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# How to deal with frenemy NRF2: Targeting NRF2 for chemoprevention and cancer therapy

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#### Abstract

Induction of antioxidant proteins and phase 2 detoxifying enzymes that neutralize reactive electrophiles are important mechanisms for protection against carcinogenesis. Normal cells provide multifaceted pathways to tightly control NF-E2related factor 2 (NRF2)-mediated gene expression in response to an assault by a range of endogenous and exogenous oncogenic molecules. Transient activation of NRF2 by its activators is able to induce ARE-mediated cytoprotective proteins which are essential for protection against various toxic and oxidative damages, and NRF2 activators thereby have efficacy in cancer chemoprevention. Because NRF2 has a cytoprotective function, it can protect normal cells from carcinogens like an angel, but when the protective effect acts on cancer cells, it will give rise to invincible cancer cells and play a devilish role in tumor progression. Indeed, aberrant activation of NRF2 has been found in a variety of cancers that create a favorable environment for the proliferation and survival of cancer cells and leads to drug resistance, ultimately leading to the poor clinical prognosis of patients. Therefore, pharmacological inhibition of NRF2 signaling has emerged as a promising approach for cancer therapy. This review aims to compile the regulatory mechanisms of NRF2 and its double-edged role in cancer. In addition, we also summarize the research progress of NRF2 modulators, especially phytochemicals, in chemoprevention and cancer therapy.

Keywords: Cancer therapy, Chemoprevention, NRF2, Phytochemicals

#### 1. Discovery of NRF2

N RF2 (<u>Nuclear factor erythroid 2-related factor</u> <u>2</u>), also known as <u>Nuclear Factor</u>, <u>Erythroid 2</u> Like <u>2</u> (NFE2L2), was discovered as a widely expressed transcription factor belonging to the CNC (cap 'n' collar)-basic leucine zipper family [1,2]. In humans, NRF2 protein is composed of 605 amino acids and is encoded by the NFE2L2 gene located at chromosome region 2q31.2 (gene ID: 4780). As the name implies, NRF2 was initially found to bind to the tandem repeats of NF-E2/AP-1 binding sequence ((c/t)gctga(g/c)tca(c/t)) in the promoter region of the  $\beta$ -globin gene [1]. Later, NRF2 was identified as a positive regulator of NAD(P) H:quinone oxidoreductase1 (NQO1) gene induced by antioxidants, such as  $\beta$ -naphthoflavone and *tert*butyl hydroquinone (*t*-BHQ) through antioxidant response element (ARE; gcagtcacagtgactcagcagaatc) [3]. NRF2 protein forms a heterodimer with small muscle aponeurotic fibrosarcoma (small Maf) protein and directly induces the transcription of phase II detoxifying enzymes through ARE binding [4]. NRF2 knockout did not affect mouse development [5], however, NRF2-deficiency leads to increased susceptibility to xenobiotic-induced toxicity due to

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the impairment of inducible expression of detoxifying enzymes [6-8]. Afterward, the cytoprotective role of NRF2-ARE pathway emerges by regulating the expression of numerous antioxidant proteins and detoxifying enzymes; all of which have been intensively studied and confirmed [9,10].

#### 2. Regulation of NRF2-ARE pathway

#### 2.1. Ubiquitination and proteasomal degradation of NRF2

NRF2-ARE pathway has been conclusively deemed pivotal in cellular defense, especially in warding off oxidative stress-induced cellular damages [11]. When cells confront stressful stimuli, NRF2 is imported into the nucleus and heterodimerizes with one of the small Maf proteins, and induces expression of genes involved in detoxification and antioxidant defense through directly binding to ARE in the promoter region of genes [12,13]. In 1999, Itoh et al. identified a novel cvtoplasmic protein, KEAP1 (Kelch-like ECH-associated protein1), which traps NRF2 in the cytoplasm and suppressed transactivation activity of NRF2 through directly interacting with the evolutionary conserved N-terminal domain (Neh2) of NRF2 protein [14].

NRF2 has a short half-life ( $T_{1/2} = 15 \text{ min}-3 \text{ h}$ ) [15,16], and the rapid turnover of NRF2 is due to ubiquitin-mediated proteasome degradation. In unstressed conditions, dimeric KEAP1 serves as a substrate adaptor protein that interacts with the Cullin3 (CUL3) ubiquitin ligase to form an active E3 ubiquitin ligase complex and triggers the degradation of NRF2 through ubiquitin-proteasome system (UPS) [12,13]. The detailed mechanisms of KAEP1mediated inhibition of NRF2-ARE pathway have been revealed in several studies via using different experimental approaches [17–21].

When confronted with stressful conditions, reactive cysteine residues within KEAP1 protein, which functions as a sensor for stressful stimulus, become modified, thereby resulting in KEAP1-NRF2 disassociation due to a conformational change in dimeric KEAP1 protein [12,13]. Subsequently, NRF2 is stabilized and transported into the nucleus to drive transcriptional activation of target genes through binding to the ARE [12,13]. Therefore, upon directly interrupting protein-protein interaction of NRF2-KEAP1 complex via competitively binding to KEAP1 protein by sequestosome 1/p62 [22-24], dipeptidyl peptidase 3 (DPP3) [25], prothymosin  $\alpha$  (ProT $\alpha$ ) [26], Wilms tumor gene on X chromosome (WTX) [27], partner and localizer of BRCA2 (PALB2) [28], tripartite motif-containing protein 29 (TRIM29, ATDC) [29], and cell-cycle related kinase 20 (CDK20) [30] or binding to NRF2 protein by p21 (Cip1/Waf1) [31], breast cancer susceptibility protein 1 (BRCA1) [32], and progestin and adipoQ receptor family member 4 (PAQR4) [33] leads to facilitating stabilization, nuclear accumulation and transcriptional activation of NRF2. Similarly, ubiquitin-processing proteases, such as ubiquitin-specific-processing protease 11 (USP11) and deubiquitinating enzyme 3 (DUB3), catalyze the removal of ubiquitin from NRF2, thereby stabilizing NRF2 protein [34,35].

