Annual Review of Pharmacology and Toxicology

Targeting NRF2 and Its Downstream Processes: Opportunities and Challenges

Laura Torrente and Gina M. DeNicola

Department of Cancer Physiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida 33612, USA; email: gina.denicola@moffitt.org

Annu. Rev. Pharmacol. Toxicol. 2022. 62:279–300

First published as a Review in Advance on September 9, 2021

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

https://doi.org/10.1146/annurev-pharmtox-052220-104025

Keywords

NRF2, KEAP1, cancer, therapeutics

Abstract

The transcription factor NRF2 coordinates the expression of a vast array of cytoprotective and metabolic genes in response to various stress inputs to restore cellular homeostasis. Transient activation of NRF2 in healthy tissues has been long recognized as a cellular defense mechanism and is critical to prevent cancer initiation by carcinogens. However, cancer cells frequently hijack the protective capability of NRF2 to sustain the redox balance and meet their metabolic requirements for proliferation. Further, aberrant activation of NRF2 in cancer cells confers resistance to commonly used chemotherapeutic agents and radiotherapy. During the last decade, many research groups have attempted to block NRF2 activity in tumors to counteract the survival and proliferative advantage of cancer cells and reverse resistance to treatment. In this review, we highlight the role of NRF2 in cancer progression and discuss the past and current approaches to disable NRF2 signaling in tumors.

1. INTRODUCTION

The transcription factor nuclear factor erythroid 2–related factor 2 (NRF2/*NFE2L2*) plays a pivotal role in the maintenance of redox, metabolic, and protein homeostasis. Historically, NRF2 has been recognized as the master regulator of the detoxification and antioxidant programs. Notably, during the last decade, multiple studies revealed new functions of NRF2 beyond the regulation of the redox balance. It is now recognized that NRF2 responds to redox alterations, growth factor signaling, proteotoxic stress, and changes in nutrient status (1–4). In response to stress inputs, NRF2 stimulates cytoprotective responses by inducing the expression of a plethora of genes involved in antioxidant signaling, metabolism, xenobiotic transformation, autophagy, proteostasis, and iron catabolism (5–7) (**Figure 1**).

Timely activation of NRF2 protects healthy tissues against environmental insults by readily inducing detoxification programs and consequently preventing the accumulation of damaged cellular components. Therefore, NRF2 signaling is essential to prevent tumor initiation. Conversely, activation of NRF2 in established tumors promotes cancer progression, confers therapeutic resistance, and correlates with poor prognosis in patients (8, 9). The dual roles of NRF2 in tumorigenesis sparked considerable interest in developing both NRF2 activators for chemoprevention and NRF2 inhibitors for cancer treatment. Herein, we expand on the consequences of NRF2 activation in malignant progression and the efforts to tackle NRF2 signaling for cancer treatment.

2. REGULATION OF NRF2 ACTIVITY

The activity of NRF2 is primarily regulated at the protein level by a constitutive cycle of synthesis and degradation. Under unstressed conditions, Kelch-like ECH-associated protein 1 (KEAP1) associates with two separate NRF2 motifs containing the amino acid sequences ^{29}DLG^{31} and

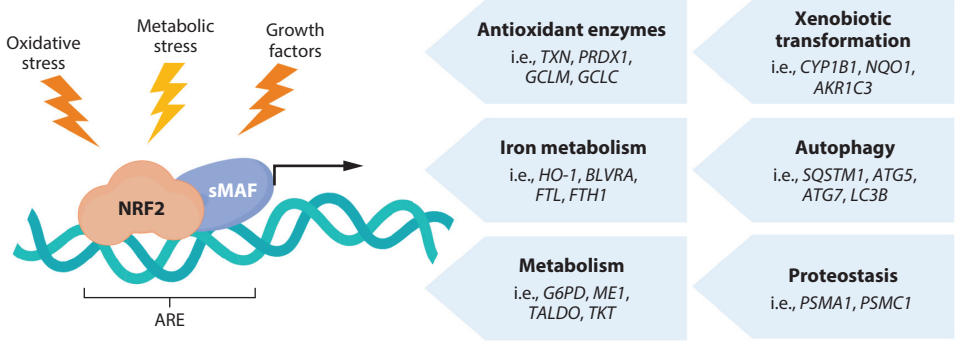

Figure 1

The nuclear factor erythroid 2–related factor 2 (NRF2) transcriptional network. NRF2 heterodimerizes with small musculoaponeurotic fibrosarcoma (sMAF) and binds to antioxidant response elements (AREs) to regulate the expression of target genes, including antioxidant enzymes [glutamate-cysteine ligase modifier subunit (*GCLM*), glutamate-cysteine ligase catalytic subunit (*GCLC*), peroxiredoxin 1 (*PRDX1*), thioredoxin (*TXN*)], xenobiotic transformation [aldo-ketoreductase 1C3 (*AKR1C3*), cytochrome P450 family 1 subfamily B member 1 (*CYP1B1*), NAD(P)H:quinone oxidoreductase 1 (NQO1)], iron metabolism [biliverdin reductase A (*BLVRA*), ferritin heavy chain (*FTH1*), ferritin light chain (*FTL*), heme oxygenase-1 (*HO-1*)], autophagy [autophagy protein 5/7 (*ATG5/7*), microtubule-associated protein 1A/1B-light chain 3B (*LC3B*), sequestosome 1 (*SQSTM1*, *p62*)], metabolism [glucose 6-phosphate dehydrogenase (*G6PD*), malic enzyme 1 (*ME1*), transaldolase 1 (*TALDO*), transketolase (*TKT*)], and proteostasis [proteasome 26S subunit ATPase 1 (*PSMC1*), proteasome subunit alpha type-1 (*PSMA1*)]. Figure adapted from images created with BioRender.com.

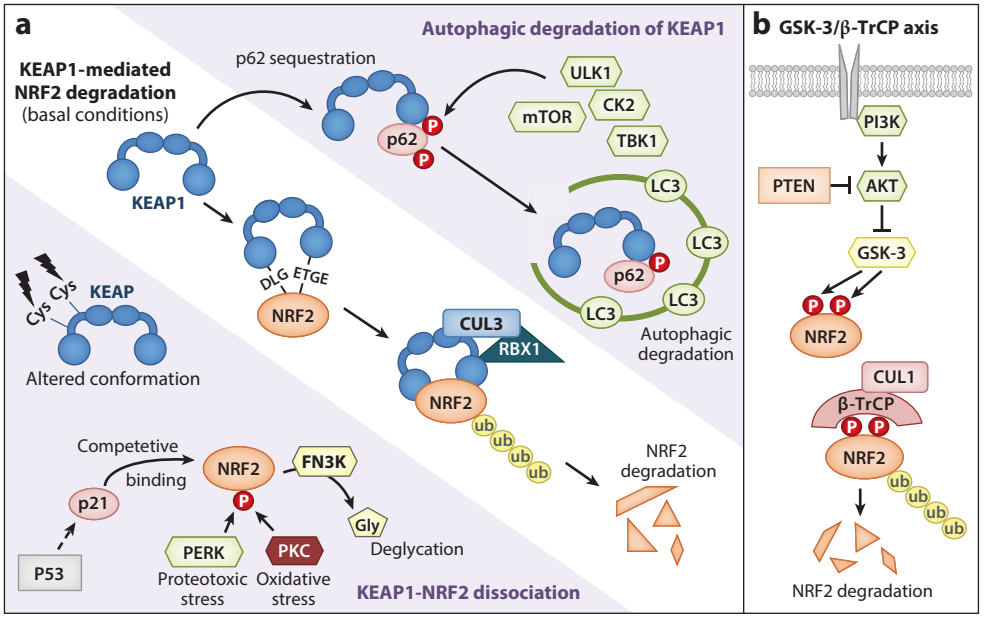

Figure 2

Regulation of NRF2 activity by KEAP1 and the GSK-3/β-TrCP axis. (*a*, *left*) Signaling pathways that impair NRF2-KEAP1 association. KEAP1 is equipped with a variety of cysteine residues that respond to oxidative, electrophilic, and metabolic stress. Modification of key cysteine thiols alters KEAP1 conformation and impedes NRF2 degradation. KEAP1 competes with p21 for NRF2 binding. Phosphorylation of NRF2 by PERK and PKC promotes NRF2 activation. FN3K promotes NRF2 deglycation and stabilizes NRF2 by preventing KEAP1 binding. (*Middle*) KEAP1 binds to the ETGE and DLG motifs on NRF2 and recruits the CUL3/RBX E3 ubiquitin ligase complex to promote the ubiquitination and proteasomal degradation of NRF2. (*Right*) Phosphorylation of p62 promotes the autophagic degradation of KEAP1. (*b*) Noncanonical degradation of NRF2 by the GSK-3/β-TrCP axis. GSK-3 phosphorylates NRF2 to create a phosphodegron that triggers the recruitment of the β-TrCP-CUL1-based E3 ubiquitin ligase complex. In response to growth factors or acute exposure to ROS, the PI3K/AKT pathway negatively regulates the activity of GSK-3. The phosphatase PTEN antagonizes the PI3K/AKT axis. Abbreviations: β-TrCP, β-transducin repeat-containing protein; CK2, casein kinase 2; CUL, Cullin; Cys, cysteine; FN3K, fructosamine-3-kinase; GSK-3, glycogen synthase kinase 3; KEAP, Kelch-like ECH-associated protein; LC3, microtubule-associated proteins 1A/1B light chain 3B; mTOR, mammalian target of rapamycin; NRF2, nuclear factor erythroid 2–related factor 2; PERK, PKR-like endoplasmic reticulum kinase; PI3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; RBX, E3 ubiquitin-protein ligase RBX1 (Ring-Box 1); ROS, reactive oxygen species; TBK1, TANK-binding kinase 1; ULK1, serine/threonine-protein kinase ULK (Unc-51-like autophagy-activating kinase 1).

^{79}ETGE^{82} (10). KEAP1 acts as a substrate adapter for the Cullin 3 (CUL3)-containing E3-ligase complex, which targets NRF2 for ubiquitination and subsequent degradation by the 26S proteasome (11, 12) (**Figure 2**). Constitutive degradation of NRF2 ensures that only a small fraction of newly synthetized NRF2 reaches the nucleus to regulate the basal expression of target genes. Multiple stress signals abrogate KEAP1-directed inhibition of NRF2 by modifying the sulfhydryl groups of key KEAP1 cysteine residues (13, 14). These stressors include reactive oxygen species (ROS), electrophiles and metabolic intermediates from the tricarboxylic acid (TCA) cycle, and glycolysis (15–17). Thus, both oxidative and metabolic stress inputs alter KEAP1 conformation due to the modification of multiple sulfhydryl groups, leading to the transient activation of NRF2. Once in the nucleus, NRF2 heterodimerizes with small musculoaponeurotic fibrosarcoma (sMAF)

proteins and binds to the antioxidant response elements (AREs) located in the promoter region of target genes (18).

