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Review

Keap1−Nrf2 pathway: A promising target towards lung cancer prevention and therapeutics

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Abstract

Objectives: Drugs for targeted therapy have become a new strategy of adjuvant therapy for treatment of lung cancer. The Keap1 (kelch-like ECH-associated protein 1)−Nrf2 (nuclear factor erythroid 2-related factor 2) pathway is recognized to be critical in regulating genes related to the cellular protective response and protecting cells from oxidative damages and toxic insult.
Methods: Pubmed, Embase, OVID, and the Cochrane Library databases were searched from the beginning of each database without any limitations to the date of publication. Search terms were "Nrf2" or "Keap1" and "Lung cancer".
Results: The upregulation of Nrf2 had been closely related to tumor protection and drug resistance. The aberrant state of Keap1 or Nrf2 that were frequently found in lung cancer conferred a poor prognosis. Nrf2 could prevent cells from undergoing oncogenesis as a tumor suppressor, while it could also promote cancer progression and resistance to chemotherapeutic drugs as an oncogene, depending on the different stages of tumor progression. Target Nrf2 signaling by specific chemicals showed it could prevent tumor growth or combat chemoresistance.
Conclusions: Increasing evidence has demonstrated the dual roles of the Keap1−Nrf2 pathway in tumor initiation and progression. In this paper, we provide a comprehensive overview of the potency of the Keap1−Nrf2 pathway as an antitumor target, and the current status of Nrf2 activators or inhibitors for therapeutic approaches. Further studies are required to clarify the role of Nrf2 in lung cancer at different tumor stages, in order to maximize the efficacy of Keap1−Nrf2 targeting agents.

Keywords: Cancer prevention; Keap1; Lung cancer; Nrf2

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Introduction

Cancer cells are frequently exposed to multiple stresses and toxicants both from intracellular and extracellular events. To protect against these insults, eukaryotic cells have developed complex defense systems for adjusting cells to oxidative and xenobiotic stresses. Nuclear factor erythroid 2-related factor 2 (Nrf2), also known as Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) and its natural inhibitor Kelch-like erythroid cell derived protein with CNC homology (ECH)-associated protein 1 (Keap1) are essential in the regulation of cytoprotective and detoxifying defense systems, including phase I (cytochrome P450s) and phase II (detoxifying, and antioxidant proteins) enzymes.[1,2] Activation of Nrf2 protects against inflammatory injury in the HTR-8/SVneo human first trimester extravillous trophoblast cell line and steatosis in nutritionally induced non-alcoholic steatohepatitis in mice.[3,4] Although the Nrf2-null mice are viable and show no obvious phenotype defect, they seem to be more sensitive than wild-type mice to oxidative damage and a toxic environment, such as exposure to ultraviolet radiation B, furosemide, acetaminophen, and cigarette smoke.[5–9] The alterations of genes in the Keap1–Nrf2 pathway were commonly found to exist in lung cancer and to be closely related with tumor progression. Therefore, fully understanding the role of Nrf2 in the regulation of cytoprotective events may provide insights into the search of novel anticancer targets, and further benefit the chemotherapy outcome in lung cancer. In reviewing the dual-role of Nrf2 in lung cancer, we focus here on discussing the potency of the Keap1–Nrf2 pathway as an antitumor target for either prevention or therapy.

The Keap1–Nrf2 pathway

Nrf2

Molecular dissection of Nrf2 identified seven Neh (Nrf2-ECH homologous structure) domains, known as Neh1-Neh7 (Fig. 1A). The Neh2 domain, located in the N-terminal region of Nrf2, is essential for regulating Nrf2 stability. It contains two binding sites (the DLG and ETGE motifs) responsible for Keap1 binding[10] and seven lysine residues important for ubiquitin conjugation.[10,11] The Neh1 domain has been shown to heterodimerize with Maf and bind to the ARE within DNA since it contains a CNC-type bZIP DNA-binding motif.[12] The Neh3, Neh4 and Neh5 domains are required for the transcriptional activation of Nrf2 target genes.[13,14] The Neh7 domain was found to interact with RXRα (retinoic X receptor α), which functions as an Nrf2 repressor and repress the target gene transcription.[15]

Keap1

Keap1 mainly contains five functional domains (Fig. 1B) including the NTR (the N-terminal region), BTB (the broad complex, tramtrack, and bric-a-brac), IVR (intervening linker domain), DGR (double glycine/Keclch repeats) and CTR (the C-terminal region).[16] The DC domain (including DGR and CTR) is responsible for binding to Neh2 of Nrf2, while the BTB domain is required for the dimerization of two Keap1 molecules.[10,17] The BTB with a part of IVR bind Cullin3 (Cul3) to form ubiquitin E3 ligase, in which Keap1 serves as a substrate adaptor for Nrf2 degradation.[18] The IVR and BTB contain several cysteine residues, modifications of which under

