ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 11 & 12, 2005

Forum Review

Regulation of Nrf2, NF-κB, and AP-1 Signaling Pathways by Chemopreventive Agents

GUOXIANG SHEN,[1,2] WOO-SIK JEONG,[3] RONG HU,[1,2] and AH-NG TONY KONG[1]

ABSTRACT

The inhibition of carcinogenesis by chemopreventive agents has been demonstrated in many tumorigenesis animal models. The chemopreventive mechanisms of those phytochemicals have been investigated extensively, though mostly in *in vitro* cell culture systems. The cellular signaling cascades mediated by transcription factors, including nuclear factor E2-related factor 2 (Nrf2), nuclear factor-κB (NF-κB), and activator protein-1 (AP-1), have been shown to play pivotal roles in tumor initiation, promotion, and progression processes. Thus, as demonstrated by previous substantive mechanistic studies, they appear to be ideal targets for cancer chemoprevention. In this review, we discuss the current progress and future challenges on our understanding of the molecular mechanisms in cancer chemoprevention by phytochemicals, focusing on the regulation of Nrf2, NF-κB, and AP-1 signaling pathways. *Antioxid. Redox Signal.* 7, 1648–1663.

INTRODUCTION

DESPITE THE TREMENDOUS IMPROVEMENTS of medical technologies and chemotherapeutic approaches, cancer has recently overtaken heart disease as the leading cause of death in many parts of the world. Although there are more than 200 types of cancer, research using animal models has produced better basic understanding of cancer development in recent decades. Cancer development is believed to be a multiple-step process including initiation, promotion, and progression (117). The initiation step is a relatively short and irreversible process triggered by carcinogens or ionizing radiation in normal cells. In contrast, promotion, and progression processes are much longer processes that are considered relatively reversible. By using various animal cancer models (14, 23, 101), scientists have found that natural or synthetic chemicals can intervene at all three cancer development stages (Fig. 1). Epidemiological evidence or population studies have also demonstrated the correlation between incidence of cancer and consumption of certain types of food (121, 132). In 1976, Dr. Michael B. Sporn first coined the term "chemoprevention" and advocated a cancer chemoprevention strategy to decrease the incidence of cancer. The basic concept of chemoprevention is to block or slow the onset of premalignant tumors by using relatively nontoxic chemical substances. Most of the nontoxic chemical substances used in cancer chemoprevention studies are natural phytochemicals found in food (117). Based on the cancer development stage targeted by a chemopreventive agent, the chemopreventive agent can be categorized either as a cancer-blocking agent that blocks the cancer initiation step or as a cancer-suppressing agent that halts or retards the promotion and progression of precancerous cells into malignant ones. If the phytochemical can act on all stages of cancer development, then it falls into both categories, and this happens quite frequently (Fig. 1).

Numerous cancer cell lines and animal cancer models have been utilized to evaluate the chemopreventive effects of phytochemicals as well as to elucidate their mechanisms of cancer prevention. These studies have resulted in the discovery of several new phytochemicals that possess cancer preventive

[1]Department of Pharmaceutics and [2]Graduate Program in Pharmaceutical Science, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854.
[3]Food Science Institute, School of Food & Life Science, College of Biomedical Science & Engineering, Inje University, Gyeongnam, South Korea.

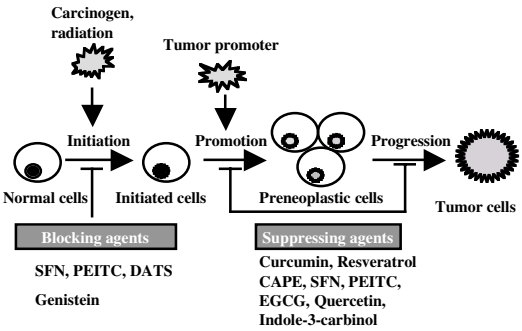

FIG. 1. Intervention of tumor development processes by chemopreventive agents. Cancer development is a multistep process including initiation, promotion, and progression. The initiation step is started by carcinogen addition and irradiation in normal cells. Many phytochemicals can block this step by inducing the detoxification and antioxidant enzyme systems that protect cells from the damage caused by initiators. Chemopreventive agents can also block or retard the progress of tumor promotion and progression by modulating the key signaling pathways elicited by tumor promoters, inflammatory cytokines, growth factors, etc. It should be mentioned that a single chemopreventive agent could act as both tumor blocking and suppressing agent. DATS, diallyl trisulfide; SFN, sulforaphane.

effects, such as flavonoids from soybeans, isothiocyanates from cruciferous vegetables, and polyphenol from green and black tea (Fig. 2) (117). Besides the discovery of new chemopreventive agents, understanding of the mechanisms by which these chemopreventive agents inhibit cancer development is also improved. Although some chemicals elicit unique signal transduction pathways, most chemicals trigger common cellular or molecular events in the cells. For example, it has been shown that cellular exposure to dietary phytochemicals can generate reactive oxygen species (ROS) and electrophiles that lead to mild oxidative or electrophilic stress (19, 71, 95). By modulating cellular signaling pathways,

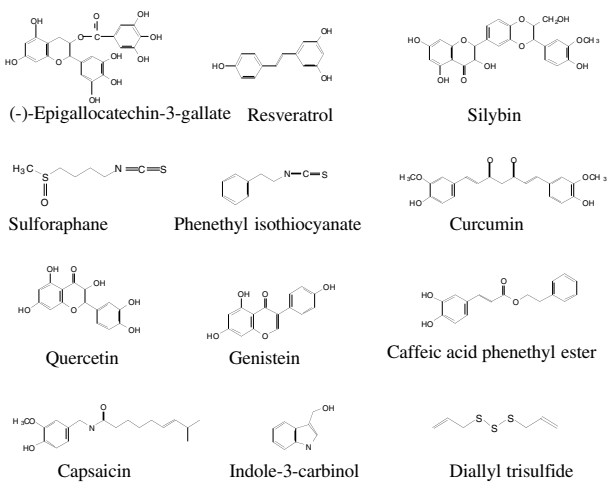

FIG. 2. Chemical structures of representative chemopreventive agents discussed in the article.

these stresses can trigger a wide variety of cellular events such as increasing expression of detoxifying enzymes and/or antioxidant enzymes, inhibiting cell cycle progression and cell proliferation, inducing differentiation and apoptosis, inhibiting expression and functional activation of oncogenes, increasing expression of tumor-suppressor genes, and inhibiting angiogenesis and metastasis (45, 117). Although the study of signal transduction pathways responsible for sensing oxidative and/or electrophilic stress has been performed extensively in recent decades, our understanding of this area is still limited. This review will focus on recent progress in the study of modulation of nuclear factor E2-related factor 2 (Nrf2), nuclear factor-κB (NF-κB), and activator protein-1 (AP-1) signaling pathways by chemopreventive agents.

REGULATION OF NRF2 SIGNALING PATHWAYS

To survive under a variety of environmental or intracellular stresses, eukaryotic cells have developed cellular defensive systems to protect themselves from oxidative or electrophilic challenges (52, 75). Among these defensive enzymes are the Phase II drug-metabolizing enzymes, such as glutathione *S*-transferase and UDP-glucuronosyltransferase (40), and antioxidant enzymes, such as heme oxygenase-1 (HO-1) (4), NADP(H):quinone oxidoreductase, and γ-glutamylcysteine synthetase (129). Many of these enzyme genes are coordinately regulated through a consensus *cis*-element at the 5′-flanking promoter region such as the antioxidant responsive element (ARE) or the electrophile response element (EpRE). ARE/EpRE-mediated gene induction plays a pivotal role in the cellular defense against the cellular damage caused by electrophiles and ROS.

Nrf2, which belongs to the Cap'n'Collar family of basic region-leucine zipper transcription factors, has been shown to be an essential component of the ARE-binding transcriptional machinery (53). Its important role in regulating the expression of many mammalian detoxifying and antioxidant enzymes under oxidative or electrophilic stress has been verified in various Nrf2-deficient mice experiments, in which the enzyme expression was dramatically abolished and the Nrf2 knockout mice were much more susceptible to carcinogen-induced carcinogenesis (7, 33, 100).

As described above, many phytochemicals exert their chemopreventive effects by blocking the initiation stage of cancer development. For a subset of these compounds, chemoprotection mainly derived from the induction of Nrf2/ARE-regulated genes. For example, the Phase II glutathione *S*-transferase gene can be induced by the phenolic antioxidant butylated hydroxyanisole and ethoxyquin (40) or by isothiocyanate (86). The redox-sensitive stress gene *HO-1* can be induced by curcumin and caffeic acid phenethyl ester (CAPE) through an Nrf2 signaling pathway (10). In oligonucleotide microarray studies (77, 120), the comparison of the change of the global gene expression profiles elicited by the chemopreventive agents sulforaphane and dithiolethiones in wild-type mice and Nrf2 knockout mice revealed that Phase II and antioxidant genes are the major group of target genes being regulated.

By inducing the Phase II detoxifying and antioxidant enzyme genes, phytochemicals increase the detoxification of pro-carcinogens or carcinogens and protect normal cells from the damages of electrophiles and reactive oxygen intermediates, thus decreasing the incidence of initiation and reducing the risk of cancer. This hypothesis is supported by the study of Kensler *et al.* (69) in which the chemoprotective effect of oltipraz was investigated in human subjects with a high incidence of liver cancer in China. In their study, oral administration of oltipraz significantly enhanced the urinary excretion of the Phase II conjugation product of the carcinogen aflatoxin, and furthermore higher doses of oltipraz significantly reduced the excretion of the primary oxidative metabolite of aflatoxin M1. Another supportive example is the study of the tumor-blocking effects of sulforaphane, which is an isothiocyanate present abundantly as a glucosinolate precursor in cruciferous vegetables. It has been shown that it could block benzo[*a*]pyrene-induced forestomach tumors in ICR mice (34). This protection was resulted from induction of Phase II detoxification and antioxidant enzymes since the blocking effect was abrogated in the Nrf2 knockout mice.

