Review

Natural Nrf2 Inhibitors: A Review of Their Potential for Cancer Treatment

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Abstract

Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates redox homeostasis, plays a pivotal role in several cellular processes such as cell proliferation and survival, and has been found to be aberrantly activated in many cancers. As one of the key oncogenes, Nrf2 represents an important therapeutic target for cancer treatment. Research has unraveled the main mechanisms underlying the Nrf2 pathway regulation and the role of Nrf2 in promoting tumorigenesis. Many efforts have been made to develop potent Nrf2 inhibitors, and several clinical trials are being conducted on some of these inhibitors. Natural products are well-recognized as a valuable source for development of novel therapeutics for cancer. So far, a number of natural compounds have been identified as Nrf2 inhibitors, such as apigenin, luteolin, and quassinoids compounds including brusatol and brucein D. These Nrf2 inhibitors have been found to mediate an oxidant response and display therapeutic effects in different types of human cancers. In this article, we reviewed the structure and function of the Nrf2/Keap1 system and the development of natural Nrf2 inhibitors with an emphasis on their biological function on cancer. The current status regarding the Nrf2 as a potential therapeutic target for cancer treatment was also summarized. It is hoped that this review will stimulate research on naturally occurring Nrf2 inhibitors as therapeutic candidates for cancer treatment.

Keywords: Natural products; Nrf2 inhibitors; Brusatol; Cancer; Molecular target

Introduction

Cancer is a condition in which the cells of an organism multiply in an uncontrollable manner. Currently, over 200 different types of cancer have been identified in humans [1], and they present a leading cause of death and a significant hindrance to life expectancy worldwide. In 2020 alone, 19,292,789 new cancer cases and 9,985,133 cancer deaths were reported globally, equating to around 27,356 deaths per day [2]. Carcinogenesis is a multistep process involving multiple genetic alterations which are necessary for a normal cell to develop into a tumor cell. This process is typically categorized into three stages: initiation, promotion and progression, and is characterized by various biological hallmarks [3]. These hallmarks include cell transformation, genome instability, hyperproliferation, immortalization, angiogenesis, epithelial-mesenchymal transition (EMT) and metastasis. Many of these processes are influenced by intracellular reactive oxygen species (ROS) [4-6]. The redox signaling process begins with the generation of ROS, which subsequently plays a role in signal transduction pathways involved in every step of carcinogenesis [7, 8]. A high level of the oxidative stress, usually induced by ROS, is a significant characteristics of cancer cells. On the other hand, genes and signal transduction pathways commonly

found in multiple cancer hallmarks are considered promising therapeutic targets.

Nrf2, also known as NFE2L2, is considered as the leading transcription factor controlling cell defense responses against various forms of cellular stresses, including oxidative stress [9]. The stability of Nrf2 is subtly regulated by KELCH-like ECH-associated protein 1 (Keap1), which facilitates its degradation through ubiquitin-proteasome pathway in the absence of oxidative stress [10-12]. As the primary stress regulator of cells, Nrf2 is intimately involved in tumor formation, progression and metastasis [13]. Notably, the persistent and aberrant activation of Nrf2, which is a frequent occurrence in many tumors, provides cancer cells with a selective advantage, leading to tumor progression, metastasis, therapy resistance, and poor prognosis [14, 15]. Over the past decades, Nrf2 has emerged as an auspicious target for cancer treatment, and numerous efforts have been made to identify therapeutic strategies that can disrupt its pro-oncogenic role [14]. Inhibiting Nrf2 activation is an attractive and promising approach for cancer prevention, treatment and drug sensitization.

Natural products and Chinese herbal medicine (CHM) are known to possess various pharmacological or biological activities, some of which are beneficial for cancer therapy. Moreover, they are a valuable source of bioactive molecules that can be used for the development of pharmaceutical drugs [16]. Several studies have demonstrated that natural products have the potential to counteract oxidative stress by modulating the Nrf2 pathway [17-23]. In addition, natural compounds such as apigenin, luteolin, chrysin and brusatol have been shown to be potent Nrf2 inhibitors. These findings suggest that natural Nrf2 inhibitors could be utilized as chemopreventive and chemotherapeutic agents, as well as tumor sensitizers for conventional radiotherapy and chemotherapy. This review provides an overview of the major naturally occurring Nrf2 inhibitors and their underlying molecular mechanisms for Nrf2 regulation. It highlights the potential of these compounds in the treatment of human cancers. We hope this review will facilitate new ideas in the development of Nrf2 inhibitors as novel anticancer drugs.

Nrf2 and Its Biological Functions and Regulation

Nrf2 is a 605 amino acid protein with a molecular weight of 68 kDa and encoded by NFE2L2 gene belonging to a transcription factor subfamily with the cnc ('cap'n'collar') structure. It contains a basic region-leucine zipper DNA binding domain (b-Zip). So far, six members of this family have been identified: NF-F2, Nrf1, Nrf2, Nrf3, Bach1 and Bach2.

Nrf2 is considered to be the primary regulator in oxidative stress [24]. It exhibits seven highly conserved Nrf2-ECH homology (Neh) domains designated as Neh1-7, respectively, which are important for Nrf2 functions [25, 26]. Among them, Neh1 contains a b-Zip domain for DNA and small musculoaponeurotic fibrosarcoma proteins (sMafs) binding. The Neh2 domain mediates the interaction with the negative regulator Keap1 within specific binding sites known as ETG and DLG motifs. Neh3-5 are required for target genes transactivation and functional interaction with several modulators, while the Neh6 domain contains a serine-rich region and a degron that is involved in the degradation of Nrf2 in oxidative stressed cells. Finally, Neh7 domain can interact with an Nrf2 repressor called "retinoic X receptor α" which inhibits NEF2L2 target gene transcription [26, 27]. Keap1 is a component of the Cullin 3 (CUL3)-based E3 ubiquitin ligase complex and controls the stability and accumulation of Nrf2. The Keap1 DC domain directly binds to Nrf2 through DLG and ETGE motifs within the N-terminal Neh2 domain of Nrf2 [28]. This two-site binding of Nrf2 and Keap1 has been shown to be the molecular basis of electrophile-induced Nrf2 accumulation [29]. Inactivation of Keap1 strongly induces Nrf2, and this phenomenon is often observed in cancer cells, enabling them to acquire malignancy by perverting Nrf2 activity [15]. Apart from Keap1-dependent mechanism, emerging evidence has revealed that Nrf2 can also be regulated through various mechanisms independent of Keap1 [30].