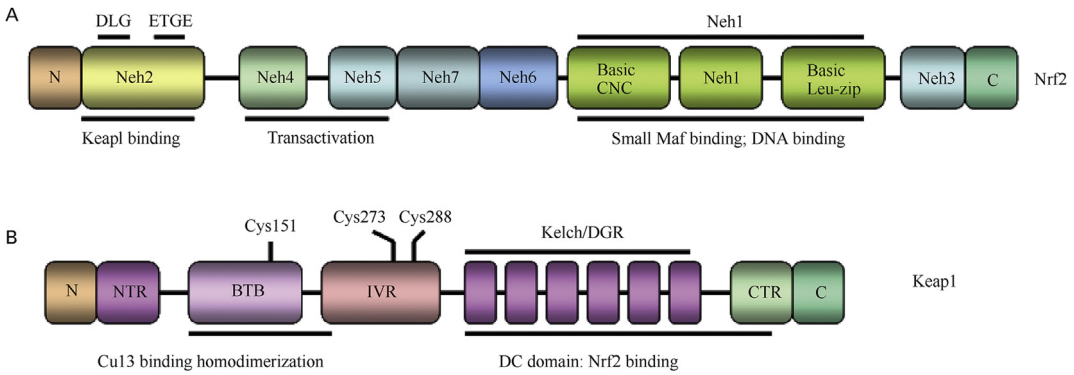

Fig. 1. Schematic representation of Nrf2 and Keap1 protein domains. (A) Nrf2 mainly contains seven conserved domains named the Neh domains. (B) The Keap1 protein mainly comprises five functional domains.

oxidative and electrophilic stress may associate with Keap1 activity and Nrf2 stability.[19]

Target genes of Nrf2

Nrf2 belongs to the cap'n'collar (CNC)-bZip (basic leucine zipper) family of transcription factor. Under basal conditions, Nrf2 is anchored in the cytoplasm and inhibited by Keap1 and maintained at a low expression level. Once activated, Nrf2 translocates into the nucleus, heterodimerizes with small Maf proteins and regulates antioxidant response element- (ARE-) mediated transcriptions of various genes encoding antioxidant enzymes and metabolic proteins classified as phase II enzymes.[20] A series of genes encoding cellular detoxification enzymes and antioxidant proteins are regulated by Nrf2, including several categories as follows: (1) proteins related to synthesis and conjugation of glutathione: glutamate cysteine ligase (GCL:GCLC and GCLM), glutathione reductase (GSR), etc;[21] (2) antioxidant proteins that maintain redox-balancing: thioredoxin (Trx), peroxidase (Prx), etc;[22,23] (3) drug metabolizing enzymes: NAD (P) H quinone oxidoreductase 1 (NQO1), etc;[21] (4) proteins involved in drug delivery: multiple drug resistant associated proteins (MRPs), etc;[24] (5) Heme and iron metabolism: Heme oxygenase 1 (HO-1), etc.[21] Thus, the Nrf2-mediated antioxidant response defends cells from multiple stresses including oxidative stresses and toxic damages.

Regulation of Keap1–Nrf2 pathway

Keap1-dependent regulation of Nrf2

Under basal conditions, Keap1 functions as a substrate adaptor protein for the Cul3-containing E3 ubiquitin ligase complex, which targets Nrf2 for ubiquitination and degradation.[25] The "hinge and latch" model proposed that two Keap1 molecules in the dimmer interact with one Nrf2 molecule at two binding sites, the high-affinity ETGE (hinge) motif and the low-affinity DLG (latch) motif, which position Nrf2 to undergo poly-ubiquitinations and degradation by the 26S proteasome.[26] Thus, Nrf2 is sequestered in the cytoplasm by Keap1 and maintained at a low level (Fig. 2).

