Redox-Sensitive Transcription Factors as Prime Targets for Chemoprevention with Anti-Inflammatory and Antioxidative Phytochemicals

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ABSTRACT Oxidative stress has been implicated in various pathological conditions including cancer. However, the human body has an intrinsic ability to fight against oxidative stress. A wide array of phase 2 detoxifying or antioxidant enzymes constitutes a fundamental cellular defense system against oxidative and electrophilic insults. Transcriptional activation of genes encoding detoxifying and antioxidant enzymes by NF-E2 related factor 2 (Nrf2), a member of the cap’n’collar family of basic leucine zipper transcription factors, may protect cells and tissues from oxidative damage. Many chemopreventive and chemoprotective phytochemicals have been found to enhance cellular antioxidant capacity through activation of this particular transcription factor, thereby blocking initiation of carcinogenesis. A new horizon in chemoprevention research is the recent discovery of molecular links between inflammation and cancer. Components of the cell signaling pathways, especially those that converge on redox-sensitive transcription factors, including nuclear factor-kappaB (NF-κB) and activator protein 1 (AP-1) involved in mediating inflammatory response, have been implicated in carcinogenesis. A wide variety of chemopreventive and chemoprotective agents can alter or correct undesired cellular functions caused by abnormal proinflammatory signal transmission mediated by inappropriately activated NF-κB and AP-1. The modulation of cellular signaling by anti-inflammatory phytochemicals hence provides a rational and pragmatic strategy for molecular target–based chemoprevention. J. Nutr. 135: 2993S–3001S, 2005.

KEY WORDS: • chemoprevention • oxidative stress • inflammation • Nrf2 • NF-κB • AP-1 • phytochemicals

Oxidative stress and inflammation contribute to multistage carcinogenesis by several distinct mechanisms, including direct damage to genomic DNA, alteration of intracellular signal transduction leading to abnormal cellular growth, and forcing damaged or initiated cells to undergo promotion and progression. Cells are endowed with an antioxidative defense system consisting of a variety of enzymatic and nonenzymatic antioxidants to combat oxidative insults, thereby protecting cellular macromolecules from detrimental effects of exogenous or endogenous reactive oxygen species (ROS). Cells and tissues are also equipped with a panel of detoxifying enzymes responsible for metabolic inactivation and subsequent elimination of carcinogens (1,2). Exposure of cells and tissues to oxidative stimuli or electrophilic carcinogens, therefore, forces the cell to turn on its antioxidant-detoxification arsenal as the first line of defense. Transcriptional regulation of antioxidant or detoxifying genes is predominantly mediated by a redox-sensitive transcription factor NF-E2 related factor-2 (Nrf2). A variety of edible phytochemicals are able to activate Nrf2 signaling thereby upregulating a set of enzymes including NADPH: quinone oxidoreductase-1 (NQO1), superoxide dismutase (SOD), glutathione S-transferase (GST), hemeoxygenase-1 (HO-1), and γ-glutamyl cysteine ligase (GCL) (1,3). On the other hand, persistently elevated ROS activate other redox-sensitive transcription factors, such as nuclear factor-kappaB (NF-κB) pathways.
(NF-κB) and activator protein-1 (AP-1), which may act as molecular switches to turn normal cells into premalignant cells, with subsequent clonal expansion to form solid tumors (4). Thus, aberrant activation of NF-κB and AP-1, which results in transcriptional activation of genes involved in inflammation, cellular proliferation, and growth, has been implicated in pathophysiology of various malignancies (5,6).

Therefore, augmenting cellular antioxidative or detoxification systems via activation of Nrf2-regulated genes, suppressing proliferation of damaged or initiated cells via inactivation of NF-κB or AP-1, or both appear to be pragmatic approaches for achieving chemoprevention. This review addresses the rationale and significance of targeting the aforementioned redox-sensitive transcription factors by dietary chemopreventive phytochemicals.

