-κB pathway can result in inflammatory cell damage which can lead to transformation (Naugler and Karin, 2008; Rial et al., 2012), and one way this pathway can be regulated is through NRF2/KEAP1 (Wardyn et al., 2015). The E3 ligase component KEAP1 directly suppresses NF-κB activity through ubiquitination-mediated degradation of the NF-κB activator IKKβ (Kim et al., 2010; Lee et al., 2009); NF-κB signaling is increased after NRF2 depletion (Pan et al., 2012). This negative regulation of the NF-κB pathway is complemented by other anti-inflammatory regulatory roles of NRF2 which culminate in protection from aberrant inflammation (Chi et al., 2015; Ryan et al., 2022; Suzuki et al., 2017; Thimmulappa et al., 2006). Additionally, the tumor suppressor *ARF* (*p14ARF*) decreases NRF2 activity and sensitizes damaged cells to ferro-

ptosis, thus decreasing survival of transformed cells (Chen et al., 2017). In general, these studies suggest that NRF2 activation protects from cellular damage that would otherwise lead to the transformation of normal cells, with some exceptions in NRF2-mediated activation of carcinogens.

There is conflicting data as to whether NRF2 promotes or inhibits tumor initiation. To our knowledge, no study has demonstrated that NRF2-activating mutations alone are sufficient to initiate cancer. Attempts to create mice for assessing effects of whole-body constitutive NRF2 activity have been unsuccessful, as homozygous knockouts of *KEAP1* are lethal in the post-natal period. In these mice, the esophagus and forestomach developed abnormal keratinization not present during the embryonic stages (Wakabayashi et al., 2003). Interestingly, genetically engineered mice have been created that express the most common NRF2-activating mutant in esophageal cancer, $NRF2^{E79Q}$, controlled by a *lox-stop-lox* (*LSL*) motif. When crossed with *KRT14*-driven cre-recombinase mice, the phenotype of *KEAP1* KO is recapitulated. These mice live far beyond the post-natal period, and no increase in cancer incidence was observed in this model (Bowman et al., 2020).

Carcinogen-induced models such as benzo[a]pyrene-induced models of gastric cancer or cadmium-initiated lung carcinogenesis are frequently used to investigate the protective roles of the NRF2 pathway (Ramos-Gomez et al., 2001; Wang et al., 2018). Hepatocellular carcinoma induced by diethylnitrosamine was prevented in mice with liver-specific deletion of the metabolic regulator *SIRT1* which increased NRF2 pathway activation, promoting glutathione metabolism and eliminating ROS (Qiu et al., 2021). Many studies support the idea that NRF2 prevents cancer initiation if activated prior to accumulation of mutations (Ramos-Gomez et al., 2001; Satoh et al., 2013; Schaue et al., 2022; Wang et al., 2021). Specifically, pharmacological and genetic NRF2 pathway activation decreases tumor burden in vinyl carbamate- or urethane-induced models of murine lung cancer (Liby et al., 2007; Satoh et al., 2013). Satoh et al. (2013) reported decreased tumor formation in NRF2 WT mice compared to NRF2 KO mice, but the tumors that formed in NRF2 WT were larger and of higher grade, consistent with the notion that NRF2 activity can provide growth advantages to tumors that do develop. These results contrast with other models in which the loss of NRF2 exacerbated lung carcinogenesis, with higher tumor burden in NRF2 KO mice, even at late stages (Zhang et al., 2018).

NRF2 activation in genetic models has also been shown to prevent tumor development. Overexpression of the *NOTCH* intracellular domain in adipocytes leads to liposarcoma-like soft tissue sarcomas, but knockout of *KEAP1* leading to NRF2 activation prevented tumor development through metabolic reprogramming (Chartoumpekis et al., 2018). CDDO-methyl ester, a potent NRF2 activator, delayed tumor development in *BRCA1*-deficient, *MMTV-neu*, and *PyMT* mouse models of breast cancers (Kim et al., 2012; Liby et al., 2008; Tran et al., 2012). Interestingly, the anti-tumor effects of NRF2 activators in these models was attributed to immune cell modulation rather than the canonical metabolic and antioxidative mechanisms described in other studies. In contrast, NRF2 activation