In addition to the destabilization of NRF2 via redox-sensitive interaction with KEAP1 and NRF2 Neh2 domain, the NRF2 Neh6 domain also possesses KEAP1-independent inhibitory activity on NRF2 protein stability [36]. The serine-rich Neh6 domain of NRF2 is recognized by a substrate receptor, β-transducin repeats-containing protein (β-TrCP), of the SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complex, resulting in ubiquitin-proteasome degradation of NRF2 [37-39]. Furthermore, Neh6 degron-directed degradation of NRF2 protein is enhanced by glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ )-dependent phosphorylation of serine residues within the Neh6 domain [37-39]. Besides KEAP1 and β-TrCP, WD40 Repeat Protein 23 (WDR23) also served as a proteasome substrate receptor for the recruitment of DDB1-CUL4-RBX1 E3 ubiquitin-protein ligase complex to induce the degradation of NRF2 protein [40,41]. Furthermore, ER-associated ubiquitin ligase Hrd1 [42,43], CR6interacting factor 1 (CRIF1) [43-45], IQ motif-containing GTPase-activating protein 1 (IQGAP1) [46,47], seven in absentia homolog 2 (Siah2) [48], and UFM1-binding and PCI domain-containing protein 1 (UFBP1) [49] were also reported to down-regulated NRF2-ARE pathway through inducing ubiquitination and proteasomal degradation of NRF2.

#### 2.2. Post-translational modification of NRF2

#### 2.2.1. Phosphorylation of NRF2

NRF2-ARE pathway is not only tightly controlled by proteasomal degradation but also regulated by post-translational modification of NRF2 protein [50–52]. In 2000, Huang et al. first demonstrated that activation of protein kinase C (PKC) had a decisive role in inducing NRF2-ARE pathway-dependent gene expression through the phosphorylation of NRF2 protein, which leads to the NRF2 nuclear translocation under antioxidant treatment [53]. Further investigation through utilizing site-directed mutational analysis identified that Ser40 within NRF2 Neh2 domain (KEAP1-interacting domain) is the specific phosphorylation site catalyzed directly

388

by PKC [54]. PKC-mediated phosphorylation of NRF2 at Ser40 enhances KEAP1-NRF2 dissociation, NRF2 stabilization, nuclear accumulation of NRF2, and therefore induces ARE-dependent gene expression [53–55].

Regulation of NRF2-ARE pathway by mitogenactivated protein kinase (MAPK) signaling cascades has been noticed [56–60]. Therefore, several studies have explored and verified the possibility that MAPKs directly phosphorylate NRF2 protein to regulate the NRF2-ARE pathway [61–64]. Furthermore, a comparative analysis of amino acid sequences indicates that several conserved MAPK phosphorylation sites (P-X-S/T-P, or S/T-P) within NRF2 protein in humans and other species (chicken, rat, and mouse) [61]. Moreover, five serine/threonine residues (Ser215, Ser408, Ser558, Thr559, and Ser577) have been identified in NRF2 protein that can be directly phosphorylated by MAPKs [62].

Although the connections between MAPKs and NRF2 activation and direct phosphorylation of NRF2 protein by MAPKs have been reported in numerous studies, the effects of MAPK-dependent phosphorvlation of NRF2 on NRF2-ARE pathway are still controversial. For instance, activation of p38 MAPK has been shown to positively regulate NRF2-ARE pathway via enhancing nuclear translocation and transactivation activity of NRF2 [56,57,65]. On the contrary, the increasing interaction between KEAP1 and NRF2, resulting in diminished transactivation activity and nuclear translocation of NRF2 has been observed when p38 MAPK is ectopically overexpressed [63,66]. Likewise, the contradictory regulatory effects of other MAPKs, such as extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), on NRF2-ARE pathway have also been observed [61,62,66,67]. The opposite effects of MAPK-mediated regulation of NRF2-ARE pathway may be related to the complexity of MAPKs signaling pathways and reciprocal actions with other signaling networks. Hence, the regulatory effects and specific mechanisms of MAPK signaling cascades on NRF2 activity via direct phosphorylation requires further investigation.

Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) has been reported to enhance the nuclear accumulation of NRF2 through directly phosphorylating serine residue at position 558 within the nuclear export signal (NES) of NRF2 protein [68]. Others have found that three serine residues (Ser374, Ser408, and Ser433) of NRF2 protein were hyper-phosphorylated by AMPK [69]. Unlike phosphorylation at Ser558 which involved in nuclear accumulation of NRF2, substitutions of Ser374, Ser408, and Ser433 by alanine had no significant effects on protein stability, nuclear import, and transactivation activity of NRF2 [69].

Additional protein kinases, like protein kinase RNA-like endoplasmic reticulum kinase (PERK), Fyn kinase, ceramide-protein kinase C zeta-casein kinase 2 (CK2), and p35/Cdk5 kinase complex, have been demonstrated to regulate NRF2-ARE pathway through direct phosphorylation of NRF2 [70-77]. PERK directly phosphorylates NRF2 and leads to the liberation of NRF2 from KEAP1 in response to ER stress [70-72]. CK2 enhances NRF2 nuclear translocation and transactivation activity directly by phosphorylating several sites in the transcription activation domains, Neh4 and Neh5, of NRF2 [73,74]. Similarly, activation of cyclin-dependent kinase 5 (Cdk5)-p35 complexes catalyze the phosphorylation of Thr395, Ser433, and Thr439 in NRF2 protein [75]. The direct phosphorylation of NRF2 by active Cdk5-p35 complex in the cytosol triggered nuclear translocation of NRF2 and leads to the induction of genes encoding enzymes involved in glutathione metabolism that protected the cells against oxidative damage [75]. On the contrary, direct phosphorylation of Tyr576 within NRF2 Neh3 domain, which contains nuclear localization sequence, by Fvn appears to suppress NRF2-ARE pathway through inducing nuclear export of NRF2 [76,77].

### 2.2.2. Acetylation, SUMOylation, methylation, and glycation of NRF2

In addition to phosphorylation, other forms of post-translational modification of the NRF2 protein, including acetylation, SUMOylation, and methylation, also contribute to the control of the NRF2-ARE pathway. CREB binding protein (CBP)/p300-mediated acetylated NRF2 protein at several specific lysine residues, which are located in NRF2 Neh1 DNA-binding domain and Neh3 transactivation domain containing a nuclear localization signal (NLS) [78]. Acetylation of these lysine residues enhanced nuclear retention and DNA-binding activity of NRF2, consequently driving transcriptional activation of ARE-mediated genes. Furthermore, NRF2 acetylation also facilitated the association of NRF2 and CBP, which functions as a transcriptional co-activator [79]. Another post-translational modification, SUMOylation, has been reported to positively regulate NRF2-ARE pathway [80-83]. Increased transcriptional activity of SUMOylated NRF2 has been observed due to the enhancement of NRF2 protein stability, nuclear localization, and promoting heterodimerization of NRF2 and MafG [80-83]. Conversely, SUMOylattion of NRF2 also serves as an inhibitory regulation of NRF2 protein stability within

the nucleus [84,85]. SUMO-modification of nuclear NRF2 is ubiquitinated by poly-SUMO-specific E3 ubiquitin ligase, RING finger protein 4 (RNF4), and to be degraded in the promyelocytic leukemia nuclear bodies [84,85]. Arg437 in NRF2 protein is methyltransferase-1 methylated by arginine (PRMT1), resulting in up-regulating DNA-binding and gene transactivation activities of NRF2 [86]. In addition, de-glycation of NRF2 protein by fructosamine-3-kinase (FN3K) increases its protein stability and heterodimerization with small Maf proteins, indicating that protein glycation is involved in modulating NRF2 activity [87,88].

