Natural products for cancer prevention associated with Nrf2–ARE pathway

Xianjuan Kou [a,1], Michael Kirberger [b,1], Yi Yang [a], Ning Chen [a,c,*]

[a] *College of Health Science, Wuhan Sports University, Wuhan 430079, China*
[b] *Department of Natural Sciences, Clayton State University, Morrow, GA 30260, USA*
[c] *The Cancer Institute of New Jersey, New Brunswick, NJ 08903, USA*

Abstract

Cancer chemoprevention involves the application of natural or synthetic compounds to reduce the risk of cancer development. One of the most effective strategies for preventing human cancers might involve inducing phase II detoxifying enzymes and antioxidant enzymes *via* natural dietary compounds. The regulatory regions of these inducible genes encode the antioxidant response element (ARE). Nuclear factor-erythroid 2-related factor 2 (Nrf2), as a transcription factor, plays a key role in the expression of ARE-mediated genes. Similarly, Nrf2 performs an essential function in the up-regulation of these genes in response to oxidative stress and treatment with dietary phytochemicals. In this article, we discuss the current state of knowledge regarding the Nrf2/ARE pathway as a potential molecular target for cancer chemoprevention and its molecular regulation mechanisms, and highlight Nrf2/ARE inducers derived from natural products, which may be used as chemopreventive agents for cancer patients.
© 2013 Beijing Academy of Food Sciences. Production and hosting by Elsevier B.V. All rights reserved.

Keywords: Natural products; Cancer prevention; Nrf2–ARE pathway; Tumorigenesis; Dietary phytochemicals

1. Introduction

Tumorigenesis is a multiple-stage and multiple-factor process, typically divided into three stages, including initiation, promotion and progression [1]. Despite extensive global research aimed at ameliorating the dismal outcomes of cancers, no obvious decline in the overall mortality rate of most cancers has been observed in the past 30 years. During this same period, chemoprevention has gained much attention as a realistic strategy to fight against cancers. Emerging evidence has shown that phytochemicals in vegetables and fruits may serve as potential chemopreventive agents that also have the advantage of exhibiting relatively low toxicity, low cost, and are recognized as important components of healthy foods [2].

These phytochemicals have also been linked with the induction of cellular defense detoxifying/antioxidant enzymes, but more importantly, dietary phytochemicals can induce apoptotic cell death in pre-neoplastic or neoplastic cells through different growth inhibitory mechanisms, including the activation of cytochrome C (Cyt C)/caspase and cell cycle arrest, and the inhibition of nuclear factor-κB (NF-κB), Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathways, resulting in the inhibition of tumor progression [3,4].

Nuclear factor-erythroid 2-related factor 2 (Nrf2), a member of the Cap "n" collar (CNC) family of basic region-leucine zipper (bZIP) proteins, plays an important role in mediating ARE-dependent gene expression [5]. In addition, previous findings have demonstrated that Nrf2 is an important modulator of susceptibility to carcinogen-induced carcinogenesis. The cytoprotective role of activated Nrf2 has been demonstrated in the susceptibility of Nrf2 knockout mice to cancers [6]; therefore, the activation of Nrf2 signaling and the induction of its target genes identify it as an important pharmacological target for cancer prevention. In addition, many phytochemicals are strong activators of Nrf2, thus they regulate the defense enzymes through the activated Nrf2 signaling pathway. Evidence continues to accumulate from both experimental and epidemiological studies demonstrating the significance of natural products in chemoprevention, which suggests that daily consumption is a promising new approach in the prevention of carcinogenesis. This review summarizes the current knowledge related to the

molecular mechanisms of Nrf2 regulation, and highlights the protective role of natural products in cancer chemoprevention *via* induction of the Nrf2–ARE-axis signaling pathway.

2. Cytoprotective enzymes regulated by Nrf2

Nrf2, also described in the literature as NF-E2 related factor-2, is a bZIP protein encoded by the nuclear factor (erythroid-derived 2)-like2 (NFE2L2) gene. NFE2L2 contains conserved JUN and FOS regions that function to form the activator protein-1 (AP-1) transcription factor for mediating various cellular processes associated with cell differentiation, proliferation and apoptosis [7–9].

Under basal resting conditions, Nrf2 appears to be associated with the repressor Kelch-like ECH-associated protein (Keap1) as an inactive Nrf2–Keap1 complex, subsequently preventing Nrf2 from entering into the nucleus. After translocation into the nucleus, Nrf2 binds to the antioxidant response element (ARE) of ARE-target genes, which leads to the enhanced expression of phase II detoxifying/antioxidant enzymes. Nrf2 protein is an important transcription factor responsible for stimulating the transcription of cytoprotective genes in response to oxidative or electrophilic stress. This process involves the binding of Nrf2 with Maf protein in the nucleus to form a heterodimer, subsequently interacting with AREs to activate gene transcription [5,10]. Downstream genes transcribed by Nrf2 may be categorized as intracellular redox balancing proteins, phase II detoxifying enzymes, or transporters responsible for the removal of harmful endogenous substances [11]. Redox balancing proteins are responsible for reducing the levels of reactive oxygen species (ROS), thioredoxin (Trx), thioredoxin reductase (TrxR), peroxiredoxin (Prx), glutamate cysteine ligase (GCL), and heme oxygenase-1 (HO-1); while the phase II detoxifying enzymes are responsible for metabolizing xenobiotics or increasing xenobiotic solubility to facilitate the removal of glutathione S transferase (GST), UDP-glucuronosyltransferase (UGT), and NAD(P)H:quinone oxidoreductase-1 (NQO1) [11,12]. Therefore, Nrf2 and its downstream genes not only guard against oxidative and electrophilic insults, but also prevent the development of cancers [13,14]. How Nrf2 and ARE-regulated genes prevent the development of cancers will be discussed in the following sections.