Under stressed conditions, it has been proposed that covalent modifications of the critical cysteine residues within Keap1 lead to Nrf2 release, since Keap1 is a thiol-rich protein and is thus sensitive to an electrophile.[27] It was found that *de novo* synthesized Nrf2 accumulated in the cytoplasm and translocates into the nucleus rather than Nrf2 dissociated from Keap1.[28] Besides, the Cul3–Keap1 interaction was disrupted due to oxidative stress as in the case of modification at Cys151 in BTB domain,[29] which also resulted in a decrease of Nrf2 degradation. As a result, the inhibition of Nrf2 was hindered. Nrf2 was then allowed to accumulate in the cytoplasm and translocate into the nucleus for transcription of target genes (Fig. 2). There are three residues (Cys151, Cys273, and Cys288) that are critical for the normal function of Keap1. Cys273 and Cys288, located in the IVR domain, were found to be required for Keap1-dependent ubiquitination of Nrf2 under basal conditions.[30] Cys151 in the BTB domain is important in the de-repression of Nrf2 both under basal culture conditions and upon exposure of cells to Nrf2 inducers. Modification of Cys151 probably impedes the Keap1–Cul3 interaction and prevents the ubiquitination of Nrf2, resulting in the termination of Nrf2 degradation.[31]

Keap1-independent regulation of Nrf2

Besides the Keap1-dependent regulation of Nrf2, there are several alternative mechanisms related to Nrf2 activation such as phosphorylation, acetylation, and cysteine modification of Nrf2. Pathways involved include protein kinase C (PKC), glycogen synthase kinase-3 beta (GSK3β), mitogen-activated protein kinase (MAPK) cascades, the phosphatidylinositol 3-kinase pathway (PI3K/AKT), and extracellular regulated protein kinases (ERK), etc. PKC phosphorylates Ser-40 of Nrf2 in its Neh2 domain, resulting in the disassociation between Nrf2 and Keap1.[32] GSK-3β phosphorylates the tyrosine kinase Fyn and induces its nuclear accumulation.[33] Fyn has been shown to phosphorylate Nrf2 at tyrosine-568, facilitating its nuclear export and degradation. In cells with mutant Nrf2 (Y568A), nuclear accumulation of Nrf2 occurs attributing to the loss of the ability to be phosphorylated at tyrosine-568.[34] Thus, the phosphorylation modification and subsequent nuclear accumulation of Fyn mediated by GSK-3β confer the nuclear export of Nrf2.[35–37] In addition, GSK3β is a downstream target of multiple kinase cascades, such as Akt and MAPK, which are involved in Nrf2 regulation. Thus, GSK3β is essential in controlling the nuclear export, ubiquitination, and subsequent proteasomal degradation of Nrf2, and is also important in the down-regulation of Nrf2-dependent transcription in cell antioxidant defense.[38] The histone acetyltransferase hMOF is another protein that can regulate Nrf2 independent of Keap1. hMOF is reported to be acetylated Nrf2 at Lys 588, which increased nuclear accumulation of Nrf2 and enhanced the transcription of its downstream genes.[39]