## 2.3. Regulation of the NRF2-ARE pathway by interacting with transcriptional co-factors or other proteins in the nucleus

Small Maf proteins, which contain basic regionleucine zipper (bZIP) motif, are transcription coactivators necessary for gearing up NRF2-mediated activation of ARE-dependent gene transcription [4,89,90]. NRF2 heterodimerizes with one of the small Maf proteins (MafF, MafG, and MafK) via Neh1 Cap'n'Collar (CNC)-bZIP domain within the NRF2 protein, and then interacts with the ARE sequences to trigger transcriptional activation [89,91]. Other bZIP transcriptional regulatory factors, such as Jun family proteins (c-Jun, JunB, and JunD) and activating transcription factor 4 (ATF4) have also been shown to induce transcriptional activation of NRF2 through direct interaction with NRF2 [92,93]. Similarly, Jun dimerization protein 2 (JDP2) [94], p300/CBP [95], Brahma-related gene 1 (BRG1) [96], nuclear-restricted protein/brain (NRP/ B) [97], receptor-associated coactivator 3 (RAC3) [98,99], mediator of RNA polymerase II transcription subunit 16 (MED16) [100], p63 [101] and chromodomain helicase DNA binding protein 6 (CHD6) [102] have been reported to function as transcriptional coactivators, contributing to the enhancement of NRF2-regulated transcriptional activity of the ARE-driven genes in the nucleus. A recent study found that NRF2 directly interacts with STAT3 dimers and enhances the transcription of interleukin 23A to promote breast cancer progression [103].

BTB domain and CNC Homolog 1 and 2 (BACH1 and BACH2) act as transcriptional repressors of ARE-regulated genes by competing with NRF2 for binding to the small Maf proteins [104–106]. Similarly, Replication Protein A1 (RPA1) has also been shown to compete for the interaction between NRF2 and small Maf proteins by forming NRF2-RPA1 heterodimers that bind AREs and adjacent 7-nt negative regulatory sequence to down-regulate MYLK transcription [107]. Homodimerization of small Maf proteins acts as a negative regulator of the NRF2-ARE pathway by competing for ARE binding due to the scarcity of transcriptional activation domains [89,91]. In addition, c-MYC [108], retinoid X receptor alpha (RXRa) [109-111], retinoic acid receptor alpha (RARa) [112], estrogen-related receptor beta (ERR $\beta$ ) [113], silencing mediator for retinoid and thyroid hormone receptors (SMRT) [114], p14ARF [115] and estrogen receptor a (ERa) [116] are involved in the repression of NRF2-mediated transcription by directly binding to NRF2. Furthermore, a controversial role for peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) in the regulation of NRF2 activity has been reported. Interaction with PPARy decreases the transcription of the thromboxane synthase gene in rat macrophages [117], while the expression of heme oxygenase-1 (HO-1) is activated by NRF2-PPARy complex in rat brain astrocytes [118]. Moreover, truncated lamin A protein progerin is implicated in Hutchinson-Gilford progeria syndrome (HGPS), which impairs NRF2 transcriptional activity by trapping NRF2 in the nuclear periphery, leading to mislocalization of NRF2 in the nucleus and then reducing access of NRF2 to ARE [86].

### 2.4. Transcriptional and translational regulation of NRF2

Regulation of NRF2 gene expression at transcriptional and post-transcriptional levels has been extensively studied [9,51,119]. Besides NRF2 itself [120,121], other transcription factors, AhR-ARNT complex [122,123], human telomerase reverse transcriptase (hTERT)-Y-Box binding protein 1 (YBX1) [124], NF-ĸB [125,126], BRCA1 [127], myocyte enhancer factor 2d (MEF2D) [128], Notch [129], KRAS, BRAF and c-MYC [130] have been reported to up-regulate the transcription of NRF2 gene. In contrast, the tumor suppressor p53 represses the promotor activity of NRF2 [131]. Epigenetic modifications (such as DNA methylation and histone modifications) within the NRF2 promoter region and post-transcriptional regulation by microRNAs have also emerged as important regulatory mechanisms for NRF2 expression [50,132–136]. Recently, direct interactions between RNA-binding proteins (RBPs), HuR and AUF1, and the 3-UTR of NRF2-mRNA have been shown to activate the NRF2 pathway by enhancing the maturation, nuclear export, and stability of NRF2 mRNA [137]. Furthermore, encoding an NRF2 protein isoform without the KEAP1 interaction domain via alternative splicing is another post-transcriptional regulation of NRF2 activity [138].

Both 5'- and 3'- untranslated region (UTR) of NRF2 mRNA transcript are involved in the regulation of NRF2 protein translation. Under basal conditions, translation of NRF2 protein is strongly suppressed by Sg3 motif within the open reading frame (ORF) of NRF2 mRNA 3'-portion in a capdependent process [139,140]. On the contrary, 5'-UTR of NRF2 mRNA transcript contains two regulatory elements, internal ribosomal entry site (IRES) and G-quadruplex, which contribute to inducing de novo NRF2 protein translation in response to oxidative stress [141-144]. Small RNA binding exonuclease protection factor La (La/SSB), eukaryotic translation elongation factor 1 alpha 1 (EF1 $\alpha$ ), and the far upstream element binding protein 1 (FUBP1) are shown to play crucial regulatory roles in oxidative stress-induced translation of NRF2 protein through facilitating the recruitment of the translation machinery by interaction with NRF2 5'-UTR [142,145].

#### 3. NRF2 and cancer progression

#### 3.1. NRF2 acts as a double-edged sword in cancer

NRF2 exhibits dual pro- and anti-tumorigenic effects in cancer cells and normal cells, respectively [10,146,147]. NRF2 activation is considered as a protector during the initiation of carcinogenesis by its protective effects on enzymatic detoxification and elimination of chemical carcinogens and re-establishing cellular redox homeostasis in normal cells [2,10,147,148]. Several in vivo studies demonstrated that NRF2 deficiency enhances susceptibility to carcinogen-induced tumorigenesis and results in a more aggressive tumor phenotype in many types of cancers [149-154]. In addition, activation of NRF2 in hepatocytes by deletion of the KEAP1 gene attenuates steatohepatitis-induced fibrosis and tumor development, indicating that NRF2 plays an important role in preventing inflammation-triggered carcinogenesis [154,155].