3. Regulation of Nrf2 activation

The core of the Nrf2 regulation is Keap1. Keap1 functions as a negative regulator of Nrf2 by promoting ubiquitination and proteasomal degradation of Nrf2 [15]. Since Keap1 has highly reactive sulfhydryl groups in its cysteine residues, it is considered as a sensor for electrophilic compounds including heavy metals and chemopreventive agents. Many xenobiotics undergoing oxidative metabolism can interact with thiol residues present in the functionally critical motifs of different proteins. Nuclear accumulation and activation of Nrf2 involves the alteration of Keap1 structure by oxidation or covalent modification of critical cysteine residues in Keap1 [16]. At present, two general mechanisms have been proposed to explain nuclear accumulation of Nrf2 in response to inducers. The first is the down-regulation of Nrf2 ubiquitination. The second mechanism involves the alteration of the nuclear import/export of Nrf2 [17]. At least one previous study has reported that a compound with high electron withdrawing potency could also function as an Nrf2 activator through the modification of cysteine residues in Keap1 [18]. For example, sulforaphane can form the thionoacyl adduct of sulforaphane–Keap1 and modify the tertiary structure of Keap1 most readily at the cysteine residues localized at the Kelch domain, thereby to stabilize Nrf2 [19]. This suggests that the selection of compounds that do not induce cellular toxicity and are capable of modifying the structure and function of Keap1 may provide important clues for the development of therapeutically applicable drugs targeting Nrf2.

In addition to covalent modification of thiol groups in Keap1, the activation of protein kinase signaling pathways including protein kinase C, mitogen activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI-3K), ER-localized pancreatic endoplasmic reticulum kinase (PERK) or casein kinase 2 (CK2), can also phosphorylate Nrf2, thus they can affect the release process of Nrf2 from the Nrf2–Keap1 complex as well as the stability and nuclear translocation of Nrf2. Research to date has suggested that the phosphorylation of Nrf2 serine and threonine residues by the above-listed kinases may be potentially involved in Nrf2-mediated signal transduction at AREs [20–23]. Previous studies have reported that PKC directly phosphorylates Nrf2 at serine 40, thereby promoting the dissociation of Nrf2 from Keap1 [24,25]. Nrf2 may also be activated by MAPKs. In MAPKs, ERK and JNK appear to enhance Nrf2 signaling pathways through the recruitment and phosphorylation of transcriptional co-activators such as p300 and PBP [26]. In contrast, p38MAPK may either stimulate or inhibit the nuclear translocation of Nrf2 depending on cell types [27,28]. Several other studies have indicated that the activation of PI-3K signaling cascades results in the activation of Nrf2 [29,30]. Moreover, Nrf2 can be a substrate for PERK that phosphorylates Nrf2 [23], and then enhances its nuclear translocation by disrupting Keap1 binding [23]. Furthermore, the phosphorylation of Nrf2 by CK2 is also a critical controlling factor in Nrf2 activity and degradation [31].

4. Potential natural chemopreventive agents

As mentioned above, the activation of the Nrf2 signaling pathway governs the expression of ARE-driven genes, and dietary chemopreventive compounds function as detoxifying enzyme inducers. For example, phase II detoxification and antioxidant enzymes act against carcinogenic insults. Currently, an increasing number of natural compounds have been found to exert chemopreventive properties against a wide spectrum of cancers by involving the Nrf2–ARE signaling pathway. The chemopreventive activities of cruciferous vegetables have been addressed in several epidemiological studies and in animal models of chemically induced carcinogenesis [32]. Phenolic and sulfur-containing compounds are two major classes of dietary components that also act as promising chemopreventive agents. Phenolic compounds, widely distributed in plants, can

be classified into polyphenols such as epigallocatechin-3-gallate (EGCG) from green tea, curcumin from turmeric, resveratrol from grapes, and flavonoids such as quercetin from citrus fruits and genistein from soybean. Sulfur-containing compounds can be generally classified into two major categories, namely, isothiocyanates including sulforaphane (SFN) from broccoli, phenethyl isothiocyanate (PEITC) from turnips and watercress, as well as allyl isothiocyanate from Brussels sprouts, and organosulfur compounds including diallyl sulfides from garlic oil [33].