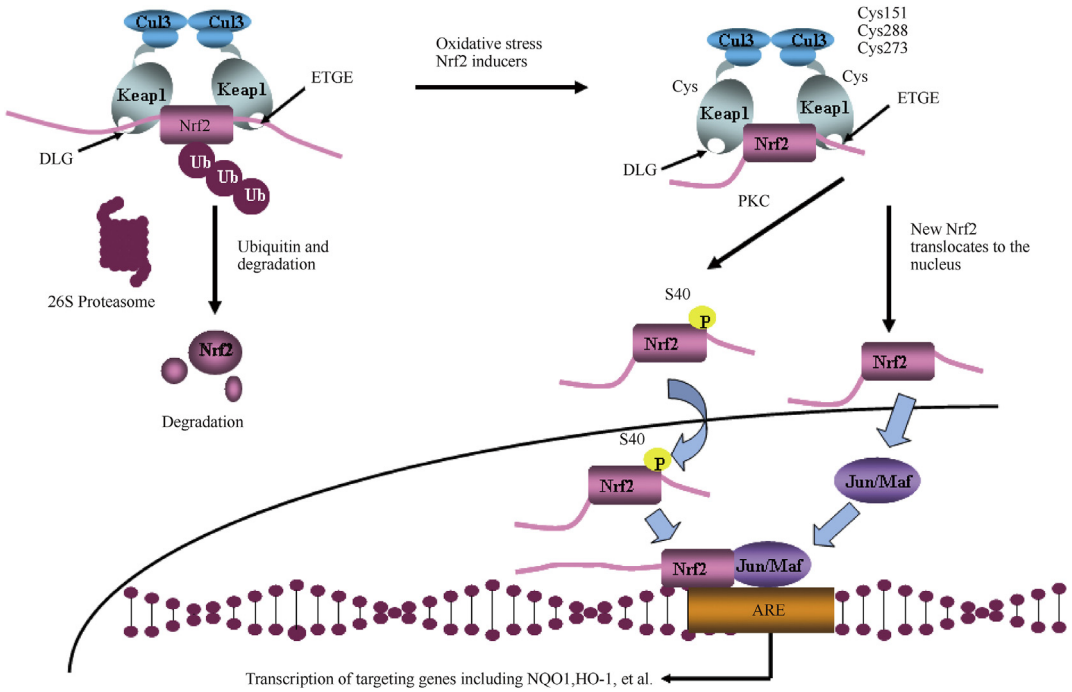

Fig. 2. The hinge-latch model of Nrf2. Nrf2 is negatively regulated by Keap1 under unstressed conditions. Two Keap1 molecules interact with one Nrf2 molecule at two binding sites, the ETGE and DLG motifs. Keap1 functions as a substrate adaptor protein for the Cullin3 (Cul3)-containing E3-ligase complex and targets Nrf2 for ubiquitination and degradation. Under stress conditions, the inhibition of Nrf2 is undermined. The *de novo* synthesized Nrf2 accumulates in the cytoplasm and translocates into the nucleus, functioning as a transcriptional factor.

The dysregulation of Nrf2–Keap1 pathway in lung cancer

Dysfunction of Keap1 in lung cancer

A growing number of studies indicate that abnormal states of the Keap1–Nrf2 pathway exist in lung cancer, including somatic mutations, loss of heterozygosity or DNA methylations in the promoter region of Keap1, and Nrf2 mutations.[40–45] Somatic alterations in Keap1 were first found both in lung cancer cell lines and in lung cancer tumor tissues.[41] The aberrant Keap1 disrupts the association between Keap1 and Nrf2, resulting in increased Nrf2 accumulation and downstream gene transcription. The Cancer Genome Atlas (TCGA) recently profile 178 lung squamous cell carcinomas (SQCC) for comprehensive genomic characterization. The data revealed that mutations or deletions in Nrf2, Keap1, and CUL3 occurred in 34% of tumors.[40] Mutant Keap1 or Cul3 have been reported to lose their normal function.[40] Another study reviewed 213 somatic mutations in Keap1, and then characterized 18 common mutations in lung SQCC. Of these 18 mutations, four (L231V, S224Y, P318L, and R71L) did not affect the inhibitory effect on NRF2, five (N469fs, P318fs, G333C, R554Q, and W544C) impacted the Keap1–Nrf2 association and the Keap1 activity of suppressing Nrf2, the remaining mutation sites showed hypomorphic suppression of Nrf2. Surprisingly, several mutations enhanced the binding of Keap1 and Nrf2, but this enhancement impaired degradation of Nrf2 while retaining the ability to ubiquitinate Nrf2.[46] Anju Singh et al. performed a systematic analysis of the Keap1 genomic locus on a Caucasian population of 54 cases of non-small cell lung cancer (NSCLC) samples and 12 lung cancer cell lines, in which deletions, insertions, missense mutations of Keap1, and loss of heterozygosity at 19p13.2 were commonly found.[41] All somatic mutations are located in the highly conserved Kelch binding or IVR domain, which are important functional regions for Keap1–Nrf2 binding or for Keap1 associating with Cul3, respectively. Another study that assessed 65 lung cancer samples of Japanese patients confirmed a similar result: non-synonymous mutations or reduced expression levels of Keap1 were identified both in patients' samples and in different lung cancer cell lines. Low Keap1 activity has been reported to lead to Nrf2 activation and subsequent up-regulation of target genes, which will favor tumor growth and drug resistance.[42] Besides, high methylation of the Keap1 promoter are found in both lung cancer cell

lines and tissues and decreased Keap1 mRNA expression is found when compared with a normal bronchial epithelial cell line.[43] In addition, Keap1 alterations are very common events in lung papillary adenocarcinoma (60%) and the rates of Keap1 mutations may vary in different histological lung tumor types.[47]

Nrf2 mutations in lung cancer

Keap1 mutations or inactivation are not the only reason for elevated Nrf2, the somatic mutations of Nrf2 itself occurs in lung cancer cells.[40,44,45] Intriguingly, it has been found that almost all mutations in Nrf2 were specifically within either the DLG or ETGE motifs, domains of Nrf2 that interact with Keap1, and were mutually exclusive with mutations in Keap1.[40] A similar result was seen in another two studies, including one study that collected 1145 cancer tissues from various histological carcinomas.[44,45] Somatic mutations were identified in the coding region of Nrf2 and all mutations found were missense substitutions.[45] Cells with mutant Nrf2 are insensitive to Keap1-mediated regulation and display aberrant cellular accumulation of Nrf2 as well as constitutive induction of cytoprotective enzymes and drug efflux pumps.[45] Interestingly, gain-of-function mutations in Nrf2 are found more frequently in multiple types of squamous cell carcinoma malignant tumors, including lung cancer.[44,48]