However, NRF2-governed cytoprotective pathways endow transformed cells with biological capabilities – the hallmarks of cancer – acquired during malignant progression [10,156–160]. In 2017, an *in vivo* study has demonstrated that activation of NRF2 before chemical carcinogen exposure suppresses tumor development, whereas activation of NRF2 after tumor initiation facilitates the malignant progression of both chemically and genetically induced tumors [148]. Before tumor initiation, inhibition of NRF2 promotes cell proliferation and activation of NRF2-induced apoptosis accompanied with the generation of oxidative DNA damage in response to carcinogen treatment [148]. Conversely, after tumor development, suppression of NRF2 increases apoptosis, along with higher oxidative DNA damage, in both genetic and chemical-induced models of lung cancer in mice [148]. These studies have clarified that NRF2-mediated antioxidant response exerts strikingly opposite effects on determining whether cells undergo apoptosis or proliferation during cancer initiation and progression.

#### 3.2. Mechanisms of NRF2 over-activation in cancer

Persistent NRF2 over-activation is frequently observed in different types of cancer and is associated with poor clinical outcomes [10,161-167]. Somatic gain-of-function mutations of NRF2 gene and somatic loss-of-function mutations of NRF2 repressors, KEAP1, and CUL3 genes, are common genetic mechanisms underlying over-activation of NRF2 pathway in cancer cells [156,168–171] (Fig. 1). Genome-scale analysis, covering gene expression levels from RNA sequencing, somatic mutations from whole-exome sequencing, DNA copy-number variation, and DNA methylation, of nearly 9000 samples on 33 cancer types from the entire collection of TCGA PanCancer Atlas revealed that 6 types of cancer exhibited higher (greater than 10%) frequency of alterations in NRF2 pathway, including lung squamous cell carcinoma (LUSC) (25% altered), esophagogastric squamous cell carcinoma (STES ESCC) (23% altered), uterine corpus endometrial carcinoma with microsatellite instability and polymerase  $\epsilon$  (UCEC MSI-POLE) (19% altered), lung adenocarcinoma (LUAD) (15% altered), headneck squamous cell carcinoma (HNSC) (HPV-) (13% altered), and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (10% altered) [171]. The gain-of-function mutations of the NRF2 gene are predominantly detected in the ETGE and DLG motifs within the Neh2 domain, which is required for NRF2-KEAP1 protein-protein interaction [168,170,172,173]. Furthermore, constitutively active forms of NRF2 protein, which are encoded by the truncated transcripts lacking exon 2 or exon 2/3, have been found in lung and head-neck squamous cell carcinoma [138,174]. These aberrant NRF2 protein isoforms lacking the Neh2 domain would cause the stabilization and persistent nuclear localization of NRF2 by preventing KEAP1-mediated proteolysis [138,174]. In contrast to NRF2, the loss-of-function mutations of KEAP1 gene are found throughout the entire gene [168,172,175]. Most inactivating mutations of the KEAP1 and CUL3 result in impairing NRF2 degradation and contribute to constitutive NRF2 activation in cancer cells [168,175–177].





Fig. 1. Schematic illustration depicts mechanisms causing the NRF2 over-activation in cancer cells.

As mentioned in Section 1-2, the cellular abundance and activity of NRF2 are rigorously controlled at the transcriptional, post-transcriptional, translational, and post-translational levels. Therefore, in addition to the genetic deregulation of NRF2 and KAEP1, aberrations in these regulatory mechanisms also play crucial roles in the constitutive activation of NRF2 in cancer cells [52,134,146,175,178] (Fig. 1). For instance, NRF2 gene transcription can be up-regulated by oncogenic transcription factors, such as c-MYC, KRAS, BRAF, and NF-κB [125,126,130,159,179]. In cancer cells, NRF2 mRNA level can also be regulated by several miRNAs [168], such as miR-28 [180], miR-144 [181], and miR-153 [182]. The reduction of these microRNAs leads to an increase in NRF2 levels. In addition to up-regulation of NRF2 expression, impairment of KEAP1-mediated NRF2 degradation due to the reduction of KEAP1 expression (through DNA methylation, miRNA-mediated gene regulation) and suppression of NRF2-KEAP1 interaction (through modification of KEAP1 protein, competing directly for NRF2-KEAP1 interaction) is an important regulatory mechanism which involves in sustained over-activation of NRF2 in cancer cells [146,168,175,183,184]. Furthermore, activation of oncogenic signaling pathways, such as EGFR, PI3K/

AKT, and MAPK signaling pathway, also contributes to the enhancement of the NRF2-ARE pathway [171,185–187].

#### 3.3. NRF2 and cancer metabolic reprogramming

#### 3.3.1. NRF2 and Warburg effect

Metabolic reprogramming is widely considered as one of the hallmarks of cancer, which not only provides cancer cells themselves with advantages for survival and proliferation but also helps create a favorable microenvironment that facilitates metastasis during cancer progression [188,189]. In 1956, Otto Warburg discovered that, unlike normal cells generating energy by oxidative phosphorylation in mitochondria, cancer cells mainly tend to produce ATP through the aerobic glycolysis pathway despite oxygen availability [190]. This metabolic adaptation supplies cancer cells with a higher rate of ATP generation enhances the carbons from glucose feeding into biosynthetic pathways, provides an advantageous microenvironment for cancer cells proliferation and metastasis as well as directly manipulates signal transductions through reactive oxidative stress (ROS) or chromatin modulation [191]. In addition to the changes in glucose metabolism, accumulating evidence verified that dysregulation of lipid and amino acid metabolism are also involved in the enhancement of malignant progression [192,193]. Abnormalities in proto-oncogenes or tumor suppressor genes directly drive the metabolic rewiring in cancer cells [194,195].

Besides the transactivation of genes involved in the maintenance of cellular redox homeostasis, increasing evidence emerges that NRF2 pleiotropically modulates cancer cell metabolism [196,197]. Over-activation of NRF2 has been reported to promote the Warburg effect in cancer cells [10,198]. NRF2 enhanced glucose uptake, induced transcriptional up-regulation of genes encoding glycolytic enzymes, such as HK2, PFKFB3, GAPDH, PGK1, and ENO1/2, and then reduced the entry of glycolysisderived pyruvate into the TCA cycle through induction of LDHA and PDK1 [198-200]. In addition to central carbon metabolism, NRF2 regulates numerous metabolic processes, including amino acid metabolism, lipid metabolism, and iron/heme metabolism, that enhance cellular plasticity of cancer cells to enable malignant progression [197] (Fig. 2).