4.1. Phenolic compounds

Phenolic compounds are well-known for their anti-carcinogenic activity in the edible plant kingdom. The molecular mechanisms of polyphenols acting as chemopreventive agents *in vitro* and *in vivo* have been extensively reported [34]. The stimulation of phase II detoxifying and antioxidant defense enzymes through Nrf2-signaling pathway has been considered as one of the critical mechanisms. Representative examples of these compounds, including EGCG, quercetin and curcumin, will be discussed, respectively.

4.1.1. EGCG

Green tea has attracted much attention for its beneficial health effects. Epidemiological studies have suggested that the consumption of green tea may reduce the risk of cancers. Accumulating evidence from numerous laboratory and epidemiological studies supports the concept that the consumption of green tea can complete the protective role against the development of various types of malignancies. EGCG, a putative chemopreventive agent and the major active catechin in green tea, is known to possess anti-oxidant, anti-inflammatory and chemopreventive properties. Therefore, EGCG has been identified as the most potent Nrf2 activator among green tea polyphenols due to its ability to induce the transactivation of the ARE-luciferase reporter gene [35]. EGCG has also been reported to activate Nrf2-mediated HO-1 expression in B lymphoblasts, epithelial and endothelial cells, and to stimulate the expression of many Nrf2-dependent genes in the liver and intestines of mice [36,37]. Similarly, EGCG has been reported to inhibit lipopolysaccharide-induced pulmonary fibrosis by enhancing the activities of antioxidant and phase II enzymes such as GST and NQO1 mediated by Nrf2–Keap1 signaling [38]. Additionally, the administration of EGCG by gavage can induce the expression of HO-1, γ-glutamyltransferase 1 and the catalytic subunit of GCL in an Nrf2-dependent manner, which has been confirmed by results demonstrating that EGCG does not induce the expression of these genes in Nrf2-deficient mice. Another mechanism responsible for EGCG-induced activation of Nrf2 involves the phosphorylation of serine/threonine residues of Nrf2. For example, EGCG can induce the nuclear localization of Nrf2 through the activation of ERK1/2 and the phosphorylation of Akt in MCF-10A breast cancer and Caco-2 colon cancer cells [39]. It is apparent from these data that the anti-inflammatory and anti-oxidative stress capabilities of EGCG can be potentially

utilized for cancer prevention in humans, particularly since chronic inflammation is correlated with 20% of human cancers.

Chemopreventive effects of EGCG are attributed to modulation of the intracellular signaling network responsible for proliferation, differentiation, apoptosis, adhesion, angiogenesis and metastasis associated with carcinogenesis [40,41], and EGCG appears to exert an inhibitory effect on the proliferation of bovine capillary endothelial cells [42]. In addition, the anti-cancer activity of EGCG may also reduce metastatic invasion, the major cause of mortality in cancer patients, by inhibiting the activity of urokinase or matrix metalloproteinases (MMPs), or by scavenging oxygen free radicals [43]. In human colon HT-29 cancer cells, EGCG treatment results in the damage of mitochondria and apoptosis through the JNK pathway [44]. Angiogenesis is necessary not only for nourishing the growth of tumors, but also for metastasis. Experimental evidence suggests that the administration of EGCG in drinking water for TRAMP mice is associated with the inhibition of tumors due to decreased expression of vascular endothelial growth factor (VEGF) and inhibition of angiogenesis [45]. Another study has also shown that EGCG inhibits both the growth of colon tumors, as well as metastasis through the liver or lungs, in orthotopically implanted nude mice, which is proposed to be partly due to the modulated activation of the Nrf2–UGT1A signal pathway [46].

4.1.2. Resveratrol

Resveratrol, a phytoalexin produced by a wide variety of plants, particularly in the skin and seeds of red grapes, exhibits multiple therapeutic effects, including anti-oxidative and anti-inflammatory effects as well as chemopreventive activity in several types of cancers. It is well known that resveratrol has been intensively investigated as a promising agent for cancer prevention. Previous reports have demonstrated that resveratrol, as a cancer chemopreventive molecule, mainly completes its function by triggering apoptosis, which has been confirmed by resveratrol-mediated cell growth inhibition and apoptosis induction in prostate cancer cells [47] as well as the reduced incidence of carcinogen-induced carcinogenesis in experimental animals [48]. With respect to corresponding mechanisms, Nrf2–Keap1–ARE has attracted much attention. Resveratrol has been shown to protect against H_2O_2-induced PC12 cell death through the activation of Nrf2 and increased HO-1 expression [49]. It is also reported to exhibit protective effects against H_2O_2-induced and β-amyloid-induced cell death in rat pheochromocytoma cells by attenuating intracellular ROS accumulation and restoring the level of some marker proteins related to apoptosis [50,51]. Resveratrol is postulated to function as a potential signaling pathway modulator and, as such, has demonstrated its ability to affect a multitude of signal transduction pathways associated with cancer-preventive effects. Resveratrol also can cause an arrest at the S/G2 phase transition of the cell cycle and is capable of inducing differentiation and apoptosis in a multitude of cancer cell lines, including leukemia, hepatoma, neuroblastoma, prostate cancer, colon cancer, gastric cancer and breast cancer cells [52]. In a postnatal day 7 rodent model of fetal alcohol spectrum disorders (FASD), the administration of resveratrol before ethanol exposure can restore the level of Nrf2,

and prevent ethanol-induced oxidative stress in the cerebellum [53]. In addition, resveratrol exhibits antioxidant properties by inducing GSH biosynthesis *via* the activation of Nrf2, and protects lung epithelial cells against cigarette smoke-mediated oxidative stress in human lung epithelial cells [54].