Stabilized Nrf2 translocates into the nucleus and forms a heterodimer with a sMaf transcription factor [31]. The Nrf2-sMaf heterodimer binds to antioxidant-responsive element (ARE) and induces transcription of numerous cytoprotective genes [15, 32]. The target genes regulated by Nrf2/ARE include phase I, II detoxification enzymes and phase III drug transporters, as well as some other transcription factors [23]. Briefly, phase I enzymes oxidize drugs or xenobiotics, while phase II enzymes conjugate products of phase I reactions, and phase III enzymes transport the final metabolites out of cells, cooperating to exert a cytoprotective function [27]. So far, more than 200 Nrf2 target genes have been identified in humans [33]. Most of them encode for metabolic enzymes that detoxify electrophiles (phase I/II/III drug metabolism) or scavenge ROS to restore the intracellular redox homeostasis and minimize the oxidative damage [34-36]. Furthermore, accumulating evidence indicates that Nrf2 can also regulate other biological processes with physio-pathological relevance in human diseases (e.g., tumors), such as proliferation, apoptosis, mitochondrial function or

biogenesis, inflammation and several metabolic pathways involved in pentose phosphate metabolism and fatty acid pathway. These biological processes are essential contributors to the maintenance of cellular redox and normal cell proliferation [14, 37, 38].

Nrf2 Activation and Its Role in Cancer Development

Many studies have amply demonstrated the physiological relationship among Nrf2, cellular redox homeostasis, and tumorigenesis [27, 39]. Redox status imbalance commonly occurs in cancer [40]. Tumor cells exhibit permanent high ROS levels owing to the oncogene activation, hypoxia, mitochondrial and/or peroxisomal dysfunction, increased metabolic rates, as well as anchorage-independent growth [41]. In this context, Nrf2 plays a crucial role by acting as a major regulator of the antioxidant response. Furthermore, studies have shown that persistent activation of Nrf2 has been frequently observed in many types of human cancers, including lung, breast, esophagus, ovarian, head and neck, skin and renal cancers [42-44]. Nrf2 activation can contribute to tumor development and progression by promoting cell survival, proliferation, and resistance to chemotherapy and radiation therapy (Figure 1) [45]. Moreover, overexpression of Nrf2 has been linked to poor prognosis and resistance to chemotherapy in several types of cancer [46].

Several mechanisms have been proposed to explain how Nrf2 activation contributes to cancer development. Normally, Nrf2 is degraded through a Keap1-Cul3-Roc1-dependent mechanism. However, in human cancer, somatic mutations occur in Nrf2, altering amino acids in the DLG or ETGE motifs, resulting in the accumulation of Nrf2. Mutant Nrf2 cells display constitutive induction of cytoprotective enzymes and drug efflux pumps that are insensitive to Keap1-mediated regulation [47]. Moreover, Nrf2 activation can increase the anti-apoptotic gene expression and promote carcinogen metabolism,

leading to cell survival and increased DNA damage and mutation rates, which can further promote cancer development [48].

Overall, while Nrf2 activation is important for cellular homeostasis and protection against oxidative stress, it can also contribute to cancer development and progression. Therefore, targeting Nrf2 signaling may represent a promising therapeutic approach for cancer treatment, e.g. temporally inhibiting Nrf2-dependent cytoprotection using Nrf2 inhibitors can be utilized to enhance the responses to anticancer drugs [49].

Natural Compounds as Nrf2 Inhibitors in Cancer

Based on the evidence concerning the role of Nrf2 in tumor cells, increasing number of natural products have been found to exert chemopreventive and therapeutic properties against different cancers via targeting Nrf2. These Nrf2 inhibitors include natural compounds extracted from plants, such as flavonoids, alkaloids and quassinoids. Moreover, some coffee extracts and vitamins have also been identified as Nrf2 inhibitors, including trigonelline and ascorbic acid (AA). We summarized the naturally occurring Nrf2 inhibitors below and discussed their biological significance related to cancer treatment in this section (**Figure 2-3** & **Table 1**).

Flavonoids compounds

Flavonoids, a diverse group of natural polyphenolic compounds commonly occurring in plants, have been found to have strong anti-proliferative activity against many types of cancer and sensitize cancer cells to anticancer agents [50, 51]. Several flavonoidal compounds, including apigenin, luteolin, wogonin and chrysin, have been reported to be Nrf2 inhibitors that can effectively reverse drug resistance.
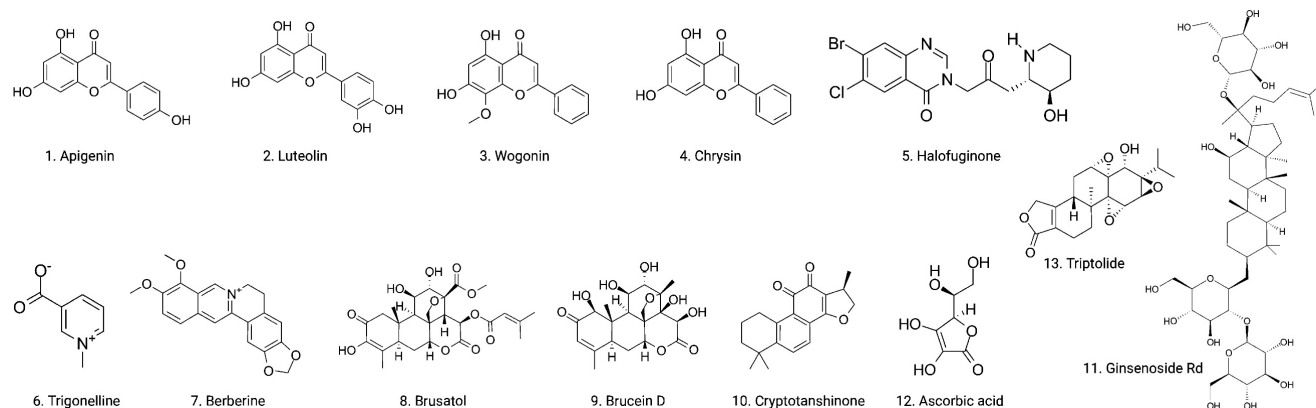