The KEAP1-NRF2 interaction can also be disrupted by other KEAP1 binding partners, such as sequestosome 1 (SQSTM1/p62), that contain amino acid motifs that resemble the ETGE and DLG domains in NRF2. Under conditions of selective autophagy, p62 is initially phosphorylated at Ser407 by the serine/threonine-protein kinase ULK1, followed by Ser403 phosphorylation by casein kinase 2 (CK2), TANK-binding kinase 1 (TBK1), or ULK1 (3, 19, 20). Consequently, p62 is translocated to ubiquitinated cargos and is then phosphorylated at Ser349 by mammalian target of rapamycin complex 1 (mTORC1) (21). Ser349 of p62 is located within a ^{349}STGE^{352} motif that, upon phosphorylation, mimics the ETGE domain of NRF2. Phosphorylation of p62 promotes the sequestration and autophagic degradation of KEAP1. Notably, mTORC1 can phosphorylate p62 to induce KEAP1 sequestration under conditions of oxidative stress, damaged mitochondria (mitophagy), and invading pathogens (xenophagy) (21). Further, because p62 is essential for mTORC1 activation in response to amino acid supplementation, it is likely that this mechanism links NRF2 activity to the cellular response to nutrient status (22). Glucose starvation also induces p62-mediated degradation of KEAP1 through the activation of the liver kinase B1 (LKB1)–adenosine monophosphate–activated kinase (AMPK) signaling pathway by an mTORC1-independent autophagy pathway (23). Of note, NRF2 induces p62 expression, implying a positive feedback loop (24). Additionally, NRF2-KEAP1 association is also challenged by cyclin-dependent kinase inhibitor 1 (p21), a major target of the tumor suppressor p53. p21 directly interacts with the DLG and ETGE domains of NRF2, which impedes KEAP1 binding in response to oxidative stress (25).

KEAP1-mediated regulation of NRF2 activity can be modulated by posttranslational modifications of NRF2, including phosphorylation and glycation. The protein kinase C (PKC) phosphorylates NRF2 at Ser40 in response to oxidative stress, which impedes KEAP1 binding and promotes nuclear accumulation of NRF2 (26). The PKR-like endoplasmic reticulum kinase (PERK) phosphorylates and activates NRF2 following protein folding stress in the endoplasmic reticulum (2). The cellular energy sensor AMPK phosphorylates NRF2 at Ser550, a residue located within the canonical nuclear export signal, which promotes its nuclear accumulation (27). NRF2 glycation enhances KEAP1-mediated NRF2 degradation and impairs NRF2 interaction with sMAF proteins (28). Fructosamine-3-kinase (FN3K) reverses NRF2 glycation by phosphorylating the attached sugars (28).

KEAP1 is not the only negative regulator of NRF2. At least three additional systems regulate NRF2 proteasomal degradation: the β-transducin repeat–containing protein (β-TrCP)-Cullin1/Rbx1 E3 ligase complex, the WD repeat–containing protein 23 (WDR23)-DDB1-Cullin4 E3 ligase complex, and the E3 ubiquitin ligase HMG-CoA reductase degradation 1 homolog (HRD1, also known as synoviolin 1). The glycogen synthase kinase 3 (GSK-3) phosphorylates NRF2 at Ser344 and Ser347 of the ^{342}DSGIS^{347} motif, which prompts the recruitment of the of the S-phase kinase–associated protein 1 (Skp1)-Cullin1-Rbx1/β-TrCP E3 ligase complex (29). In response to insulin and growth factors, GSK-3 activity is repressed by the phosphatidylinositol-3 kinase (PI3K) and the protein kinase B (PKB/AKT) axis. Thus, PI3K/AKT activates, while GSK-3 inhibits, NRF2 activity. Remarkably, the phosphatase and tensin homolog (PTEN) is a major antagonist of the PI3K/AKT signaling cascade and acts as a redox sensor. The contribution of the WDR23 and HRD1 systems to NRF2 degradation is less understood, as only one study of each system has been published (30, 31). Altogether, multiple signaling cascades are involved in both the repression and activation of NRF2, which permits the fine-tuning of NRF2 signaling under distinct physiological conditions.

3. RATIONALE FOR THE DEVELOPMENT OF NRF2 INHIBITORS IN CANCER

The transient and inducible nature of NRF2 signaling is frequently lost in cancer. Instead, constitutive NRF2 activity has been reported across different cancer types, including non-small-cell lung cancer (NSCLC), endometrial carcinoma, hepatocellular carcinoma (HCC), pancreatic cancer, esophageal and head and neck squamous cell carcinoma (32, 33). Hyperactivation of NRF2 in cancer cells is driven by multiple factors, including somatic mutations of *NFE2L2*, *KEAP1*, or *CUL3* genes; epigenetic alterations in the *KEAP1* and *NFE2L2* promoters; transcriptional upregulation of NRF2; and accumulation of KEAP1-associated proteins (i.e., p62).

At the DNA level, aberrant activation of NRF2 in cancer cells is frequently driven by loss-of-function mutations in *KEAP1* or *CUL3* and gain-of-function mutations in *NFE2L2* (32, 34, 35). Somatic mutations that disrupt the NRF2-KEAP1 interaction are particularly prevalent in NSCLC, as it is estimated that 34% of lung squamous cell carcinoma and 18% of lung adenocarcinoma patients harbor mutations in *NFE2L2* or *KEAP1* (8, 35, 36). While inactivating mutations in KEAP1 are dispersed across the full length of KEAP1 protein, *NFE2L2* mutations are found in two distinct hot-spot regions, within the KEAP1 binding domains (DLG and ETGE motifs), thus preventing KEAP1 association. Further, changes of *NFE2L2* and *KEAP1* copy number in cancer cells can also impact NRF2 and KEAP1 abundance. Epigenetically, the *KEAP1* promoter is often hypermethylated in breast, colon, and lung carcinomas, while demethylation of the *NFE2L2* promoter frequently occurs in lung and colorectal cancers (37).

Beyond NRF2/KEAP1/CUL3 genetic alterations, the activation of oncogenes or inactivation of tumor suppressors also can contribute to the hyperactivation of NRF2. DeNicola et al. (38) reported that NRF2 is transcriptionally activated by the oncogenic mutants K-RAS$^{G12V/D}$, B-RAF^{V619E}, and MYC overexpression. Rojo et al. (39) reported that loss of the tumor suppressor PTEN, frequently altered in prostate and endometrial tumors, stabilizes NRF2 due to the inactivation of the GSK-3/β-TrCP axis. KEAP1 is also inactivated by fumarate and succinate, which accumulate in tumors harboring mutations of the tumor suppressor fumarate hydratase (FH) (40). Germline mutations of FH are characteristic of the hereditary leiomyomatosis and renal cell carcinoma cancer syndrome; yet the contribution of KEAP1 inactivation to the pathogenesis of this disease is not fully understood. Overexpression of the oncogene ataxia-telangiectasia group D–associated gene (*ATDC*) in pancreatic cancer binds to and sequesters KEAP1, thereby promoting the stabilization of NRF2 (41). The ubiquitin-specific-processing protease 11 (USP11), which is highly expressed in NSCLC, deubiquitinylates NRF2 and promotes its stabilization (42). NRF2 can be also activated in cancer in a p62-dependent manner due to the impairment of autophagy, abnormal expression of p62, or persistent phosphorylation of p62 (43).

The large variety of genetic alterations that drive NRF2 activation may suggest that these mutations provide a survival advantage in response to selective pressures during tumor development (i.e., oxidative stress, hypoxia, nutrient shortage). It is interesting to note that the vast majority of NRF2 activating mechanisms disrupt KEAP1-mediated degradation and, to some extent, the GSK-3/β-TrCP axis. Additional work is needed to understand the contribution of WDR23 and HRD1 to NRF2 degradation in cancer tissues.

3.1. NRF2 Inhibitors in Cancer Therapy: Advantages

The protumorigenic effects of NRF2 activation have been thoroughly described in the literature. It is well established that NRF2 contributes to tumor progression by inducing the expression of antioxidant and detoxification enzymes (**Figure 3**). Indeed, cancer cells harboring aberrant

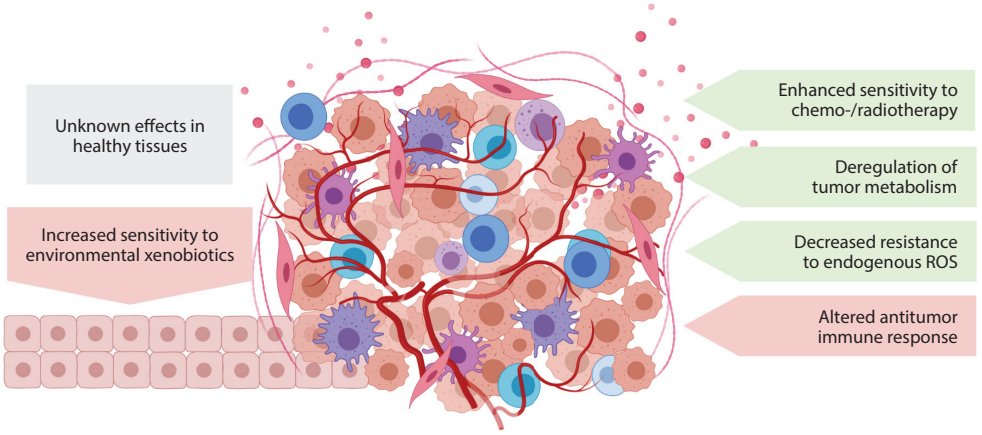

Figure 3

Potential benefits and side effects of systemic inhibition of nuclear factor erythroid 2–related factor 2 (NRF2) in tumor-bearing hosts. NRF2 inhibition in cancer cells impairs tumor metabolism, impedes the detoxification of reactive oxygen species (ROS) and decreases resistance to chemotherapeutic agents and radiotherapy. NRF2 inhibition in the tumor microenvironment and healthy tissues enhances susceptibility to environmental carcinogens and hampers antitumor immune responses. Figure adapted from "Tumor Microenvironment 2" by BioRender.com (2021), retrieved from **https://app.biorender.com/biorender-templates**.

activation of NRF2 are equipped with a reinforced antioxidant capacity that allows them to counteract elevated ROS levels produced by various endogenous sources, including high metabolic rate and mitochondrial dysfunction. NRF2 also induces the expression of enzymes involved in xenobiotic metabolism and drug efflux pumps, which cooperate with the antioxidant defense to render cancer cells resistant to commonly used chemotherapeutic agents and radiotherapy (44–46).