Nrf2 plays a complicated role in lung cancer

The dual roles of Nrf2 in lung cancer: the role of tumor suppressor

At first glance, Nrf2 was thought to be a protective gene and could prevent humans from many oxidative stress related diseases such as inflammation,[6] neurodegenerative disorders,[49] and pulmonary fibrosis.[50] Nrf2 deficient (Nrf2−/−) mice exhibit increased susceptibility to tumorigenesis.[51] Notably, many chemopreventive drugs have been identified as Nrf2 inducers, such as sulforaphane,[52] curcumin,[53] EGCG (epigallocatechin-3-gallate),[54] resveratrol,[55] oltipraz,[56] the synthetic triterpenoid CDDO (2-cyano-3,12-dioxooleana-1,9 (11)-dien-28-oic acid) and its imidazolide (CDDO-Im), and methyl ester (CDDO-Me) derivatives.[57] All these compounds have been shown to defend cells from carcinogens by inducing the Nrf2-dependent transcriptions, including multiple phase II detoxifying enzymes and antioxidants. The protective role of Nrf2 was also verified in the promotion or progression phase of lung cancer. Suppression of Nrf2 by Keap1-directed ubiquitylation or by Nrf2 shRNA/siRNA enhanced cell migration and plasticity in A549 cells.[58] Additionally, Nrf2-deficient mice display increased susceptibility to pulmonary metastasis of the mouse Lewis lung carcinoma tumors.[59]

The dual roles of Nrf2 in lung cancer: the role of tumor promoter

Although Nrf2 showed its protective role in tumorigenesis, accumulating evidence has started to point out the "dark" side of Nrf2 in cancers. The elevation of Nrf2 protein levels were found in multiple types of human cancers and were associated with tumor promotion and progression,[60–62] which was reasonable since elevated Nrf2 was favored by malignant cells to protect them from the environmental stress. As a matter of fact, the mutations of either Keap1 or Nrf2 commonly existed in lung cancer,[41–45,47,63] contributing to tumor growth advantages,[42,64,65] chemoresistance,[41,42,65–68] radio resistance,[69,70] and a poor outcome.[71] The increased levels and activities of Nrf2 have been shown to promote cancer cell proliferation, since the cell growth was stimulated in lung cancer-derived cell lines with Nrf2 activation due to mutations or low expression of Keap1.[42] Additionally, silencing of Nrf2 using shRNA or siRNA caused an inhibition of the proliferation of A549 cells,[64,65] a human lung cancer cell line with mutated Keap1 and elevated levels of Nrf2.[41] Cells with dysfunctional Keap1 showed less sensitivity to etoposide and carboplatin than those with wild-type Keap1, suggesting the abnormal state of Keap1–Nrf2 confers chemoresisrance.[41] A later similar study in 65 Japanese patients with lung cancer also verified that loss of Keap1 function (due to mutations or low-level expression) increased nuclear accumulation and induced constitutive activation of Nrf2, leading to the expression of its target genes, and thus resulted in growth advantages and cisplatin resistance of lung cancer cells.[42] Silencing of Nrf2 through RNAi sufficiently reversed resistance to cisplatin in NSCLC cells[65,67,68] and improved sensitivity of A549 to doxorubicin and etoposide.[67] The elevated level of Nrf2 also led to radioresistance through regulation of reactive oxygen species (ROS) in human lung cancer.[69,70] Thus, all these growth advantages conferred by Nrf2 over-activation result in poor outcomes of lung cancer and make Nrf2 an independent prognostic factor for survival analysis of patients with lung cancer.[71]

Could Nrf2 be a therapeutic target for lung cancer?