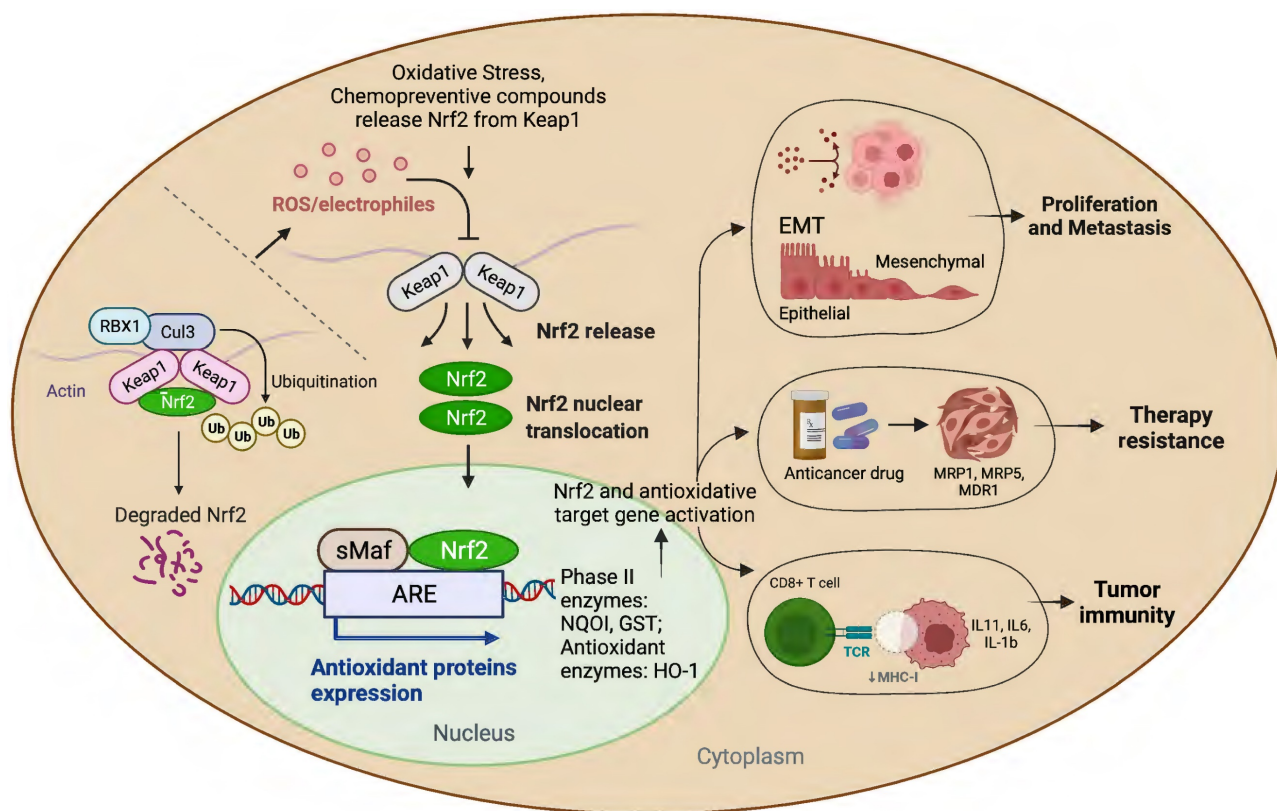**Figure 1.** Chemical structures of naturally occurring Nrf2 inhibitors.

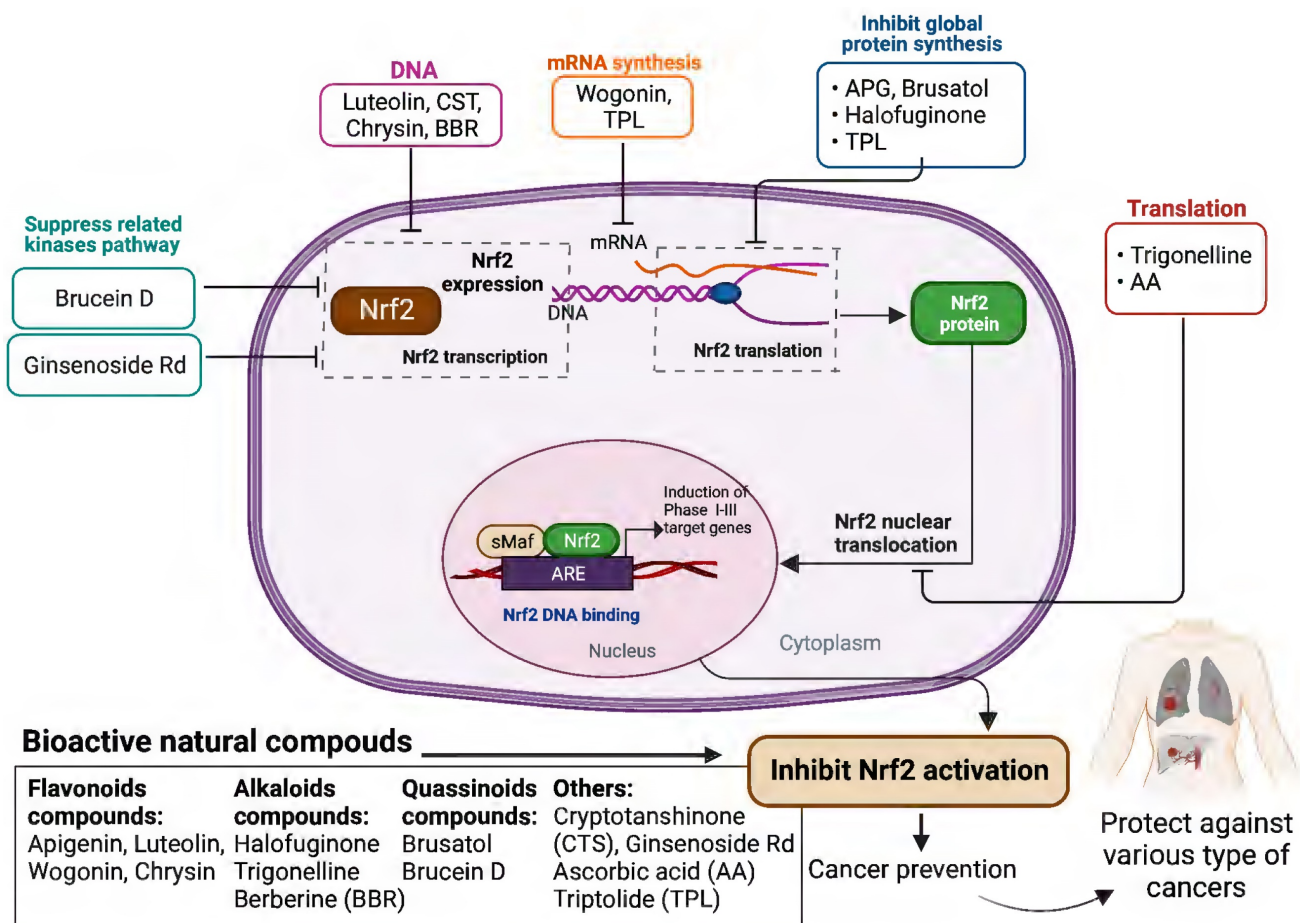**Figure 2**. Schematic presentation of the role of Nrf2 in cancer progression.