failed to alter adenoma development in a $GSTP^{-/-}:APC^{Min/+}$ mouse model, suggesting that expression of the NRF2 transcriptional target, the phase II enzyme glutathione S-Transferase pi (GSTP), is required for NRF2-mediated protection from cancer. Tao et al. (2018) compared carcinogen-induced and genetic models of lung cancer and found that sulforaphane-mediated NRF2 activation was protective against vinyl carbamate-induced lung cancer but was ineffective in a $KRAS^{G12D}$ genetic model. Treatment with sulforaphane prior to initiation using vinyl carbamate decreased lung tumor burden in mice, but treatment post-initiation was ineffective. Similarly, NRF2 inhibition prior to carcinogenic initiation increased the tumor number, while sulforaphane treatment of $KRAS^{G12D}$ mice after initiation also increased tumor number. *KRAS* mutations, including $KRAS^{G12D}$, are known drivers of oncogenesis, but they also indirectly activate NRF2 through the RAF-MEK-ERK-AP1 pathway (DeNicola et al., 2011). In $KRAS^{G12D}$-driven pancreatic cancer, NRF2 protects tumor cells by reducing oxidative stress (DeNicola et al., 2011). The inability of NRF2 activation to delay tumorigenesis in $KRAS^{G12D}$-driven lung cancer could be related to ROS levels, but Tao et al. (2018) posit that constitutive KRAS activation robustly drives proliferation, thus surpassing any protective effect of NRF2 activation. Regardless, the mutational burden and timing of NRF2 activation appear to be important factors for the efficacy of prevention.

Despite numerous studies showing that NRF2 activation prevents tumor formation, a considerable body of literature reports the opposite result. In agreement with the *KRAS*-driven lung adenocarcinoma model described previously, tumor development and tumor burden increased following the introduction of *KEAP1* mutations via inhaled Cre-adenovirus in LSL-$KRAS^{G12D/+}$ mice. Inflammation and macrophage numbers were reduced in these tumors, permitting tumor growth (Best et al., 2019). Supporting the immune-regulatory effect of *KEAP1* mutant tumors, enhanced lung adenocarcinoma formation was found in $KEAP1^{fl/fl};PTEN^{fl/fl}$ mice initiated by inhaled Cre-adenovirus, while $KEAP1^{fl/fl}$ or $PTEN^{fl/fl}$ mice had no malignancy (Best et al., 2018). This model is characterized by an immunosuppressive microenvironment, although tumors regressed in response to immune checkpoint blockade.

The complexity of the story continues since many models of NRF2-mediated malignancy require co-mutation of other oncogenic drivers and/or tumor suppressors, but different combinations yield disparate results. There was no increase in tumor incidence in small cell lung cancer initiated by inhaled Cre-adenovirus in $TRP53^{fl/fl};P16^{fl/fl}$ mice when LSL-$NRF2^{E79Q/+}$ was activated, although the tumor histology subtype was altered (Hamad et al., 2022). In fact, a large subset of tumors failed to express the mutant NRF2 despite recombination, and the authors concluded that it was silenced due to a deleterious effect on tumor development. Conversely, tumor burden increased when *KEAP1* was deleted by CRISPR editing *in vivo* in a model of hepatocellular carcinoma promoted by insertional mutagenesis with a *MYC* transposon (Sanghvi et al., 2019). The same group reported that deglycation of NRF2 by fructosamine-3-kinase (FN3K) is required for mediation of its oncogenic function, and if FN3K is inhibited, tumors regress. These studies provide evidence for the great diversity of ge-

netically engineered murine cancer models and how they can lead to different results. In addition, the function of accessory metabolic proteins like FN3K or co-mutations in tumor suppressors like *STK11* have defined roles in tumor development. The triple mutant *LSL-KRAS^{G12D};STK11^{fl/fl};KEAP1^{fl/fl}* mouse lung adenocarcinoma model had poor prognosis, a more aggressive phenotype, and earlier tumor onset compared to non-*KEAP1* mutant counterparts (Singh et al., 2021), mimicking observations in human lung cancer patients.