Considering the great structural diversity of the inducers that regulate the Nrf2 signaling pathway (99), a mechanism of activation requiring the interaction of phytochemical Nrf2 inducers with a structurally complementary receptor appears to be quite unlikely. Therefore, many phytochemicals might regulate Nrf2-mediated gene transcription by different mechanisms. It is important to note that many chemopreventive agents have pro-oxidant properties, meaning they can generate oxidative/electrophilic stress by themselves in the cells. The stress generated by chemopreventive agents appears to be dose-dependent and mild at low concentrations but strong enough to activate the cellular defense mechanisms that lead to coordinated activation of the Nrf2 signaling pathway. However, this "low stress" is believed to be at a subtoxic level that would not cause adverse effects such as DNA damage, mutagenicity, and degeneration of tissues as caused by carcinogens.

As a sensor regulating ARE-mediated gene expression in response to oxidative stress, Nrf2 resides mainly in the cytoplasm bound to a cytoskeleton-related protein called Kelch-like ECH-associated protein 1 (Keap1) (55) (Fig. 3). Keap1 is a cysteine-rich protein that interacts with the ETGE motif within the N-terminal Neh2 domain of Nrf2 (72). Many studies have shown that challenges with chemopreventive agents can lead to the nuclear accumulation of Nrf2, thereby activating Nrf2-dependent gene transcription (10, 40, 100, 120). However, the exact mechanisms by which these phytochemicals or the generated exogenous/endogenous oxidative/electrophile stress trigger the Nrf2 transactivation are still unclear. The binding of Keap1 to Nrf2 represses Nrf2-mediated gene transcription under homeostatic conditions. Upon exposure to above threshold levels of chemopreventive chemicals or oxidative stress, Nrf2 is able to escape Keap1-mediated repression, translocate to the nucleus, and activate expression of its target genes. Several mechanisms have been proposed for the ability of Nrf2 to escape the Keap1-medi-

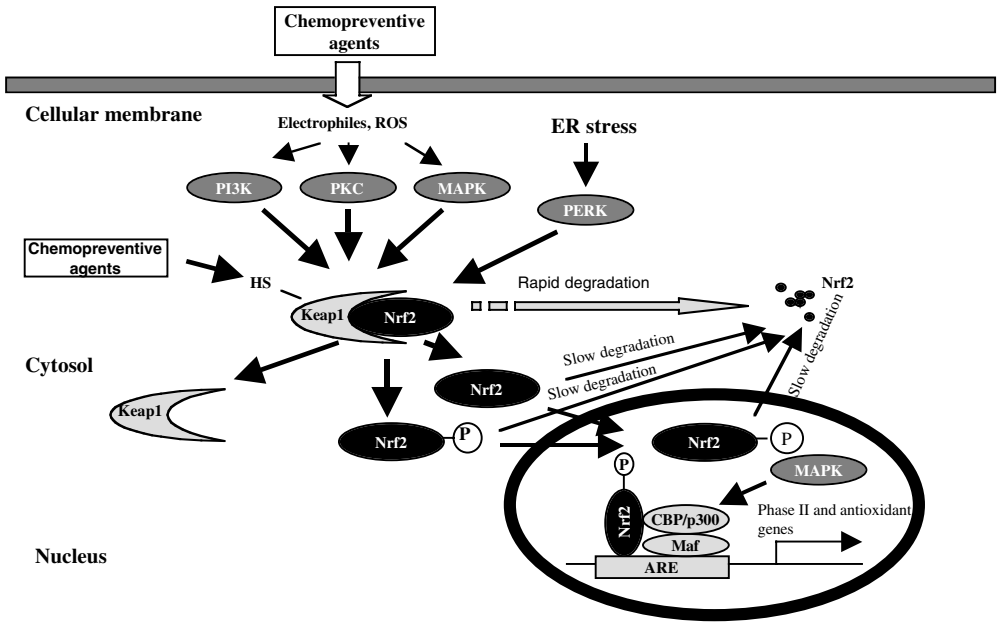

FIG. 3. Regulation mechanism of Nrf2-mediated gene transcription by chemopreventive agents. Under homeostatic condition, Nrf2 is retained in the cytoplasm by Keap1 protein. Chemopreventive agents can interact directly with the cysteine residues of Keap1 to trigger the release of Nrf2 from the complex. Alternatively, chemopreventive agent-generated electrophiles or ROS can activate a wide variety of kinase signaling pathways, including PI3K, PKC, and MAPK cascades, which can also trigger the release and nuclear translocation of Nrf2. ER stress-activated PERK kinase is also involved in the phosphorylation of Nrf2 and causes Nrf2 release, though its activation is not related to chemoprevention. MAPK kinases such as Raf may also regulate Nrf2-mediated transcription by modulating the activity of Nrf2-interacting co-activators such as CBP/p300.

ated repression after cellular exposure to the Phase II enzyme inducers.

The first proposed mechanism involves the cysteine-rich Keap1 protein directly sensing oxidative stress *via* thiol modification on its cysteine residues, which may cause a conformational change and thereby release Nrf2 from the complex. In an *in vitro* purified Keap1–Nrf2 interacting system (27), researchers found direct evidence that the most reactive cysteine residues of Keap1 (C257, C273, C288, C297) can be modified by an irreversible modifier of thiols, [^{3}H]dexamethasone mesylate. The chemopreventive agent sulforaphane showed a concentration-dependent disruption effect on the interaction between Keap1 and the Neh2 domain of Nrf2 in a native gel electrophoresis assay. Since the Neh2 domain of Nrf2 lacks cysteine residues and sulforaphane reacts rapidly but reversibly with thiols to give rise to a dithiocarbamate, the above observations suggest that sulforaphane might also trigger the release of Nrf2 from the Keap1–Nrf2 complex *in vivo* by targeting the cysteines on Keap1. Subsequent studies by the same group showed that mutation of both C273 and C288 could disrupt the repressive effect of Keap1 on Nrf2 (125), suggesting that modification of these two cysteines is critical for the repression of Nrf2.

Under homeostatic conditions, Keap1 not only sequesters Nrf2 in the cytoplasm, but also actively targets Nrf2 for ubiquitination and degradation by the 26S proteasome (92). Furthermore, Keap1-dependent Nrf2 degradation is faster than the Keap1-independent degradation process; therefore the second mechanism proposed involves release of Nrf2 from Keap1 in response to chemopreventive agents or oxidative/electrophilic stress. This may dramatically increase its protein stability, resulting in accumulation of Nrf2 in the cells and enhancement of Nrf2-dependent transcription activity. In addition to their potential to react with thiol-specific chemopreventive agents *in vivo*, the cysteines C273 and C288 of Keap1 are also required for Keap1-dependent ubiquitination of Nrf2. In pulse-chase labeling experiments conducted by Zhang and Hannink (137), mutations of these cysteines increased the half-life of Nrf2 up to twofold compared to wild type. These two cysteines may assist Nrf2 degradation by allowing binding of Keap1 to either an E2 ubiquitin conjugating enzyme or another component of a multisubunit E3 ubiquitin ligase complex. Alternatively, C273 and C288 may actively participate in ubiquitin transfer from the E2 protein to Nrf2. Interestingly, cysteine C151 was found to be uniquely required for inhibition of Keap1-dependent degradation of Nrf2 by sulforaphane and oxidative stress since sulforaphane has no effect on the stability of Nrf2 in the presence of the Keap1-C151S mutant protein. Thus, prior modification of Keap1 at C151 by chemopreventive agents may induce a conformational change that either alters the accessibility of C273 and C288 to the cytoplasmic environment to inhibit the Keap1 degradation or facilitates further thiol modification on these residues by chemical inducers. The results reported in this article, in conjunction with a number of other recent reports that the Nrf2 protein is stabilized by chemical inducers of Nrf2-dependent transcription, have provided one important mechanism for Keap1-mediated repression as well as the ability of chemical inducers to allow Nrf2 to escape Keap1-mediated repression.

Additional regulatory mechanisms are likely to co-operate with Keap1-dependent ubiquitination of Nrf2 to achieve precise regulation of Nrf2-dependent transcription. One possibility is that inhibition of Keap1-dependent degradation simply stabilizes Nrf2 and that additional posttranslational modifications to the Nrf2/Keap1 complex are required for dissociation of Nrf2 from Keap1. Phosphorylation of the Nrf2/Keap1 complex, triggered by kinase signaling pathways, might be another possibility for the release of Nrf2 from the complex. Mitogen-activated protein kinases (MAPKs) can be regulated by a wide variety of chemopreventive agents *in vitro* and *in vivo*. MAPKs have been shown to be involved in Nrf2-mediated gene regulation elicited by chemopreventive agents (73, 135). The activation of extracellular signal-regulated kinase (ERK) is required for the nuclear translocation of Nrf2 during the pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells (140). Huang *et al.* (46) first showed that protein kinase C (PKC) was involved in the activation of Nrf2 in response to antioxidants such as *tert*-butylhydroquinone (tBHQ) and β-naphthoflavone by showing that PKC inhibitors such as staurosporine could block Nrf2 activation. Further studies demonstrated that PKC could directly phosphorylate Nrf2 at Ser^{40} in response to tBHQ challenge, and that the phosphorylation of Nrf2 by PKC decreased the affinity of Nrf2 to Keap1, thus releasing Nrf2 from the complex and translocating it to the nucleus (13, 47). By using chemical inhibitors to investigate signaling pathways, phosphatidylinositol 3-kinase (PI3K) was shown to be involved in the activation of Nrf2-mediated gene expression triggered by antioxidants such as tBHQ or by oxidative stress, and treatment with PI3K inhibitors, such as LY294002 or wortmannin, significantly decreased the nuclear translocation of Nrf2 (63, 80). However, there is no evidence that activation of the PI3K signaling pathway leads to the phosphorylation of Nrf2. One hypothesis is that activation of the PI3K signaling pathway by antioxidants or by oxidative stress regulates the rearrangement of actin microfilaments and that the depolymerization of actin disrupts the Nrf2–Keap1 complex and increases the nuclear translocation of Nrf2 (63).

Agents that affect calcium homeostasis and protein glycosylation as well as physiological stresses such as hypoxia and glucose deprivation can disrupt the proper protein folding within the endoplasmic reticulum (ER). The unfolded protein response or ER stress can specifically activate PKR-like ER eIF2α kinase (PERK), which belongs to the eIF2α family and phosphorylates its putative substrate, eIF2α. Interestingly, Cullinan and Diehl (24) and Cullinan *et al.* (25) demonstrated that Nrf2 is a direct PERK substrate and that PERK-dependent phosphorylation is both necessary and sufficient to trigger dissociation of the Nrf2/Keap1 complex, thereby promoting Nrf2 nuclear import. Furthermore, the Nrf2 nuclear translocation activated by PERK is independent of the phosphorylation of eIF2α and is unlikely to result from the generation of ROS following ER stress since the antioxidant *N*-acetylcysteine did not block Nrf2 nuclear translocation.