Figure 3. Schematic presentation of the molecular mechanisms underlying the cancer prevention and treatment of natural compounds via inhibiting Nrf2 pathway.

Table 1. Anticancer effects of naturally occurring Nrf2 inhibitors and associated action mechanisms.

Active compounds	Cancer type	Model	Dose	Mechanisms of action	Ref.
Apigenin	HCC	HCC cells (BEL-7402) and BEL-7402/ADM cells	20 µM;50 mg/kg	↓Nrf2, ↓HO-1, AKR1B10, MRP5; ↓p-Akt	[53]
Luteolin	Lung cancer	A549 cells	1-20 µM;	↓Nrf2, ↓HO-1, NQO1, AKR1C	[55]
	CRC	HCT116 and SW620	10 µM; 40 mg/kg	↓Nrf2, ↓HO-1, NQO1, GSTα1/2	[56]
	Breast CSCs	MDA-MB-231	1-2 µM	↓Nrf2, ↓ABCG2, Nanog, Oct4, Cripto1, Sirt3, HO-1	[57]
Wogonin	CML	K562 cells	40 µM; 40 mg/kg	↓Nrf2, ↓P65, P50, p-Stat3, NF-κB pathway	[59]
	CML	K562 cells	40 µM;	↓Nrf2, ↓MRP1, NQO1, HO-1, PI3K, p-Akt,	[60]
Chrysin	Glioblastoma	T98, U251, U87 cells	30/60 µM; 40/80 mg/kg	↓Nrf2, ↓HO-1, NQO1, ↓p-JNK, p-P38, p-ERK	[62]
	HCC	BEL-7402 cells	10 µM	↓Nrf2, ↓HO-1, AKR1B10, MRP5; ↓p-Akt, p-JNK, p-ERK, p-P38	[63]
Halofuginone	Lung cancer	A549, KYSE70 and ABC1 cells	50-100 nM; 0.25 mg/kg	↓Nrf2, ↓NQO1	[69]
Trigonelline	Pancreatic cancer	MiaPaca2, Panc1 and Colo357 cells	0.1/0.5 µM; 0.02 mg/kg	↓Nrf2	[71]
Berberine	HCC	Huh7, HepG2 and HHL-5 cells	20 µM; 5 mg/kg	↓Nrf2, ↓HO-1, NQO1	[73]
	Breast cancer	BT-474 and AU-565 cells	2 µM; 5 mg/kg	↓Nrf2, ↓p-GSK3β, C-Myc	[74]
Brusatol	AML	THP1, Molm13, U937, HL60 cells	1 µM	↓Nrf2	[80]
	Lung cancer	A549 cells	40 nM; 2 mg/kg	↓Nrf2, ↑Keap-1 ↓MRP1, MRP2, NQO1, γ-GCS,	[78]
		A549 cells	40 nM	↓Nrf2, ↓NQO1, GCLC, GCLM	[83]
	CRC	HCT116, CT26	300 nM; 2 mg/kg	↓Nrf2, ↑Keap-1	[84]
	Hepatoma	Mouse Hepa-1c1c7 cells	300 nM;	↓Nrf2, ↓NQO1	[85]
	Melanoma	A357 cells	100 nM; 2 mg/kg	↓Nrf2, ↓HO-1, NQO1, GSTP1	[86]
	Breast cancer	MCF-7, MDA-MB-231	40 nM	↓Nrf2, ↑Keap-1, ↓NQO1, GCLM, HO-1	[87]
	Glioma	U-251	2 ng/mL	↓Nrf2, ↓NQO1, GCLC	[88]
Brucein D	PDAC	Miapaca-2, Capan2 and PANC1 cells	1.5 µM; 2 mg/kg	↓Nrf2, ↓HO-1, NQO1, AKR1B10 γGCSm, MRP1 and MRP5	[96]
Cryptotanshinone	Lung cancer	A549 cells	10 µM	↓Nrf2, ↓MRP1, GCLC, GCLM, HO-1, NQO1, ↓p-Akt, p-STAT3, p-JNK, p-ERK	[97]
Ginsenoside Rd	NSCLC	A549 cells	80 µM	↓Nrf2, ↓HO-1, NQO1, GCLC, MRP1,	[100]
Ascorbic acid	CML	KCL22 cells	0.125 mM	↓Nrf2	[102]
Triptolide	NSCLC/liver cancer	A549 cells and HepG2	0.5 µM; 0.25 mg/kg	↓Nrf2	[104]
	Heart	H9c2 cells	1.2 mg/kg	↓Nrf2, ↓Bax/Bcl-2, ↑Caspase 3	[105]
	Glioblastoma	U251 MG cells	30 nM	↓Nrf2, ↓GCLC, GCLM	[106]

Apigenin

Apigenin (4',5,7-trihydroxyflavone, APG) is a naturally occurring flavonoid widely present in fruits, vegetables and herbs [52]. It has diverse biological activities such as anti-inflammatory, antioxidant and anti-carcinogenic properties. Studies have shown that APG as a potent Nrf2 inhibitor significantly sensitizes doxorubicin-resistant hepatocellular carcinoma (HCC) BEL-7402 (BEL-7402/ADM) cells to doxorubicin, increases intracellular concentration of ADM and suppresses tumor growth in a mouse xenograft model. Mechanistically, APG dramatically reduced the expressions of Nrf2 and its downstream genes through downregulating phosphatidylinositol-3 kinase (PI3K)/Akt pathway, leading to a reversal of drug-resistant phenotype [53].

Luteolin

Luteolin (3',4',5,7-tetrahydroxyflavone) is a polyphenolic flavonoid found in high concentrations in celery, parsley, green pepper, perilla leaf and chamomile tea [54]. It has been shown to exhibit multiple pharmacological effects, such as antioxidative, anti-inflammatory, autophagy-regulatory, apoptotic and antitumor effects [54]. Luteolin was found to be a potent Nrf2 inhibitor using a cell-based ARE-reporter assay. Data indicated that luteolin dramatically sensitized A549 cells to the anticancer drugs oxaliplatin, bleomycin, and doxorubicin, suggesting its potential application as a natural sensitizer in chemotherapy [55]. Studies have suggested that luteolin could sensitize oxaliplatin-resistant colorectal cancer (CRC) cells to chemotherapeutic drugs through inhibition of the Nrf2 pathway [56]. Studies have also demonstrated that luteolin could suppress the sphere formation of breast cancer stem cells (CSCs) and further enhance the chemosensitivity of anticancer drug taxol through downregulating Nrf2 expression via inhibiting the antioxidant genes HO-1 and Cripto-1, which have been known to contribute to CSC features. These findings indicate that luteolin may be a potential therapeutic agent for the cancer stemness-targeted breast cancer treatment [57].