In addition to metabolic advantages afforded by constitutive NRF2 activation, NRF2 can cause enhancer remodeling of oncogenic drivers to promote tumor initiation. This remodeling can partially explain how NRF2 switches from an anti-cancer to a pro-tumorigenic phenotype (Okazaki et al., 2020). NRF2 activation also impacts cancer cell differentiation. In melanoma cells, NRF2 activation led to de-differentiation and promoted tumor formation through COX2-mediated immune evasion (Jessen et al., 2020). Increased IL-11 expression in NRF2-activated tumorigenic fibroblasts promotes cancer development, possibly through regulation of the immune system (Kitamura et al., 2017). Taken altogether, the dual roles of NRF2 in promoting or preventing the initiation of cancer remains a complex topic [see (Robertson et al., 2020) for an excellent review on NRF2 and cancer initiation] that requires carefully designed studies and prudent interpretation of data.

PROMOTION AND PROGRESSION

- *Promotion*: "Process that stimulates or accelerates tumor progression, usually presumed to do so without directly damaging the genomes of cells."
- *Progression*: "Process of multi-step evolution of a normal cell into a tumor"; "Evolution of a benign into a malignant cancer cell"; "Evolution of a premalignant cell from a promoter-dependent to a promoter-independent state."
  Taken from Weinberg (2014).

Cytoprotective mechanisms enable cancer cells to survive their harsh environments, and NRF2 is activated after cells undergo transformation (Wu et al., 2019). Some cancer subtypes develop mutations within the NRF2 pathway, which lead to constitutive pathway activation (Taguchi and Yamamoto, 2017). Most notably, the hypoxic nature of tumors creates ROS within cancer cells that activates NRF2, regardless of mutational status (Toth and Warfel, 2017). While NRF2 activation alone is not sufficient for the initiation of cancer, it can facilitate proliferation of existing cancer cells initiated by other carcinogenic processes (Vartanian et al., 2019). Activation of oncogenes including *IGF-1*, *KRAS*, *c-MYC*, and others cause cellular stress which in turn activates NRF2 (Lim and Leprivier, 2019; Riis et al., 2020; Vafa et al., 2002), in part because of mitochondrial hyperactivity that occurs within rapidly proliferating cells (Sabharwal and Schumacker, 2014; Sotgia et al., 2011). However, this positive feedback loop between oncogenes such as *KRAS* may also provide opportunities for therapeutic intervention, exemplified by NRF2 activation sensitizing pancreatic cancer cells to glutaminase inhibition *in vitro* (Hamada et al., 2021).

Activation of NRF2 by direct mutation or increased oxida-

tive stress modulates a variety of other processes facilitating progression. The direct gene targets of oncogene-mediated NRF2 activation include not only an extensive array of antioxidant genes (Kavian et al., 2018) but also genes encoding metabolic enzymes (He et al., 2020) and drug efflux pumps (Jeddi et al., 2018; del Vecchio et al., 2014) which collaborate in tumor-promoting effects ranging from increased cancer cell survival to drug resistance. Tumor cells can escape autophagy inhibition by switching to macropinocytosis, a process that is dependent on NRF2 (Su et al., 2021; Towers et al., 2019). NRF2 activation also protects cells from ferroptosis (Fiore et al., 2022; Liu et al., 2020; Nishizawa et al., 2022) and apoptosis (Niture and Jaiswal, 2012; Xie et al., 2020), allowing cancer cells to escape death. Cellular stress induced by anti-cancer drugs and radiation is alleviated by NRF2 activation, leading to therapeutic resistance (Kamble et al., 2021; Noh et al., 2021; Silva et al., 2019). However, the cancer-promoting activation of NRF2 occurs mainly as a response to the high-stress environment within tumors and therefore should be characterized as an enabler, rather than an active driver of cancer progression. For a more comprehensive evaluation of NRF2 and cancer progression, please see (Schmidlin et al., 2021) or (He et al., 2020).

METASTASIS

- *Metastasis*: "Malignant growth forming at one site in the body, the cells of which derive from a malignancy located elsewhere in the body."
  Taken from Weinberg (2014).