**Oxidative stress and inflammation: a deadly duo in carcinogenesis**

ROS, such as superoxide radical anion, hydroperoxyl radical, hydrogen peroxide, and hydroxyl radical, are constantly generated in cells as unwanted by-products of aerobic metabolism. Under physiological conditions, a low level of ROS is scavenged effectively by the cellular antioxidant defense system. However, an imbalance between the generation of ROS and cellular antioxidant capacity leads to a state of oxidative stress that contributes to various pathological conditions including cancer (7–9). Although certain stimuli such as growth factors, hormones, and neurotransmitters use ROS as a second messenger to execute normal physiological response (10,11), an excessive generation of ROS by external stimuli including redox chemicals, ultraviolet and ionizing radiation, and bacterial or viral infection, has a deleterious effect on human health. Oxidative stress contributes to tumorigenesis either by a direct mechanism involving damage to DNA or indirectly by modulating cellular signal transduction pathways (7,12,13).

Substantial evidence supports the protective role of antioxidant and detoxification enzymes in chemically induced carcinogenesis (14–16). A higher degree of oxidative DNA damage and a dramatic increase in the tumor incidence were noted in mice lacking MnSOD (17). In addition, mouse epidermal JB6 cells transfected with MnSOD exhibited a slower growth rate and a reduced rate of colony formation in soft agar on exposure to a prototype tumor promoter 12-O-tetradecanoyl-phorbol-13 acetate (TPA) (18). The overexpression of MnSOD, a representative antioxidant enzyme, suppressed papilloma formation in a 2-stage mouse skin carcinogenesis model (16). Mice lacking CuZnSOD developed more hepatic nodules, either as hyperplasia or hepatocellular carcinoma, than the wild type counterpart (15). Similarly, the deletion of murine GST-P gene cluster led to increased papillomagenesis in GST-P1/P2−/− mice in a chemically induced multistage skin carcinogenesis model (14).

The inhibition of ROS generation has been paralleled by a decrease in rat foot pad inflammation induced by Freund's complete adjuvant (19), suggesting that accumulation of ROS in vivo leads to inflammation. It has long been suspected that inflammation is causally linked to carcinogenesis. According to an estimate, ~15% of all cancers are somehow linked to inflammation and about 5% of all human colorectal cancer is associated with ulcerative colitis (20). Growing evidence indicates that chronic inflammation may cause cancers of different organs including stomach, colon, breast, skin, prostate, and pancreas (21–24). A distinct set of proinflammatory mediators, such as cytokines, chemokines, prostaglandins (PGs), nitric oxide (NO), and leukotrienes, promote neoplastic transformation of cells by altering normal cellular signaling cascades (25) (Fig. 1). Mounting evidence from laboratory and population-based studies suggests that prolonged use of non-steroidal anti-inflammatory drugs reduces the risk of certain malignancies (26,27) that frequently occur in persistently inflamed tissues (28).

Proinflammatory cytokines, such as interleukins (ILs) and tumor necrosis factor-alpha (TNF-α), have been implicated in tumor promotion in various experimental models of tumorigenesis (29). The incidence and the multiplicity of mouse skin papillomas are remarkably low in TNF-α−/− mice compared with mice overexpressing TNF-α (30). In response to inflammatory stimuli, PGs are produced in abundance through metabolic conversion of arachidonic acid by cyclooxygenase-2 (COX-2). Recent studies suggest that PGs, especially PGE2 and PGF2α, are functionally related to tumor promotion (31–33). Another proinflammatory mediator, NO, produced by inducible nitric oxide synthase (iNOS), was implicated in tumor promotion as evidenced by a suppressive effect of aminoguanidine, an inhibitor of iNOS, on TPA-induced mouse skin papilloma formation (34).

Chronic inflammation contributes to cancer not only as a consequence of a direct effect of proinflammatory mediators on cellular signaling but also by creating a state of oxidative stress. The transformed cells are often surrounded by innate immune cells, inflammatory macrophages, fibroblasts, and endothelial cells, which release a distinct set of proinflammatory mediators and hence exacerbate the generation of ROS (25). This can create a vicious loop between oxidative stress and inflammation, which in turn favors tumorigenesis.

**Roles of redox-regulated transcription factors in the causation and prevention of oxidative stress- and inflammation-associated cancer**

Recently, attention has been focused on intracellular signal transduction pathways regulating cell proliferation and differentiation as the molecular basis of carcinogenesis. Compo-
ments of intracellular signaling networks include the family of proline-directed serine and threonine kinases named mitogen-activated protein kinases (MAPks); protein kinase C (PKC); phosphoinositide 3-kinase (PI3K); glycogen synthase kinase, protein kinase B; and tyrosine kinases (e.g., growth factor receptor and soluble Src kinase). Most of these upstream kinases are aberrantly turned on by diverse stimuli provoking oxidative and proinflammatory stress and often amplified via activation of a battery of redox-sensitive transcription factors including Nrf2 and NF-κB/AP-1.