Wogonin

Wogonin (5,7-dihydroxy-8-methoxyflavone), one of the major flavonoids isolated from the root of *Scutellaria baicalensis*, is a promising anticancer candidate owing to its anti-proliferative, apoptotic, cell migration-inhibitory, angiogenesis-inhibitory and differentiation-inducing activities [58]. Studies have

shown that wogonin could effectively combat chemoresistance in the Adriamycin (ADR)-induced resistant human chronic myelogenous leukemia (CML) K562/A02 and potentiated the inhibitory effect of ADR on leukemia *in vivo* through inhibiting Nrf2 signaling by Stat3/NF-kB inactivation [59]. Similarly, other studies also reported that wogonin had strong reversal potency in the ADR-induced resistant human CML K562/A02 cells through inhibition of MRP1, decrease of the binding ability of Nrf2 to ARE and modulation of Nrf2 through reduction of Nrf2 mRNA in transcriptional processes [60]. These studies strongly suggest that being a Nrf2 inhibitor wogonin holds good potential as an efficient natural sensitizer for anti-neoplastic resistant human myelogenous leukemia.

Chrysin

Chrysin (5,7-dihydroxyflavone), a natural flavonoid, is derived from many plant extracts such as blue passionflower, propolis and honey, all of which are widely used as herbal medicine in China. Chrysin has been demonstrated to possess multiple bioactivities such as antioxidant, anti-inflammatory, antibacterial and antitumor actions through experiments using a variety of human cancer cell lines [61]. Chrysin could inhibit the cell proliferation, migration and invasion capacity in glioblastoma multiforme and inhibit tumor growth in U87 xenografts through inhibiting nuclear localization of Nrf2 and suppressing the expression of hemeoxygenase-1 (HO-1) and NAD(P)H quinine oxidoreductase-1 [62]. In doxorubicin resistant HCC BEL-7402/ADM cells, chrysin could significantly enhance their sensitivity to doxorubicin via inhibiting Nrf2 expression and downregulating the downstream genes such as HO-1, AKR1B10 and MRP5 [63]. These results indicate the effectiveness of using chrysin as an adjuvant sensitizer to combat chemoresistance.

Alkaloid compounds

Alkaloids are a highly diverse group of compounds containing cyclic structures with at least one basic nitrogen atom being incorporated within, and they are important chemical compounds that serve as a rich reservoir for drug discovery [64]. Numerous alkaloids have been screened from medicinal plants and herbs for their anticancer effects [65]. Among them, febrifugine derivative halofuginone, trigonelline and berberine are well known as Nrf2 inhibitors and possess potent chemotherapeutic actions.

Halofuginone

Febrifugine, a naturally occurring alkaloid, is the active ingredient found in the root of *Dichroa febrifuga*

Lour (Blue Evergreen Hydrangea), and has been used as a remedy to treat malaria, cancer, fibrosis, and inflammatory diseases [66]. Febrifugine was identified as a potential Nrf2 inhibitor through a high-throughput chemical screening programme using lung adenocarcinoma A549 cells [67]. Halofuginone is a less-toxic febrifugine-derivative that has been used as an antibiotic for animals [68]. Halofuginone also exhibited Nrf2 inhibitory activity in a dose-dependent manner, and a study showed that the Nrf2-addicted cancer cells were more susceptible to its treatment than the normal epithelial cells. For instance, the IC_{50} values of halofuginone in normal epithelial cell lines BEAS-2B and NCC16-P11were 436.3 and 442.7 nM, respectively, while the IC_{50} values for the Nrf2-addicted cancer cells KYSE70 and A549 were 114.6 and 58.9 nM, respectively. It was also shown to enhance the anticancer effects of cisplatin *in vivo* [69]. Taken together, halofuginone enhanced the chemo-sensitivity of cancer cells via suppressing Nrf2 activation. The results provide preclinical proof-of-concept evidence for halofuginone as a Nrf2 inhibitor which could be developed into therapeutic agent for chemoresistance.

Trigonelline

Coffee extracts were found to be modulators of Nrf2 nuclear translocation and ARE-dependent gene expression [70]. Trigonelline, a coffee alkaloid that is abundantly present in coffee beans and many plants, efficiently decreased the basal and tBHQ-induced Nrf2 activity in several pancreatic carcinoma cell lines. In addition to inhibition of Nrf2, trigonelline also prevented the Nrf2-dependent expression of proteasomal genes. The sensitivity of pancreatic cancer cell lines to anticancer drugs, and tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis was potentiated by trigonelline [71].

Berberine (BBR)

BBR is an isoquinoline alkaloid derived mainly from Coptidis Rhizoma, which is officially listed in the Chinese Pharmacopoeia and commonly used for the treatment of diabetes [72]. BBR was recently found to exert antineoplastic activity in HCC by inducing oxidative stress and enhancing radiosensitivity via suppressing Nrf2 signaling pathway in HCC cells. Later, BBR was also found to enhance the growth inhibitory effect of radiation in a Nrf2-dependent manner in a xenograft mouse model [73]. Further studies also suggested that BBR could reverse lapatinib resistance of Her2-positive breast cancer cells by upregulating the level of ROS through inhibiting Nrf2 pathway [74]. The above results have

amply demonstrated that BBR is a promising sensitizer for chemotherapy.

Quassinoid compounds

Quassinoids are a group of compounds extracted from plants of the Simaroubaceae family, which have been used for many years in folk medicine. Quassinoids have attracted increasing attention due to their promising biological activities, especially in relation to active anticancer principles [75]. Among them, brusatol and brucein D (BD) are the most studied quassinoids, and have been identified as Nrf2 inhibitors with potent anti-tumor activities.

Brusatol

Brusatol, a quassinoid first isolated from the seeds of *Brucea javanica* (Yadanzi in Chinese), which is known to possess heat-clearing and detoxification, and anti-malarial effects in Chinese medicine practice. As a quassinoid compound, brusatol specifically suppresses Nrf2 signaling, and sensitizes a broad spectrum of cancer cells to anticancer agents [76, 77]. Additionally, it has been identified as a Nrf2 inhibitor and inhibited the Nrf2 transcriptional signature to enhance the chemosensitivity to several chemotherapeutics [78]. Brusatol was also evaluated as an experimental therapeutic for P-388 lymphocytic leukemia model, and the results showed that brusatol exhibited potent inhibitory effect on tumor metabolism and proliferation through interfering with protein synthesis [79]. A follow-up study further indicated that treatment with brusatol could augment the sensitivity of anticancer drug cytarabine and daunorubicin to acute myeloid leukemia (AML) cells, and reduce its colony formation ability by downregulating the protein expression of Nrf2. Brusatol exerted minimal effects on early apoptosis in AML cells at high concentration [80]. It has also been shown that brusatol sensitizes lung cancer cell A549 and xenografts to chemotherapeutic drugs, including carboplatin, etoposide, 5-fluorouracil and paclitaxel. The effects were found to be associated with downregulation of the protein expression of Nrf2 and accumulation of ROS, resulting in DNA damage and enhancement of radiation-sensitivity [67, 81].