In addition to promoting cancer cell survival and progression, the ratio of *HMOX1/NRF2* mRNA expression in tumors is predictive of metastasis to distant sites. The ratio in the tumor tumor tissue was lower in patients with distant metastasis (97.4%) than in those without (101%) (Chang et al., 2016). NRF2 enables metastatic dissemination through multiple mechanisms (Lignitto et al., 2019; Wiel et al., 2019). Competition for KEAP1 binding by HBXIP, part of a c-Fos complex that drives gene expression, elicits a cytoprotective effect by detoxifying ROS through NRF2 activation, enabling cancer cells to tolerate the stress encountered during metastasis (Zhou et al., 2019). A more direct pro-invasive role is mediated through an NRF2/heme oxygenase-1 (HO-1)/BACH1 axis. Constitutive NRF2 activation promotes cancer cell migration and metastasis through accumulation of the BACH1 transcriptional regulator. NRF2 upregulates expression of HO-1, increasing heme catabolism, and thereby preventing FBXO22-mediated degradation of BACH1 (Lignitto et al., 2019). In lung cancer, BACH1 accumulation promotes a transcriptional shift toward pro-metastatic gene profiles (Lignitto et al., 2019). The importance of BACH1 was corroborated by a complementary study in which chronic administration of the antioxidants N-acetylcysteine and vitamin E downregulate the NRF2 pathway. Antioxidant treatment increased tumor metastases through a metabolic switch to glycolysis that was advantageous to invading tumor cells (Wiel et al., 2019). Antioxidant treatment was a surrogate for NRF2-mediated detoxification, which increased BACH1-dependent metastases.

However, in tumors with constitutive NRF2 pathway activation, it is conceivable that the BACH1-mediated mechanism for metastasis would be overactivated.

Additional evidence for the involvement of NRF2 in metastasis can be found in the promotion of the epithelial to mesenchymal transition (EMT). In glioblastoma multiforme, NRF2 acts in conjunction with p62 to activate EMT and subsequently increases tumor invasiveness (Pölönen et al., 2019). This effect was also observed in hepatocellular carcinoma with MCUR1-induced mitochondrial calcium uptake that induced NRF2/NOTCH-mediated EMT (Jin et al., 2019). Additionally, NRF2 works in conjunction with NOTCH/EMT signaling in breast cancer. NRF2 promotes the upregulation of G6PD/HIF-1α, and in turn, activates NOTCH-mediated EMT of breast cancer cells (Zhang et al., 2019). Further, in non-small cell lung cancer NRF2 activates the RhoA-ROCK1 signaling pathway that increases expression of mesenchymal-type markers and increases cell motility, which was prevented by NRF2 inhibition (Ko et al., 2021).

In combination with increased cancer cell motility through EMT, angiogenesis, a critical feature that enhances dissemination of metastatic cancer cells, is increased by NRF2 (Huang et al., 2021; Liu et al., 2021; Shahcheraghi et al., 2022). This increased angiogenesis has been attributed to NRF2-mediated stabilization of HIF-1α and activation of its transcriptional program (Ji et al., 2014; Zheng et al., 2023). Finally, there is a connection between mitochondrial stress and NRF2. Tumor cells interact with the extracellular matrix to induce intra-tumoral mechanical signaling that increases mitochondrial ROS, which in turn increases oxidative stress, and thus NRF2-mediated cytoprotection (Romani et al., 2022). Romani et al. (2022) discovered that soft extracellular metastatic niches promote NRF2-mediated chemoresistance through increased mitochondrial ROS. Activation of NRF2 is permissive for cancer cell tolerance to oxidative insults in both the invading primary tumor cells and those disseminated to distant metastatic niches.

CONCLUSIONS AND FUTURE DIRECTIONS

Knowledge gaps persist in defining the precise function of NRF2 in cancer transformation, initiation, promotion, and metastasis (Fig. 1). Moreover, the lack of definitive conclusions is compounded by the disparate effects observed at different stages of carcinogenesis and in the different cancer models used (genetic versus carcinogen-induced and immune competent versus immunodeficient). These observations, detailed above, lead to a primary conclusion that the functions of NRF2 are highly context dependent. Context encompasses the stage of cancer, the experimental models, and the type of cancer.