Persistent NRF2 activation also rewires cellular metabolism to fulfill the specific metabolic requirements necessary to sustain cancer cell proliferation and the antioxidant defense (47). In brief, NRF2 enhances cystine uptake and promotes the use of cysteine for synthesis of the antioxidant glutathione (GSH) (continued in Section 6.1). NRF2 induces the expression of enzymes involved in the pentose phosphate pathway (PPP) and de novo nucleotide synthesis, which leads to increased production of NAD(P)H and nucleotides (continued in Section 6.2) (48). In addition, NRF2 regulates the expression of the activating transcription factor 4 (ATF4), which transcriptionally activates serine biosynthetic genes (49). Metabolism of serine supplies substrates for GSH and nucleotide production, thus synergizing with the PPP to supply ribose for nucleotide production (49).

Beyond the redox and metabolic aspects, NRF2 directly regulates the expression of multiple components of the autophagy system (7). Autophagy supports tumor survival and growth by recycling intracellular components to supply metabolic substrates, hence promoting resistance to nutrient starvation. In turn, NRF2 also promotes adaptation to autophagy inhibition in cancer cells by inducing the expression of proteasome subunits (50). NRF2 also improves the growth of the vascular endothelia in tumors by activating and sustaining the hypoxia-inducible factor (HIF-1) response (51). Some studies have also reported that NRF2 contributes to the epithelial–mesenchymal transition (52).

Because of the multiple advantageous aspects, cancer cells harboring aberrant NRF2 activation frequently lapse into NRF2 addiction status, meaning that survival and proliferation of these cancer cells rely on the constitutive NRF2 activity. Several studies have confirmed that NRF2 silencing is sufficient to disturb the redox balance, hamper proliferation, and alter the survival

of NRF2 hyperactive cancer cells (48, 53). Therefore, from a cell-autonomous standpoint, direct inhibition of NRF2 is the most effective strategy to reverse the protumoral effects caused by constitutive NRF2 activity.

3.2. Potential Detrimental Effects of Systemic NRF2 Inhibition

It still remains unclear whether systemic delivery of NRF2 inhibitors for cancer treatment would offer a therapeutic benefit or, conversely, promote tumor progression (**Figure 3**). First of all, it is not known whether inhibition of NRF2 in normal healthy tissues could trigger adverse side effects. From animal models, we have learned that Nrf2-knockout ($Nrf2^{-/-}$) mice are viable and grossly normal (54); yet, $Nrf2^{-/-}$ mice have decreased fertility compared to wild-type (WT) and heterozygous littermates and display a lupus-like autoimmune syndrome (55, 56). Remarkably, Nrf2-null mice do not develop cancer spontaneously, but decreased antioxidant capacity renders $Nrf2^{-/-}$ mice more susceptible to acute and prolonged exposure to both toxic compounds and carcinogens (57). These data suggest that transient inhibition of NRF2 in healthy individuals may be tolerable.

Negative consequences of systemic NRF2 inhibition in cancer patients may arise from the loss of NRF2 in the tumor microenvironment and/or alterations in the immune response. The Yamamoto lab (58) conducted several studies that provide evidence that NRF2 activity is essential to promote antitumor immune responses. First, Satoh et al. (58) investigated the metastatic potential of Lewis lung carcinoma (3LL) cells in WT, Nrf2-deficient, and KEAP1 knockdown (Keap1-KD) mice. Following 3LL inoculation, Nrf2-deficient mice exhibited a higher number of pulmonary metastatic nodules than did WT mice. Lack of Nrf2 expression led to the recruitment of myeloid-derived suppressor cells that retained elevated levels of ROS, which attenuated $CD8^+$ T cell immunity. In contrast, Keap1-KD mice displayed decreased susceptibility to lung metastasis of 3LL cancer cells, thus suggesting that NRF2 activation in the host microenvironment restricts tumor invasiveness. A follow-up study examined the role of NRF2 in tumor initiation and progression using urethane to induce lung tumors in Keap1-KD and Keap1-WT mice. Keap1-KD mice were resistant against urethane-induced lung carcinogenesis, consistent with the chemoprotective role of Nrf2 (59). However, when tumors of Keap1-KD and Keap1-WT mice were transplanted into immunodeficient mice, tumors derived from Keap1-KD mice grew much more aggressively than did tumors derived from WT mice. Thus, the authors concluded that global activation of NRF2 prevents tumor initiation by enhancing anticancer immunity, while NRF2 confers tumorigenic ability on cancer cells.

Later, a sophisticated animal study using genetically engineered mouse models of lung cancer demonstrated that microenvironmental activation of Nrf2 represses the growth of Nrf2-hyperactive lung tumors (60). In this study, the $Kras^{G12D}$ mouse model of adenocarcinoma was crossed with two distinct *Keap1*-flox mouse models. Mice harboring the $Keap1^{FA}$ allele exhibit systemic suppression of the *Keap1* gene expression before Cre recombination, which results in systemic Nrf2 activation. Conversely, Keap1 expression from the $Keap1^{FB}$ allele is normal before Cre recombination. Intriguingly, while Nrf2 accumulation in tumors was comparable in both *Keap1*-floxed models after Cre recombination, sustained activation of Nrf2 in the microenvironment of $Keap1^{FA/FA}$ mice restricted tumor progression. Nrf2 activation in immune cells contributed to these suppressive effects, specifically through the restoration of the $CD8^+$ T cell immunity. These data suggest that systemic activation of NRF2 in cancer patients may restrict tumor growth due to potentiation of the host immunity, regardless of KEAP1/NRF2 mutation status.

In summary, these findings suggest that NRF2 activation in cancer cells enhances their malignant potential, while activation of NRF2 in the host cells enhances anticancer immunity.

Therefore, these results argue against the systemic delivery of NRF2 inhibitors, as they could hamper the immune system's ability to restrain cancer cells from spreading. In addition, sustained NRF2 inhibition may decrease the protection of healthy tissues against environmental insults, leading to increased susceptibility to carcinogens. Specifically, NRF2 plays a critical protective role in the detoxification against environmental xenobiotics in the respiratory tract (i.e., cigarette smoke exposure) and the gastrointestinal tract (i.e., alcohol and acetaminophen consumption). NRF2 signaling is also important for the resolution of persistent inflammation due to the modulation of redox homeostasis, crosstalk with nuclear factor-κB, and regulation of inflammatory genes (61). Hence, NRF2 inhibition could increase the risk of proinflammatory diseases and worsen autoimmune and cardiovascular disorders (62). Accordingly, at this point in time, inhibition of NRF2 for cancer treatment is still in a proof-of-concept stage, as the systemic consequences of this approach are largely unknown.

4. EFFORTS TO IDENTIFY DIRECT NRF2 INHIBITORS

The development of therapies to combat NRF2/KEAP1 mutant tumors has been focused on the development of direct NRF2 inhibitors. Transcription factors have historically been difficult to target directly, partly due to the challenges associated with targeting either the protein-DNA or protein-protein interactions that mediate their function, as opposed to active sites of kinases. Indeed, NRF2 inhibitors identified to date lack either specificity or potency, and none have entered clinical trials. Herein, we classify and describe some of the compounds identified to date with the ability to repress NRF2 signaling according to their mechanism of action (**Table 1**).

4.1. Inhibition of Protein Synthesis

Because NRF2 is a very short-lived protein under nonstressed conditions (15–30 min), inhibitors of protein synthesis can significantly impact NRF2 activity within minutes and may be easily mistaken by direct NRF2 inhibitors (63). In 2011, the Zhang laboratory (64) identified brusatol as a potential inhibitor of NRF2 signaling. This compound was identified in a drug screen, in which the efficacy of a large number of natural products was evaluated (65). Brusatol was shown to deplete NRF2 protein levels within 2 h in the nanomolar range in a KEAP1-independent fashion. Additionally, brusatol treatment sensitized cancer cells, A549 xenograft models, and $Kras^{G12D}$-driven lung tumors to cisplatin (64, 66, 67). However, in 2016, the Stokoe laboratory (68) revealed that brusatol functions as a global inhibitor of protein synthesis using a mass-spectrometry-based approach. The authors also showed that brusatol treatment induces cytotoxicity in a vast array of cancer cell lines independent of their NRF2/KEAP1 status, and it displays a similar cytotoxic profile to silvestrol, a well-characterized protein translation inhibitor. Shortly after (2017), the Yamamoto laboratory (69) screened 5,861 chemical compounds aiming to identify NRF2 inhibitors. In this study, febrifugine and derivatives, including halofuginone, were reported to exhibit NRF2 inhibitory properties. Halofuginone suppresses NRF2 activity by repressing global protein synthesis (69). Although halofuginone does not directly inhibit NRF2, the authors showed that NRF2-addicted lung cancer cells are more sensitive to halofuginone treatment than are immortalized normal epithelial cells. Further, halofuginone also increased cisplatin and doxorubicin efficacy in in vitro and in vivo models.

Although these inhibitors have yielded promising anticancer effects in vitro and in preclinical models, inhibition of global protein translation may limit their clinical applicability because of potential toxicities. Indeed, early Phase I clinical studies with bruceantin, a translation inhibitor with a chemical structure similar to brusatol, reported systemic toxicity (70). Further, limited efficacy was found against metastatic breast cancer and malignant melanoma in Phase II clinical trials,

Table 1 Potential therapeutic strategies to target NRF2 hyperactive cancer cells described in this review

Inhibitor class	Target (compound)	Mechanism of action
NRF2 inhibitors	NRF2 (brusatol)	Global inhibitor of protein translation
	NRF2 (halofuginone)	Global inhibitor of protein translation
	NRF2 (trigonelline)	Blocks NRF2 phosphorylation (Ser40) and nuclear import
	NRF2 (ML-385)	Prevents NRF2-MAFG association to ARE
	NRF2 (AEM1)	Unknown
NRF2 regulatory pathways	KEAP1-p62 (K67)	Inhibits p62-KEAP1 interaction
	PI3K inhibitor (e.g., BKM120, BYL719, or BAY 80-6946)	Promotes NRF2 degradation via the GSK-3/β-TrCP axis
	PTEN activators (e.g., rituximab, lovastatin)	Antagonize PI3K signaling to promote NRF2 degradation via the GSK-3/β-TrCP axis
	FN3K inhibitor (unavailable)	Inhibits NRF2 deglycation
	BRSK inhibitor (unavailable)	Blocks translation
Metabolic inhibitors	GLS (e.g., CB-839)	Inhibits metabolism of glutamine to glutamate
	ASCT2 (e.g., GPNA, V-9302)	Blocks glutamine import
	xCT (e.g., erastin)	Blocks cystine import
	Cyst(e)ine availability [cyst(e)inase]	Depletes extracellular cyst(e)ine
	G6PD (e.g., 6-AN)	Blocks the flux of glucose into the PPP
NQO1 bioactivatable agents	DNA oxidation (e.g., β-lapachone)	Redox cyclers, generation of ROS
	DNA alkylation (e.g., mitomycin C)	DNA alkylating agents
	HSP90 (e.g., 17-AAG, 17-DMAG)	HSP90 inhibitors
ARK1C3 bioactivatable agents	DNA cross-linking (PR-104A)	DNA cross-linking agent

Abbreviations: ARE, antioxidant response element; BRSK, brain-specific kinase; β-TrCP, β-transducin repeat-containing protein; FN3K, fructosamine-3-kinase; G6PD, glucose 6-phosphate dehydrogenase; GLS, glutaminase; GPNA, L-γ-glutamyl-*p*-nitroanilide; GSK-3, glycogen synthase kinase 3; HSP90, heat shock protein 90; KEAP1, Kelch-like ECH-associated protein 1; MAFG, V-maf musculoaponeurotic fibrosarcoma oncogene homolog G; NRF2, nuclear factor erythroid 2–related factor 2; PI3K, phosphatidylinositol-3 kinase; PPP, pentose phosphate pathway; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; xCT, cystine/glutamate antiporter.

which were subsequently terminated (70). Interestingly, halofuginone has been tested in Phase I and II clinical trials for Duchenne muscular dystrophy with limited toxicity, although its efficacy against NRF2-active tumors remains to be evaluated. Further, inhibitors of translation (i.e., EIF4A inhibitors, EIF4E inhibitors) are being developed for cancer therapy, and their effect on NRF2 protein remains to be evaluated.