### 3.3.2. NRF2 plays a pivotal role in the satisfaction of NADPH demand in cancer cells

Maintaining the optimal balance of redox status is a crucial requisite within the cells of living organisms. Compared with normal cells, cancer cells commonly encounter greater oxidative stress in virtue of environmental stress as well as the upregulation of growth signals, metabolic activity, mitochondrial function, and integrin activation [201]. A large part of NRF2-regulated metabolic pathways, such as NADPH generation, glutathione synthesis, and cysteine metabolism, is hijacked by cancer cells to cope with excessive ROS production to adapt and survive under such stressful conditions [196]. NRF2 directly regulated major cytosolic NADPH-producing enzymes, two of the pentose phosphate pathway (glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (PGD)), one of TCA cycle (isocitrate dehydrogenase 1 (IDH1), and one is a link between glycolysis and citric acid cycle (malic enzyme 1 (ME1)) [202,203]. Furthermore, a mitochondrial enzyme, methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), involved in mitochondrial NADPH production is also regulated by NRF2 [202,204]. Conversely, pyruvate kinase, a key glycolytic enzyme that controls glucose carbon toward oxidaphosphorylation or pentose phosphate tive pathway, is negatively regulated by NRF2 [205]. Suppression of pyruvate kinase promoted the influx of glucose into the pentose phosphate pathway, thereby increasing NADPH production through G6PD and PGD [206].

De novo fatty acid synthesis is one of the most NADPH-consuming metabolic processes in the cells [207,208]. Genetic (KEAP1 knockout) and chemical activation of NRF2 in vivo reduce the genes encoding the key enzymes for de novo lipogenesis, like ATPcitrate lyase (ACLY), Acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FASN), fatty acid desaturases (FADS), fatty acid elongases (ELOVL), and stearoyl CoA desaturase (SCD1) [205]. This evidence has indicated that NRF2 increases the intracellular NADPH pool not only through induction of NADPHgenerating enzymes and enforced carbon-flux toward NADPH generation but also suppressed NADPH-consuming metabolic pathway. Up-regulation of the de novo fatty acid biosynthetic pathway is a common metabolic feature of cancer cells [207,208], however, the exact role of NRF2-regulated de novo fatty acid biosynthesis in tumor progression remains uncertain and requires systematic investigations.

#### 3.3.3. NRF2 and fatty acid oxidation

Unlike suppression of *de novo* fatty acid biosynthesis, several in vivo studies have demonstrated that NRF2 enhances fatty acid oxidation (FAO) via upregulation of FAO-related enzymes expression, such as carnitine palmitoyltransferase (CPT1/2) and acyl-CoA oxidase (ACOX1/2) [196,209,210]. Further, peroxisome proliferator-activated receptor  $\delta$  (PPAR $\delta$ ), a transcription factor with key roles in regulating the expression of several genes encoding main enzymes involved in fatty acid oxidation and cholesterol metabolism, is upregulated by the constitutivelyactive mutant form of NRF2 (NRF2 E79Q) in the hyperplastic mouse squamous epithelial cells [211]. In addition to inducing the expression of FAO-related genes, NRF2 has been verified to enhance lipid uptake through activation of cluster of differentiation 36 (CD36), a fatty acid translocase in macrophages [212,213] and hepatocellular carcinoma (Huh-7) cells [214]. Although CD36-dependent lipid metabolism exhibits a tumor-promoting role in cancer cells [215], the dual effects of NRF2 in CD36-mediated enhancement of tumor progression are still unclear. Accordingly, even though the role of NRF2 in fatty acid metabolism has been identified, further investigations are needed to clarify the influences of NRF2-directed lipid metabolism on malignant progression.

#### 3.3.4. NRF2 and amino acid metabolism

In addition to increasing intracellular NADPH amount, NRF2-mediated activation of thioredoxin (Trx) and glutathione (GSH) systems is the principal mechanism for coping with ROS overproduction in



Fig. 2. Schematic illustration depicts mechanisms of actions of NRF2 on cancer metabolic reprogramming.

cancer cells [216]. NRF2 enhances *de novo* synthesis of glutathione from glutamate, cysteine, and glycine via inductions in the expression of glutamatecysteine ligase modifier subunit (GCLM), the glutamate-cysteine ligase catalytic subunit (GCLC), and glutathione synthetase (GSS) [217]. NRF2 promotes transcriptional upregulations of the cystine/ glutamate antiporter, SLC7A11 (xCT), to enhance cystine uptake [218] as well as glutathione reductase (GR) and glutathione peroxidase (GPx) to facilitate glutathione recycling [9]. Furthermore, NRF2 also up-regulates the expression of genes encoding enzymes involved in the thioredoxin systems to increase the reduction of cystine to cysteine [219].

Glutamine, a conditionally essential amino acid, is an important amino acid with pleiotropic functions for tumor progression [220]. Glutaminolysis, a metabolic process composed of several steps for catalyzing glutamine, has been considered as one of the hallmarks of cancer metabolism [220] and a potential therapeutic target for cancer therapy [221]. Persistent activation of NRF2 enhanced the dependency of cancer cells on glutaminolysis and thereby resulted in sensitizing tumors to glutaminase inhibition [222,223]. A significantly higher requirement for exogenous glutamine has been observed in the NSCLC cells with loss-of-function mutations in KEAP1 than in the KEAP1-wild-type NSCLC cells [224]. The enhanced dependency of exogenous glutamine in NRF2-hyperactive cancer cells is due to the decreased intracellular glutamate pools induced by the induction of glutathione synthesis and glutamate excretion via cystine/glutamate antiporter [224]. NRF2 facilities glutaminolysis through transcriptional activation of the enzymes involved in glutamine uptake (solute carrier family 1 member 5 (SLC1A5) [222]), hydrolysis of glutamine forming glutamate (glutaminase 2 (GLS2) [225]), and reversible transamination between alanine and 2-oxoglutarate to form pyruvate and glutamate (glutamic-pyruvic transaminase 2 (GPT2) [225]). Enhanced glutaminolysis not only provides glutamate for increased glutathione biosynthesis but also fuels the TCA cycle through replenishing intermediates from glutamine [222,224], and promotes the synthesis of nonessential amino acids through

aminotransferases in cancer cells [220]. Furthermore, up-regulation of glutaminolysis also fulfills the needs of *de novo* nucleic acid and lipid biosynthesis in cancer cells [202,226].