Once in the nucleus, Nrf2 can bind not only to the specific ARE sequence on the target genes but also to other *trans*-acting factors that can coordinately regulate gene transcription with Nrf2. Among these *trans*-acting factors, the small

Maf proteins such as MafG and MafK have been reported to dimerize specifically with Nrf2 to regulate its transcriptional activity negatively (54, 84, 91). Recent studies indicated that cyclic AMP responsive element binding protein (CBP) can bind directly to the Nrf2 transactivation domain or to another member of the p160 protein family, ARE-binding protein-1 (68, 139). Furthermore, previous reports have shown that CBP can be phosphorylated by MAPK and that phosphorylation of the C-terminal activation domain of CBP by the MAPK cascades leads to enhanced transcriptional activity. Recently, MAPK signaling molecules, *e.g.,* Raf, have been shown to up-regulate Nrf2 transactivation synergistically with CBP in the reporter transient transfection systems (109), suggesting another role of MAPK in the regulation of Nrf2-mediated gene transcription.

The last proposed mechanism of regulating the Nrf2 signaling pathway involves Nrf2 autoregulation through an ARE-like element located in the proximal region of its promoter, leading to persistent nuclear accumulation of Nrf2 and subsequent induction of phase II genes. Using murine keratinocyte PE cells, Kwak *et al.* (76) found that Nrf2 mRNA levels increased approximately twofold at 6 h after treatment of the anticarcinogen ^{3}H-1,2-dithiole-3-thione. Further studies identified two ARE-like sequences in the promoter region of the Nrf2 gene, and a chromatin immunoprecipitation assay demonstrated the direct binding of Nrf2 to its own promoter.

In summary, chemopreventive agents activate Nrf2-mediated gene expression either by directly modifying the cysteine residues on Keap1 to disrupt the Nrf2–Keap1 complex, or by activating kinase signaling pathways such as MAPKs, PKC, and PI3K to phosphorylate the Nrf2/Keap1 complex and facilitate the release of Nrf2, or to increase the nuclear translocation of Nrf2 and regulate the transcriptional activity of Nrf2 nuclear co-activators. Although there is no direct evidence that chemopreventive agents can interact with the PERK signaling pathway, the identification of the PERK regulation pathway provided additional insight into the complexity of the cellular kinase signaling pathways that regulate Nrf2-mediated gene expression. It should also be noted that there are controversial results regarding the role of specific kinase signaling pathway. Because most studies use kinase inhibitors to perform the experiments, it is possible that some kinase inhibitors themselves may directly affect the regulation of Phase II or antioxidant gene transcription in addition to blocking kinase activation (64).

REGULATION OF NF-κB SIGNALING PATHWAYS

NF-κB is a family of transcription factors originally recognized in regulating gene expression in B cell lymphocytes. The basic characteristics and regulation mechanisms of NF-κB have been extensively reviewed elsewhere (37, 106). The NF-κB/Rel family includes six members: NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), RelA (p65), RelB (p68), c-Rel (p75), and v-Rel. Although all the members can bind DNA, only p65, c-Rel, and RelB contain a transactivation domain. Except for RelB, all NF-κB members can form homodimers as well as heterodimers with each other; however, the most prevalent activated form found in eukaryotes is the p50/p65 (NF-κB1/RelA) heterodimer. All RelA family members share the 300-amino acid Rel homology domain that is involved in dimerization, nuclear translocation, sequence-specific consensus DNA recognition, and interaction with inhibitory-κB (IκB) proteins (IκBα, IκBβ, IκBϵe). In resting or unstimulated cells, the NF-κB heterodimer, composed mainly of NF-κB1 (p50) and RelA (p65), is sequestered in the cytoplasm by the binding of inhibitor IκBs (Fig. 4). The binding of IκBs to NF-κB masks the nuclear localization sequence on NF-κB, retaining it in the cytosol. When cells receive the signals that activate NF-κB signaling pathways, IκBα is phosphorylated by the IκB kinase (IKK) complex (consisting of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ) at its Ser/Thr residues, causing subsequent ubiquitination and proteolytic degradation (50, 60). The exposure of the nuclear localization sequence of the NF-κB complex results in nuclear translocation mediated by the nuclear import machinery, leading to activation of transcription of NF-κB target genes.

Although the role of NF-κB signaling pathways in regulating immunoregulatory functions has been well accepted, accumulated evidence has also indicated the involvement of NF-κB signaling pathways in tumorigenesis/carcinogenesis (12, 106). In addition, inappropriate regulation of NF-κB is also involved in neurodegenerative diseases, ataxia telangiectasia, arthritis, and asthma (66). Activation of NF-κB has been associated with apoptotic cell death, either promoting or inhibiting apoptosis depending on the cell type and conditions (8, 15). In most cells, however, activation of NF-κB is believed to protect cells from apoptotic stimuli, presumably *via* the induction of survival genes (66). Therefore, the NF-κB pathway has become an important target of cancer therapeutic/chemopreventive approaches. Inhibition of this pathway by some phytochemicals and synthetic drugs can either prevent cancer development or inhibit cancer cell growth in animal models (12).

Regulation of NF-κB is a complicated process. In addition to being activated by canonical cytokine-mediated pathways, NF-κB can also be activated by other stimuli such as tumor necrosis factor (TNF-α), T and B cell mitogens, bacterial lipopolysaccharide (LPS), viruses, ultraviolet (UV) light, γ-rays, and oxidative stress (66, 89). Many of these stimuli trigger the NF-κB cascade primarily by activating the IKK complex and largely through signaling cross-talk involving the MAPK pathway (56, 105), PKC pathway (87), TNF-α receptor (94), and G-protein-coupled receptor signaling pathway (110). Moreover, there are other signaling factors that act more directly *via* the IκBs or phosphorylation of the NF-κB subunit p65 (123). Finally, some signaling pathways achieve their regulatory effects by modulating the interaction of NF-κB and its co-activators, such as CBP/P300, on the promoter of target genes (138). In many cell lines derived from human cancers, such as leukemia (39), prostate (49), breast (70), and pancreatic (126) cancer, the NF-κB signaling pathway is constitutively activated. All the above observations suggest that NF-κB could be a promising target for cancer chemopreventive agents. In trying to elucidate the mechanism by which dietary constituents exert their chemopreventive effects, re-

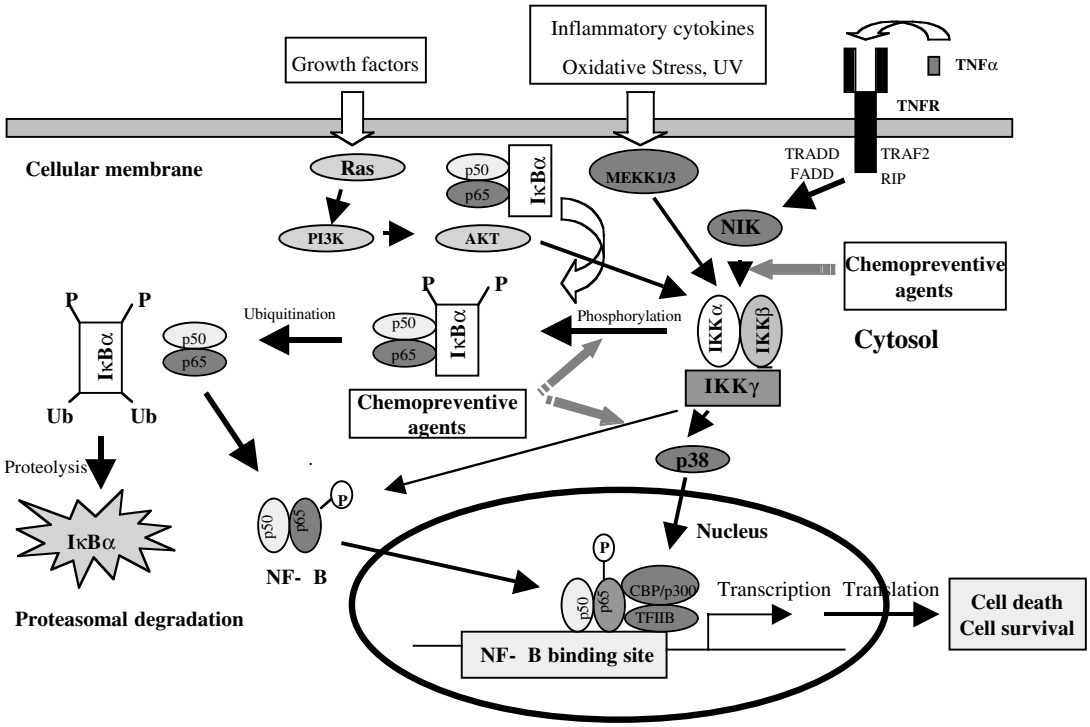

FIG. 4. Regulation of NF-κB signaling pathway by chemopreventive agents. NF-κB activation can be induced by the binding of TNF-α to its receptor, the inflammatory cytokine- and oxidative stress-triggered MAPK cascade, and the growth factor-activated PI3K cascade. Activation of the IκB kinase (IKK) complex by NF-κB-inducing kinase (NIK) and MEK kinases (MEKKs) results in the phosphorylation and degradation of IκBα and the release of the NF-κB dimer. The NF-κB then translocates into the nucleus and activates the transcription of target genes involved in cell survival or death events. IKKs can also modulate NF-κB activity by directly phosphorylating the Rel/p65 subunit. Chemopreventive agents can suppress the activation of NF-κB by blocking the activation of IKKs, blocking phosphorylation of IκBα and the direct phosphorylation of p65 subunit. Alternatively, chemopreventive compounds by themselves might activate the NF-κB pathway, especially at low concentrations. FADD, Fas-associated death domain; RIP, regulated intramembrane proteolysis; TNFR, TNF receptor; TRADD, TNF receptor-associated death domain; TRAF2, TNF receptor-associated factor 2; Ub, ubiquinone.

searchers have noted that several chemopreventive phytochemicals can either abrogate the activation of NF-κB signaling induced by carcinogens or tumor-promoting agents or suppress the basal level of NF-κB activity in many cancer cell lines (12). Most interestingly, these chemopreventive agents can sensitize tumors to chemotherapeutic drugs by blocking the activation of NF-κB elicited by chemotherapeutic agents (12). However, since the NF-κB pathway may involve many converging signaling pathways, it is important to understand how different chemopreventive agents differentially modulate the NF-κB pathway. Below, we discuss our current knowledge and understanding of the modulatory activities of natural chemopreventive agents on NF-κB pathways.