4.2. Inhibitors of NRF2 Transcriptional Activity

To date, the largest high-throughput screen to identify novel NRF2 inhibitors was conducted by Biswal and colleagues (71), in which approximately 400,000 small molecules were investigated. This study led to the identification of ML-385, a small molecule that binds to the NRF2 DNA binding domain and prevents the association of the NRF2-V-maf musculoaponeurotic fibrosarcoma oncogene homolog G (MAFG) protein complex with ARE enhancer sequences. In addition, ML-385 treatment significantly reduced NRF2 messenger RNA and protein levels, suggesting that ML-385 might alter NRF2 function through other unknown mechanisms. ML-385 exhibited potent antitumor effects in tumor xenograft experiments, suggesting that it might have clinical applicability. However, it still remains unclear whether ML-385 can interact with other CNC-bZIP transcription factors. Another large-scale screen by Schultz and colleagues (72) of 30,000 compounds against an NRF2 activity reporter 3T3 cell line identified AEM1, which decreased the

expression of NRF2 target genes and sensitized A549 cells to chemotherapeutics. However, NRF2 protein levels were not affected by AEM1, and its mechanism of action remains to be determined.

4.3. Trigonelline Inhibits the Nuclear Accumulation of NRF2

Trigonelline is a pyridine alkaloid found in many dietary food plants and edible seeds, including fenugreek and coffee seeds. The inhibitory effects of trigonelline on NRF2 activity were first described by Boettler et al. (73) in 2011 in a study that aimed to characterize the effect of multiple coffee constituents on the NRF2/ARE pathway in human colon carcinoma cells. In 2013, Arlt et al. (74) investigated the effects of trigonelline on multiple pancreatic carcinoma cell lines and immortalized normal human pancreatic duct cells. Trigonelline reduced basal NRF2 activity and prevented nuclear accumulation of NRF2 following stimulation with tert-butylhydroquinone, a known NRF2 inducer. Further, trigonelline prevented the induction of NRF2-dependent proteasomal genes and enhanced the efficacy of etoposide in in vitro and murine subcutaneous xenograft tumor models (74). Another independent study found that trigonelline inhibits epidermal growth factor receptor (EGFR) signaling in NSCLC cell lines, which correlated with diminished NRF2 Ser40 phosphorylation and reduced nuclear translocation (75). Further, trigonelline treatment sensitized two different KEAP1 mutant cell lines, A549 and H460, to etoposide and cisplatin in vitro. Despite these promising results, the therapeutic potential of trigonelline in NRF2-hyperactive tumors is understudied and requires further characterization.

5. TARGETING NRF2 REGULATORY PATHWAYS

Selective targeting of signaling pathways that modulate NRF2 stability or activity might represent a valuable strategy to circumvent the need to develop direct NRF2 inhibitors. Herein, we highlight some of the emerging strategies to indirectly modulate NRF2 activity (**Table 1**).

5.1. Inhibitors of p62-KEAP1 Association

Previous reports demonstrated that p62 phosphorylation sequesters KEAP1, which is eventually removed by selective autophagy, and leads to NRF2 activation. Notably, p62 accumulation in cancer cells can be driven by amplified copy number of p62 on chromosome 5q as well as defective autophagy, and it is associated with malignancy in various cancers (43). p62-driven activation of NRF2 in cancer is particularly relevant in human HCCs, as p62-KEAP1 aggregates are present in more than 25% of patients (76, 77). Remarkably, Saito et al. (78) identified a specific inhibitor for the Keap1/phospho-p62 (Ser349) interaction, termed K67. Treatment with K67 attenuates resistance to sorafenib and cisplatin in HCC cell lines with intact KEAP1 activity (78). However, to our knowledge, K67 has not been tested in animal models.

5.2. PI3K/AKT Inhibition

PI3K/AKT activation promotes NRF2 accumulation via GSK-3 inactivation; therefore, inhibition of PI3K/AKT might trigger NRF2 degradation via the GSK-3/β-TrCP axis (**Figure 2**). PI3K inhibitors have shown substantial suppression of NRF2 in KEAP1 mutant cancer cell lines as well as increased sensitivity to chemotherapeutic agents and radiation in vitro (79, 80). Interestingly, *NFE2L2* amplification and *KEAP1* mutations frequently co-occur with PIK3CA activation in lung tumors, esophageal carcinomas, head and neck squamous cell carcinoma, and uterine carcinoma (33). These mutation patterns suggest that NRF2 pathway activation is synergistic with active PI3K signaling. Indeed, loss of PTEN and Keap1, but not loss of either alone, is sufficient for the initiation and progression of lung adenocarcinoma in a genetically engineered mouse model (81).

Notably, multiple PI3K inhibitors (including BKM120, BYL719, and BAY 80–6946) have reached clinical trials; currently, 248 clinical trials listed in ClinicalTrials.gov include PI3K inhibitors. Alternatively, PTEN agonists or mimetics may be employed to antagonize PI3K signaling (82).

5.3. FN3K Inhibition

NRF2 deglycation is essential to permit NRF2 binding to sMAF proteins and prevents KEAP1 degradation (28). The kinase FN3K promotes NRF2 deglycation. FN3K depletion prevents the removal of these sugar adducts, which either decreases NRF2 stability by promoting KEAP1 degradation or promotes the accumulation of a nonfunctional form of NRF2 in the absence of functional KEAP1. Thus, FN3K might represent a valuable candidate drug target in NRF2-driven cancers (28). Remarkably, FN3K-deficient mice develop and reproduce normally, despite showing high levels of glycated proteins, suggesting that FN3K inhibition may be safe and/or well tolerated (83).

5.4. Activation of the Brain-Specific Kinase 2

The Major lab (84) recently found that the understudied brain-specific kinases 1/2 (BRSK1/2) repress NRF2 activity. The authors performed a gain-of-function screen in which the activity of 385 kinases on NRF2 transcriptional activity was investigated using an ARE reporter system. This study revealed that BRSK2 activates AMPK signaling and suppresses mTOR, which represses global protein translation, hence decreasing NRF2 protein levels. However, additional studies will be required to assess the therapeutic potential of BRSK in tumorigenesis, as in vivo studies are still lacking. In addition, selective BRSK inhibitors are currently under development (85).

6. ALTERNATIVE STRATEGIES TO TARGET NRF2/KEAP1 MUTANT TUMORS: METABOLIC AND REDOX LIABILITIES OF NRF2-ACTIVE CANCER CELLS

6.1. Metabolic Imbalance of the Intracellular Cysteine and Glutamate Levels

It is now clear that NRF2 metabolic reprogramming in cancer cells results in the accumulation of intracellular cysteine, while triggering a chronic glutamate-deficient state. This metabolic imbalance is initiated by the upregulation of the cystine/glutamate antiporter system x_c^-, also known as xCT. The system x_c^- is composed of a heterodimer of *SCL7A11*, a bona fide NRF2 target gene, and *SLC3A2* (86). Intracellularly, cystine is reduced to its monomeric form, cysteine, by the thioredoxin (TXN)-dependent system at the expense of nicotinamide adenine dinucleotide phosphate (NADPH) (**Figure 4**). NRF2 activation also induces the expression of TXN and thioredoxin reductase 1 (TRXR1) and provides NADPH-reducing equivalents, facilitating the accumulation of intracellular cysteine. In NRF2-addicted cancer cells, increased cysteine uptake is beneficial to fuel the production of GSH. In fact, NRF2 diverts cysteine toward GSH synthesis through the direct regulation of both the regulatory and catalytic subunits of the glutamate-cysteine ligase (GCL) and glutathione synthase (GSS). Increased levels of GSH production in cancer cells provide a survival advantage by reinforcing the antioxidant capacity, which in turn is linked to the resistance to multiple chemotherapeutic agents.

Direct inhibition of xCT has been investigated as a therapeutic strategy for cancer, given that elevated xCT expression on cancer cells correlates with poor prognosis. Inhibition of xCT in preclinical studies suppresses tumor growth and sensitizes cancer cells to radiation and cisplatin (87–91). As such, the development of strategies to target xCT and cysteine metabolism for cancer

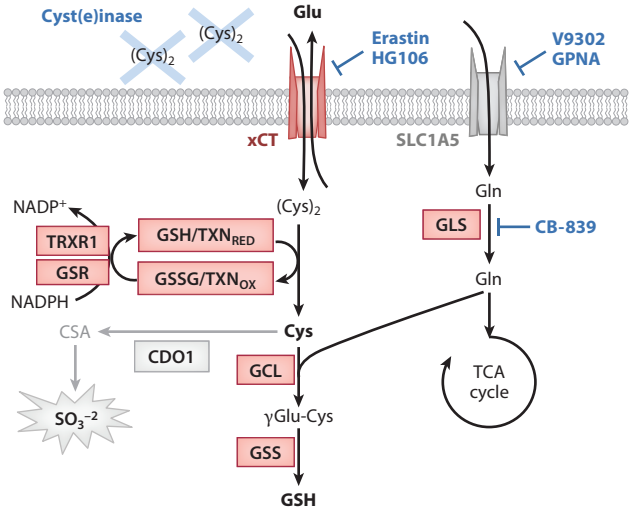

Figure 4

Aberrant activation of NRF2 imbalances cysteine (Cys) and glutamate (Glu) pools. NRF2 upregulates xCT, which increases $(Cys)_2$ uptake at the expense of Glu, which creates a deficit in intracellular Glu. GLS catalyzes the conversion of Gln to Glu. NRF2 redirects Glu and Cys to glutathione synthesis, while limiting Glu as a carbon source for TCA cycle activity (100). CDO1 silencing prevents the futile metabolism of Cys to the toxic byproducts CSA and SO_3^{2-} and the depletion of cellular NADPH (98). Enzymes transcriptionally regulated by NRF2 are indicated in the figure within red boxes. Emerging strategies to target Cys/Glu metabolism are shown in blue. Abbreviations: CDO1, cysteine dioxygenase; CSA, cysteine sulfinic acid; Cys, cysteine; $(Cys)_2$, cystine; GCL, glutamate-cysteine ligase; Gln, glutamine; GLS, glutaminase; Glu, glutamate; γGlu-Cys, gamma-L-glutamyl-L-cysteine; NADP, nicotinamide adenine dinucleotide phosphate (NADP+ is the oxidized form and NADPH is the reduced form); SO_3^{2-}, sulfite; TCA, tricarboxylic acid; TRXR1, thioredoxin reductase; xCT, cystine/glutamate antiporter.