Nrf2. A large variety of xenobiotic metabolizing enzymes, which catalyze phase I and phase II metabolic reactions, are involved in carcinogen activation and deactivation. The balance between carcinogen activating enzymes and detoxifying enzymes determines the ultimate risk of chemically induced carcinogenesis (35). An overall shift toward carcinogen inactivation or elimination by a panel of detoxifying and antioxidant enzymes, such as GST, NQO1, UDP-glucuronosyltransferase (UGT), microsomal epoxide hydrolase, GCL, glutathione synthetase, γ-glutamyl transpeptidase, and HO-1, protects cellular components from oncogenic insults. The induction of these enzymes facilitates inactivation and subsequent elimination of electrophilic and oxidative carcinogens (1,3).

Genomic analysis has revealed the presence of a cis-acting element known as antioxidant response element (ARE) or electrophile response element (EpRE) [5'-[(G/A)TGA(G/C)nnGC(G/A)-3'] located in the promoter region of many of the genes encoding antioxidant and detoxifying enzymes. Nrf2, a member of the cap'n'collar family of bZIP transcription factors, can act as a master regulator of ARE-driven transactivation of antioxidant genes (36). A distinct set of Nrf2-regulated proteins detoxify xenobiotics, reduce oxidized proteins, maintain cellular reducing equivalents, disrupt redox cycling reactions, and counteract the noxious effects of ROS (13,37).

Nrf2 is sequestered in the cytoplasm as an inactive complex with its cytosolic repressor Kelch-like ECH associated protein 1 (Keap1). Dissociation of Nrf2 from the inhibitory protein Keap1 is a prerequisite for nuclear translocation and subsequent DNA binding of Nrf2. After forming a heterodimer with small Maf protein inside the nucleus, the active Nrf2 binds to cis-acting ARE or EpRE, also alternatively known as Maf recognition element (38) (Fig. 2). Besides the dissociation of the Nrf2-Keap1 complex that is facilitated by upstream kinase-mediated signals, covalent modification of multiple cysteine residues on Keap1 by electrophiles or inducers of detoxifying enzymes is also considered to release Nrf2 from the Keap1 repression (39). Multiple mechanisms of Nrf2 activation by signals mediated via one or more of the upstream kinases, including MAPks, PI3K, PKC, and Akt, were recently reviewed (1,3,40).

The genetic ablation of the Nrf2 results in severe airway inflammation (41) and development of emphysema in mice (41). The Nrf2-null mice failed to induce many of the genes responsible for carcinogen detoxification and protection against oxidative stress (3). Moreover, the deletion of the Nrf2 gene in mice resulted in a decrease in the basal expression level of genes, including those for epoxide hydrolase, GCL, GST, HO-1, NQO1, and UGT (42-44). The Nrf2-null mice also have defects in detoxifying carcinogens such as aflatoxin B1 (40). Fibroblasts from Nrf2-null mice express only about 15% as much GCL mRNA as wild type cells (45). The significance of Nrf2 activation as a measure of chemoprevention was evident from a remarkably higher incidence of benzo[a]pyrene-induced gastric neoplasia in Nrf2-deficient mice, which were less responsive to the phase II enzyme inducer olipraz (46). Therefore, targeted activation of Nrf2 is considered to be a rational approach for chemoprevention, especially at the initiation stage of carcinogenesis.

NF-κB. Since the discovery of leukocytes in neoplastic tissues by Rudlof Virchow in 1863, inflammation and cancer are thought to be closely associated. Virchow’s early observation is now more evident from multiple lines of studies suggesting an inflammatory microenvironment of malignant tissues. Several recent studies have identified NF-κB as a critical component to bridge inflammation and cancer (47-49). The heterodimeric protein NF-κB is a ubiquitous redox-regulated transcription factor that remains sequestered in the cytoplasm as an inactive complex with its inhibitory counterpart IκB. Exposure to oxidative and inflammatory stimuli, such as TNF-α, IL-1, phorbol ester, ultraviolet radiation or microbial infection, leads to phosphorylation and subsequent proteasomal degradation of IκBα, thereby releasing free NF-κB dimers for translocation to the nucleus (50,51), as illustrated in Figure 3.