Of the natural chemopreventive agents, isothiocyanates and phenolic compounds have gained much attention. Isothiocyanates such as sulforaphane, phenylethyl isothiocyanate (PEITC), and allyl isothiocyanate (AITC) are a group of dietary phytochemicals derived from cruciferous vegetables. These compounds are well known for their ability to induce Phase II enzymes and chemopreventive effects. Our study (58) has shown these three isothiocyanates dose-dependently inhibited the LPS-induced NF-κB-luciferase reporter activity in HT-29 cells stably expressing the NF-κB-luciferase reporter. Specifically, this inhibition was associated with the blocking of LPS-induced IκBα phosphorylation. Studies by another group have produced similar results with sulforaphane, confirming its ability to reduce DNA binding of NF-κB in macrophages without interfering with LPS-induced degradation of the IκBα or with nuclear translocation of NF-κB (41). However, benzyl isothiocyanate (BITC) at 25 μ*M* increases NF-κB binding to its binding site in unstimulated HT-29 cells, reaching a maximum at around 6 h (97).

Studies have shown that green tea, black tea, or tea polyphenol preparations have inhibitory effects on carcinogenesis in rodent animal models (67, 131). A major component of green tea extracts is (−)-epigallocatechin gallate (EGCG). EGCG is an antioxidant that efficiently scavenges $^{\cdot}OH$ and $O_2^{\cdot}$ radicals (111), and it is also well known for its cancer chemopreventive effects. Many *in vitro* studies have shown that EGCG can modulate cell cycle progression and induce apoptosis (3, 19). The anti-promoting effects of EGCG have been attributed to its ability to modulate the NF-κB signaling pathway. Nomura *et al.* (93) demonstrated that EGCG and theaflavins inhibit 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced NF-κB activity by blocking the TPA-induced phosphorylation of IκBα at Ser^{32}. Moreover,

the NF-κB and target DNA binding activity induced by TPA was also blocked by tea-derived polyphenols in the JB6 mouse epidermal cell line. In HT-29 cells, EGCG was shown to diminish the LPS-induced IκBα phosphorylation and cause dramatic activation of caspase-3 (58). In this study, however, tea catechins including epicatechin, epicatechin gallate, epigallocatechin, and EGCG had little effect or slightly stimulatory effects on the LPS-induced NF-κB-luciferase activity at concentrations up to 100 μ*M*.

Resveratrol, derived from grape skin, has been studied for its role in the regulation of NF-κB in several experimental systems. In female Sprague-Dawley rats (11), receiving a resveratrol-supplemented diet in addition to 7,12-dimethylbenz[*a*]anthracene significantly reduced tumor incidence as well as average number of tumors compared with those treated with 7,12-dimethylbenz[*a*]anthracene alone. The latency to onset of tumor development was prolonged by 3 weeks relative to the control animals. Gel shift assay of the mammary tissue indicated resveratrol significantly suppresses the NF-κB activity. An *in vitro* study using human breast MCF-7 cells indicated that resveratrol could suppress TNF-induced NF-κB activation. Like most NF-κB inhibitors, resveratrol blocks the phosphorylation and degradation of IκBα (44). More directly, resveratrol also blocks the phosphorylation of the p65 subunit, which is required for transactivation function (83). It has been postulated that activation of NF-κB expression may be associated with the progression of epithelial cells to a malignant phenotype (70), and aberrant expression of NF-κB in human breast cancer specimens has been reported (115). Resveratrol may attenuate the early critical steps involved in carcinogen-driven transformation of mammary epithelial cells. In another study, resveratrol was shown to protect SKH-1 hairless mice from UVB-mediated cutaneous damage. Mechanistic studies in normal epidermal keratinocytes indicated that resveratrol blocked the UVB-mediated activation of NF-κB in a dose- and time-dependent manner, probably by inhibiting UVB-mediated phosphorylation and degradation of IκBα and activation of IKKα (1). However, our *in vitro* studies using the stable human colon carcinoma HT-29 cells expressing an NF-κB-luciferase reporter indicated that resveratrol treatment dramatically induced the LPS-induced NF-κB-luciferase at doses between 25 and 100 μ*M* and showed an inhibition of LPS-induced phosphorylation of IκBα at 50 μ*M*. This suggests the involvement of other mechanism(s) that might be independent of IκBα phosphorylation (58). This result was supported in part by the inability of resveratrol to reduce cell viability at 50 μ*M*, indicating the involvement of a protective mechanism due to activation of NF-κB by resveratrol at the given concentration.

Curcumin, the main yellow coloring component of turmeric, has displayed anticarcinogenic effects in many animal models (118). In human non-small cell lung carcinoma H1299 cells, pretreatment with curcumin abolished DNA binding of NF-κB, IκBα kinase activation, and IκBα phosphorylation and degradation induced by cigarette smoke, a major cause of malignancies including esophageal and lung cancers (112). In the study performed by Chun *et al.* (21), curcumin treatment attenuated NF-κB activation following topical application of TPA in ICR mouse skin by blocking the phosphorylation and degradation of the inhibitory protein IκBα. The curcumin treatment also decreased the NF-κB DNA binding activity as indicated by gel shift assay. Further study indicated that the MAPK ERK pathway positively regulated NF-κB activity by inhibiting the phosphorylation of IκBα, and that curcumin blocked the activation of ERK induced by TPA. Therefore, curcumin might exert its inhibitory effect on NF-κB activity by inhibiting the degradation of IκBα as well as the activation of the ERK.

Quercetin, a major flavonoid present in the human diet, has been widely studied for its protective role in cardiovascular systems. In human aortic smooth muscle cells (85), quercetin potently inhibited NF-κB binding activities to repress TNF-α-induced matrix metalloproteinase-9 expression and induced G1 cell cycle arrest. Baicalein and wogonin are flavonoids isolated from the root of the Chinese herb *Scutellaria baicalensis*, which exert anti-inflammatory and antioxidant effects. In the studies performed by Lee *et al.* (79) and Suk *et al.* (116), both baicalein and wogonin inhibited LPS-induced nitric oxide production and suppressed inducible NO synthase induction and NF-κB activation in BV-2 microglial cells. Although the exact mechanism by which baicalein and wogonin inhibit NF-κB activation was not elucidated in these two studies, the authors suggested that the antioxidant properties of the two flavonoids might contribute to the blocking effect.

Silymarin is another polyphenolic flavonoid mixture known to have hepatoprotective, anti-inflammatory, and anticarcinogenic effects. In the murine macrophage-like cell line RAW 264, silymarin blocked the LPS-induced DNA binding activity of NF-κB/Rel (62), and this effect was mediated by inhibiting the degradation of the inhibitory factor IκBα. Further study showed that silymarin suppressed the production of ROS generated by H_2O_2. Thus, the radical-scavenging activity of silymarin may explain its inhibitory effect on NF-κB/Rel activation.

CAPE is a natural honeybee product exhibiting a wide variety of biological effects including antioxidant and antitumor functions. CAPE has chemopreventive effects in intestinal, colon, and skin cancer models. CAPE also protects against the induction of γ-glutamyl transpeptidase-positive altered hepatic foci during promotion in hepatocarcinogenesis. Additionally, CAPE decreases the nuclear localization of the p65 subunit of NF-κB by up to 85%. While this decrease was independent of IκBα phosphorylation or degradation, the mechanism of the down-regulation of p65 subunit in the nucleus by CAPE remains unclear (17).

Lupeol is a triterpene found in common fruits such as olive and mango. It has been recognized as a chemopreventive agent because of its biological properties, including strong antioxidant, antimutagenic, and anti-inflammatory effects. In a mouse skin tumorigenesis model (102), topical application of lupeol significantly reduced TPA-mediated tumor incidence and tumor body burden as well as delayed the latency period for tumor appearance. These effects were associated with the inhibition of TPA-induced activation of NF-κB and IKKα and degradation and phosphorylation of IκBα.

Capsaicin is a pungent component of red pepper that exhibits profound anticarcinogenic and antimutagenic activities. Topical application of capsaicin onto the dorsal skin of female ICR mice prior to TPA application significantly attenuates TPA-induced NF-κB activation in the skin (119). Capsaicin can also block the TNF-mediated activation of NF-κB

in human myeloid ML-1a cells and block the degradation of IκB (113).

Vitamin C (ascorbic acid) is one of the most important physiological antioxidants. When administered at high doses, vitamin C has been suggested to have both a preventative and therapeutic role in a number of pathologies including cancer, atherosclerosis, and viral infections such as those with human immunodeficiency virus. Vitamin C was shown to block interleukin-1- and TNF-mediated degradation and phosphorylation of IκBα by inhibiting IKK activation (16). Inhibition of TNF-driven IKK activation is mediated by the rapid and sustained activation of p38 MAPK. Moreover, inhibition of NF-κB activation by vitamin C is probably not due to an antioxidant effect because redox-insensitive pathways to NF-κB are also blocked (16).

Carnosol is a phytopolyphenol found in rosemary that accounts for most of the herb's antioxidant activities. Carnosol inhibited the binding of benzo[*a*]pyrene to epidermal DNA, tumor initiation by 7,12-dimethylbenz[*a*]anthracene and tumor promotion by TPA in the mouse skin experimental model (48, 114). Treatment of mouse macrophage RAW 264.7 cell line with carnosol remarkably reduced LPS-stimulated NF-κB activation by blocking NF-κB subunit translocation and NF-κB DNA binding activity. Similar to vitamin C, carnosol also inhibited LPS-induced phosphorylation, degradation of IκBα, and p38 kinase activation (82). The contradictory role of p38 kinase in the regulation of NF-κB activation in the vitamin C and carnosol studies suggests that the effects of p38 in NF-κB signaling pathways may be cell line dependent, stimuli dependent, and time dependent.