Asparagine and aspartate play important roles in the maintenance of cancer cell proliferation, and survival under stress conditions, such as nutrientlimiting conditions (especially glucose and glutamine deprivation), ETC dysfunction and hypoxia [227–230]. Decreasing the bioavailability of asparagine through knockdown of asparagine synthetase (ASNS) reduces the transcription of EMT-related genes and metastasis of breast cancer cells, indicating that asparagine bioavailability may be an important regulatory mechanism in cancer metastasis [231]. Asparagine synthetase (ASNS) catalyzes the synthesis of the asparagine from aspartate and glutamine that require ATP hydrolysis [232]. The expression of ASNA is induced by activating transcription factor 4 (ATF4), thereby maintaining intracellular asparagine levels in response to nutrient-limiting conditions [227,233]. NRF2 promoted ATF4-regulated ASNS expression induced by activating PI3K-AKT pathway during glutamine deprivation in a KRAS<sup>G12D</sup>-driven mouse lung cancer model [234]. Thus, asparagine biosynthesis was enhanced and then glutamine deprivationinduced apoptosis was suppressed. In addition, NRF2 may transactivated ATF4 gene expression through direct binding to its promoter, thus allowing amplification of ATF4-driven ASNS expression to contribute to the development of esophageal squamous cell carcinoma (ESCC) under glucosedeprived conditions [227].

Besides regulation of *de novo* asparagine synthesis via ATF4, NRF2 has also been reported to increase serine/glycine biosynthesis from glucose through enhancing ATF4-mediated phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and serine hydroxymethyltransferase 2 (SHMT2) expressions in NSCLC cells [235]. Activation of serine/glycine biosynthesis pathway replenishes carbons from glycolysis to fuel GSH, nucleotides, and NADPH synthesis and then promoted lung cancer progression [235]. Serine biosynthesis is dependent on GLUT8 (SLC2A8)-mediated glucose uptake in the KRAS-mutant KEAP1 deficient NSCLC cell lines, indicating that glucose availability played an important role in NRF2-mediated serine biosynthesis in lung cancer [236]. In addition to NSCLC, NRF2 is also involved in the regulation of de novo serine synthesis in hepatocellular carcinoma (HCC) [80]. Under serine-deprived conditions, an increased SUMOylation of NRF2 enhances de novo

serine synthesis through up-regulating the translation of the first rate-limiting enzyme, PHGDH, of the pathway, contributing to the maintenance of HCC. This enhancement of serine biosynthesis by NRF2 SUMOylation is partly dependent on the intracellular ROS level in HCC.

#### 3.3.5. NRF2 and de novo nucleotide synthesis

Over-activation of NRF2 empowers de novo nucleotide synthesis to satisfy the constant demands of rapid proliferation in cancer cells through metabolic network rewiring to provide building blocks for fueling nucleotide synthesis and transactivation of the genes that encode for nucleotide synthesis enzymes [196,202,235]. For instance, increased activity of the pentose phosphate pathway caused by constitutive activation of NRF2 promoted nucleotide synthesis by raising the availabilities of the sugar backbone of the nucleotide (ribose 5-phosphate (R5P)) [179,196,206]. Up-regulation of intracellular NADPH level by NRF2 provides advantages for deoxyribonucleotides synthesis via supporting the enzymatic reaction of ribonucleotide reductase [196]. Furthermore, NRF2 directly transactivates the expression of phosphoribosyl pyrophosphate amidotransferase (PPAT), an important rate-limiting enzyme for the de novo nucleotide biosynthetic pathway [202,237]. Up-regulation of the expression of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), a mitochondrial enzyme involved in folate-mediated one-carbon metabolism, by NRF2 also promotes de novo synthesis of purine in cancer cells [202,237]. Another mitochondrial enzyme, methylenetetrahydrofolate dehydrogenase 1-like (MTHFD1L), which is downstream of MTHFD2 in the mitochondrial folate cycle, is also found to be directly regulated by NRF2 in HCC [238,239]. In addition, oxidative pentose phosphate pathway (oxPPP)-generated NADPH plays a critical role in supporting folate metabolism by regulation of dihydrofolate reductase (DHFR) activity, indicating that NRF2 promotes folate-mediated one-carbon metabolism to support de novo nucleotide synthesis not only directly through transactivation of MTHFD2 and MTHFD1L gene expressions but also indirectly through up-regulation of PPP [240].

#### 3.3.6. NRF2 and iron/heme metabolism

The role of NRF2 in iron/heme metabolism has been well established [241]. NRF2 controls the intracellular iron homeostasis through regulating the expression of genes involved in heme biosynthesis (ferrochelatase (FECH) [242]), heme catabolism (heme oxygenase (HMOX-1) [243], biliverdin reductase A/B (BLVR A/B) [244]), heme/iron transport (ATP binding cassette subfamily B member 6 (ABCB6) [245], transferrin receptor-1 (TFR1), ferroportin-1 (FPN1) [246]), and heme transporter (HRG1) [242]) and intracellular iron storage (ferritin heavy chain 1 (FTH1) and ferritin light chain [247]). Ferroptosis is iron-dependent programmed necrosis, which is induced by ferrous iron  $(Fe^{2+})$ -mediated lipid peroxidation [248]. Recently, triggering ferroptosis is considered as a novel therapeutic strategy in cancer treatment [249]. Owing to the regulatory role in iron/heme metabolism and ROS detoxification, NRF2 functions as the protector of cancer cells against ferroptosis [250]. Notably, the activity of glutathione peroxidase 4 (GPX4), the gatekeeper for ferroptosis, is directly or indirectly up-regulated by NRF2 [250]. Another ferroptosisinhibiting protein, cystine/glutamate antiporter, SLC7A11 (xCT), which maintains intracellular cysteine availability for glutathione production, is also induced by NRF2 activation [250]. Therefore, inducing ferroptosis via inhibition of NRF2 has been viewed as a potential therapeutic strategy for the treatment of cancer, especially for re-sensitization of treatment-resistant cancer cells [251].

### 4. Chemopreventive effects and therapeutic potentials of NRF2 modulators

### 4.1. Applications of NRF2 activators in cancer chemoprevention

One of the most prominent strategies in cancer chemoprevention is to protect cells or tissues from various carcinogens and carcinogenic metabolites, both exogenous and endogenous, by inducing detoxifying enzymes and antioxidant proteins. NRF2 plays an important role in preventing carcinogenesis through antioxidant response element (ARE)-mediated transcriptional activation of several detoxifying and antioxidant enzymes [120,252,253]. The exploration of phytochemicals and food constituents which exerted chemopreventive potential via the elimination of electrophile-induced carcinogenesis by Nrf2-driven antioxidants and detoxifying proteins are hot areas in cancer prevention research [252–256].