Nrf2 activators: defenders of cancer prevention

Nrf2 activators are chemoprevention agents due to their role of oxidation protection. Plenty of compounds with anticarcinogenic activities have been identified as potent Nrf2 inducers, most of which are from natural plants and foods (Fig. 3). One of them is sulforaphane (SFN), an isothiocyanate isolated from broccoli (cruciferous vegetables).[72] Sulforaphane has been proven to inhibit the growth of lung carcinomas in A/J mice treated with carcinogens.[73] Sulforaphane modulates the genes involved in tumor proliferation, apoptosis, angiogenesis and the metastatic process, thus it has proved effective in different stages of carcinogenesis.[74] The chemoprevention of sulforaphane may be due to its induction of the expressions of Nrf2 and its target genes. At first, sulforaphane was identified as the most potent inducer of NQO1.[52] Later, the induction of NQO1 by sulphoraphane was recognized to actually be regulated through the Keap1–Nrf2–ARE signaling pathway.[75] Sulforaphane elevates expression of Nrf2 protein rapidly in less than 30 min with the transcriptional activation of its target genes up-regulated concomitantly; genes such as HO-1, NQOs, GSTs, and UGTs.[76] Keap1 is a sulfhydryl-rich protein, thus electrophiles, like sulforaphane, can react with Cys residues in the Keap1 protein to form thionoacyl adducts.[77] The thionoacyl adducts at several particular Cys residues may disrupt the association between Cul3 and Keap1, resulting in the failure of Nrf2 ubiquitination.[78] As a consequence, newly synthesized Nrf2 escape degradation and translocates to the nucleus as the active form and facilitates the transcription of its target genes. In addition, sulforaphane might regulate intracellular localization and protein stability of Nrf2 through a series of intracellular kinases.[79] Several clinical trials have been completed or are underway to evaluate the bioavailability, safety, tolerance, and metabolism in healthy people, or the effect and safety in humans with different cancers (*https://www.clinicaltrials.gov/ct2/results?term=sulforaphane&Search=Search*). Although all these clinical trials are still in relatively early phases, they will prepare the way for sulforaphane to become a new safe drug and the Keap1–Nrf2–ARE pathway can thereby become an attractive target for treating chemoprevention during carcinogenesis.

The famous polyphenol compound curcumin, that has antitumor activity, is derived from the Indian spice turmeric plant[80] and has also been found to be an Nrf2 inducer.[53] Numerous and constant studies recognized curcumin as a chemotherapeutic drug, whose inhibitory activities on tumor growth work throughout the stages of tumor progression; including initiation, promotion, proliferation, angiogenesis, and metastasis.[80] Daily oral curcumin (80 mg/kg for 2 weeks) administered to Wistar rats exposed to cigarette smoke (CS) attenuated the CS-induced changes of pulmonary histology and decreased inflammation.[81] Curcumin also exerts its protective effect against nicotine-induced lung toxicity by modulating the biochemical marker enzymes; including nicotine-induced alkaline phosphatase, lactate dehydrogenase, and lipid peroxidation levels, decreasing the lipid peroxidation and augmenting the antioxidant defense system.[82] More recent reports showed that supplementation of curcumin effectively modulates premature mitochondria senescence and related ultrastructural changes during benzo [a]pyrene (BP)-induced lung carcinogenesis in mice.[83] Moreover, curcumin reversed cisplatin (DDP) resistance in A549/DDP cells (DDP-resistant lung adenocarcinoma cells).[84] Although the anticancer effects of curcumin have been revealed to be achieved through regulating multiple biochemical pathways and various molecules involved in carcinogenesis, several researches verified that Nrf2 plays a vital role in carcinogenesis prevention as well as the antitumor activity of curcumin. In epithelial cells, curcumin has been showed to stimulate the expression of Nrf-2 in a concentration- and time-dependent manner resulting in a significant increase in HO-1 protein expression and activity.[85] Curcumin has been proven to be pharmacologically safe in the early-phase clinical trials. The effects of curcumin on cancer patients with NSCLC or other cancers have also been investigated in relevant clinical trials (*https://www.clinicaltrials.gov/ct2/results?term=curcumin&Search=Search*).[86]

In addition, several other natural compounds such as (−)-epigallocatechin-3-gallate (EGCG), caffeic acid phenethyl ester (CAPE), and resveratrol show the ability to inhibit cancer growth via the Nrf2-dependent pathway. EGCG is the most abundant one of the major polyphenolic catechins presented in green tea, with potent antioxidant and chemopreventive activities.[87] The antitumor effect of EGCG has been validated in different types of cancers including lung cancer.[88] The antitumor effect of EGCG is partly through inducing the NRF2 signaling pathway.[54] Caffeic acid phenethyl ester (CAPE), an active component extracted from propolis, has the activities of cancer prevention and antitumor action in various types of cancer.[89] Nrf2 is also proven to be one of the molecular targets of CAPE in cancer