4.1.3. Curcumin

Curcumin is a naturally occurring yellow pigment, isolated from the rhizomes of the plant *Curcuma longa* (Linn), which is commonly used in Asian cooking as a coloring and flavoring agent. Studies have shown that curcumin has a wide range of biological effects, including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activities. As summarized by Hatcher et al., curcumin protects against various types of carcinomas [55]. Pleiotropic effects of curcumin are derived from its ability to affect multiple survival and cytoprotective signaling pathways, including the pathways that inhibit inflammatory responses and those regulated by NF-κB, AKT, growth factors and Nrf2 transcription factor [56]. Curcumin exerts both direct and indirect antioxidant effects by scavenging reactive oxygen species (ROS) [57] and inducing the expression of cytoprotective proteins in an Nrf2-dependent way [28]. Curcumin has been shown to induce GSTP1 expression with the involvement of transcription factor Nrf2 in human hepatic cells [58]. Pretreatment with curcumin can protect against H_2O_2-induced cell death by up-regulating HO-1 and thioredoxin that are mediated by Nrf2 transcription factor in retina-derived cell lines (661W and ARPE-19) [56]. In addition, curcumin can activate Nrf2–ARE signaling by stimulating upstream kinases. For example, curcumin can induce the expression of HO-1 by a pathway involving the transcription factor Nrf2 and PI-3K/Akt-mediated signaling pathway in mouse β cells. Moreover, curcumin can activate ARE-mediated expression of antioxidant defense genes in human monocytes *via* PKC-δ, p38MAPK and Nrf2 [17].

4.2. Sulfur-containing compounds

4.2.1. Isothiocyanates

Isothiocyanates (ITCs) are found in cruciferous vegetables such as broccoli, Brussels sprouts, cauliflower and cabbage. Sulforaphane (SFN) is a type of ITC found in cruciferous vegetables, with particularly higher levels in broccoli and Brussels sprouts. Epidemiological evidence suggests a reduced risk for prostate, lung, breast and colon cancers for people who consume cruciferous vegetables [59]. SFN has proven to be an effective chemoprotective agent in cell cultures, carcinogen-induced and genetic animal cancer models, as well as in xenograft models of cancer.

SFN was identified as a chemopreventive agent over a decade ago on the basis of its capability to induce phase II detoxification enzymes, and to inhibit phase I enzymes involved in the activation of carcinogens. SFN augments various pathways known to facilitate carcinogen detoxification and excretion. For example, mice treated with SFN at a daily dosage of 15 μmol/mouse by gavage for 5 consecutive days exhibited an increase in quinone reductase (QR) and glutathione-S-transferase (GST) in

the liver, forestomach, glandular stomach, proximal small intestine, and lungs [60], suggesting that SFN also can induce phase II enzymes *in vivo* following oral ingestion. Although many cell lines reveal a response to SFN *in vitro*, the induction and type of phase II enzymes reveal variations dependent on the different cell types. In human HepG2 cells, SFN up-regulates mRNA expression of UDP-glucuronosyltranferase (UGT) 1A1 and GSTA1 [61,62]. Similarly, upon treatment with SFN, a significant increase in GST and QR activities is also observed in murine Hepa1c1c7 cells and human prostate cells, although, an apparent decrease in GST activity in HT29 cells has also been reported [63,64]. Importantly, the effects of SFN in cell cultures can be extrapolated to *in vivo* situations. *In vivo* experiments have demonstrated the SFN-induced increase in QR and GST activities in the liver, colon and pancreas [65]. Moreover, oral treatment with SFN increases the gene expression of phase II antioxidant enzymes such as GSTM1, GSTP1, NQO1, and HO-1 in the human upper respiratory tract [66]. Additionally, the induction effects of SFN on cell cycle arrest and/or apoptosis have also been explored and confirmed in different experiments. In HT-29 human colon cancer cells, treatment with 100 μM SFN for 24 or 48 h can result in a decrease in cell viability [67], a decrease in the population of the cells in G1 phase and an increase in the population in G2/M phase, as well as an obvious increase in protein levels of cyclin A and cyclin B1 [68].

In recent years, two major mechanisms have been proposed to explain the induction of phase II enzymes by SFN. One is the disruption of Nrf2–Keap1 interaction and the other is the activation of mitogen-activated protein kinase (MAPK). The disruption of Nrf2–Keap1 interaction, translocation of Nrf2 to the nucleus, and induction of ARE-associated genes due to the application of SFN have been extensively discussed. The importance of Nrf2 is illustrated through Nrf2 knockout mice, in which the response to SFN in the induction of the aforementioned genes is abrogated. Conversely, SFN can activate ARE-driven genes by activating the MAPK pathway, however, the application of MAPK inhibitors can significantly attenuate QR activity and ARE reporter activity in HepG2 cells [69].