In order to discover more novel NRF2 modulators, we have established a cell-based NRF2/ARE-driven luciferase reporter system that can efficiently and accurately screen large compound libraries [257]. This platform has helped us to successfully develop many NRF2 activators [257–263]. Among them, we first identified 4-ketopinoresinol as a novel NRF2 activator. 4-Ketopinoresinol was isolated from adlay and exhibited a potent cytoprotective effect against

oxidative stress-induced cell injury through activation of PI3K/AKT/NRF2/HO-1 axis [257]. In addition to 4-Ketopinoresinol, several phytochemicals isolated from adlay, such as trans-coniferylaldehyde and sinapaldehyde, were also identified to possess free-radical scavenging and antimutagenic activities by inducing NRF2/ARE pathway [259]. We noticed that trans-coniferylaldehyde increased the level of Nrf2-mediated detoxifying/antioxidant proteins in vitro and in vivo, and attenuated carcinogen-induced oxidative stress by activating Nrf2 via p38a/ MAPKAPK-2- and PK-N3-dependent signaling pathways [260]. Adlay has been recognized as a chemopreventive blocking and suppressing agent against carcinogenesis [261]. Enhancement of NRF2mediated antioxidant and detoxifying enzyme induction is one of the important mechanisms underlying the cancer chemopreventive effects of adlay [261].

By exploiting the NRF2/ARE-driven luciferase reporter system, we also noted that resveratrol, hydroquinone, ethyl ferulate, tert-Butylhydroquinone, apigenin, piceatannol, ebselen, curcumin, n-octyl caffeate, carnosic acid, tanshinone IIA, and bisdemethoxycurcumin possessed significant abilities to induce NRF2/ARE-driven luciferase activities [263]. In fact, some of the several phytochemicals mentioned above are known NRF2 activators and potent cancer chemopreventive agents, such as resveratrol, piceatannol, pterostilbene, curcumin, and bis-demethoxvcurcumin. Resveratrol and its hydroxyl derivative piceatannol exert significant cancer preventive benefits through activation of NRF2 and NRF2-mediated cellular defense system [264,265]. A natural methoxylated analog of resveratrol, pterostilbene has been found to be more effective than resveratrol in reducing chemically induced mouse colon carcinogenesis by induction of NRF2/ARE pathway [266]. Furthermore, pterostilbene also effectually prevents chemical-induced skin and lung tumorigenesis [267,268]. Curcumin and its analogues such as bisdemethoxycurcumin possess chemopreventive ability through activation of NRF2 [269-273]. In addition, other natural polyphenolic compounds, such as apigenin and carnosic acid, activate NRF2mediated antioxidant signaling thereby providing significant cancer preventive benefits [274–277].

Although some of the NRF2 activators identified from our study [263] are not directly linked to the chemopreventive capability of cancer, however, their ability to induce NRF2-mediated protection against oxidative stress and inflammation has been reported in previous studies. For example, tanshinone IIA, a lipophilic diterpene isolated from the root of *Salvia miltiorrhiza* Bunge (Lamiaceae),

exerts renal- and neuron-protective actions and anti-fibrotic effects in the silica-induced lung fibrosis model via increasing the induction of NRF2 [278,279]. The n-Octyl caffeate facilitates NRF2mediated cytoprotection through increasing nuclear accumulation of NRF2, thus proving the multiple countervailing effects of oxidative hepatotoxicity [280]. Ebselen effectively protects auditory and retinal Müller cells against oxidative damages by eliciting activation of NRF2 pathway [281,282]. Although ebselen has cancer chemopreventive activity in inflammation-related carcinogenesis, there is no evidence that this is related to NRF2 activation [283]. Ethyl ferulate, a naturally lipophilic polyphenol, guards against inflammation-induced acute lung injury and renal damage caused by hyperglycemia-induced oxidative stress through NRF2 activation [284,285]. Ethyl ferulate has been reported to be a chemopreventive agent through targeting the mTOR signaling pathway [286].

Based on the above discussion, we believe that our screening platform is efficient and accurate. It accurately presented consistent results to validate NRF2 activators identified by others, and has also helped us to discover many novel and potent NRF2 activators. The role of some above-mentioned NRF2 activators in NRF2-mediated cancer chemoprevention has been validated, but more studies are still needed to elucidate other phytochemicals that have not been systematically studied in NRF2-mediated cancer chemoprevention.

### 4.2. Applications of NRF2 inhibitors in cancer therapy

Several lines of evidence have demonstrated that elevated NRF2 activity is strongly correlated with tumor progression. Cancer cells acquire resistance by producing high levels of detoxifying enzymes that destroy chemotherapy drugs, pumping chemotherapy drugs out by efflux pumps, or by producing antioxidants that protect cells from oxidative damage caused by certain chemotherapy drugs. Our previous studies demonstrated that activation of NRF2-ARE pathway is critical for the development of chemoresistance of tumor cells [159,160,263]. Considering the role of Nrf2 in regulating a battery of genes that act to detoxify anticancer drugs and/or attenuate drug-induced oxidative stress or induced drug efflux, it is noted that Nrf2/ARE pathway plays an important role in cancer etiology and developing the chemoresistance in the clinical setting [287,288].

The diversity of changes in metabolic programs within the cancer cells can determine how

proliferative rewiring is driven. Therefore, cancer cells must rewire cellular metabolism to meet the demands of growth and proliferation, and cancer metabolism has long been considered a hallmark of cancer [289]. Recent studies have identified novel functions of NRF2 in cancer metabolic reprogramming [10], and has been explored and confirmed in different studies (Please refer Section 3-3. NRF2 and cancer metabolic reprogramming). Recently, we revealed that carcinogens, such as nicotine and arecoline, trigger c-MYC-directed NRF2 activation in head and neck cancer. Over-activation of NRF2 promotes malignant progression of HNSCC through reprogramming a wide range of cancer metabolic pathways, including pentose phosphate pathway (PPP), valine leucine and isoleucine degradation, pyrimidine metabolism, glycolysis and gluconeogenesis, purine metabolism, oxidative phosphorylation, xenobiotic metabolism, heme metabolism, fatty acid metabolism, and adipogenesis, in head and neck cancer. Targeting NRF2-directed cellular metabolism could be an effective strategy for the development of novel treatments for head and neck cancer [179].