Mechanistically, brusatol inhibits Nrf2 to overcome chemoresistance through inhibition of protein translation [82]. Also, brusatol decreased A549 cell viability and induced apoptosis independent of caspase-3 via the inhibition of Nrf2 activity [83]. Further research also showed that brusatol decreased the viability and sensitized CRC cells to irinotecan toxicity, and abrogated CRC tumor growth in subcutaneously and orthotopically-allografted mice through inhibiting Nrf2 [84]. Additionally, brusatol

provokes a rapid and transient depletion of Nrf2 protein through a posttranscriptional mechanism in mouse Hepa-1c1c7 hepatoma cells and inhibits Nrf2 in freshly isolated primary human liver cancer cells [85]. In another study conducted on melanoma A375 cells, co-treatment with brusatol and ultraviolet A resulted in an inhibition of A375 cell proliferation and reduction of melanoma-derived tumor burden, and the anticancer effects were through triggering ROS-dependent apoptosis and blocking AKT-Nrf2 pathway [86]. Furthermore, brusatol demonstrated significantly inhibitory effects against breast cancer MCF-7 cells with IC$_{50}$ 0.08 μM/L and improved the sensitivity of MCF-7 cells to Taxol via suppressing Nrf2 pathway and enhancing ROS levels of the breast cancer cells [87]. Brusatol was also found to exert inhibitory effect on glioma, and its mechanisms were associated with the Nrf2 pathway blockage via suppressing glutathione synthesis and accumulating ROS, eventually leading to tumor cell damage [88]. These findings have amply demonstrated that the inhibitory effect of brusatol on Nrf2 is associated with the global inhibition of protein synthesis, suggesting that Nrf2 signaling can be used as an anticancer therapeutic target, and brusatol as a naturally occurring potent Nrf2 inhibitor is worthy of further development into anticancer therapeutics.

Brucein D (BD)

BD, another quassinoid compound, was also first isolated from the seeds of *Brucea javanica.* This herbal plant has been used in Chinese medicine practice to treat inflammation, malaria, warts and cancers [89]. BD has been demonstrated in a number of studies to exhibit excellent anticancer activities owing to its ability to inhibit proliferation, induce apoptosis and improve chemoresistance [89-91]. For instance, BD was able to inhibit antiapoptotic activity elicited by NF-κB activation in pancreatic cancer cells [92], and BD treatment also induced pancreatic cell apoptosis through the activation of p38-mitogen activated protein kinase and mitochondrial pathways [93, 94]. Besides, BD exerted anti-migratory activity by repressing PI3K/Akt signaling in breast cancer cells [95]. Study has also shown that BD could effectively inhibit human pancreatic ductal adenocarcinoma (PDAC) cell proliferation, induce apoptosis, and enhance the chemosensitivity of gemcitabine in a genetically engineered Kras^{tm4Tyj} Trp53^{tm1Brn} Tg (Pdx1-cre/Esr1*) #Dam/J (KPC) mouse model through inhibiting Nrf2 expression via promoting the ubiquitin-proteasome-dependent degradation and downregulating its downstream genes such as HO-1, NQO1, AKR1B10 and γGCSm [96].

Other promising compounds

Other natural compounds, such as cryptotanshinone (CTS), ginsenoside Rd (GS-Rd), ascorbic acid (AA), triptolide (TPL), have also been found to exert antineoplastic effects through suppressing Nrf2 activities.

Cryptotanshinone (CTS)

CTS, also named cryptotanshinone or tanshinone C, is a cell-permeable diterpene quinone. It is also one of the major tanshinones (including tanshinone I, tanshinone IIA, dihydrotanshinone and cryptotanshinone) derived from the root of *Salvia miltiorrhiza* (Danshen in Chinese). CTS has been demonstrated to be a potent antitumor agent due to its ability to induce cell death and apoptosis in human lung carcinoma A549 (A549/DDP) cells and enhance the sensitivity of cisplatin by down-regulating Nrf2 pathway [97].

Ginsenoside Rd (GS-Rd)

Ginsenosides are the major active ingredients of ginseng and display a wide variety of pharmacological effects, including antioxidant, anti-inflammatory, immunomodulatory, vascular protective, neuroprotective, and anticancer activities [98]. GS-Rd, one of the principal active constituents of ginsenosides, is a potent antitumor agent [99]. Studies conducted on non-small-cell lung cancer (NSCLC) A549 cells demonstrated that GS-Rd could inhibit the proliferation of NSCLC A549 cells in a dose and time-dependent manners, and renders cells more sensitive to anticancer drug cisplatin, with combination index values much smaller than 1.0, indicating a synergistic action. Collectively, treatment with GS-Rd led to the inhibition of A549 cells through a marked depletion of the expression of Nrf2 and its target genes, resulting in an enhanced sensitivity of A549 cells to cisplatin and amelioration of chemoresistance [100]. Considering its effective medicinal use, GS-Rd and other ginseng compounds have good potential for combination therapy of NSCLC.

Ascorbic acid (AA)

AA, also known as vitamin C, is a powerful antioxidant and cofactor that participates in diverse enzymatic reactions, and has been shown to have anticancer property by selectively inducing ROS in cancer cells [101]. AA suppresses oxidative stress to inhibit the nuclear translocation of Nrf2, and treatment with AA sensitizes cells to imatinib in imatinib-resistant chronic myelogenous leukemia KCL22/SR cells [102]. However, in another study, AA was shown to induce the production of high levels of hydrogen peroxide, resulting in inhibition rather than activation of Nrf2 and HO-1 expression in Hug7 liver cancer cells [103]. Thus, further work is needed to determine the involvement of Nrf2 pathway associated with the anticancer effect of AA.