The prevalence of high NRF2 expression (increased transcription and translocation to the nucleus) and activation (downstream effectors) is relevant in cancers that arise in organs with high exposure to environmental insults or that function in detoxification, such as the lung, digestive system, pancreas, and liver (Gao et al., 2015; Liby et al., 2008; Pillai et al., 2022). As such, it is likely that pharmacological interventions will be first used in cancers of these organs. However, whether NRF2 activators or inhibitors are appropriate is still
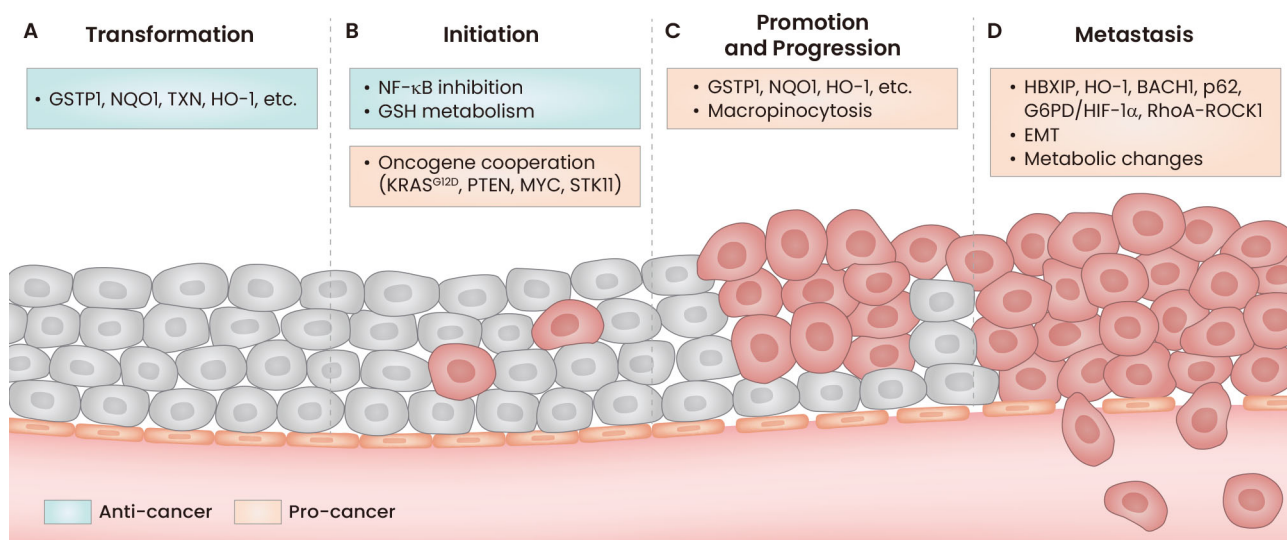

Fig. 1. NRF2 activation throughout carcinogenesis. In the initial stages of carcinogenesis, NRF2 has anti-tumor effects through transcription of antioxidant and cytoprotective genes. As transformed cells progress, they utilize these same cytoprotective effects in cooperation with other pro-tumor mechanisms to facilitate drug resistance, cell survival, and metastasis. (A) Activation of NRF2 in the transformation stage of carcinogenesis increases cytoprotective genes that prevent cancer formation. (B) Regulation of inflammation and redox balance can prevent increases in mutational burden thereby preventing initiation, but co-occurring mutations in oncogenes or tumor suppressors increase cancer initiation by utilizing NRF2-mediated cytoprotection. (C) Transformation-preventing genes that are upregulated by NRF2 are similarly upregulated in the promotion and progression phase, although protect the existing cancer. (D) NRF2 upregulates a variety of pro-metastatic gene pathways, enabling cancer metastasis.