4.2.2. Diallyl sulfide

Garlic is a bulbous root with a strong taste and smell. Diallyl sulfide (DAS) is a flavor compound derived from garlic and is sequentially converted to diallyl sulfoxide (DASO) and diallyl sulfone (DASO2) by cytochrome $P_{450}2E1$ (CYP2E1). Epidemiological and animal studies have shown that frequent consumption of garlic and other allium vegetables may be associated with decreased incidence of gastric, chemical-induced skin, cervical, forestomach, lung, colon and esophageal cancers [70].

Research over the years has revealed that the chemopreventive activity of garlic targets multiple pathways to inhibit the growth of cancer cells *via* carcinogen metabolism impairment, cell cycle arrest, apoptosis induction, and angiogenesis inhibition. It is well known that allyl sulfur compounds can modulate drug metabolism systems, especially different phase II detoxifying enzymes. DAS has also been shown to inhibit phase I enzymes and induce phase II enzymes such as GST-π,

GST-α and GST-μ in rat liver [71,72]. Another study has also described the roles of DAS in the expression of ARE-mediated genes and Nrf2, NQO1 and HO-1 proteins [73]. Wattenberg and colleagues have demonstrated that organosulfur compounds (OSCs) in garlic may prevent benzopyrene-induced forestomach and lung cancers in mice, and this process is accompanied by observed elevation of hepatic and target organ glutathione transferase activity [74]. One recent study has shown that DAS can cause a striking increase in GSTMu2 and epoxide hydrolase [75]. Collectively, these investigations suggest that OSCs can execute preventive functions for chemical-induced cancers not only by inhibiting the activation of carcinogens, but also by enhancing the detoxification of activated carcinogenic intermediates through the induction of phase II enzymes.

Currently, the underlying mechanism of DAS on the inductive effect of phase II enzymes is still unclear although several hypotheses have been proposed. Extensive studies have demonstrated that diallyl sulfides could induce drug-metabolizing enzymes such as NQO1 and HO-1 in an Nrf2/ARE-dependent manner because DATS-induced expression of several detoxifying enzyme genes is observed in wild-type mice, but not in Nrf2 knockout mice [76]. Similarly, other investigators have reported that DAS can induce HO-1 through the production of ROS that is mediated by Nrf2 and MAPK.

4.2.3. Others

Other natural chemopreventive agents that may induce phase II detoxifying enzymes through the Nrf2/ARE pathway include dithiolethiones and anethole dithiolethione. Dithiolethione has been reported to exert chemopreventive properties in the lung, colon and liver. Similarly, dithiolethiones appear to exhibit chemopreventive activity against aflatoxin-mediated hepatocarcinogenesis [77].

5. Conclusion

Two classes of potential natural chemopreventive agents as described in this review have been extensively studied and many other compounds are under investigation for their regulation of this protective mechanism. Gaining a better understanding of the molecular mechanisms associated with these compounds will provide beneficial guidance for developing new strategies in cancer prevention. An increasing body of evidence supports the premise that the Nrf2/ARE pathway plays a critical role in the protective mechanism of cells through the induction of phase II detoxifying and antioxidant enzymes. Thus, the identification of factors influencing the Nrf2 pathway provides new insight into fighting cancers. Natural compounds represent an effective and rational strategy for cancer prevention due to their ability to induce the expression of phase II detoxification *via* Nrf2–ARE pathway, as well as their relatively low toxicity, low cost and resource abundance. Due to the structural diversity among these chemopreventive agents targeting the Nrf2/ARE signaling pathway and the complexity of the upstream cellular signaling events, substantial research is still needed to elucidate the complex regulation of the Nrf2/ARE signal transduction pathway. Moreover, most investigations of natural products are focused on *in vitro* experiments, but the improved understanding of the roles of natural products in chemoprevention will necessarily include data from *in vivo* studies in future, as well.

References

[1] S.H. Moolgavkar, The multistage theory of carcinogenesis and the age distribution of cancer in man, Journal of the National Cancer Institute 61 (1) (1978) 49–52.

[2] W.S. Jeong, I.W. Kim, R. Hu, et al., Modulatory properties of various natural chemopreventive agents on the activation of NF-kappaB signaling pathway, Pharmaceutical Research 21 (4) (2004) 661–670.

[3] Y.S. Keum, W.S. Jeong, A.N. Kong, Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms, Mutation Research 555 (1/2) (2004) 191–202.

[4] C. Chen, A.N. Kong, Dietary cancer-chemopreventive compounds: from signaling and gene expression to pharmacological effects, Trends in Pharmacological Sciences 26 (6) (2005) 318–326.

[5] D.D. Zhang, Mechanistic studies of the Nrf2–Keap1 signaling pathway, Drug Metabolism Reviews 38 (4) (2006) 769–789.