Schubert *et al.* (104) investigated the mechanism of the inhibition of NF-κB activation in vascular endothelial cells by the natural antioxidants pomegranate, red winem and *N*-acetylcysteine. Although these antioxidants could potently inhibit TNF-α-induced NF-κB activation by blocking NF-κB nuclear translocation and by inhibiting DNA binding activity, pomegranate and *N*-acetylcysteine were unable to inhibit TNF-α-induced $Ser^{32/36}$ phosphorylation and degradation of IκBα. Surprisingly, these antioxidants alone could induce the phosphorylation of IκBα at sites other than $Ser^{32/36}$, and this phosphorylation did not affect the degradation of IκBα or NF-κB nuclear translocation. Additionally, *N*-acetylcysteine could block the TNF-α-induced direct phosphorylation of p65 on Ser^{536}. Further studies would be needed in the future.

Taken together, NF-κB signaling involves several complex mechanisms, and different chemopreventive agents differentially regulate this signaling pathway. Additional intensive studies on the regulation of NF-κB and other signaling pathways by these compounds are required. In addition, the biological consequences elicited by these natural agents need further elucidation of the chemopreventive mechanisms and functions that are involved.

REGULATION OF AP-1 SIGNALING PATHWAYS

Although AP-1 was one of the first mammalian transcription factors identified, its physiological function has yet to be fully understood. AP-1 activity can be regulated by a large variety of stimuli including pro-inflammatory cytokines, growth factors, oxidative stress, and tumor promoters (6). AP-1-mediated gene transcription is involved in a wide range of cellular events such as cell proliferation, cell cycle control, apoptosis, differentiation, and tumorigenesis. AP-1 is a dimeric transcription factor which composed basic leucine-zipper family members including Jun (c-Jun, JunB, and JunD), Fos (c-Fos, FosB, Fra1, and Fra2), ATF (ATF2, B-ATF, JDP1, and JDP2), and Maf (MafA, MafB, c-Maf, and MafG/F/K) protein families (31, 108). The AP-1 dimer complex can recognize the TPA response element or cyclic AMP response element within the promoter region of target genes. AP-1 dimer activity is regulated by transcriptional regulation of individual subunits, dimer composition, post-translational modification of the subunits, and the interactions between the dimer and other proteins (Fig. 5). Two of the components, Jun and Fos, are the most common members in this group. Unlike Jun proteins, Fos proteins cannot homodimerize but form heterodimers with Jun proteins to enhance their DNA binding activities. Among the Fos protein subfamilies, only c-Fos and FosB contain transcriptional activation domains, while Fra1 and Fra2 do not. c-Jun, c-Fos, and FosB are very potent mammalian transcription factors, and their roles in tumorigenesis have been widely investigated. Studies based on cell culture models indicate that all of them can efficiently transform cells (6, 59) and that the activation of AP-1 is important in traversing tumor promotion and/or progression stages (30). Inhibition of Fos and Jun expression in mouse fibroblasts and erythroleukemia cells has demonstrated their requirement for proliferation and cell cycle progression (107). In mice overexpressing c-Fos, Wang *et al.* (127) demonstrated the correlation between c-Fos expression levels and chondrogenic tumor development. c-Jun was shown to be more important in the development of skin and liver tumors (28). For example, down-regulation of c-Jun/AP-1 activity using a dominant-negative c-Jun (TAM67) in basal keratinocytes or conditional inactivation of c-Jun in the liver interferes with the development of chemically induced papillomas and liver tumors, respectively (32, 134). However, some Jun (JunB and JunD) and Fos family proteins suppress tumor formation (26, 96, 98). Whether AP-1 is oncogenic or anti-oncogenic depends on the cell type, its differentiation state, its tumor stage, and the genetic background of the tumor (5, 81).

With regards to the signaling pathways that regulate AP-1 activity in response to pro-inflammatory cytokines or exogenous stress, the major MAPK signaling pathways that include ERK, c-Jun NH_2-terminal kinase (JNK) and p38 cascades appear to be critical (18). For instance, once activated, JNKs translocate to the nucleus where they phosphorylate c-Jun and ATF2 to potentiate their transcription activities. The increased activity of c-Jun and ATF2 may result in the induction of c-Jun transcription as well as that of AP-1 target genes. Induction of AP-1 activity by the p38 MAPK pathway is mediated by its direct phosphorylation and activation of ATF2. The ERK pathway is also involved in the regulation of AP-1 activity since constitutive expression of activated oncogenes such as Ha-*ras* results in elevated AP-1 activity (43, 103). PI3K has also been shown to cross-talk with JNK to regulate AP-1 activity in response to interleukin-1 (36). MAPK cascades can also directly modulate the expression of c-Fos. c-Fos contains a serum response element in the

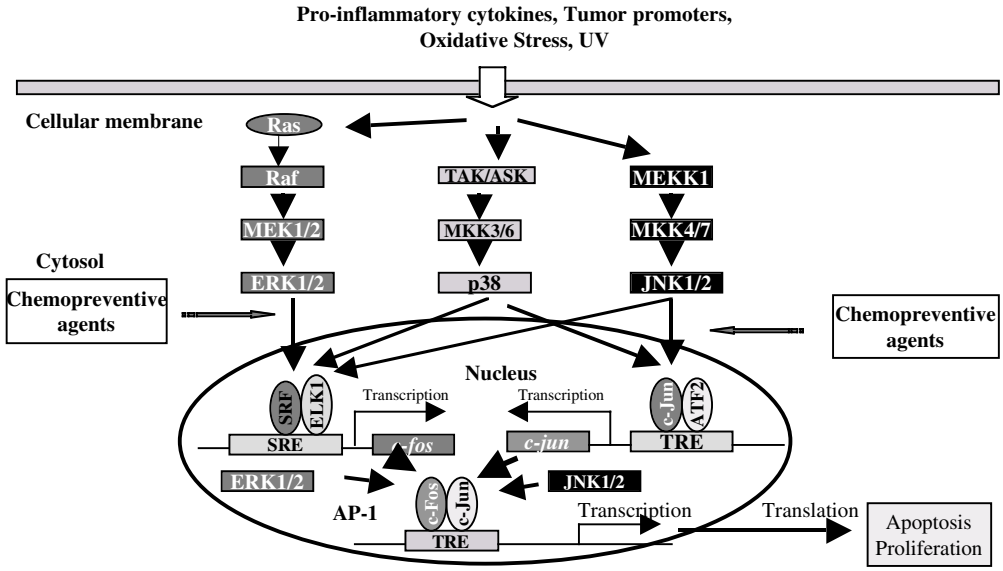

FIG. 5. The AP-1 signaling pathway and possible modulation by chemopreventive agents. MAPK signaling pathways such as ERK, JNK, and p38 cascades can phosphorylate and regulate the transcriptional activities of transcription factors such as ELK1, c-Jun, and ATF2 that are involved in the transcription of AP-1 genes, including c-*fos* and c-*jun*. Activation of ERK and JNK pathways can also increase the activities of the AP-1 proteins c-Fos and c-Jun, respectively. By blocking the activation of the MAPK cascades elicited by tumor promoters, oxidative stress, or UV irradiation, chemopreventive agents can suppress the resulting AP-1 activation. Nevertheless, some chemopreventive compounds by themselves can also activate the AP-1 signaling pathway at low concentrations. MKK, MEK kinase; SRE, serum response element; SRF, serum response factor; TAK/ASK, transforming growth factor-β-activated kinase/apoptosis signal-regulating kinase; TRE, transforming growth factor-β response element.

promoter region that interacts with a transcription factor complex containing dimeric serum response factor and ELK1. The MAPKs can phosphorylate and activate ELK1, thereby inducing the expression of c-Fos (65, 128). Activation of MAPK pathways also appears to stimulate the transcriptional activities of AP-1 components as well as to increase the abundance of their proteins (128). Since regulation of the AP-1 pathway is highly complicated and activation of AP-1 can contribute to cell survival or cell death by regulating different set of genes involved in cell proliferation, differentiation, apoptosis, angiogenesis, and tumor invasion, many researchers are currently extensively studying the modulation of AP-1 activity by various chemopreventive agents.

Several isothiocyanates have been studied for their regulatory effects on AP-1. BITC treatment at 25 μ*M* in HT-29 cells results in increased AP-1 DNA binding activity and induction of AP-1 activity associated with an earlier activation of JNK (97). This result is consistent with our recent study showing that PEITC and sulforaphane induced AP-1-luciferase activity, though induction only occurs at lower concentrations in HT-29 C-4 cells (57). Interestingly, AITC dose-dependently increased AP-1-luciferase activity at concentrations up to 50 μ*M*. In addition, the expression of endogenous cyclin D1, a gene under the control of AP-1, positively correlated with AP-1 luciferase activity induced by these isothiocyanates. Since some of the Phase II genes such as NADP(H):quinone reductase have AP-1 binding sites in the promoter region, the induction of AP-1 activity by low concentrations of isothiocyanates may be involved in the induction of Phase II or antioxidant enzymes such as HO-1 (74). A recent study on *N*-acetylcysteine conjugates of BITC and PEITC compounds, which inhibit lung tumorigenesis in A/J mice after administration of the carcinogen benzo[*a*]pyrene, provided the first possible *in vivo* evidence that activation of MAPKs and AP-1 transcription factors may be involved in the chemopreventive activity of these compounds.

EGCG and theaflavins inhibit TPA- and epidermal growth factor-induced transformation of JB6 mouse epidermal cells at doses of 5–25 μ*M* (29). This finding correlates with the inhibition of AP-1 DNA binding and transcriptional activity. The inhibition of AP-1 activity by EGCG was associated with inhibition of JNK activation but not ERK activation. Interestingly, in another study where EGCG blocked the UVB-induced c-Fos activation in a human keratinocyte cell line HaCaT (20), inhibition of p38 activation was suggested as the major mechanism underlying the effects of EGCG. The role of MAPK pathways in the regulation of AP-1 activity by EGCG was further investigated in the Ha-*ras*-transformed JB6 cell line (22). Treatment of 30.7b Ras 12 cells (*Ras*-transformed mouse epidermal cells) with EGCG resulted in decreased levels of phosphorylated ERK1/2 and MAPK kinase (MEK) 1/2 without affecting JNK phosphorylation or p38 pathways. EGCG was also found to inhibit the association between Raf-1 (an upstream protein kinase) and MEK1. Similarly, treatment of Ha-*ras*-transformed human bronchial cells (21BES) with EGCG was shown to inhibit c-Jun and ERK1/2 phosphorylation as well as the phosphorylation of ELK1 and MEK1/2 (133). In contrast to these reports, EGCG has been shown to markedly increase AP-1 factor-associated responses through an MAPK signaling mechanism in normal

human keratinocytes, suggesting that the signaling mechanism of EGCG action could be markedly different in different cell types (9). Similarly, we found that EGCG treatment resulted in a dose-dependent increase in AP-1-luciferase activity at concentrations ranging from 20 to 100 μ*M*, and both 46- and 54-kDa isoforms of phospho-JNKs were activated by EGCG treatment in HT-29 C-4 cells (19, 57). Antioxidants, such as glutathione and *N*-acetyl-L-cysteine, can block the activation of the three MAPKs including JNK, ERK, and p38, suggesting that EGCG-induced oxidative stress might activate MAPK cascades as well as AP-1 activity (19). While *in vitro* studies showed that regulation of MAPKs by EGCG is highly cell line dependent, *in vivo* studies showed UVB-induced phosphorylation of ERK, JNK, and p38 can be blocked by EGCG treatment in the SKH-1 hairless mouse skin model (2, 122). This suggests that EGCG might inhibit the tumor initiator/promoter-induced AP-1 activity by blocking the activation of all three MAPK cascades.