treatment is rapidly expanding. At the molecular level, inhibition of cysteine uptake reduces GSH synthesis, which leads to the accumulation of lipid peroxides, ultimately triggering cell death via ferroptosis (92). Several pharmacological inhibitors for xCT have already been characterized, including erastin, sulfasalazine, and, more recently, HG106 (92–94) (**Table 1**). Further, antibody-based strategies to target xCT for cancer treatment are currently under development (AgilVax Inc.). Alternatively, cystine may be depleted from the extracellular microenvironment to prevent its uptake. Cramer et al. (95) reported the development of an engineered human cyst(e)inase that provides unprecedented opportunity to deplete both extracellular cystine and cysteine in vivo. However, it remains unclear whether cysteine deprivation represents a valuable therapeutic strategy to target NRF2/KEAP1 tumors, as NRF2 activation protects NSCLC and HCC cells against cysteine starvation–induced ferroptosis (96, 97).

While xCT upregulation is metabolically advantageous in NRF2-addicted tumors, cancer cells must adapt and evolve to cope with an increased flux of cystine and achieve a metabolic equilibrium. First, the continuous reduction of cystine to cysteine depletes cellular NADPH, limiting its availability for cellular processes. Second, GCL competes with cysteine dioxygenase 1 (CDO1) for intracellular cysteine, which limits the production of GSH. Third, CDO1-mediated cysteine catabolism increases the production of the toxic products cysteine sulfinic acid and sulfite. Notably, we recently demonstrated that human NSCLC cells with aberrant NRF2 activity evolve to shut down CDO1, which is silenced by promoter methylation (98). CDO1 silencing limits the futile metabolism of cysteine to wasteful and toxic byproducts and prevents depletion of cellular

NADPH. Therefore, restoration of CDO1 function in NRF2/KEAP1 mutant cancer cells is an attractive therapeutic strategy to counteract the advantageous aspects of NRF2-driven metabolic reprogramming. However, to date, strategies to reactivate or mimic CDO1 in vivo are lacking.

Aberrant activation of NRF2 in cancer cells also promotes dependence on an exogenous supply of glutamine to satisfy glutamate requirements (99). In fact, KEAP1 mutant cancer cells display decreased intracellular glutamate pools due to continuous glutamate secretion by the xCT antiporter system and the increased demand of glutamate for GSH synthesis (86, 100). Decreased availability of glutamate limits the anaplerosis of the TCA cycle and other biosynthetic reactions, which creates a metabolic bottleneck. The dependency of cancer cells on glutamine can be exploited therapeutically by blocking either glutamine uptake or glutaminase activity. Glutamine enters into cells via the solute carrier family 1 neutral amino acid transporter member 5 (SLC1A5, also known as ASCT2), and it is converted to glutamate by the mitochondrial glutaminase (GLS). A number of ASCT2 inhibitors have been identified to date, including GPNA (L-γ-glutamyl-*p*-nitroanilide) and V-9302 (101, 102). Promisingly, CB-839, a GLS inhibitor, is currently being tested in Phase I and II clinical trials for solid and hematological tumors. Further, it has been shown to reverse the radioresistance of cultured NRF2-active lung cancer cells (103), suggesting that glutaminase inhibition also has potential in combination with standard-of-care treatments.

6.2. The Pentose Phosphate Pathway

NRF2 diverts the flux of glucose into the PPP by directly regulating the expression of glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (PGD), transaldolase (TALDO), and transketolase (TKT) (48, 104) (**Figure 5**). In cancer cells, upregulation of the PPP flux promotes the regeneration of NADPH, which provides reducing power for anabolic processes and antioxidant defense and provides cells with ribose-5-phosphate (R-5-P), which is utilized for nucleotide biosynthesis. NRF2 also directly regulates the expression of the de novo purine synthesis enzymes phosphoribosyl pyrophosphate amidotransferase (PPAT) and methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) (48). Thus, NRF2 rewires glucose utilization toward the PPP and de novo purine synthesis to enhance cytoprotection and support cell proliferation. Mitsuishi et al. (48) reported that depletion of G6PD and TKT in A549 cells ($KEAP1^{MUT}$) effectively represses cell proliferation in vitro and tumor growth in xenograft experiments. In line with these data, the Sutherland lab (105) reported that KEAP1 loss in $KRas^{G12D}$-driven lung tumors accelerated tumor growth and activated the PPP, while treatment with the G6PD inhibitor 6-aminonicotinamide (6-AN) abrogated tumor growth. Another independent study found that resistance to cisplatin in A549 cells can be reversed by G6PD inhibition, using small interfering RNA or 6-AN, in vitro (106). Therefore, these data suggest that cancer cells harboring aberrant NRF2 activity are dependent on the PPP, and inhibition of this pathway might represent a valuable therapeutic approach to tackle KEAP1/NRF2 mutant tumors. Excitingly, the Rabinowitz lab (107) recently identified a novel small molecule that inhibits G6PD, termed G6PDi-1. In addition, purine synthesis pathway inhibitors are used in the clinic for the treatment of cancer, although their efficacy against NRF2-active tumors needs to be tested.

6.3. NQO1 Bioactivatable Drugs

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a phase II detoxification enzyme that catalyzes the two-electron reduction of quinone substrates, leading to the generation of hydroquinones. NQO1-mediated reduction of quinones exerts a cytoprotective function, as it prevents formation of highly unstable semiquinones, generated by one-electron reductases (108). Following NQO1 reduction, hydroquinones are conjugated with GSH or glucuronic acid and excreted from the

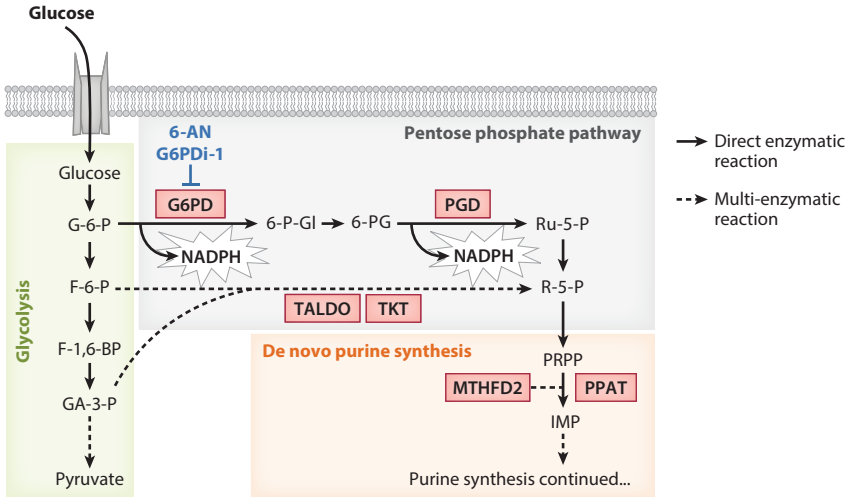

Figure 5

NRF2 diverts the flux of glucose to the PPP and facilitates the synthesis of purine nucleotides. NRF2 directly regulates the expression of G6PD, PGD, TALDO, and TKT, which enhances the flux of glucose to the PPP. The PPP generates NADPH and precursors for the synthesis of nucleotides. NRF2 also regulates MTHFD2 and PPAT, which are involved in de novo purine synthesis. Enzymes transcriptionally regulated by NRF2 are indicated in the figure within red boxes. Strategies to target the PPP are shown in blue. Abbreviations: 6-P-Gl, 6-phosphogluconolactone; 6-PG, 6-phosphogluconate; F-1,6-BP, fructose-1, 6-bisphosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; G6PD, glucose 6-phosphate dehydrogenase; GA-3-P, glyceraldehyde-3-phosphate; IMP, inosine 5′-monophosphate; MTHFD2, methylenetetrahydrofolate dehydrogenase 2; NADP, nicotinamide adenine dinucleotide phosphate (NADP+ is the oxidized form and NADPH is the reduced form); NRF2, nuclear factor erythroid 2–related factor 2; PGD, 6-phosphogluconate dehydrogenase; PPAT, phosphoribosyl pyrophosphate amidotransferase; PPP, pentose phosphate pathway; PRPP, 5-phosphoribosyl pyrophosphate; R-5-P, ribose-5-phosphate; Ru-5-P, ribulose 5-phosphate; TALDO, transaldolase; TKT, transketolase.

cells. Paradoxically, a number of quinones induce toxicity following NQO1 reduction through three different mechanisms: (*a*) initiation of a futile redox cycling that induces oxidative stress [e.g., β-lapachone, isobutyl-deoxynyboquinone (IB-DNQ), and streptonigrin], (*b*) generation of hydroquinones with DNA alkylating properties (e.g., mitomycin C, AZQ, E09, and RH1), and (*c*) formation of stable hydroquinones with enhanced pharmacological properties (geldanamycin, 17-AAG, and 17-DMAG) (**Table 1**) (109).

The ability of NQO1 to generate cytotoxic hydroquinones has been exploited for the development of anticancer agents, given that NQO1 is overexpressed in many cancer types (110–115), compared to normal tissues (116). Therefore, it was postulated that the systemic delivery of NQO1 bioactivatable agents would selectively target tumors, while sparing normal cells. Remarkably, NQO1 is a bona fide NRF2 target gene. In fact, NQO1 immunohistochemistry staining is commonly used as a surrogate marker of NRF2 activity (98, 99). Despite the robust association between NRF2 activity and NQO1 expression, NQO1 bioactivatable agents have only recently sparked the interest of the NRF2 cancer field. Consequently, studies aimed at understanding the therapeutic potential of these compounds in NRF2/KEAP1 mutant tumors are now emerging.