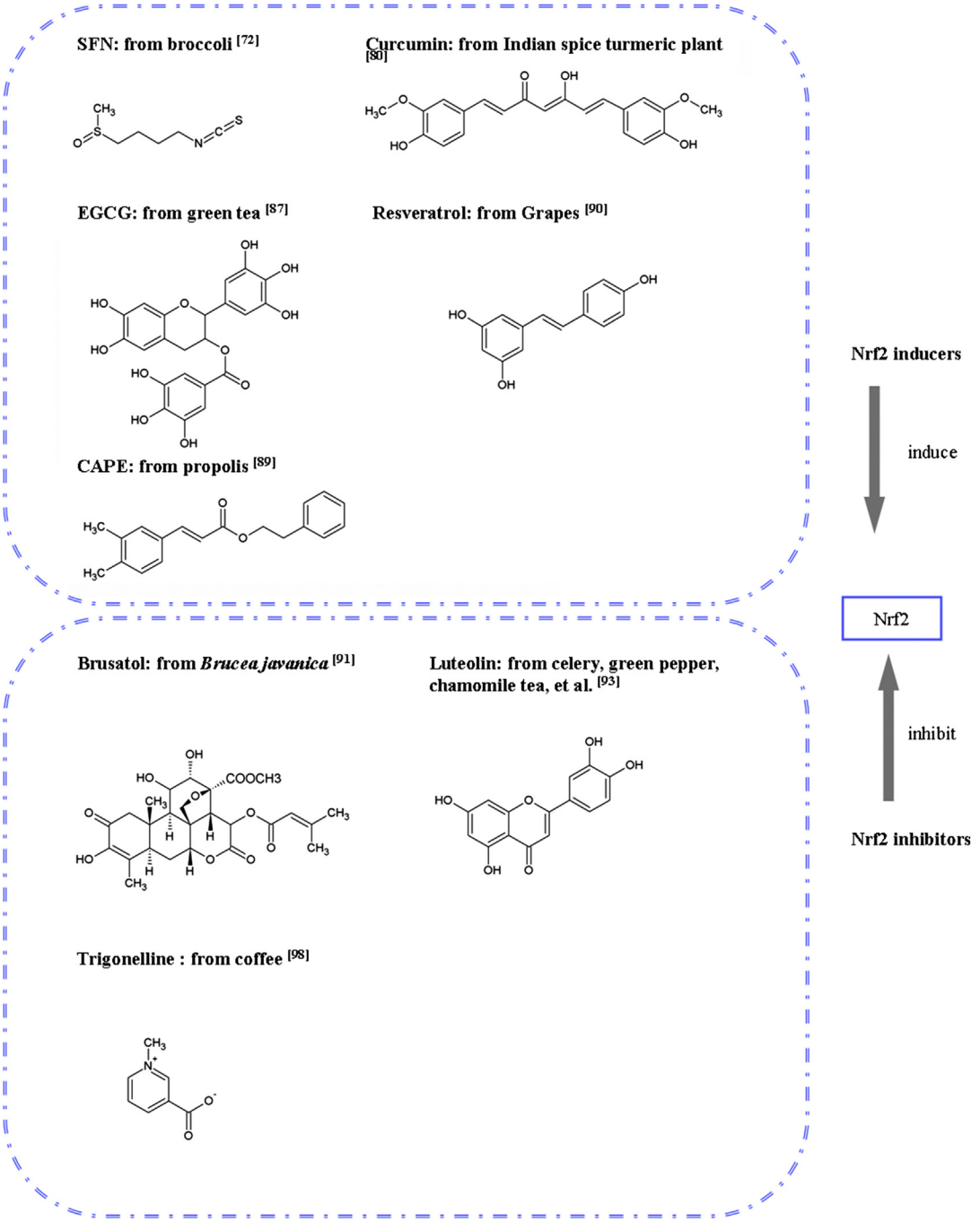

Fig. 3. The structure and origins of natural molecules that have activity to incite or inhibit Nrf2.

progression pathways.[85] Resveratrol, presented in grapes and peanuts, has been shown to prevent or delay the development of malignancies in a broad variety of *in vivo* preclinical models of carcinogenesis.[90] Likewise, this stilbenoid, polyphenol phytochemical is one of the widely known natural Keap1–Nrf2 activators.[55] These Nrf2 inducers described above have all been in several clinical evaluations for safety and bioavailability, which promote the Keap1–Nrf2 activators from preclinical experiments to clinical application (*https://www.clinicaltrials.gov/ct2/results?term=EGCG&Search=Search*; *https://www.clinicaltrials.gov/ct2/show/NCT02114892?term=Resveratrol&rank=3*).