Lagarrigue *et al.* (78) showed that the flavonoid quercetin could inhibit the transformation of the rat liver epithelial (REL) cell line overexpressing c-Fos, suggesting that regulation of c-Fos/AP-1 complexes might be involved in the anti-transforming mechanism of quercetin. Pretreatment of RAW 264.7 macrophages with quercetin blocked LPS-induced TNF-α transcription. This effect of quercetin was mediated by inhibiting the phosphorylation and activation of JNK/stress-activated protein kinase, by suppressing AP-1 DNA binding, and by down-regulating the TNF-α transcription. Although quercetin can block the LPS-induced activation of ERK and p38, it has been suggested that these kinase pathways might not be involved in the quercetin-mediated inhibition of TNF-α transcription and AP-1 activity (124). Quercetin and other flavonoids, such as chalcone, flavone, and apigenin, stimulate AP-1 activity in AP-1-luciferase stable human endometrial adenocarcinoma Ishikawa cells and human embryonic kidney 293 cells. Further study using the GAL4 transcriptional activation system of c-Jun demonstrated that basal c-Jun activity is not strongly stimulated by flavonoids such as apigenin and that the phorbol 12-myristate-13-acetate-induced Gal4-c-Jun activity can be strongly blocked by apigenin (35). Interestingly, we found that low concentrations (<50 μ*M*) of flavonoids such as quercetin and apigenin increased AP-1 activity in a prostate cancer cell line stably expressing AP-1-luciferase reporter (authors unpublished data); however, at high flavonoid concentrations, the luciferase activity of the AP-1 reporter was inhibited. These studies suggest that regulation of basal AP-1 activity by flavonoids is also dependent on the concentration and chemical structure. Further *in vitro* and *in vivo* studies would be needed.

Resveratrol has been shown to inhibit the activity of AP-1 as demonstrated by several studies. One of the earliest reports used a gel shift assay to show that resveratrol inhibits TNF-dependent AP-1 activation in U-937 cells, and that pretreatment with resveratrol strongly attenuates TNF-activated JNK and MEK kinases (83). We have previously reported that pretreatment of HeLa cells with resveratrol inhibits the AP-1-luciferase reporter activity as well as the activation of ERK, JNK, and p38 kinases elicited by UVC and phorbol 12-myristate 13-acetate (136). Furthermore, the effects of resveratrol on MAPK and AP-1 may involve the inhibition of both protein tyrosine kinases and PKC. More recently, Woo *et al.* (130) also reported that in CaSki cells resveratrol inhibited phorbol 12-myristate 13-acetate-induced matrix metalloproteinase-9 expression, which is mediated by the regulation of transcription factor AP-1. Moreover, resveratrol inhibited the activation of the kinases JNK and PKC-δ, which regulate matrix metalloproteinase-1 expression and converge at AP-1 signaling pathways. In the HT-29 model, AP-1-luciferase activity following treatment with resveratrol increased in a dose-dependent manner (1–50 μ*M*) but decreased at higher doses (75–100 μ*M*), while JNK activity was unaffected (57). The effects of resveratrol on AP-1 may appear to be cellular dependent, external stimuli dependent, as well as concentration dependent.

Curcumin has been shown to suppress the activation of TPA-induced AP-1 in HL-60 cells and Raji DR-LUC cells (38, 42). Curcumin treatment also suppresses constitutive AP-1 activity in the prostate cancer cell line LNCaP, PC-3 DU145 (88, 90). In our recent study (57), curcumin increased AP-1-luciferase activity dose-dependently and then decreased it at higher doses in the presence or absence of TPA stimulation in a HT-29 cell line stably transfected with a AP-1 luciferase reporter. Inhibition of AP-1 transcriptional activity by curcumin administration also correlated with inhibition of Lewis lung carcinoma (LLC) invasion in the orthotopic implantation model (51). More recently, curcumin was reported to suppress LPS-induced cyclooxygenase-2 gene expression by inhibiting AP-1 DNA binding in BV2 microglial cells (61).

SUMMARY

Chemopreventive agents derived from the human diet have gained much attention recently, as the cancer incidence remains high and the concept of cancer intervention by phytochemicals is more widely accepted. Because of the rapid progress in our understanding of the genetic and epigenetic mechanisms of tumorigenesis at the signal transduction level, more molecular targets have been identified for intervention at the initiation, promotion, and progression steps of tumor development. Among these are transcription factors such as Nrf2, NF-κB, and AP-1. The signaling pathways mediated by these transcription factors in response to pro-inflammatory cytokines, growth factors, xenobiotics, oxidative stress, and tumor promoters can regulate a wide array of genes involved in many cellular events such as cell cycle control, differentiation, transformation, apoptosis, and tumorigenesis. Because of their potential role in preventing cancer development, the mechanisms by which phytochemicals regulate signaling pathways have been extensively studied. While many chemopreventive agents directly act on the Nrf2/ARE signaling pathway to induce detoxification and antioxidant enzyme systems, which may block tumor initiation in normal cells, others may exert anti-promotion or anti-progression effects by modulating the NF-κB and AP-1 signaling pathways that are usually des-regulated in initiated or pre-cancerous cells.

Although experimental data from cell culture models provide valuable information about the molecular and cellular

mechanisms involved in the modulation of Nrf2, NF-κB, and AP-1 signaling pathways, the signal transduction cascades between phytochemicals and transcription factors are still not fully understood both in cell culture models, and, more importantly, in *in vivo* tumor models. For instance, diametrically opposite theories exist in the current understanding of the relationship between regulation of these signaling pathways and tumorigenesis, especially the regulation of NF-κB and AP-1 signaling pathways. One explanation for the observed difference in the literature could be related to the use of different cell lines or animal models and different concentration and type of chemopreventive agents; therefore different stimuli would have impinged upon the system. In the final analysis, it is quite likely that the roles of NF-κB and AP-1 signaling pathways in response to chemoprevention are cell type and signal dependent and that the level and duration of inhibition and/or activation may also be important. While chemoprevention studies using animal models certainly provide promising results for the chemopreventive agents discussed in this article, future affirmative human clinical trials as well as epidemiological data would be needed to support their eventual chemopreventive potentials.

ACKNOWLEDGMENTS

We thank our collaborators and colleagues for their helpful discussions and suggestions. We regret that authors whose work relates to this discussion could not be directly cited because of space limitation. This work is supported in part by National Institutes of Health grants R01-CA73674, R01-CA92515, and R01-CA94828.

ABBREVIATIONS

AITC, allylisothiocyanate; AP-1, activator protein-1; ARE, antioxidant response element; BITC, benzyl isothiocyanate; CAPE, caffeic acid phenethyl ester; CBP, cyclic AMP responsive element binding protein binding protein; EGCG, (−)-epigallocatechin-3-gallate; EpRE, electrophile response element; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; HO-1, heme oxygenase-1; IκB, inhibitory-κB; IKK, IκB kinase; JNK, c-Jun NH_2-terminal kinase; Keap1, kelch-like ECH-associated protein-1; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; NF-κB, nuclear factor-κB; Nrf2, nuclear factor E2-related factor 2; PEITC, phenylethyl isothiocyanate; PERK, PKR-like ER eIFα kinase; ROS, reactive oxygen species; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; tBHQ, *tert*-butylhydroquinone; TNF, tumor necrosis factor; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; UV, ultraviolet.

REFERENCES

1. Adhami VM, Afaq F, and Ahmad N. Suppression of ultraviolet B exposure-mediated activation of NF-kappaB in normal human keratinocytes by resveratrol. *Neoplasia* 5: 74–82, 2003.
2. Afaq F, Ahmad N, and Mukhtar H. Suppression of UVB-induced phosphorylation of mitogen-activated protein kinases and nuclear factor kappa B by green tea polyphenol in SKH-1 hairless mice. *Oncogene* 22: 9254–9264, 2003.
3. Ahmad N, Gupta S, and Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells. *Arch Biochem Biophys* 376: 338–346, 2000.
4. Alam J, Stewart D, Touchard C, Boinapally S, Choi AM, and Cook JL. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem* 274: 26071–26078, 1999.
5. Angel P and Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1072: 129–157, 1991.
6. Angel P and Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1072: 129–157, 1991.
7. Aoki Y, Sato H, Nishimura N, Takahashi S, Itoh K, and Yamamoto M. Accelerated DNA adduct formation in the lung of the Nrf2 knockout mouse exposed to diesel exhaust. *Toxicol Appl Pharmacol* 173: 154–160, 2001.
8. Baichwal VR and Baeuerle PA. Activate NF-kappa B or die? *Curr Biol* 7: R94–R96, 1997.
9. Balasubramanian S, Efimova T, and Eckert RL. Green tea polyphenol stimulates a Ras, MEKK1, MEK3, and p38 cascade to increase activator protein 1 factor-dependent involucrin gene expression in normal human keratinocytes. *J Biol Chem* 277: 1828–1836, 2002.
10. Balogun E, Hoque M, Gong P, Killeen E, Green CJ, Foresti R, Alam J, and Motterlini R. Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. *Biochem J* 371: 887–895, 2003.
11. Banerjee S, Bueso-Ramos C, and Aggarwal BB. Suppression of 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-kappaB, cyclooxygenase 2, and matrix metalloprotease 9. *Cancer Res* 62: 4945–4954, 2002.
12. Bharti AC and Aggarwal BB. Nuclear factor-kappa B and cancer: its role in prevention and therapy. *Biochem Pharmacol* 64: 883–888, 2002.
13. Bloom DA and Jaiswal AK. 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. *J Biol Chem* 278: 44675–44682, 2003.
14. Bosland MC. Use of animal models in defining efficacy of chemoprevention agents against prostate cancer. *Eur Urol* 35: 459–463, 1999.
15. Bours V, Bentires-Alj M, Hellin AC, Viatour P, Robe P, Delhalle S, Benoit V, and Merville MP. Nuclear factor-kappa B, cancer, and apoptosis. *Biochem Pharmacol* 60: 1085–1089, 2000.
16. Bowie AG and O'Neill LA. Vitamin C inhibits NF-kappa B activation by TNF via the activation of p38 mitogen-activated protein kinase. *J Immunol* 165: 7180–7188, 2000.