To date, β-lapachone is probably one of the best-characterized NQO1 bioactivatable agents, and it has been widely investigated for cancer treatment (117–122). β-Lapachone enters into a redox futile cycle following NQO1 reduction, which ultimately induces cell death via oxidative

DNA damage and NAD(P)H depletion. However, the cytotoxic properties of β-lapachone rely on the production of ROS, which are readily detoxified in NRF2/KEAP1 mutant cells. Indeed, we recently demonstrated that, despite overexpression of NQO1, KEAP1 mutant cells were resistant to β-lapachone due to enhanced detoxification of ROS, which prevented DNA damage and cell death (123). Remarkably, inhibition of the TXN-dependent system or SOD1 was sufficient to sensitize KEAP1 mutant NSCLC cells to β-lapachone exposure. These findings suggest that β-lapachone treatment in combination with selective inhibitors of antioxidant enzymes might be exploited to target cancer cells with aberrant activation of NRF2. However, the systemic effects, which are potentially harmful, of this combinatorial therapeutic approach remain unknown, as in vivo studies are still lacking. In parallel, β-lapachone has entered multiple clinical trials as the analogs ARQ501 and ARQ761; yet, the contribution of NRF2/KEAP1 mutations in the patient outcomes has not been investigated.

As mentioned above, the third class of NQO1 bioactivatable agents are those that, upon NQO1 bioreduction, acquire enhanced pharmacological properties. The benzoquinone ansamycins (BQAs), which include geldanamycin, 17-AAG, and 17-DMAG, are a group of quinone-containing polyketide antibiotics that exert antitumor activities by binding to the ATP pocket in heat shock protein 90 (HSP90) (109). The hydroquinone forms of BQAs exhibit higher affinity for HSP90 than does the parent quinone and are resistant to GSH conjugation (124, 125). BQAs are well-known NQO1 substrates. In melanoma and NSCLC cells, with or without KEAP1 mutations, 17-AAG toxicity was shown to correlate with NQO1 levels (126). Another independent study using a paired WT/Keap1-knockout Hepa1 cell-screening system reported that activation of NRF2 in lung, liver, and esophagus cancer cells increases sensitivity to 17-AAG, 17-DMAG, and IPI-504 (127). Using the same cell-based system, it was later reported that activation of NRF2 in cancer cells also confers sensitivity to the mitomycin C, a quinone-containing antineoplastic agent (128). However, in this scenario, bioreductive activation of mitomycin C can be mediated by multiple reductases, including NQO1, xanthine oxidoreductase (XOR), cytochrome b5 (CYB5R), and cytochrome P450 (CYPOR).

In summary, NQO1 bioactivatable agents represent an attractive strategy to selectively target NRF2/KEAP1 mutant tumors. However, it is important to assess the effect of other drug-metabolizing and antioxidant enzymes activated by NRF2 as well as the impact of co-occurring cancer mutations.

6.4. Aldo-Ketoreductases

The aldo-ketoreductases (AKRs) are a family of NADP(H)-dependent oxidoreductases that catalyze the interconversion of aldehydes and ketones to primary and secondary alcohols, respectively, for subsequent conjugation reactions. The expression of several *AKR* genes is regulated by NRF2 as part of its cytoprotective program. Several transcriptional signatures for predicting NRF2 activity in tumors are rich in *AKR* genes, which emphasizes the consistent induction of these genes in NRF2-hyperactive cancers (34, 49, 129). Thus, AKR enzymatic activity might be exploited for the bioactivation of prodrugs in tumors.

PR-104A is dinitrobenzamide mustard that undergoes nitro reduction to PR-104H and PR-104M, which are DNA cross-linking agents. While PR-104A is primarily bioactivated under hypoxic conditions by CYPOR (130), AKR1C3 is capable of promoting the bioreductive activation of PR-104A under aerobic conditions (131) (**Table 1**). Remarkably, pretreatment with sulforaphane, a well-known NRF2 inducer, was shown to increase the cytotoxic potential of PR-104A in multiple cancer cells under aerobic conditions due to the induction of AKR1C3 (132). Therefore, while NRF2 activation in human cancers might induce the expression of AKR1C3, it is unclear whether,

given that most solid tumors contain hypoxic regions, AKR1C3 bioactivation of PR-104A would be favored. Additional research using relevant animal models to investigate the antitumor effects of PR-104 in NRF2-hyperactive solid tumors will be required to address this question.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

While the efforts on developing personalized therapies for cancer patients bearing NRF2 activation were previously focused on the identification of direct NRF2 inhibitors, recent findings indicate that systemic inhibition of NRF2 could accelerate tumor progression. However, more specific and potent inhibitors as well as additional mechanistic studies are needed to understand how NRF2 inhibition will differentially impact both NRF2-addicted tumor cells and cells within the microenvironment. Meanwhile, the field is evolving toward the identification of novel upstream regulators of NRF2 as well as redox and metabolic vulnerabilities. This new focus and the recent advances in understanding the pathways regulated by NRF2 are rapidly expanding the possibilities to tackle these types of tumors. Targeting NRF2 metabolism is sparking the interest of multiple research groups, as disturbances in the cysteine/glutamate metabolism as well as in the PPP are druggable. Indeed, it is from this area that the more promising advances have been made, particularly in the area of glutaminase inhibitors, which have now advanced into clinical trials. As we gain a deeper understanding of NRF2-regulated metabolism and the potential of bioactivatable compounds, personalized therapy for NRF2-active tumors will become possible.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Lee SB, Sellers BN, DeNicola GM. 2018. The regulation of NRF2 by nutrient-responsive signaling and its role in anabolic cancer metabolism. *Antioxid. Redox Signal.* 29:1774–91
2. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA. 2003. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol. Cell. Biol.* 23:7198–209
3. Pajares M, Cuadrado A, Rojo AI. 2017. Modulation of proteostasis by transcription factor NRF2 and impact in neurodegenerative diseases. *Redox Biol.* 11:543–53
4. Hayes JD, Chowdhry S, Dinkova-Kostova AT, Sutherland C. 2015. Dual regulation of transcription factor Nrf2 by Keap1 and by the combined actions of β-TrCP and GSK-3. *Biochem. Soc. Trans.* 43:611–20
5. Hayes JD, Dinkova-Kostova AT. 2014. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem. Sci.* 39:199–218
6. Kerins MJ, Ooi A. 2018. The roles of NRF2 in modulating cellular iron homeostasis. *Antioxid. Redox Signal.* 29:1756–73
7. Pajares M, Jimenez-Moreno N, Garcia-Yague AJ, Escoll M, de Ceballos ML, et al. 2016. Transcription factor NFE2L2/NRF2 is a regulator of macroautophagy genes. *Autophagy* 12:1902–16
8. Solis LM, Behrens C, Dong W, Suraokar M, Ozburn NC, et al. 2010. Nrf2 and Keap1 abnormalities in non-small cell lung carcinoma and association with clinicopathologic features. *Clin. Cancer Res.* 16:3743–53
9. Goeman F, De Nicola F, Scalera S, Sperati F, Gallo E, et al. 2019. Mutations in the KEAP1-NFE2L2 pathway define a molecular subset of rapidly progressing lung adenocarcinoma. *J. Thorac. Oncol.* 14:1924–34

10. Tong KI, Katoh Y, Kusunoki H, Itoh K, Tanaka T, Yamamoto M. 2006. Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. *Mol. Cell. Biol.* 26:2887–900
11. Zhang DD, Lo SC, Cross JV, Templeton DJ, Hannink M. 2004. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell. Biol.* 24:10941–53
12. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, et al. 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev*. 13:76–86
13. Dayalan Naidu S, Dinkova-Kostova AT. 2020. KEAP1, a cysteine-based sensor and a drug target for the prevention and treatment of chronic disease. *Open Biol*. 10:200105
14. Dinkova-Kostova AT, Kostov RV, Canning P. 2017. Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. *Arch. Biochem. Biophys.* 617:84–93
15. Bollong MJ, Lee G, Coukos JS, Yun H, Zambaldo C, et al. 2018. A metabolite-derived protein modification integrates glycolysis with KEAP1-NRF2 signalling. *Nature* 562:600–4
16. Adam J, Hatipoglu E, O'Flaherty L, Ternette N, Sahgal N, et al. 2011. 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* 20:524–37
17. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, et al. 2002. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *PNAS* 99:11908–13
18. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, et al. 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* 236:313–22
19. Matsumoto G, Wada K, Okuno M, Kurosawa M, Nukina N. 2011. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol. Cell* 44:279–89
20. Pilli M, Arko-Mensah J, Ponpuak M, Roberts E, Master S, et al. 2012. TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. *Immunity* 37:223–34
21. Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, et al. 2013. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Mol. Cell* 51:618–31
22. Duran A, Amanchy R, Linares JF, Joshi J, Abu-Baker S, et al. 2011. p62 is a key regulator of nutrient sensing in the mTORC1 pathway. *Mol. Cell* 44:134–46
23. Endo H, Owada S, Inagaki Y, Shida Y, Tatemichi M. 2018. Glucose starvation induces LKB1-AMPK-mediated MMP-9 expression in cancer cells. *Sci. Rep.* 8:10122
24. Jain A, Lamark T, Sjottem E, Larsen KB, Awuh JA, et al. 2010. *p62/SQSTM1* is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J. Biol. Chem.* 285:22576–91
25. Chen W, Sun Z, Wang XJ, Jiang T, Huang Z, et al. 2009. Direct interaction between Nrf2 and p21(Cip1/WAF1) upregulates the Nrf2-mediated antioxidant response. *Mol. Cell* 34:663–73
26. Bloom DA, Jaiswal AK. 2003. Phosphorylation of Nrf2 at Ser^{40} 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. *J. Biol. Chem.* 278:44675–82
27. Joo MS, Kim WD, Lee KY, Kim JH, Koo JH, Kim SG. 2016. AMPK facilitates nuclear accumulation of Nrf2 by phosphorylating at serine 550. *Mol. Cell. Biol.* 36:1931–42
28. Sanghvi VR, Leibold J, Mina M, Mohan P, Berishaj M, et al. 2019. The oncogenic action of NRF2 depends on de-glycation by fructosamine-3-kinase. *Cell* 178:807–19.e21
29. Cuadrado A. 2015. Structural and functional characterization of Nrf2 degradation by glycogen synthase kinase 3/β-TrCP. *Free Radic. Biol. Med.* 88:147–57
30. Wu T, Zhao F, Gao B, Tan C, Yagishita N, et al. 2014. Hrd1 suppresses Nrf2-mediated cellular protection during liver cirrhosis. *Genes Dev*. 28:708–22
31. Lo JY, Spatola BN, Curran SP. 2017. WDR23 regulates NRF2 independently of KEAP1. *PLOS Genet*. 13:e1006762