Excessive oxidative or inflammatory stress may activate NF-κB by distinct mechanisms in a cell type- or stimuli-specific manner (4,50,52). Although it is generally accepted that degradation of IκBα is a pivotal step in NF-κB activation,
several recent studies have reported that NF-κB may be activated independently of IkBα degradation (53,54). Stimuli such as H₂O₂ or hypoxia followed by reoxygenation may cause phosphorylation of IkBα at a tyrosine residue, which facilitates the dissociation of IkBα from NF-κB without proteasomal degradation of IkBα (55–57). Moreover, translocation of NF-κB to nucleus is not necessarily essential for transactivating target genes because inhibitors of several upstream kinases, such as P38K, p38 MAPK, and protein kinase A (PKA), could block the transcriptional activity of NF-κB without affecting its nuclear translocation (58–61). It has been suggested that the transcriptional activation of NF-κB depends on the phosphorylation of its active subunit p65 (RelA) (62). The proinflammatory cytokines, TNF-α and IL-1, have been shown to stimulate p65 phosphorylation and subsequent NF-κB transactivation via mechanisms distinct from those that involve the IkBα phosphorylation and subsequent nuclear translocation of NF-κB (59,63–65). An upstream kinase, IkB kinase (IKK), which was recently recognized as a potential link between inflammation and cancer, regulates the transcriptional activity of NF-κB through phosphorylation of both IkBα and NF-κB (66,67). Besides IKK, the regulation of NF-κB activation was reported to be mediated by extracellular signal-regulated protein kinase (68) and p38 MAPK (69) because specific inhibitors of these MAPKs abrogated phosphorylation of both IkBα and p65 in TPA-stimulated mouse skin. Certain isoforms of PKC (e.g., ε and ζ) were also reported to regulate transcriptional activation of NF-κB (70,71). One of the key steps in NF-κB transactivation is the interaction of p65 with transcriptional coactivator cyclic AMP response element binding protein-binding protein, which makes a bridge between basal transcriptional machinery and DNA-bound NF-κB. Induction of a wide array of genes regulating proinflammatory mediators such as TNF-α, IL-8, IL-1, iNOS, and COX-2 is transcriptionally regulated by NF-κB (72). Earlier studies reported that genes encoding c-myc, TNF-α, and FasL are activated by NF-κB, suggesting a proapoptotic function of this transcription factor. However, subsequent studies provided evidence for an antiapoptotic role of NF-κB in response to a variety of proapoptotic stimuli including severe oxidative stress (73). The role of NF-κB in carcinogenesis was further corroborated by the fact that NF-κB antagonizes the function of the tumor suppressor protein p53 (73). Moreover, activation of NF-κB promotes transcriptional upregulation of genes involved in cell cycle progression (73). Tumor cells can attain survival benefit via upregulation of NF-κB-driven antiapoptotic gene products such as cIAP1, cIAP2, XIAP, Bcl-2, and Bcl-XL (73). Therefore, turning off improper activation of NF-κB by chemopreventive phytochemicals would prevent initiated cells from undergoing further proliferation at the state of tumor promotion.

**AP-1.** AP-1 is another redox-sensitive transcription factor that plays a critical role in tumorigenesis. AP-1 exists as different dimeric combinations of basic leucine zipper proteins from the Jun (c-Jun, JunB, and JunD) and Fos (c-Fos, FosB, Fra-1, and Fra-2) family, Jun dimerization partners (JDP1 and JDP2), and the closely related activating transcription factor (ATF2, LRF1/ATF3, and B-ATF) subfamilies (5,74,75). Although Jun proteins can form stable homodimers that bind to AP-1 DNA recognition elements (5′-TGAG/CTCA-3′) known as TPA response element (76), Fos family proteins do not form stable homodimers. However, heterodimers composed of Jun and Fos family proteins can form a more stable AP-1–DNA complex than Jun:Jun homodimers (77,78). In response to oxidative and proinflammatory stimuli, the activation of AP-1 is mediated predominantly via the MAPK signaling pathways (Fig. 3). The major MAPK-responsive element in the c-fos promoter is the serum response element, which is bound by a transcription factor complex including dimeric serum response factor and the ternary complex factors Elk-1, Sap1, and Sap2. Extracellular signal-regulated protein kinase, c-Jun-N-terminal kinase, and p38 MAPK phosphorylate and activate Elk-1, resulting in enhanced serum-response-element-dependent c-fos expression (79,80). The heterodimers of c-Jun and ATF2 are phosphorylated by c-Jun-N-terminal kinase and preferentially bind to TPA response element. Because transactivation of AP-1 promotes induction of proinflammatory and proliferative gene products, targeted inhibition of this transcription factor is recognized as a molecular basis of chemoprevention by antioxidative and anti-inflammatory phytochemicals.