Triptolide (TPL)

TPL is a diterpenoid epoxide originated from *Tripterygium wilfordii*. It has been used in Chinese medicine to treat autoimmune and inflammatory diseases, such as rheumatoid arthritis and lupus erythematosus, for a long time. Recent studies have demonstrated that TPL exhibits antitumor properties against various cancer types, such as breast, lung, and pancreatic cancer. Of significance, TPL has been found to increase the sensitivity of a drug-resistant human leukemia cell line to chemotherapeutic agents such as doxorubicin and imatinib by reducing the expression of Nrf2. Further studies identified that TPL significantly reduced Nrf2 expression and transcriptional activity in NSCLC and liver cancer cells, which resulted in chemosensitivity to antitumor drugs *in vitro* and in a xenograft tumor model system using lung carcinoma cells [104]. Additionally, TPL was shown to induce cardiotoxicity through oxidative stress, downregulate Nrf2 activation, and the mitochondria-mediated apoptotic signaling pathway [105]. However, inhibiting Nrf2 activity in combination with a neomorphic IDH1 mutation resulted in synergistic lethality. TPL was also found to be a potent Nrf2 inhibitor, and could induce apoptotic changes in IDH1-mutated cells through redox catastrophe [106]. Above studies indicated that TPL could target the Nrf2 pathway, thereby preventing cancer progression. Further investigation is warranted to evaluate the efficacy and safety of TPL in clinical cancer trials.

Collectively, these data manifest that natural products and their derivatives might be promising anticancer agents. Although additional studies are required to better characterize the toxicity profile as well as the efficacy of these agents in specific clinical contexts, there is undisputable evidence that these compounds hold great promises in the treatment of malignant tumors, particularly in those cancer types with Nrf2 aberrant expression.

Recent Insights in the Clinical Investigation of Natural Nrf2 Inhibitors

Nrf2 inhibitors are natural or synthetic compounds that are designed to target the Nrf2 pathway in order to exert therapeutic effects in various diseases, including cancer. Although no Nrf2 targeted therapy has been approved for cancer treatment, the results of some early-phase clinical trials on targeting Nrf2 have revealed promising results for cancer

treatment. For instance, halofuginone is being tested in Phase I/II clinical trials for the treatment of AIDS-related Kaposi Sarcoma and recurrent Kaposi Sarcoma (NCT00027677 and NCT00064142, respectively). Other clinical trials have assessed the safety and antitumor activity of BBR in patients with colorectal, lung and gastric carcinoma (NCT03281096, NCT03486496, NCT04697186, NCT03609892, respectively). **Table 2** presents the Nrf2 inhibitors that have entered into clinical trials (http://clinicaltrials.gov/).

Table 2. Overview of the clinical trials on natural Nrf2 inhibitors for cancer treatment.

Compounds	Study design	Intervention/treatment	Tumor types	Phase	Status	Primary endpoint	Clinical trial identifier
Apigenin	17 participants, Non-Randomized, Crossover Assignment, Open label	Chamomile Tea	Health	N/A	Completed	Metabolites in plasma/urine	NCT03526081
Luteolin	4 participants, Non-Randomized, Parallel Assignment,	Luteolin, nano-luteolin	Tongue Neoplasms Carcinoma	Early I	N/R	gene expression of Caspase 3 to detect apoptosis	NCT03288298
Wogonin	/	/	/	/	N/R	/	N/A
Chrysin	/	/	/	/	N/R	/	N/A
Halofuginone	25 participants	halofuginone hydrobromide	Unspecified Adult Solid Tumor, Protocol Specific	I	Completed	N/R	NCT00027677
	30 participants, Randomized, Parallel Assignment,	halofuginone hydrobromide , placebo	AIDS-related Kaposi Sarcoma, Recurrent Kaposi Sarcoma	II	Completed	Safety, RR	NCT00064142
Trigonelline	/	/	/	/	N/R	/	N/A
Berberine	1000 participants, Randomized, Parallel Assignment,	Berberine hydrochloride, placebo	Colorectal Adenomas	II-III	Completed	Incidence rate	NCT03281096
	50 participants, Single Group Assignment, open label	Gefitinib, berberine	Lung Adenocarcinoma EGFR Mutation	II	N/R	PFS; Safety	NCT03486496
	18 participants, Randomized, Parallel Assignment	Berberine chloride, laboratory biomarker analysis, placebo administration	Ulcerative Colitis	I	Completed	Safety	NCT02365480
	524 participants, Randomized, Parallel Assignment	Berberine, amoxicillin, rabeprazole, clarithromycin, bismuth	Gastric Cancer	IV	Enrolling by invitation	Helicobacter pylori, symptoms effective rate	NCT04697186
	658 participants, Randomized, Parallel Assignment	Berberine, amoxicillin, esomeprazole bismuth, etracycline, furazolidone	Gastric Cancer	IV	Completed	Helicobacter pylori, symptoms effective rate	NCT03609892
	1108 participants, Randomized, Parallel Assignment	Berberine hydrochloride, placebo	Colorectal Adenoma	II-III	Completed	Recurrence rates	NCT02226185
	100 participants, Randomized, Parallel Assignment	Berberine hydrochloride	Colorectal Adenomas	II-III	Completed	Measured tumor	NCT03333265
Brusatol	/	/	/	/	N/R	/	N/A
Brucein D	/	/	/	/	N/R	/	N/A
Cryptotanshinone	/	/	/	/	N/R	/	N/A
Ginsenoside Rd	/	/	/	/	N/R	/	N/A
Ascorbic acid	30 participants, Randomized, Parallel Assignment	Ascorbic acid	Breast Cancer	I-II	N/R	Safety, tolerability and RR	NCT03175341
	21 participants, Single Group Assignment, open label	Ascorbic acid	Bladder Cancer	I-II	Recruiting	Pathological Staging	NCT04046094
	200 participants, Randomized, Parallel Assignment	Ascorbic acid in combination with mFOLFOX6	Gastric Cancer	III	N/R	PFS, OS, RR	NCT03015675
	31 participants, Single Group Assignment, open label	Vitamin C	Prostatic Neoplasms	II	Completed	Biomarkers change (PSA, bALP, NTX, PINP)	NCT01080352
	15 participants, Single Group Assignment, open label	Gemcitabine with escalating ascorbic acid	Pancreatic Neoplasms	I	Completed	Blood cell counts	NCT01049880
	97 participants, Randomized, Parallel Assignment	Vitamin C + supportive care	Stage IIIA-B, IV NSCLC Recurrent NSCLC	I-II	Completed	Safety, tolerability, anti-tumor activity, PFS, OS	NCT02655913
	5 participants,	Ascorbic acid in combination	Metastatic Hepatocellular	I-II	Completed	Safety, overall	NCT01754987