17. Carrasco-Legleu CE, Marquez-Rosado L, Fattel-Fazenda S, Arce-Popoca E, Perez-Carreon JI, and Villa-Trevino S. Chemoprotective effect of caffeic acid phenethyl ester on promotion in a medium-term rat hepatocarcinogenesis assay. *Int J Cancer* 108: 488–492, 2004.
18. Chang L and Karin M. Mammalian MAP kinase signalling cascades. *Nature* 410: 37–40, 2001.
19. Chen C, Shen G, Hebbar V, Hu R, Owuor ED, and Kong AN. Epigallocatechin-3-gallate-induced stress signals in HT-29 human colon adenocarcinoma cells. *Carcinogenesis* 24: 1369–1378, 2003.
20. Chen W, Dong Z, Valcic S, Timmermann BN, and Bowden GT. Inhibition of ultraviolet B-induced c-fos gene expression and p38 mitogen-activated protein kinase activation by (−)-epigallocatechin gallate in a human keratinocyte cell line. *Mol Carcinog* 24: 79–84, 1999.
21. Chun KS, Keum YS, Han SS, Song YS, Kim SH, and Surh YJ. Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappaB activation. *Carcinogenesis* 24: 1515–1524, 2003.
22. Chung JY, Park JO, Phyu H, Dong Z, and Yang CS. Mechanisms of inhibition of the Ras-MAP kinase signaling pathway in 30.7b Ras 12 cells by tea polyphenols (−)-epigallocatechin-3-gallate and theaflavin-3,3′-digallate. *FASEB J* 15: 2022–2024, 2001.
23. Corpet DE and Pierre F. Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system. *Cancer Epidemiol Biomarkers Prev* 12: 391–400, 2003.
24. Cullinan SB and Diehl JA. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem* 279: 20108–20117, 2004.
25. Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, and Diehl JA. Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol* 23: 7198–7209, 2003.
26. Deng T and Karin M. JunB differs from c-Jun in its DNA-binding and dimerization domains, and represses c-Jun by formation of inactive heterodimers. *Genes Dev* 7: 479–490, 1993.
27. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, and Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci U S A* 99: 11908–11913, 2002.
28. Domann FE, Levy JP, Birrer MJ, and Bowden GT. Stable expression of a c-JUN deletion mutant in two malignant mouse epidermal cell lines blocks tumor formation in nude mice. *Cell Growth Differ* 5: 9–16, 1994.
29. Dong Z. Effects of food factors on signal transduction pathways. *Biofactors* 12: 17–28, 2000.
30. Dong Z, Birrer MJ, Watts RG, Matrisian LM, and Colburn NH. Blocking of tumor promoter-induced AP-1 activity inhibits induced transformation in JB6 mouse epidermal cells. *Proc Natl Acad Sci U S A* 91: 609–613, 1994.
31. Eferl R and Wagner EF. AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 3: 859–868, 2003.
32. Eferl R, Ricci R, Kenner L, Zenz R, David JP, Rath M, and Wagner EF. Liver tumor development. c-Jun antagonizes the proapoptotic activity of p53. *Cell* 112: 181–192, 2003.
33. Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, Harada T, and Yamamoto M. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. *Toxicol Sci* 59: 169–177, 2001.
34. Fahey JW, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, and Lozniewski A. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. *Proc Natl Acad Sci U S A* 99: 7610–7615, 2002.
35. Frigo DE, Duong BN, Melnik LI, Schief LS, Collins-Burow BM, Pace DK, McLachlan JA, and Burow ME. Flavonoid phytochemicals regulate activator protein-1 signal transduction pathways in endometrial and kidney stable cell lines. *J Nutr* 132: 1848–1853, 2002.
36. Funakoshi-Tago M, Tago K, Sonoda Y, Tominaga S, and Kasahara T. TRAF6 and C-SRC induce synergistic AP-1 activation via PI3-kinase-AKT-JNK pathway. *Eur J Biochem* 270: 1257–1268, 2003.
37. Haddad JJ. Antioxidant and prooxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. *Cell Signal* 14: 879–897, 2002.
38. Han SS, Keum YS, Seo HJ, and Surh YJ. Curcumin suppresses activation of NF-kappaB and AP-1 induced by phorbol ester in cultured human promyelocytic leukemia cells. *J Biochem Mol Biol* 35: 337–342, 2002.
39. Hatta Y, Arima N, Machino T, Itoh T, Hashimoto S, Takeuchi J, Sawada U, Hayakawa S, Yamamoto T, and Horie T. Mutational analysis of IκBα in hematologic malignancies. *Int J Mol Med* 11: 239–242, 2003.
40. Hayes JD, Chanas SA, Henderson CJ, McMahon M, Sun C, Moffat GJ, Wolf CR, and Yamamoto M. The Nrf2 transcription factor contributes both to the basal expression of glutathione S-transferases in mouse liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin. *Biochem Soc Trans* 28: 33–41, 2000.
41. Heiss E, Herhaus C, Klimo K, Bartsch H, and Gerhauser C. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J Biol Chem* 276: 32008–32015, 2001.
42. Hergenhahn M, Soto U, Weninger A, Polack A, Hsu CH, Cheng AL, and Rosl F. The chemopreventive compound curcumin is an efficient inhibitor of Epstein-Barr virus BZLF1 transcription in Raji DR-LUC cells. *Mol Carcinog* 33: 137–145, 2002.
43. Hibi M, Lin A, Smeal T, Minden A, and Karin M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7: 2135–2148, 1993.
44. Holmes-McNary M and Baldwin AS Jr. Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. *Cancer Res* 60: 3477–3483, 2000.

45. Hu R and Kong AN. Activation of MAP kinases, apoptosis and nutrigenomics of gene expression elicited by dietary cancer-prevention compounds. *Nutrition* 20: 83–88, 2004.
46. Huang HC, Nguyen T, and Pickett CB. Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. *Proc Natl Acad Sci U S A* 97: 12475–12480, 2000.
47. Huang HC, Nguyen T, and Pickett CB. Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. *J Biol Chem* 277: 42769–42774, 2002.
48. Huang MT, Ho CT, Wang ZY, Ferraro T, Lou YR, Stauber K, Ma W, Georgiadis C, Laskin JD, and Conney AH. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Res* 54: 701–708, 1994.
49. Huang S, Pettaway CA, Uehara H, Bucana CD, and Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. *Oncogene* 20: 4188–4197, 2001.
50. Huang TT, Kudo N, Yoshida M, and Miyamoto S. A nuclear export signal in the N-terminal regulatory domain of IkappaBalpha controls cytoplasmic localization of inactive NF-kappaB/IkappaBalpha complexes. *Proc Natl Acad Sci U S A* 97: 1014–1019, 2000.
51. Ichiki K, Mitani N, Doki Y, Hara H, Misaki T, and Saiki I. Regulation of activator protein-1 activity in the mediastinal lymph node metastasis of lung cancer. *Clin Exp Metastasis* 18: 539–545, 2000.
52. Ishii T, Itoh K, Sato H, and Bannai S. Oxidative stress-inducible proteins in macrophages. *Free Radic Res* 31: 351–355, 1999.
53. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, Katoh Y, Bannai S, and Yamamoto M. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J Biol Chem* 275: 16023–16029, 2000.
54. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, and Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 236: 313–322, 1997.
55. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, and Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86, 1999.
56. Janssen-Heininger YM, Poynter ME, and Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. *Free Radic Biol Med* 28: 1317–1327, 2000.
57. Jeong WS, Kim IW, Hu R, and Kong AN. Modulation of AP-1 by natural chemopreventive compounds in human colon HT-29 cancer cell line. *Pharm Res* 21: 649–660, 2004.
58. Jeong WS, Kim IW, Hu R, and Kong AN. Modulatory properties of various natural chemopreventive agents on the activation of NF-kappaB signaling pathway. *Pharm Res* 21: 661–670, 2004.
59. Jochum W, Passegue E, and Wagner EF. AP-1 in mouse development and tumorigenesis. *Oncogene* 20: 2401–2412, 2001.
60. Johnson C, Van Antwerp D, and Hope TJ. An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of IkappaBalpha. *EMBO J* 18: 6682–6693, 1999.
61. Kang G, Kong PJ, Yuh YJ, Lim SY, Yim SV, Chun W, and Kim SS. Curcumin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression by inhibiting activator protein 1 and nuclear factor kappab bindings in BV2 microglial cells. *J Pharmacol Sci* 94: 325–328, 2004.
62. Kang JS, Jeon YJ, Kim HM, Han SH, and Yang KH. Inhibition of inducible nitric-oxide synthase expression by silymarin in lipopolysaccharide-stimulated macrophages. *J Pharmacol Exp Ther* 302: 138–144, 2002.
63. Kang KW, Lee SJ, Park JW, and Kim SG. Phosphatidylinositol 3-kinase regulates nuclear translocation of NF-E2-related factor 2 through actin rearrangement in response to oxidative stress. *Mol Pharmacol* 62: 1001–1010, 2002.
64. Kang KW, Park EY, and Kim SG. Activation of CCAAT/enhancer-binding protein beta by 2′-amino-3′-methoxyflavone (PD98059) leads to the induction of glutathione S-transferase A2. *Carcinogenesis* 24: 475–482, 2003.
65. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 270: 16483–16486, 1995.
66. Karin M and Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol* 18: 621–663, 2000.
67. Katiyar SK and Mukhtar H. Tea consumption and cancer. *World Rev Nutr Diet* 79: 154–184, 1996.
68. Katoh Y, Itoh K, Yoshida E, Miyagishi M, Fukamizu A, and Yamamoto M. Two domains of Nrf2 cooperatively bind CBP, a CREB binding protein, and synergistically activate transcription. *Genes Cells* 6: 857–868, 2001.
69. Kensler TW, Curphey TJ, Maxiutenko Y, and Roebuck BD. Chemoprotection by organosulfur inducers of phase 2 enzymes: dithiolethiones and dithiins. *Drug Metab Drug Interact* 17: 3–22, 2000.
70. Kim DW, Sovak MA, Zanieski G, Nonet G, Romieu-Mourez R, Lau AW, Hafer LJ, Yaswen P, Stampfer M, Rogers AE, Russo J, and Sonenshein GE. Activation of NF-kappaB/Rel occurs early during neoplastic transformation of mammary cells. *Carcinogenesis* 21: 871–879, 2000.
71. Kim SG, Cho MK, Choi SH, Kim HJ, Kwak MK, and Kim ND. Molecular basis for hepatic detoxifying enzyme induction by 2-(allylthio)pyrazine in rats in comparison with oltipraz: effects on prooxidant production and DNA degradation. *Drug Metab Dispos* 27: 667–673, 1999.
72. Kobayashi M, Itoh K, Suzuki T, Osanai H, Nishikawa K, Katoh Y, Takagi Y, and Yamamoto M. Identification of the interactive interface and phylogenic conservation of the Nrf2-Keap1 system. *Genes Cells* 7: 807–820, 2002.
73. Kong AN, Owuor E, Yu R, Hebbar V, Chen C, Hu R, and Mandlekar S. Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). *Drug Metab Rev* 33: 255–271, 2001.