[6] J.W. Fahey, X. Haristoy, P.M. Dolan, et al., Sulforaphane inhibits extracellular, intracellular, and antibiotic–resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors, Proceedings of the National Academy of Sciences of the United States of America 99 (11) (2002) 7610–7615.

[7] M. Ameyar, M. Wisniewska, J.B. Weitzman, A role for AP-1 in apoptosis: the case for and against, Biochimie 85 (8) (2003) 747–752.

[8] E. Shaulian, M. Karin, AP-1 as a regulator of cell life and death, Nature Cell Biology 4 (5) (2002) E131–E136.

[9] R. Eferl, E.F. Wagner, AP-1: a double-edged sword in tumorigenesis, Nature Reviews Cancer 3 (11) (2003) 859–868.

[10] Y.J. Surh, J.K. Kundu, H.K. Na, Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals, Planta Medica 74 (13) (2008) 1526–1539.

[11] A. Lau, N.F. Villeneuve, Z. Sun, et al., Dual roles of Nrf2 in cancer, Pharmacological Research 58 (5/6) (2008) 262–270.

[12] Y. Li, J.D. Paonessa, Y. Zhang, Mechanism of chemical activation of Nrf2, PLoS ONE 7 (4) (2012) e35122.

[13] X.L. Chen, C. Kunsch, Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases, Current Pharmaceutical Design 10 (8) (2004) 879–891.

[14] J.S. Lee, Y.J. Surh, Nrf2 as a novel molecular target for chemoprevention, Cancer Letters 224 (2) (2005) 171–184.

[15] M. Furukawa, Y. Xiong, BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase, Molecular and Cellular Biology 25 (1) (2005) 162–171.

[16] J.M. Lee, J.A. Johnson, An important role of Nrf2–ARE pathway in the cellular defense mechanism, Journal of Biochemistry and Molecular Biology 37 (2) (2004) 139–143.

[17] C.R. Zhao, Z.H. Gao, X.J. Qu, Nrf2–ARE signaling pathway and natural products for cancer chemoprevention, Cancer Epidemiology 34 (5) (2010) 523–533.

[18] H.K. Na, Y.J. Surh, Transcriptional regulation via cysteine thiol modification: a novel molecular strategy for chemoprevention and cytoprotection, Molecular Carcinogenesis 45 (6) (2006) 368–380.

[19] A.T. Dinkova-Kostova, W.D. Holtzclaw, T.W. Kensler, The role of Keap1 in cellular protective responses, Chemical Research in Toxicology 18 (12) (2005) 1779–1791.

[20] D.A. Bloom, A.K. Jaiswal, Phosphorylation of Nrf2 at Ser40 by protein kinase C in response to antioxidants leads to the release of Nrf2 from INrf2, but is not required for Nrf2 stabilization/accumulation in the nucleus and transcriptional activation of antioxidant response element-mediated

NAD(P)H:quinone oxidoreductase-1 gene expression, Journal of Biological Chemistry 278 (45) (2003) 44675–44682.

[21] R. Yu, C. Chen, Y.Y. Mo, et al., Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism, Journal of Biological Chemistry 275 (51) (2000) 39907–39913.

[22] K.W. Kang, S.H. Choi, S.G. Kim, Peroxynitrite activates NF-E2-related factor 2/antioxidant response element through the pathway of phosphatidylinositol 3-kinase: the role of nitric oxide synthase in rat glutathione S-transferase A2 induction, Nitric Oxide 7 (4) (2002) 244–253.

[23] S.B. Cullinan, J.A. Diehl, PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress, Journal of Biological Chemistry 279 (19) (2004) 20108–20117.

[24] H.C. Huang, T. Nguyen, C.B. Pickett, Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription, Journal of Biological Chemistry 277 (45) (2002) 42769–42774.

[25] S. Numazawa, M. Ishikawa, A. Yoshida, et al., Atypical protein kinase C mediates activation of NF-E2-related factor 2 in response to oxidative stress, American Journal of Physiology – Cell Physiology 285 (2) (2003) C334–C342.

[26] P. Misra, E.D. Owuor, W. Li, et al., Phosphorylation of transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)-binding protein (PBP), Stimulation of transcriptional regulation by mitogen-activated protein kinase, Journal of Biological Chemistry 277 (50) (2002) 48745–48754.

[27] R. Yu, S. Mandlekar, W. Lei, et al., p38 mitogen-activated protein kinase negatively regulates the induction of phase II drug-metabolizing enzymes that detoxify carcinogens, Journal of Biological Chemistry 275 (4) (2000) 2322–2327.

[28] E. Balogun, M. Hoque, P. Gong, et al., Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element, Biochemical Journal 371 (Pt 3) (2003) 887–895.

[29] K.W. Kang, M.K. Cho, C.H. Lee, et al., Activation of phosphatidylinositol 3-kinase and Akt by tert-butylhydroquinone is responsible for antioxidant response element-mediated rGSTA2 induction in H4IIE cells, Molecular Pharmacology 59 (5) (2001) 1147–1156.