**Chemopreventive phytochemicals targeting signal transduction mediated by Nrf2, NF-κB, and AP-1.**

A wide variety of chemopreventive phytochemicals prevents carcinogenesis either by enhancing cellular antioxidative and detoxification enzymes via activation of Nrf2 or by suppressing induction or overamplification of proinflammatory and growth promoting gene expression driven by NF-κB or AP-1 (1,40,51). Phytochemicals capable of activating Nrf2...
inhibit the tumor initiation process by ameliorating oxidative DNA damage, promoting carcinogen detoxification, or both, thereby protecting important cellular macromolecules from damage, are known as blocking agents. Phytochemicals that can alter abnormal cellular signaling mediated via NF-κB and or AP-1 prevent tumor promotion or progression and are known as suppressing agents. The mechanistic basis of chemoprevention by representative antioxidative and anti-inflammatory phytochemicals that target aforementioned transcription factors is presented in subsequent sections.

Curcumin. Curcumin, the yellow pigment isolated from the rhizomes of Curcuma longa Linn (Zingiberaceae), inhibits chemically induced carcinogenesis in multiple organ sites, including forestomach, duodenum, colon, and skin, in various experimental animal models (81–86). The compound strongly inhibited TPA-induced inflammation, hyperplasia, proliferation, activity and expression of ornithine decarboxylase, generation of ROS, and oxidative DNA damage in mouse skin (83,87) and reduced anchorage-independent colony formation in mouse epidermal JB6 cells (88).

As a mechanism of anti-initiation, curcumin disrupted the Nrf2–Keap1 complex, leading to increased Nrf2 binding to ARE and subsequent increase in the expression and activity of HO-1 in cultured porcine renal epithelial proximal tubule (LLC-PK1) and rat kidney epithelial (NRK-52E) cells (89). Curcumin also increased the nuclear translocation of Nrf2; its ARE-DNA binding activity; and expression of both protein and the mRNA transcript of another phase II enzyme, GCL, in immortalized human bronchial epithelial (HBE1) cells (90).

Curcumin contains 2 α,β unsaturated carbonyl moieties, each of which by acting as a Michael reaction acceptor may covalently modify cysteine thiois of Keap1, thereby releasing Nrf2 for nuclear translocation.

Molecular mechanisms underlying the anti-tumor promoting effect of curcumin have largely been attributed to its inhibitory effect on tumor promoter-induced activation of NF-κB and AP-1. Curcumin suppressed the expression of c-Jun and c-Fos in CD-1 mouse skin after treatment with TPA (88). Previous studies from this laboratory demonstrated that curcumin inhibited activation of AP-1 and NF-κB in TPA-stimulated mouse skin in vivo as well as in cultured HL-60 cells (68,91). Curcumin inhibited the expression of COX-2, which is predominantly regulated by NF-κB and AP-1, and the generation of PGE2 in TPA-stimulated mouse skin (68) and human pancreatic cancer cells (92). The nuclear translocation of p65 was suppressed by curcumin via blockade of phosphorylation-dependent degradation of IkBα (68,93). Similarly, inhibition of IkBα degradation via downregulation of NF-κB–inducing kinase and IKK by curcumin contributes to its blockade of TNF-α–induced COX-2 gene transcription and NF-κB activation in human colonic epithelial cells (94). Moreover, curcumin targeted IKK in Helicobacter pylori-treated gastric epithelial (95), multiple myeloma (96), and pancreatic cancer cells (97) to exert chemopreventive activities.

Resveratrol. Resveratrol, a phytoalexin present in grapes and other plant species, exerts antioxidant, anti-inflammatory, and chemopreventive activities by modulating diverse events in cellular signaling. The compound was reported to interfere with the initiation, promotion, and progression stages of carcinogenesis (97). Subsequent studies demonstrated that resveratrol prevented chemically induced tumorigenesis in various experimental models (98–100).