32. Kerins MJ, Ooi A. 2018. A catalogue of somatic NRF2 gain-of-function mutations in cancer. *Sci. Rep.* 8:12846
33. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, et al. 2018. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell* 173:321–37.e10
34. Goldstein LD, Lee J, Gnad F, Klijn C, Schaub A, et al. 2016. Recurrent loss of NFE2L2 exon 2 is a mechanism for Nrf2 pathway activation in human cancers. *Cell Rep*. 16:2605–17
35. Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, et al. 2006. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. *PLOS Med*. 3:e420
36. Cancer Genome Atlas Res. Netw. 2012. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 489:519–25
37. Cloer EW, Goldfarb D, Schrank TP, Weissman BE, Major MB. 2019. NRF2 activation in cancer: from DNA to protein. *Cancer Res*. 79:889–98
38. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, et al. 2011. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475:106–9
39. Rojo AI, Rada P, Mendiola M, Ortega-Molina A, Wojdyla K, et al. 2014. The PTEN/NRF2 axis promotes human carcinogenesis. *Antioxid. Redox Signal.* 21:2498–514
40. Kinch L, Grishin NV, Brugarolas J. 2011. Succination of Keap1 and activation of Nrf2-dependent antioxidant pathways in FH-deficient papillary renal cell carcinoma type 2. *Cancer Cell* 20:418–20
41. Purohit V, Wang L, Yang H, Li J, Ney GM, et al. 2021. ATDC binds to KEAP1 to drive NRF2-mediated tumorigenesis and chemoresistance in pancreatic cancer. *Genes Dev*. 35:218–33
42. Meng C, Zhan J, Chen D, Shao G, Zhang H, et al. 2021. The deubiquitinase USP11 regulates cell proliferation and ferroptotic cell death via stabilization of NRF2 USP11 deubiquitinates and stabilizes NRF2. *Oncogene* 40:1706–20
43. Ichimura Y, Komatsu M. 2018. Activation of p62/SQSTM1-Keap1-nuclear factor erythroid 2-related factor 2 pathway in cancer. *Front. Oncol*. 8:210
44. Zhou S, Ye W, Shao Q, Zhang M, Liang J. 2013. Nrf2 is a potential therapeutic target in radioresistance in human cancer. *Crit. Rev. Oncol. Hematol.* 88:706–15
45. Shibata T, Kokubu A, Saito S, Narisawa-Saito M, Sasaki H, et al. 2011. *NRF2* mutation confers malignant potential and resistance to chemoradiation therapy in advanced esophageal squamous cancer. *Neoplasia* 13:864–73
46. Wang XJ, Sun Z, Villeneuve NF, Zhang S, Zhao F, et al. 2008. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. *Carcinogenesis* 29:1235–43
47. DeBlasi JM, DeNicola GM. 2020. Dissecting the crosstalk between NRF2 signaling and metabolic processes in cancer. *Cancers* 12:3023
48. Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, et al. 2012. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* 22:66–79
49. DeNicola GM, Chen PH, Mullarky E, Sudderth JA, Hu Z, et al. 2015. NRF2 regulates serine biosynthesis in non-small cell lung cancer. *Nat. Genet.* 47:1475–81
50. Towers CG, Fitzwalter BE, Regan D, Goodspeed A, Morgan MJ, et al. 2019. Cancer cells upregulate NRF2 signaling to adapt to autophagy inhibition. *Dev. Cell* 50:690–703.e6
51. Kim TH, Hur EG, Kang SJ, Kim JA, Thapa D, et al. 2011. NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1α. *Cancer Res*. 71:2260–75
52. Rojo de la Vega M, Chapman E, Zhang DD. 2018. NRF2 and the hallmarks of cancer. *Cancer Cell* 34:21–43
53. Singh A, Boldin-Adamsky S, Thimmulappa RK, Rath SK, Ashush H, et al. 2008. RNAi-mediated silencing of nuclear factor erythroid-2-related factor 2 gene expression in non-small cell lung cancer inhibits tumor growth and increases efficacy of chemotherapy. *Cancer Res*. 68:7975–84
54. Chan K, Lu R, Chang JC, Kan YW. 1996. NRF2, a member of the NFE2 family of transcription factors, is not essential for murine erythropoiesis, growth, and development. *PNAS* 93:13943–48
55. Nakamura BN, Lawson G, Chan JY, Banuelos J, Cortes MM, et al. 2010. Knockout of the transcription factor NRF2 disrupts spermatogenesis in an age-dependent manner. *Free Radic. Biol. Med.* 49:1368–79

56. Ma Q, Battelli L, Hubbs AF. 2006. Multiorgan autoimmune inflammation, enhanced lymphoproliferation, and impaired homeostasis of reactive oxygen species in mice lacking the antioxidant-activated transcription factor Nrf2. *Am. J. Pathol.* 168:1960–74
57. Slocum SL, Kensler TW. 2011. Nrf2: control of sensitivity to carcinogens. *Arch. Toxicol.* 85:273–84
58. Satoh H, Moriguchi T, Taguchi K, Takai J, Maher JM, et al. 2010. Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung. *Carcinogenesis* 31:1833–43
59. Satoh H, Moriguchi T, Saigusa D, Baird L, Yu L, et al. 2016. NRF2 intensifies host defense systems to prevent lung carcinogenesis, but after tumor initiation accelerates malignant cell growth. *Cancer Res.* 76:3088–96
60. Hayashi M, Kuga A, Suzuki M, Panda H, Kitamura H, et al. 2020. Microenvironmental activation of Nrf2 restricts the progression of Nrf2-activated malignant tumors. *Cancer Res.* 80:3331–44
61. Saha S, Buttari B, Panieri E, Profumo E, Saso L. 2020. An overview of Nrf2 signaling pathway and its role in inflammation. *Molecules* 25:5474
62. Cuadrado A, Rojo AI, Wells G, Hayes JD, Cousin SP, et al. 2019. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat. Rev. Drug Discov.* 18:295–317
63. McMahon M, Itoh K, Yamamoto M, Hayes JD. 2003. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J. Biol. Chem.* 278:21592–600
64. Ren D, Villeneuve NF, Jiang T, Wu T, Lau A, et al. 2011. Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. *PNAS* 108:1433–38
65. Du Y, Villeneuve NF, Wang XJ, Sun Z, Chen W, et al. 2008. Oridonin confers protection against arsenic-induced toxicity through activation of the Nrf2-mediated defensive response. *Environ. Health Perspect.* 116:1154–61
66. Olayanju A, Copple IM, Bryan HK, Edge GT, Sison RL, et al. 2015. Brusatol provokes a rapid and transient inhibition of Nrf2 signaling and sensitizes mammalian cells to chemical toxicity—implications for therapeutic targeting of Nrf2. *Free Radic. Biol. Med.* 78:202–12
67. Tao S, Wang S, Moghaddam SJ, Ooi A, Chapman E, et al. 2014. Oncogenic KRAS confers chemoresistance by upregulating NRF2. *Cancer Res.* 74:7430–41
68. Vartanian S, Ma TP, Lee J, Haverty PM, Kirkpatrick DS, et al. 2016. Application of mass spectrometry profiling to establish brusatol as an inhibitor of global protein synthesis. *Mol. Cell Proteom.* 15:1220–31
69. Tsuchida K, Tsujita T, Hayashi M, Ojima A, Keleku-Lukwete N, et al. 2017. Halofuginone enhances the chemo-sensitivity of cancer cells by suppressing NRF2 accumulation. *Free Radic. Biol. Med.* 103:236–47
70. Cuendet M, Pezzuto JM. 2004. Antitumor activity of bruceantin: an old drug with new promise. *J. Nat. Prod.* 67:269–72
71. Singh A, Venkannagari S, Oh KH, Zhang YQ, Rohde JM, et al. 2016. Small molecule inhibitor of NRF2 selectively intervenes therapeutic resistance in KEAP1-deficient NSCLC tumors. *ACS Chem. Biol.* 11:3214–25
72. Bollong MJ, Yun H, Sherwood L, Woods AK, Lairson LL, Schultz PG. 2015. A small molecule inhibits deregulated NRF2 transcriptional activity in cancer. *ACS Chem. Biol.* 10:2193–98
73. Boettler U, Sommerfeld K, Volz N, Pahlke G, Teller N, et al. 2011. Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression. *J. Nutr. Biochem.* 22:426–40
74. Arlt A, Sebens S, Krebs S, Geismann C, Grossmann M, et al. 2013. Inhibition of the Nrf2 transcription factor by the alkaloid trigonelline renders pancreatic cancer cells more susceptible to apoptosis through decreased proteasomal gene expression and proteasome activity. *Oncogene* 32:4825–35
75. Fouzder C, Mukhuty A, Mukherjee S, Malick C, Kundu R. 2021. Trigonelline inhibits Nrf2 via EGFR signalling pathway and augments efficacy of Cisplatin and Etoposide in NSCLC cells. *Toxicol. In Vitro* 70:105038
76. Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, et al. 2011. Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J. Cell Biol.* 193:275–84
77. Umemura A, He F, Taniguchi K, Nakagawa H, Yamachika S, et al. 2016. p62, upregulated during preneoplasia, induces hepatocellular carcinogenesis by maintaining survival of stressed HCC-initiating cells. *Cancer Cell* 29:935–48