Nrf2 inhibitors: potential therapeutic target for cancer

Since Nrf2 also plays a role in oncogenesis under given situations, it is thereby considered that inhibition of Nrf2 could turn into a promising strategy for cancer treatment. In contrast to the numerous chemicals and molecules reported to be Nrf2 inducers, the ones identified as Nrf2 inhibitors are very few. Several natural compounds known as Nrf2 inhibitors are listed in Fig. 3. Brusatol, a quassinoid isolated from the *Brucea javanica* shrub, was identified as a unique inhibitor of the Nrf2 pathway. Treatment with brusatol sensitized a broad spectrum of cancer cells to the antitumor drugs, reduced tumor burden, and improved survival in murine A549 xenograft models.[68,91] The inhibitory effect of brusatol toward Nrf2 was shown to be rapid, transient,[92] and acted through enhanced ubiquitination and degradation of Nrf2.[91]

Luteolin (3′,4′,5,7-tetrahydroxyflavone), a flavonoid present in many types of plants like celery, perilla leaf, peppers, broccoli, and parsley, was demonstrated to be a strong inhibitor of Nrf2 *in vitro*.[93] Treatment with luteolin significantly enhanced the antitumor efficacy of chemotherapeutics including oxaliplatin, doxorubicin, and bleomycin on A549 cells.[93] The study performed on murine A549 xenograft models showed that luteolin greatly inhibited the growth of A549 cells and enhanced anti-cancer efficacy of cisplatin *in vivo*.[94] Inhibition of the Nrf2 pathway using luteolin reversed the resistance of colorectal cancer cell lines toward chemotherapeutic drugs.[95] In addition, luteolin is able to inhibit the proliferation, cell cycle progression, and induce apoptosis of cancer cells derived from nearly all types of tumors; including gastric cancer, prostate cancer, melanoma, epidermoid carcinoma, leukemia, pancreatic tumor, hepatoma, and so on.[96] Although no clinical trial of luteolin treatment for cancer has been carried out to our knowledge, there is a Phase II study completed on the effects of luteolin on behavior in children with autism spectrum disorders (*https://www.clinicaltrials.gov/ct2/show/NCT01847521?term=Luteolin&rank=1*).

The alkaloid trigonelline is another molecule identified recently as an Nrf2 inhibitor with antitumor activity. Treatment of trigonelline combined with etoposide enhanced the antitumor efficacy of etoposide and reduced tumor sizes in Colo357 and Panc1 tumor bearing-SCID-beige mice.[97] In a rat ascites hepatoma cell line, trigonelline showed inhibitory effects on the invasion of AH109A hepatoma cells.[98] Studies have revealed Nrf2 as an important contributor to tumor growth and chemoresistance, thus these Nrf2 inhibitors have emerged as useful therapeutic tools for the effective improvement of anticancer therapy.

Perspectives

Lung cancer has been the first leading cause of cancer-related death around the world.[99] The overall prognosis for NSCLC patients has been greatly improved owing to the development of targeting therapy.[100] Although tyrosine kinase inhibitors (TKIs) have attracted tremendous attention in the last ten years, acquired resistance has put the further development of TKIs into a dilemma. Multi-target therapy shows its advantage in the immediate response that abolishes tumor before the occurrence of resistance. Undoubtedly, the Keap1–Nrf2 signaling pathway is a potential target mediating tumor progression and survival. Targeting Nrf2 has been found to be a promising strategy for the treatment of lung cancer associated with altered functions of Nrf2, yet further studies are needed to fully exploit the role of Nrf2 in tumor progression and prevention. On one side, Nrf2 activators may prevent tumor initiation and progression. The activation of the Nrf2 pathway directly impedes tumor growth and causes tumor regression. On the other side, Nrf2 activators may also lead to the acquisition of tumor resistance to cancer therapies, because the elevated level of Nrf2 prevents tumor cells from being destroyed due to the harsh microenvironment or chemotherapy treatment. In this context, once we decide to target Nrf2 for treatment, problems will come about as to which one to choose and when to use the Nrf2 activators or inhibitors. From our current perspective, Nrf2 activators are preferred in an early stage tumor, and Nrf2 inhibitors will be beneficial in treating late stage neoplasms. Nevertheless, a widely approved criterion or guideline is desperately needed to standardize chemotherapies targeting Nrf2.

Both Nrf2 activators and inhibitors used in the ongoing experiments lack high specificity, resulting in a weak relationship between Nrf2 regulation and tumor growths. For example, plant-derived activator of Nrf2 such as curcumin actually regulate through alternative pathways other than Nrf2. Small molecule activators or inhibitors with ideal specificity of Nrf2 would directly target this signaling pathway, and the promising effects would be easier to illustrate. The Nrf2 inhibitors identified to date are far fewer than Nrf2 activators, thus more chemicals and molecules that inhibit Nrf2 need to be designed and explored. With regard to the clinical application, intensive studies on Nrf2

inhibitors are needed to define the bioavailability, safety and clinical effects.

Conflicts of interest

All authors declare no potential conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of this manuscript.

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