There are no clinically available inhibitors of NRF2 in cancer therapy. Generally, except for the ligandinducible nuclear receptors, directly modulating the activities of transcription factors remains extremely challenging [290]. Therefore, similar to most transcription factors, the development of NRF2 inhibitors, which directly and specifically targeting of NRF2 itself, is a tremendous obstacle for researchers. Several natural compounds have been reported to exert their tumor-suppressive functions through inhibition of NRF2 activity. Notably, procyanidins isolated from cinnamomi cortex promoted nuclear degradation of NRF2 through insulin-like growth factor-1 receptorinduced activation of cysteine proteases in lung cancer cells resulting in inhibition of cell proliferation and increased doxorubicin- and etoposide-induced cytotoxicity [291-293]. Convallatoxin, derived from Adonis amurensis Regel et Radde, enhances GSK-3\beta\beta-TrCPmediated NRF2 degradation, thereby contributing to the enhancement of 5-fluorouracil cytotoxicity [294]. Hinokitiol (β-Thujaplicin), isolated from Chymacyparis obtuse, has been demonstrated to attenuate selfrenewal and invasiveness of glioblastoma stem cells by reducing both mRNA and protein expression of NRF2 [295]. Brusatol and halofuginone, which can enhance cancer cell sensitivity to chemotherapeutic agents, have been demonstrated to act as NRF2 inhibitors through reducing NRF2 protein levels [296,297]. Furthermore, some natural compounds play ambiguous roles in the regulation of NRF2 activity. For example, luteolin was shown to induce



Fig. 3. Representative naturally occurring NRF2 activators and inhibitors.

apoptosis in colorectal cancer cells by reducing nuclear localization of NRF2 [298]; and to increase NRF2 mRNA expression by reducing the methylation status of the NRF2 promoter [299]. Furthermore, Dolastatin 12, a marine natural product isolated from marine cyanobacteria, inhibits ARE-mediated reporter gene activity and exhibits potent inhibition of the NRF2/ NQO1 axis in cancer cells [300].

Recently, drug repurposing is considered an emerging strategy to identify NRF2 inhibitors for the effective treatment of cancer. For example, all-trans retinoic acid (ATRA), currently used to treat acute promyelocytic leukemia (APL) and dermatologic diseases, has been found to block nuclear translocation of NRF2, enhance NRF2 degradation, and suppress NRF2 recruitment to ARE, resulting to reduce expression of NRF2/ARE-driven genes and diminish stem cell characteristics of ALDHhigh ovarian cancer [112,301]. Clobetasol propionate, a corticosteroid used in the treatment of inflammatory skin diseases, inhibits the NRF2 pathway by triggering GSK3/β-TrCP-mediated degradation of NRF2, contributing to inhibiting growth of NRF2overactive lung cancer cells [302]. Isoniazid, a tuberculosis drug, has been shown to prevent nuclear translocation of NRF2 via reducing karyopherin β1-mediated nuclear import of NRF2 [303] and

ERK1-dependent NRF2 phosphorylation [304] in human hepatoma cells.

To identify specific inhibitors that could directly target NRF2, several research groups have performed screening tests to identify active compounds using ARE-driven cell-based reporter assays from small molecule compound libraries [300,305-307]. 4-(2-Cyclohexylethoxy)aniline (IM3829) has been discovered to have a significant inhibitory effect on the NRF2/ARE pathway and can be used as an effective lung cancer radiosensitizer [307]. Later, thienopyrimidine-containing compound ARE Expression Modulator 1 (AEM1) is found to inhibit ARE-driven luciferase activity, NRF2-regulated gene expression, and has the ability to reduce tumor growth and increase chemosensitivity in lung cancer cells [305]. Furthermore, small molecule ML385 has been discovered to directly target NRF2 itself by binding to Neh1 domain, which is involved in heterodimerization with small Maf proteins and DNA binding [306]. ML385 inhibits the binding of the NRF2/small Maf complex to ARE, causing repression of NRF2-regulated gene expression, thereby sensitizing KEAP1mutant NSCLC cells to chemotoxicity.

Over the past few decades, several studies have been done to identify potential inhibitors of NRF2; not surprisingly, a number of compounds have been

shown to exhibit anti-NRF2 activity through reducing its intracellular content, impairing nuclear localization, blocking NRF2 binding to its cooperators, and interrupting DNA binding activity [308,309]. To date, NRF2 inhibitors that have been reported so far lack specificity and selectivity, which poses a major limitation for subsequent clinical application. By utilizing a cell-based ARE reporter gene system, we have screened a small-molecule compound library and discovered that HBED, U83836E, GERIBP002A, CDC, hinokitiol, and gossypol are potential NRF2 inhibitors [263]. Among them, gossypol displayed the highest ARE-driven luciferase inhibitory activity, and the second most potent compound was hinokitiol. Hinokitiol is a known NRF2 inhibitor [295]. Notably, HBED, U83836E, GERIBP002A, CDC, and gossypol have never been reported to have NRF2 inhibitory activity before, and this role was elucidated for the first time by our group [263]. This novel NRF2 inhibitor, gossypol, has been demonstrated to enhance the therapeutic effects of etoposide and cisplatin in chemo-resistant cancer cells by inhibiting the NRF2/MRP1 and NRF2/G6PD axis, respectively. These results suggest that gossypol has a high potential to improve clinical efficacy in chemo-refractory tumors by blocking NRF2 signaling to overcome drug pumping and reprogram cancer

Based on the above discussion, one can find many NRF2 activators are natural products and belong to dietary phytochemicals. However, naturally occurring NRF2 inhibitors are mostly isolated from inedible plants or microorganisms (Fig. 3). These results reflect the recognized role of phytochemicals in edible foods that helps us build good antioxidant and detoxification defenses and support the use of NRF2 activators for chemoprevention. Conversely, NRF2 inhibitors are mostly found in non-edible plants, which indicates their use is not for daily health care, but rather as a strategy for treatment or adjuvant treatment of diseases, such as cancer.

#### 5. Conclusion

metabolism [263].

As NRF2 acts as a double-edged sword in cancer, it protects normal cells from the initiation of carcinogenesis induced by carcinogens, but it may also prompt the cancer cells to become aggressive and resistant to treatment. Therefore, the use of NRF2 activators or inhibitors in the correct and precise context is critical to the success of cancer prevention or treatment. For example, supplementation of a large amount of antioxidants with NRF2-activating capacity during the treatment phase may raise concerns about the diminished efficacy of chemoand radiotherapy. It must be noted that NRF2 plays an important regulatory role in cancer metabolic reprogramming, in addition to activating detoxifying enzymes, antioxidant proteins, and drug efflux transporters. Reprogramming metabolic pathways in cancer cells is critical for increasing energy production and supporting the biosynthesis of precursors required for tumor progression. In the future, it is worth exploring the mechanism of action of NRF2 inhibitors to achieve therapeutic effects by reprogramming tumor metabolism.

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402

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406

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