[30] K. Nakaso, H. Yano, Y. Fukuhara, et al., PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells, FEBS Letters 546 (2/3) (2003) 181–184.

[31] J. Pi, Y. Bai, J.M. Reece, et al., Molecular mechanism of human Nrf2 activation and degradation: role of sequential phosphorylation by protein kinase CK2, Free Radical Biology and Medicine 42 (12) (2007) 1797–1806.

[32] P. Talalay, J.W. Fahey, Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism, Journal of Nutrition 131 (11 Suppl.) (2001) 3027S–3033S.

[33] C. Chen, A.N. Kong, Dietary chemopreventive compounds and ARE/EpRE signaling, Free Radical Biology and Medicine 36 (12) (2004) 1505–1516.

[34] Y.J. Surh, Cancer chemoprevention with dietary phytochemicals, Nature Reviews Cancer 3 (10) (2003) 768–780.

[35] C. Chen, R. Yu, E.D. Owuor, et al., Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death, Archives of Pharmacal Research 23 (6) (2000) 605–612.

[36] C.K. Andreadi, L.M. Howells, P.A. Atherfold, et al., Involvement of Nrf2, p38, B-Raf, and nuclear factor-kappaB, but not phosphatidylinositol 3-kinase, in induction of hemeoxygenase-1 by dietary polyphenols, Molecular Pharmacology 69 (3) (2006) 1033–1040.

[37] C.C. Wu, M.C. Hsu, C.W. Hsieh, et al., Upregulation of heme oxygenase-1 by Epigallocatechin-3-gallate via the phosphatidylinositol 3-kinase/Akt and ERK pathways, Life Sciences 78 (25) (2006) 2889–2897.

[38] N. Sriram, S. Kalayarasan, G. Sudhandiran, Epigallocatechin-3-gallate augments antioxidant activities and inhibits inflammation during bleomycin-induced experimental pulmonary fibrosis through Nrf2–Keap1 signaling, Pulmonary Pharmacology and Therapeutics 22 (3) (2009) 221–236.

[39] H.K. Na, E.H. Kim, J.H. Jung, et al., (−)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells, Archives of Biochemistry and Biophysics 476 (2) (2008) 171–177.

[40] H.K. Na, Y.J. Surh, Intracellular signaling network as a prime chemopreventive target of (−)-epigallocatechin gallate, Molecular Nutrition & Food Research 50 (2) (2006) 152–159.

[41] C.S. Yang, J.D. Lambert, Z. Hou, et al., Molecular targets for the cancer preventive activity of tea polyphenols, Molecular Carcinogenesis 45 (6) (2006) 431–435.

[42] Y. Cao, R. Cao, Angiogenesis inhibited by drinking tea, Nature 398 (6726) (1999) 381.

[43] Y.D. Jung, L.M. Ellis, Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea, International Journal of Experimental Pathology 82 (6) (2001) 309–316.

[44] C. Chen, G. Shen, V. Hebbar, et al., Epigallocatechin-3-gallate-induced stress signals in HT-29 human colon adenocarcinoma cells, Carcinogenesis 24 (8) (2003) 1369–1378.

[45] V.M. Adhami, I.A. Siddiqui, N. Ahmad, et al., Oral consumption of green tea polyphenols inhibits insulin-like growth factor-I-induced signaling in an autochthonous mouse model of prostate cancer, Cancer Research 64 (23) (2004) 8715–8722.

[46] J.H. Yuan, Y.Q. Li, X.Y. Yang, Inhibition of epigallocatechin gallate on orthotopic colon cancer by upregulating the Nrf2–UGT1A signal pathway in nude mice, Pharmacology 80 (4) (2007) 269–278.

[47] T.C. Hsieh, J.M. Wu, Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines, Experimental Cell Research 249 (1) (1999) 109–115.

[48] Z. Dong, Molecular mechanism of the chemopreventive effect of resveratrol, Mutation Research 523/524 (2003) 145–150.

[49] C.Y. Chen, J.H. Jang, M.H. Li, et al., Resveratrol upregulates heme oxygenase-1 expression via activation of NF-E2-related factor 2 in PC12 cells, Biochemical and Biophysical Research Communications 331 (4) (2005) 993–1000.

[50] J.H. Jang, Y.J. Surh, Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells, Mutation Research 496 (1/2) (2001) 181–190.

[51] J.H. Jang, Y.J. Surh, Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death, Free Radical Biology and Medicine 34 (8) (2003) 1100–1110.

[52] B.L. Liu, X. Zhang, W. Zhang, et al., New enlightenment of French Paradox: resveratrol's potential for cancer chemoprevention and anti-cancer therapy, Cancer Biology and Therapy 6 (12) (2007) 1833–1836.

[53] A. Kumar, C.K. Singh, H.A. Lavoie, et al., Resveratrol restores Nrf2 level and prevents ethanol-induced toxic effects in the cerebellum of a rodent model of fetal alcohol spectrum disorders, Molecular Pharmacology 80 (3) (2011) 446–457.