under investigation due to the dual roles of NRF2 in promoting and inhibiting cancer. The use of either type of pharmacological agent will likely be stage- and cancer-dependent. Currently there are 4 clinical trials targeting tumors with either *NRF2* or *KEAP1* mutations (Pillai et al., 2022; Yagishita et al., 2020). These pharmacological interventions mainly take advantage of the downstream metabolic vulnerabilities generated by *NRF2* and *KEAP1* mutations (Dinkova-Kostova and Copple, 2023). In the future it is likely that NRF2 signaling will be targeted directly, either for activation or inhibition. The NRF2 activator dimethyl fumarate is already approved for clinical use in relapsing forms of multiple sclerosis (Faissner and Gold, 2019; Schimrigk et al., 2006). The triterpenoid CDDO-Methyl ester (CDDO-Me or bardoxolone methyl), another NRF2 activator, is currently being tested in clinical trials for chronic kidney disease (Chin et al., 2018). Moreover, CDDO-Me has been tested in preclinical models of lung, pancreas, and breast cancer. Other small molecules activators, such as curcumin, resveratrol, and sulforaphane, have been tested in cancer cells and pre-clinical mouse models, alone or in combination with chemotherapies (Ashrafizadeh et al., 2020; Dinkova-Kostova et al., 2017; Farkhondeh et al., 2020; Giordano and Tommonaro, 2019; Mansouri et al., 2020; Singh et al., 2014; Tao et al., 2018).

The development of direct pharmacological inhibitors of the NRF2 protein has been hampered primarily due to the lack of a druggable binding pocket (Karunatilleke et al., 2021). NRF2 has been considered an undruggable protein, in the same category as KRAS, one of the most prevalent oncogenes in solid tumors. Development of pharmacological inhibitors or activators of NRF2 is still in its infancy, although recent advances in medicinal chemistry have led to the development of small molecules targeting NRF2 and related proteins (Bar-Peled et al., 2017). Other approaches have been developed to target NRF2-mediated transcription and DNA binding of NRF2/MAFG complexes (Simov et al., 2021). Biological insights into how the NRF2 pathway promotes or inhibits different stages of carcinogenesis provide new opportunities for drug development (Hou et al., 2023; Pouremamali et al., 2022; Robledinos-Antón et al., 2019; Zhang et al., 2021). Additionally, with distinct functions evident in different cancers, precision medicine can be used to specifically target vulnerabilities based on mutations or upregulation within the NRF2 pathway. Combination therapies, either with chemotherapy or immunotherapy, are other possible avenues to augment the effects of small molecule inhibitors of the NRF2 pathway.

Despite advances in our knowledge of the NRF2 pathway in recent years, the indirect effects of *NRF2* and *KEAP1* mutations in the tumor microenvironment as well as how these mutations cooperate with common co-occurring mutations, such as *KRAS* and *STK11/LKB1*, are still incompletely understood (Table 1). With the development of immunotherapy, many studies in the past decade have focused on the tumor microenvironment. *NRF2* and *KEAP1* mutations as well as co-occurring mutations may influence the regulation of the NRF2 pathway in the tumor microenvironment (Best et al., 2018; Cristescu et al., 2018) and consequently influence therapeutic responses to chemotherapy and/or immunotherapy (Kobayashi et al., 2016; Taguchi and Yamamoto, 2017; Taniguchi et al., 2020). Moreover, overexpression or over activation of NRF2 through epigenetic changes or by hijacking of the pathway by other tumor promoting mutations, such as *KRAS* and *ALK*, are still not well understood biologically or mechanistically. Elucidating these biological processes will likely reveal new pharmacological vulnerabilities that can improve therapeutic options for patients with aggressive cancers where NRF2 is abnormally expressed.

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AUTHOR CONTRIBUTIONS

C.J.O. and J.A.M. surveyed the literature and wrote the main body of the manuscript. A.S.L. wrote the abstract, conclusions, and future directions. K.A.G. edited the manuscript and assisted in preparation for submission. K.T.L. provided overall direction and edited the manuscript.

CONFLICT OF INTEREST

K.T.L. is a named inventor on patents issued and filed for synthetic triterpenoids and NRF2 pathway inhibitors. Other authors have no potential conflicts of interest to disclose.

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Table 1. Areas requiring further investigation

- Characterization of NRF2-activated tumor microenvironments
- Epigenetics of NRF2-activated tumors
- Implications of tumor origin and location on NRF2-mediated tumor biology
- Clarification on pharmacologic intervention for tumor prevention and treatment

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