Compounds	Study design	Intervention/treatment	Tumor types	Phase	Status	Primary endpoint	Clinical trial identifier
	Non-Randomized, Parallel Assignment	with sorafenib	Carcinoma Advanced Liver Cancer			tumor response rate	
	60 participants, Randomized, Parallel Assignment	Vitamin C, E and Zinc	Skin Neoplasms	N/R	Completed	Oxidative stress biomaker	NCT02248584
	42 participants, Randomized, Parallel Assignment	Nutritional supplement	Head and Neck Cancer	N/R	Completed	POSAS	NCT03531190
Triptolide	30 participants, Single Group Assignment, open label	Procedure: Biopsy; osimertinib, triptolide analog	Advanced lung non-small cell carcinoma	I	Recruiting	MTD, PFS, OS	NCT05166616
	66 participants, Non-Randomized, Parallel Assignment,	Minnelide™Capsules	Gastric, breast, pancreatic, prostate, colorectal cancer, solid tumor, solid carcinoma of stomach, cancer of stomach	I	Recruiting	Safety, anti-tumor activity	NCT03129139
	55 participants, Single Group Assignment, open label	Minnelide	Adenosquamous carcinoma of the pancreas	II	Recruiting	DCR, PFS, safety, tolerability, OS	NCT04896073
	19 participants, Single Group Assignment, open label	Minnelide	Pancreatic cancer	II	Completed	DCR, PFS, OS, RR	NCT03117920

The list above did not include those studies that were either suspended or terminated prematurely. (N/A, not applicable; N/R, not reported; NSCLC, non-small cell lung cancer; POSAS, The patient and observer scar assessment scale; MTD, Maximum tolerated dose; PFS, Progression-free survival; OS, Overall survival; DCR, Disease control rate; RR, Response rate).

However, the safety of Nrf2 inhibitors is an area of ongoing research and investigation. Some studies have raised concerns about potential adverse effects of Nrf2 inhibitors, including liver toxicity, adverse cardiovascular effects, and potential interactions with other medications. Additionally, the safety and efficacy of any natural products or synthetic compounds can vary depending on the specific dose, route of administration, and individual patient factors. It is worth bearing in mind that the safety and efficacy of Nrf2 inhibitors need to be carefully evaluated before they can be used as cancer therapeutics.

Discussion and Conclusions

Transcription factor Nrf2 constitutes an attractive drug target for the treatment of various cancers owing to its capacity of controlling the intracellular redox homeostasis and its ability to activate cytoprotective antioxidant genes. Hence, attention should be paid to the potential role of Nrf2/Keap1 pathway in interaction with anticancer drugs. Notably, natural compounds are well known for their multiple-targets, multi-pathways and relatively less toxic in cancer treatment. Nrf2 inhibitors have shown potential advantages as a cancer therapy, including sensitizing cancer cells to chemotherapy and overcoming drug resistance. The exact molecular mechanisms underlying these effects may vary depending on the specific inhibitor used and the type of cancer being targeted. For example, some Nrf2 inhibitors may work by directly binding to and blocking Nrf2 activity, while others may affect upstream regulators or downstream effectors of the pathway. Moreover, different cancer types may have unique dependencies on Nrf2, which can influence the therapeutic response to Nrf2 inhibition. Studies have shown that Nrf2 is overexpressed in a variety of cancer types, including lung, breast, ovarian, pancreatic, colorectal, and liver cancer. Therefore, Nrf2 blocking therapy may be more effective in treating these types of cancer. However, further research is needed to fully understand the molecular mechanisms of Nrf2 inhibitors in different cancers. Direct comparisons between different inhibitors and cancer types would be helpful in elucidating the specific mechanisms of action and identifying optimal treatment strategies.

This review has outlined the current state of Nrf2 inhibitors derived from natural sources and summarize their corresponding molecular mechanisms in regulating Nrf2 for cancer treatment. The natural compounds discussed in this review possess promising anticancer activity as evidenced by *in vitro* and *in vivo* experimental findings, and some of them have already been subjected to clinical trials. However, clinical research on selective Nrf2 inhibitors is still in its infancy, and some limitations to their use must be noted. One such limitation is their potential lack of specificity [107], since naturally occurring Nrf2 inhibitors may interact with multiple pathways. Therefore, Nrf2 inhibitors may possess a potential risk of unintended side effects or toxicity. The lack of specificity can give rise to difficulty in identifying the exact mechanism by which they exert their effects [108]. Another limitation is the potency and bioavailability of natural product-derived Nrf2 inhibitors, which can vary widely. Some natural

products may have low potency, while others may have limited bioavailability due to factors such as poor solubility and rapid metabolism. These issues can affect the efficacy of the Nrf2 inhibitor in a clinical setting [27]. This can create challenges for regulatory approval and commercialization of natural product-derived Nrf2 inhibitors. These limitations need to be addressed before their safe and effective use in clinical settings.

Taken together, Nrf2 pathway is essential for tumorigenesis, tumor progression and resistance to therapy, and is clearly a promising target for cancer therapy, especially in cancers with high Nrf2 activity. The development of more specific and effective Nrf2 inhibitors will be necessary to improve the therapeutic potential of these drugs. Particularly, molecular docking model, a critical approach in drugs discovery and development, can be used to predict the binding affinity and orientation of ligands (natural inhibitors) with the target protein Nrf2 based on their 3D chemical structures [109]. We anticipate that further in-depth studies such as biological and structure-activity relationship investigations on the natural Nrf2 inhibitors may eventually help develop naturally occurring Nrf2 inhibitors into anticancer therapeutics.

Abbreviations

AA: ascorbic acid; ADM: doxorubicin; ADR: adriamycin; APG: Apigenin; ARE: antioxidant-responsive element; AML: acute myeloid leukemia; BBR: berberine; BD: brucein D; b-Zip: basic leucine Zipper domain; CHM: Chinese herbal medicine; CML: chronic myelogenous leukemia; CRC: colorectal cancer; CSCs: cancer stem cells; CTS: cryptotanshinone; CUL3: Cullin 3; EMT: epithelial-mesenchymal transition; GS-Rd: ginsenoside Rd; HCC: hepatocellular carcinoma; HO-1: hemeoxygenase-1; Keap1: KELCH-like ECH-associated protein 1; Neh: Nrf2-ECH homology; Nrf2: Nuclear factor erythroid 2-related factor 2; NSCLC: non-small-cell lung cancer; PDAC: pancreatic ductal adenocarcinoma; PI3K: phosphatidylinositol-3 kinase; ROS: reactive oxygen species; sMafs: small musculoaponeurotic fibrosarcoma proteins; TPL: triptolide; TRAIL: tumor necrosis factor-related apoptosis inducing ligand.

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Authors contributions

JZ, ZXL, HXX and YFX were the major contributors to design the study. JZ was the major contributor to manuscript writing, and figures creating. JQZ assisted in the data collection. YXD drew the chemical structures of compounds. ZXL and YFX revised the manuscript. All authors have read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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