[54] A. Kode, S. Rajendrasozhan, S. Caito, et al., Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells, American Journal of Physiology – Lung Cellular and Molecular Physiology 294 (3) (2008) L478–L488.

[55] H. Hatcher, R. Planalp, J. Cho, et al., Curcumin: from ancient medicine to current clinical trials, Cellular and Molecular Life Sciences 65 (11) (2008) 1631–1652.

[56] M.N. Mandal, J.M. Patlolla, L. Zheng, et al., Curcumin protects retinal cells from light-and oxidant stress-induced cell death, Free Radical Biology and Medicine 46 (5) (2009) 672–679.

[57] Sreejayan, M.N. Rao, Nitric oxide scavenging by curcuminoids, Journal of Pharmacy and Pharmacology 49 (1) (1997) 105–107.

[58] T. Nishinaka, Y. Ichijo, M. Ito, et al., Curcumin activates human glutathione S-transferase P1 expression through antioxidant response element, Toxicology Letters 170 (3) (2007) 238–247.

[59] M.C. Myzak, R.H. Dashwood, Chemoprotection by sulforaphane: keep one eye beyond Keap1, Cancer Letters 233 (2) (2006) 208–218.

[60] Y. Zhang, P. Talalay, C.G. Cho, et al., A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure, Proceedings of the National Academy of Sciences of the United States of America 89 (6) (1992) 2399–2403.

[61] J.R. Bacon, G. Williamson, R.C. Garner, et al., Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes, Carcinogenesis 24 (12) (2003) 1903–1911.

[62] G.P. Basten, Y. Bao, G. Williamson, Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells, Carcinogenesis 23 (8) (2002) 1399–1404.

[63] Z.Q. Jiang, C. Chen, B. Yang, et al., Differential responses from seven mammalian cell lines to the treatments of detoxifying enzyme inducers, Life Sciences 72 (20) (2003) 2243–2253.

[64] J.D. Brooks, V.G. Paton, G. Vidanes, Potent induction of phase 2 enzymes in human prostate cells by sulforaphane, Cancer Epidemiology, Biomarkers and Prevention 10 (9) (2001) 949–954.

[65] R. Munday, C.M. Munday, Induction of phase II detoxification enzymes in rats by plant-derived isothiocyanates: comparison of allyl isothiocyanate with sulforaphane and related compounds, Journal of Agricultural and Food Chemistry 52 (7) (2004) 1867–1871.

[66] M.A. Riedl, A. Saxon, D. Diaz-Sanchez, Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway, Clinical Immunology 130 (3) (2009) 244–251.

[67] L. Gamet-Payrastre, S. Lumeau, N. Gasc, et al., Selective cytostatic and cytotoxic effects of glucosinolates hydrolysis products on human colon cancer cells in vitro, Anti-Cancer Drugs 9 (2) (1998) 141–148.

[68] L. Gamet-Payrastre, P. Li, S. Lumeau, et al., Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells, Cancer Research 60 (5) (2000) 1426–1433.

[69] R. Yu, W. Lei, S. Mandlekar, et al., Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals, Journal of Biological Chemistry 274 (39) (1999) 27545–27552.

[70] C.S. Yang, S.K. Chhabra, J.Y. Hong, et al., Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sulfide (DAS) and related compounds from garlic, Journal of Nutrition 131 (3s) (2001) 1041S–1045S.

[71] Y. Shukla, N. Kalra, Cancer chemoprevention with garlic and its constituents, Cancer Letters 247 (2) (2007) 167–181.

[72] A. Herman-Antosiewicz, A.A. Powolny, S.V. Singh, Molecular targets of cancer chemoprevention by garlic-derived organosulfides, Acta Pharmacologica Sinica 28 (9) (2007) 1355–1364.

[73] W.S. Jeong, M. Jun, A.N. Kong, Nrf2: a potential molecular target for cancer chemoprevention by natural compounds, Antioxidants and Redox Signalling 8 (1/2) (2006) 99–106.

[74] V.L. Sparnins, G. Barany, L.W. Wattenberg, Effects of organosulfur compounds from garlic and onions on benzo[a]pyrene-induced neoplasia and glutathione S-transferase activity in the mouse, Carcinogenesis 9 (1) (1988) 131–134.

[75] R.A. Lubet, R. Yao, C.J. Grubbs, et al., Induced expression of drug metabolizing enzymes by preventive agents: role of the antioxidant response element, Chemico-Biological Interactions 182 (1) (2009) 22–28.

[76] C.D. Fisher, L.M. Augustine, J.M. Maher, et al., Induction of drug-metabolizing enzymes by garlic and allyl sulfide compounds via activation of constitutive androstane receptor and nuclear factor E2-related factor 2, Drug Metabolism and Disposition: The Biological Fate of Chemicals 35 (6) (2007) 995–1000.

[77] T.W. Kensler, P.A. Egner, P.M. Dolan, et al., Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1, 2-dithiol-3-thione (oltipraz) and related 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones, Cancer Research 47 (16) (1987) 4271–4277.