The inhibition of tumor initiation by resveratrol has been attributed to its suppressive effect on cytochrome p450 1A1/1A2 in murine hepatoma (Hepa1c1c7) cells (101), mammary epithelial (MCF-10A) cells treated with 2,3,7,8, tetrachlorodibenzo-p-dioxin (102), human breast cancer MCF-7 cells treated with dimethylbenz[a]anthracene (DMBA) (103), and human hepatoma (HepG2) cells stimulated with benzo[a]pyrene (103). Resveratrol induced NQO, an Nrf2-regulated detoxifying enzyme, in Hepa1c1c7 cells (101). In addition, several recent studies demonstrated that the compound can induce HO-1 expression and activity in human aortic smooth muscle (38) and rat pheochromocytoma (PC12) cells (104) via activation of NF-κB and Nrf2, respectively.

The inhibition of cytokine release and proinflammatory gene expression and the downregulation of intracellular signal transducing enzymes and transcription factors that regulate expression of proinflammatory genes are key molecular mechanisms underlying anti-inflammatory and anti-tumor promoting activities of resveratrol (105). The induction of proinflammatory gene products such as COX-2 and iNOS, which have been implicated in tumor promotion (9,34), by diverse stimuli including bacterial lipopolysaccharide, TPA, and interferon-γ was attenuated by resveratrol (105,106). Resveratrol suppressed activation of NF-κB and AP-1 in cell-, tissue-, and stimuli-specific fashions (105). The compound inhibited TPA-stimulated activation of AP-1 in mouse skin in vivo (105) and U937 cells (107) in culture. Moreover, resveratrol ablated TPA-induced transcriptional activity of AP-1 in human mammary epithelial cells (108,109). Resveratrol also suppressed activation of NF-κB in acute myeloid leukemia (OCI2) cells (110) and mouse epidermal JB6 cells stimulated with IL-1β and Cr (VI) (111), respectively. Our recent study also revealed that topical application of resveratrol attenuated NF-κB activation by blocking both IKKβ activity in TPA-treated mouse skin (J. K. Kundu and Y.-J. Surh, unpublished observation, 2005). Resveratrol attenuated TNF-α–induced activation of NF-κB in U937 cells by suppressing phosphorylation and nuclear translocation of p65 without affecting IkBα degradation (112). In normal human epidermal keratinocytes, resveratrol–inhibited UVB-induced activation of NF-κB by blocking the activation of IKKα and the phosphorylation and degradation of IkBα (113).

Epigallocatechin gallate. Green tea is one of the extensively investigated dietary sources of chemopreventive agents. The antioxidant phenolic compound epigallocatechin gallate (EGCG) is the major chemopreventive agent present in green tea (114). EGCG protected against UV-induced depletion of glutathione and glutathione peroxidase activity in human skin (115). The compound also restored detoxification enzymes GST, glutathione peroxidase, SOD, and catalase that were depleted as a result of DMBA treatment in mouse skin in vivo (116) and induced the ARE luciferase activity in human hepatoma HepG2 cells (117).

As an antitumor promoting agent, EGCG suppressed malignant transformation in TPA-stimulated mouse epidermal JB6 cells through inactivation of AP-1 (118,119) or NF-κB (120). EGCG also inhibited AP-1 activity in the H-ras–transformed epidermal JB6 cells (121) and in the epidermis of transgenic mice bearing an AP-1–driven luciferase reporter gene (122). In contrast, oral administration of EGCG failed to affect TPA-induced AP-1 DNA binding (123) but inhibited activation of NF-κB by blocking degradation of IkBα in mouse skin (J. K. Kundu and Y.-J. Surh, unpublished observation, 2005). The inactivation of NF-κB by EGCG was reported to be mediated via inhibition of IKK activity, leading to blockade of phosphorylation-dependent degradation of IkBα and subsequent decrease in nuclear localization of p65 protein (124,125). However, the modulation of NF-κB transcriptional activity by EGCG does not solely depend on IkBα degradation and subsequent release of NF-κB subunits because EGCG
inhibited lipopolysaccharide-induced phosphorylation of IkBα without affecting NF-κB luciferase activity in human colon cancer (HT-29) cells (126). Besides interference with the IKK-IκB signaling, suppression of signal transduction mediated by MAPKs (127–129) and PI3K-Akt (130) by EGCG has been implicated in the inactivation of NF-κB and suppression of COX-2 induction.