78. Saito T, Ichimura Y, Taguchi K, Suzuki T, Mizushima T, et al. 2016. p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming. *Nat. Commun.* 7:12030
79. Chowdhry S, Zhang Y, McMahon M, Sutherland C, Cuadrado A, Hayes JD. 2013. Nrf2 is controlled by two distinct β-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. *Oncogene* 32:3765–81
80. Abazeed ME, Adams DJ, Hurov KE, Tamayo P, Creighton CJ, et al. 2013. Integrative radiogenomic profiling of squamous cell lung cancer. *Cancer Res.* 73:6289–98
81. Best SA, De Souza DP, Kersbergen A, Policheni AN, Dayalan S, et al. 2018. Synergy between the KEAP1/NRF2 and PI3K pathways drives non-small-cell lung cancer with an altered immune microenvironment. *Cell Metab.* 27:935–43.e4
82. Boosani CS, Agrawal DK. 2013. PTEN modulators: a patent review. *Expert Opin. Ther. Pat.* 23:569–80
83. Veiga da-Cunha M, Jacquemin P, Delpierre G, Godfraind C, Theate I, et al. 2006. Increased protein glycation in fructosamine 3-kinase-deficient mice. *Biochem. J.* 399:257–64
84. Tamir TY, Bowman BM, Agajanian MJ, Goldfarb D, Schrank TP, et al. 2020. Gain-of-function genetic screen of the kinome reveals BRSK2 as an inhibitor of the NRF2 transcription factor. *J. Cell Sci.* 133(14):jcs241356
85. Tamir TY, Drewry DH, Wells C, Major MB, Axtman AD. 2020. PKIS deep dive yields a chemical starting point for dark kinases and a cell active BRSK2 inhibitor. *Sci. Rep.* 10:15826
86. Sasaki H, Sato H, Kuriyama-Matsumura K, Sato K, Maebara K, et al. 2002. Electrophile response element-mediated induction of the cystine/glutamate exchange transporter gene expression. *J. Biol. Chem.* 277:44765–71
87. Ji X, Qian J, Rahman SMJ, Siska PJ, Zou Y, et al. 2018. xCT (SLC7A11)-mediated metabolic reprogramming promotes non-small cell lung cancer progression. *Oncogene* 37:5007–19
88. Li Y, Yan H, Xu X, Liu H, Wu C, Zhao L. 2020. Erastin/sorafenib induces cisplatin-resistant non-small cell lung cancer cell ferroptosis through inhibition of the Nrf2/xCT pathway. *Oncol. Lett.* 19:323–33
89. Shibata Y, Yasui H, Higashikawa K, Miyamoto N, Kuge Y. 2019. Erastin, a ferroptosis-inducing agent, sensitized cancer cells to X-ray irradiation via glutathione starvation in vitro and in vivo. *PLOS ONE* 14:e0225931
90. Sato M, Kusumi R, Hamashima S, Kobayashi S, Sasaki S, et al. 2018. The ferroptosis inducer erastin irreversibly inhibits system xc- and synergizes with cisplatin to increase cisplatin's cytotoxicity in cancer cells. *Sci. Rep.* 8:968
91. Guo W, Zhao Y, Zhang Z, Tan N, Zhao F, et al. 2011. Disruption of xCT inhibits cell growth via the ROS/autophagy pathway in hepatocellular carcinoma. *Cancer Lett.* 312:55–61
92. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, et al. 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149:1060–72
93. Hu K, Li K, Lv J, Feng J, Chen J, et al. 2020. Suppression of the SLC7A11/glutathione axis causes synthetic lethality in *KRAS*-mutant lung adenocarcinoma. *J. Clin. Investig.* 130:1752–66
94. Gout PW, Buckley AR, Simms CR, Bruchovsky N. 2001. Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x_c^- cystine transporter: a new action for an old drug. *Leukemia* 15:1633–40
95. Cramer SL, Saha A, Liu J, Tadi S, Tiziani S, et al. 2017. Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth. *Nat. Med.* 23:120–27
96. Kang YP, Mockabee-Macias A, Jiang C, Falzone A, Prieto-Farigua N, et al. 2021. Non-canonical glutamate-cysteine ligase activity protects against ferroptosis. *Cell Metab.* 33:174–89.e7
97. Sun X, Ou Z, Chen R, Niu X, Chen D, et al. 2016. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology* 63:173–84
98. Kang YP, Torrente L, Falzone A, Elkins CM, Liu M, et al. 2019. Cysteine dioxygenase 1 is a metabolic liability for non-small cell lung cancer. *eLife* 8:e45572
99. Romero R, Sayin VI, Davidson SM, Bauer MR, Singh SX, et al. 2017. *Keap1* loss promotes *Kras*-driven lung cancer and results in dependence on glutaminolysis. *Nat. Med.* 23:1362–68
100. Sayin VI, LeBoeuf SE, Singh SX, Davidson SM, Biancur D, et al. 2017. Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. *eLife* 6:e28083

101. Schulte ML, Khodadadi AB, Cuthbertson ML, Smith JA, Manning HC. 2016. 2-Amino-4-bis(aryloxybenzyl)aminobutanoic acids: a novel scaffold for inhibition of ASCT2-mediated glutamine transport. *Bioorg. Med. Chem. Lett.* 26:1044–47
102. Schulte ML, Fu A, Zhao P, Li J, Geng L, et al. 2018. Pharmacological blockade of ASCT2-dependent glutamine transport leads to antitumor efficacy in preclinical models. *Nat. Med.* 24:194–202
103. Binkley MS, Jeon YJ, Nesselbush M, Moding EJ, Nabet BY, et al. 2020. KEAP1/NFE2L2 mutations predict lung cancer radiation resistance that can be targeted by glutaminase inhibition. *Cancer Discov.* 10:1826–41
104. Heiss EH, Schachner D, Zimmermann K, Dirsch VM. 2013. Glucose availability is a decisive factor for Nrf2-mediated gene expression. *Redox Biol.* 1:359–65
105. Best SA, Ding S, Kersbergen A, Kersbergen A, Dong X, et al. 2019. Distinct initiating events underpin the immune and metabolic heterogeneity of KRAS-mutant lung adenocarcinoma. *Nat. Commun.* 10(1):4190
106. Hong W, Cai P, Xu C, Cao D, Yu W, et al. 2018. Inhibition of glucose-6-phosphate dehydrogenase reverses cisplatin resistance in lung cancer cells via the redox system. *Front. Pharmacol.* 9:43
107. Ghergurovich JM, Garcia-Canaveras JC, Wang J, Schmidt E, Zhang Z, et al. 2020. A small molecule G6PD inhibitor reveals immune dependence on pentose phosphate pathway. *Nat. Chem. Biol.* 16:731–39
108. Dinkova-Kostova AT, Talalay P. 2000. Persuasive evidence that quinone reductase type 1 (DT diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. *Free Radic. Biol. Med.* 29:231–40
109. Siegel D, Yan C, Ross D. 2012. NAD(P)H:quinone oxidoreductase 1 (NQO1) in the sensitivity and resistance to antitumor quinones. *Biochem. Pharmacol.* 83:1033–40
110. Yang Y, Zhou X, Xu M, Piao J, Zhang Y, et al. 2017. β-Lapachone suppresses tumour progression by inhibiting epithelial-to-mesenchymal transition in NQO1-positive breast cancers. *Sci. Rep.* 7:2681
111. Yang Y, Zhang Y, Wu Q, Cui X, Lin Z, et al. 2014. Clinical implications of high NQO1 expression in breast cancers. *J. Exp. Clin. Cancer Res.* 33:14
112. Ma Y, Kong J, Yan G, Ren X, Jin D, et al. 2014. NQO1 overexpression is associated with poor prognosis in squamous cell carcinoma of the uterine cervix. *BMC Cancer* 14:414
113. Siegel D, Franklin WA, Ross D. 1998. Immunohistochemical detection of NAD(P)H:quinone oxidoreductase in human lung and lung tumors. *Clin. Cancer Res.* 4:2065–70
114. Awadallah NS, Dehn D, Shah RJ, Russell Nash S, Chen YK, et al. 2008. NQO1 expression in pancreatic cancer and its potential use as a biomarker. *Appl. Immunohistochem. Mol. Morphol.* 16:24–31
115. Siegel D, Ross D. 2000. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. *Free Radic. Biol. Med.* 29:246–53
116. Li Z, Zhang Y, Jin T, Men J, Lin Z, et al. 2015. NQO1 protein expression predicts poor prognosis of non-small cell lung cancers. *BMC Cancer* 15:207
117. Beg MS, Huang X, Silvers MA, Gerber DE, Bolluyt J, et al. 2017. Using a novel NQO1 bioactivatable drug, beta-lapachone (ARQ761), to enhance chemotherapeutic effects by metabolic modulation in pancreatic cancer. *J. Surg. Oncol.* 116:83–88
118. Bey EA, Bentle MS, Reinicke KE, Dong Y, Yang CR, et al. 2007. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by β-lapachone. *PNAS* 104:11832–37
119. Blanco E, Bey EA, Khemtong C, Yang SG, Setti-Guthi J, et al. 2010. β-Lapachone micellar nanotherapeutics for non-small cell lung cancer therapy. *Cancer Res.* 70:3896–904
120. Gerber DE, Beg MS, Fattah F, Frankel AL, Fatunde O, et al. 2018. Phase 1 study of ARQ 761, a β-lapachone analogue that promotes NQO1-mediated programmed cancer cell necrosis. *Br. J. Cancer* 119:928–36
121. Huang X, Dong Y, Bey EA, Kilgore JA, Bair JS, et al. 2012. An NQO1 substrate with potent antitumor activity that selectively kills by PARP1-induced programmed necrosis. *Cancer Res.* 72:3038–47
122. Huang X, Motea EA, Moore ZR, Yao J, Dong Y, et al. 2016. Leveraging an NQO1 bioactivatable drug for tumor-selective use of poly(ADP-ribose) polymerase inhibitors. *Cancer Cell* 30:940–52
123. Torrente L, Prieto-Farigua N, Falzone A, Elkins CM, Boothman DA, et al. 2020. Inhibition of TXNRD or SOD1 overcomes NRF2-mediated resistance to β-lapachone. *Redox Biol.* 30:101440

124. Guo W, Reigan P, Siegel D, Ross D. 2008. Enzymatic reduction and glutathione conjugation of benzoquinone ansamycin heat shock protein 90 inhibitors: relevance for toxicity and mechanism of action. *Drug Metab. Dispos.* 36:2050–57
125. Guo W, Reigan P, Siegel D, Zirrolli J, Gustafson D, Ross D. 2005. Formation of 17-allylamino-demethoxygeldanamycin (17-AAG) hydroquinone by NAD(P)H:quinone oxidoreductase 1: role of 17-AAG hydroquinone in heat shock protein 90 inhibition. *Cancer Res.* 65:10006–15
126. Kasai S, Arakawa N, Okubo A, Shigeeda W, Yasuhira S, et al. 2016. NAD(P)H:Quinone oxidoreductase-1 expression sensitizes malignant melanoma cells to the HSP90 inhibitor 17-AAG. *PLOS ONE* 11:e0153181
127. Baird L, Suzuki T, Takahashi Y, Hishinuma E, Saigusa D, Yamamoto M. 2020. Geldanamycin-derived HSP90 inhibitors are synthetic lethal with NRF2. *Mol. Cell. Biol.* 40:e00377-20
128. Baird L, Yamamoto M. 2021. NRF2-dependent bioactivation of mitomycin C as a novel strategy to target KEAP1-NRF2 pathway activation in human cancer. *Mol. Cell. Biol.* 41:e00473-20
129. Namani A, Matiur Rahaman M, Chen M, Tang X. 2018. Gene-expression signature regulated by the KEAP1-NRF2-CUL3 axis is associated with a poor prognosis in head and neck squamous cell cancer. *BMC Cancer* 18:46
130. Guise CP, Wang AT, Theil A, Bridewell DJ, Wilson WR, Patterson AV. 2007. Identification of human reductases that activate the dinitrobenzamide mustard prodrug PR-104A: a role for NADPH:cytochrome P450 oxidoreductase under hypoxia. *Biochem. Pharmacol.* 74:810–20
131. Guise CP, Abbattista MR, Singleton RS, Holford SD, Connolly J, et al. 2010. The bioreductive prodrug PR-104A is activated under aerobic conditions by human aldo-keto reductase 1C3. *Cancer Res.* 70:1573–84
132. Erzinger MM, Bovet C, Hecht KM, Senger S, Winiker P, et al. 2016. Sulforaphane preconditioning sensitizes human colon cancer cells towards the bioreductive anticancer prodrug PR-104A. *PLOS ONE* 11:e0150219

Annual Review of Pharmacology and Toxicology

Volume 62, 2022

Contents