**Caffeic acid phenethyl ester.** Caffeic acid phenethyl ester (CAPE) is the major chemopreventive principle of honey bee propolis. Treatment of rat renal epithelial cells with CAPE resulted in the increase in nuclear translocation and ARE binding of Nrf2 as well as induction of HO-1 activity (89). CAPE given during promotion of experimentally induced rat hepatocarcinogenesis suppressed the nuclear localization of p65 independently of IκBα degradation (131). Similarly, Marquez et al. (132) demonstrated that CAPE specifically inhibited both gene transcription and synthesis of IL-2 in stimulated T cells by suppressing the NF-κB-dependent transcriptional activity without affecting IκBα degradation. The compound significantly decreased the lipopolysaccharide-induced NF-κB transcriptional activity in RAW 264.7 cells (133) and attenuated NO production and iNOS expression (134,135). CAPE suppressed TPA-induced MMP-9 expression by inhibiting NF-κB but not AP-1 in HepG2 cells (136).

**Isothiocyanates.** Isothiocyanates are major chemopreventive components present in broccoli sprouts and mature broccoli. Sulforaphane [1-isothiocyanato-(4R,S)-(methylsulfinyl) butane] and its analogues inhibited chemically induced carcinogenesis in mouse skin (137) and lung (138,139). Isothiocyanates are potent inducers of ARE-regulated detoxifying enzymes (140–142). It has been reported that sulforaphane as well as phenethyl isothiocyanate regulate the activation of MAPKs and Nrf2 and the induction of phase II enzymes (143,144). Sulforaphane induced Nrf2 nuclear translocation, thereby enhancing the expression and activity of UGT in human colon cancer (Caco-2) cells (145). Moreover, sulforaphane induced Nrf2 protein expression and ARE-mediated transcriptional activation of Nrf2 resulting in HO-1 expression, partly by blocking Keap1-mediated degradation of Nrf2 (146). An Nrf2-dependent induction of GSTA1/2, GSTA3, NQO-1, and catalytic subunit of GCL by sulforaphane was recently reported (147). Sulforaphane also elevated the levels of glutathione and NQO in retinal pigment cells following an Nrf2-dependent mechanism (148). A direct covalent binding of sulforaphane with cysteine residues on Keap1 leads to thiol modification, thereby activating Nrf2 (39). Gene microarray analysis revealed that sulforaphane upregulated the expression of detoxifying enzymes including NQO1, GST, and GCL in the small intestine of wild type mice whereas Nrf2-null mice displayed lower levels of these enzymes (149). A sulforaphane analogue, 6-(methylsulphonyl)hexyl isothiocyanate, derived from Wasabia japonica or Extrema wasabi Maxim, also stimulated nuclear translocation of Nrf2, which subsequently activated ARE. This analogue given by gavage resulted in the induction of hepatic phase II detoxifying enzymes to a greater extent than did sulforaphane, which was abolished in Nrf2-null mice (150). In a recent study, sulforaphane was found to inhibit DNA binding of NF-κB and transcriptionactivation of its target genes in human prostate cancer (PC3) cells (151).

**Conclusion**

Although there is no magic bullet to completely cure cancer at this moment, we are now aware that many forms of cancers are at least avoidable or preventable. Remarkable progress in unfolding cancer biology in recent years led us to find several ways to intervene in carcinogenic process. Because oxidative and inflammatory stress contributes to malignant transformation, substances with antioxidative and anti-inflammatory properties would be good candidates for preventing most human malignancies. Some chemopreventive phytochemicals have been shown to modulate such redox-sensitive transcription factors as Nrf2, NF-κB, and AP-1, thereby fortifying cellular antioxidant capacity or suppressing inflammatory response. The activation of Nrf2 leading to the upregulation of cellular detoxifying and antioxidant enzymes is an effective way to block oxidative DNA damage and related events. On the other hand, targeted suppression of inappropriate activated NF-κB or AP-1 can ameliorate proinflammatory stress, thereby interfering with the tumor promotion or progression. Although a wide variety of phytochemicals has been identified as chemopreventive agents, studies directed to identify precise molecular targets are still limited. The modulation of aforementioned transcription factors by antioxidative and anti-inflammatory phytochemicals would provide ample opportunities for chemoprevention based on molecular targeting.

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