74. Kurata S and Nakajima H. Transcriptional activation of the heme oxygenase gene by TPA in mouse M1 cells during their differentiation to macrophage. *Exp Cell Res* 191: 89–94, 1990.
75. Kwak MK, Egner PA, Dolan PM, Ramos-Gomez M, Groopman JD, Itoh K, Yamamoto M, and Kensler TW. Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutat Res* 480–481: 305–315, 2001.
76. Kwak MK, Itoh K, Yamamoto M, and Kensler TW. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. *Mol Cell Biol* 22: 2883–2892, 2002.
77. Kwak MK, Wakabayashi N, Itoh K, Motohashi H, Yamamoto M, and Kensler TW. Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J Biol Chem* 278: 8135–8145, 2003.
78. Lagarrigue S, Chaumontet C, Heberden C, Martel P, and Gaillard-Sanchez I. Suppression of oncogene-induced transformation by quercetin and retinoic acid in rat liver epithelial cells. *Cell Mol Biol Res* 41: 551–560, 1995.
79. Lee H, Kim YO, Kim H, Kim SY, Noh HS, Kang SS, Cho GJ, Choi WS, and Suk K. Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. *FASEB J* 17: 1943–1944, 2003.
80. Lee JM, Hanson JM, Chu WA, and Johnson JA. Phosphatidylinositol 3-kinase, not extracellular signal-regulated kinase, regulates activation of the antioxidant-responsive element in IMR-32 human neuroblastoma cells. *J Biol Chem* 276: 20011–20016, 2001.
81. Li J, Ma C, Huang Y, Luo J, and Huang C. Differential requirement of EGF receptor and its tyrosine kinase for AP-1 transactivation induced by EGF and TPA. *Oncogene* 22: 211–219, 2003.
82. Lo AH, Liang YC, Lin-Shiau SY, Ho CT, and Lin JK. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-kappaB in mouse macrophages. *Carcinogenesis* 23: 983–991, 2002.
83. Manna SK, Mukhopadhyay A, and Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol* 164: 6509–6519, 2000.
84. Marini MG, Chan K, Casula L, Kan YW, Cao A, and Moi P. hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2. *J Biol Chem* 272: 16490–16497, 1997.
85. Moon SK, Cho GO, Jung SY, Gal SW, Kwon TK, Lee YC, Madamanchi NR, and Kim CH. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2, cell-cycle regulation, and matrix metalloproteinase-9. *Biochem Biophys Res Commun* 301: 1069–1078, 2003.
86. Morimitsu Y, Nakagawa Y, Hayashi K, Fujii H, Kumagai T, Nakamura Y, Osawa T, Horio F, Itoh K, Iida K, Yamamoto M, and Uchida K. A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. *J Biol Chem* 277: 3456–3463, 2002.
87. Moscat J, Diaz-Meco MT, and Rennert P. NF-kappaB activation by protein kinase C isoforms and B-cell function. *EMBO Rep* 4: 31–36, 2003.
88. Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, and Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene* 20: 7597–7609, 2001.
89. Muller JM, Ziegler-Heitbrock HW, and Baeuerle PA. Nuclear factor kappa B, a mediator of lipopolysaccharide effects. *Immunobiology* 187: 233–256, 1993.
90. Nakamura K, Yasunaga Y, Segawa T, Ko D, Moul JW, Srivastava S, and Rhim JS. Curcumin down-regulates AR gene expression and activation in prostate cancer cell lines. *Int J Oncol* 21: 825–830, 2002.
91. Nguyen T, Huang HC, and Pickett CB. Transcriptional regulation of the antioxidant response element. Activation by Nrf2 and repression by MafK. *J Biol Chem* 275: 15466– 15473, 2000.
92. Nguyen T, Sherratt PJ, Huang HC, Yang CS, and Pickett CB. Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element. Degradation of Nrf2 by the 26 S proteasome. *J Biol Chem* 278: 4536–4541, 2003.
93. Nomura M, Ma W, Chen N, Bode AM, and Dong Z. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced NF-kappaB activation by tea polyphenols, (−)-epigallocatechin gallate and theaflavins. *Carcinogenesis* 21: 1885–1890, 2000.
94. Osborn L, Kunkel S, and Nabel GJ. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. *Proc Natl Acad Sci U S A* 86: 2336– 2340, 1989.
95. Paolini M, Perocco P, Canistro D, Valgimigli L, Pedulli GF, Iori R, Croce CD, Cantelli-Forti G, Legator MS, and Abdel-Rahman SZ. Induction of cytochrome P450, generation of oxidative stress and in vitro cell-transforming and DNA-damaging activities by glucoraphanin, the bioprecursor of the chemopreventive agent sulforaphane found in broccoli. *Carcinogenesis* 25: 61–67, 2004.
96. Passegue E, Jochum W, Schorpp-Kistner M, Mohle-Steinlein U, and Wagner EF. Chronic myeloid leukemia with increased granulocyte progenitors in mice lacking junB expression in the myeloid lineage. *Cell* 104: 21–32, 2001.
97. Patten EJ and DeLong MJ. Temporal effects of the detoxification enzyme inducer, benzyl isothiocyanate: activation of c-Jun N-terminal kinase prior to the transcription factors AP-1 and NFkappaB. *Biochem Biophys Res Commun* 257: 149–155, 1999.
98. Pfarr CM, Mechta F, Spyrou G, Lallemand D, Carillo S, and Yaniv M. Mouse JunD negatively regulates fibroblast growth and antagonizes transformation by ras. *Cell* 76: 747–760, 1994.
99. Prestera T, Holtzclaw WD, Zhang Y, and Talalay P. Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Natl Acad Sci U S A* 90: 2965–2969, 1993.
100. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, and Kensler TW. Sensitivity to

carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proc Natl Acad Sci U S A* 98: 3410–3415, 2001.
101. Reddy BS. Studies with the azoxymethane-rat preclinical model for assessing colon tumor development and chemoprevention. *Environ Mol Mutagen* 44: 26–35, 2004.
102. Saleem M, Afaq F, Adhami VM, and Mukhtar H. Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. *Oncogene* 23: 5203–5214, 2004.
103. Schonthal A, Herrlich P, Rahmsdorf HJ, and Ponta H. Requirement for fos gene expression in the transcriptional activation of collagenase by other oncogenes and phorbol esters. *Cell* 54: 325–334, 1988.
104. Schubert SY, Neeman I, and Resnick N. A novel mechanism for the inhibition of NF-kappaB activation in vascular endothelial cells by natural antioxidants. *FASEB J* 16: 1931–1933, 2002.
105. Schulze-Osthoff K, Ferrari D, Riehemann K, and Wesselborg S. Regulation of NF-kappa B activation by MAP kinase cascades. *Immunobiology* 198: 35–49, 1997.
106. Schwartz SA, Hernandez A, and Mark Evers B. The role of NF-kappaB/IkappaB proteins in cancer: implications for novel treatment strategies. *Surg Oncol* 8: 143–153, 1999.
107. Shaulian E and Karin M. AP-1 in cell proliferation and survival. *Oncogene* 20: 2390–2400, 2001.
108. Shaulian E and Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol* 4: E131–E136, 2002.
109. Shen G, Hebbar V, Nair S, Xu C, Li W, Lin W, Keum YS, Han J, Gallo MA, and Kong AN. Regulation of Nrf2 transactivation domain activity: the differential effects of mitogen-activated protein kinase cascades and synergistic stimulatory effect of Raf and CREB-binding protein. *J Biol Chem* 279: 23052–23060, 2004.
110. Shi CS and Kehrl JH. PYK2 links G(q)alpha and G(13)alpha signaling to NF-kappa B activation. *J Biol Chem* 276: 31845–31850, 2001.
111. Shi X, Ye J, Leonard SS, Ding M, Vallyathan V, Castranova V, Rojanasakul Y, and Dong Z. Antioxidant properties of (−)-epicatechin-3-gallate and its inhibition of Cr(VI)-induced DNA damage and Cr(IV)- or TPA-stimulated NF-kappaB activation. *Mol Cell Biochem* 206: 125–132, 2000.
112. Shishodia S, Potdar P, Gairola CG, and Aggarwal BB. Curcumin (diferuloylmethane) down-regulates cigarette smoke-induced NF-kappaB activation through inhibition of IkappaBalpha kinase in human lung epithelial cells: correlation with suppression of COX-2, MMP-9 and cyclin D1. *Carcinogenesis* 24: 1269–1279, 2003.
113. Singh S, Natarajan K, and Aggarwal BB. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a potent inhibitor of nuclear transcription factor-kappa B activation by diverse agents. *J Immunol* 157: 4412–4420, 1996.
114. Singletary KW and Nelshoppen JM. Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumorigenesis and of in vivo formation of mammary DMBA-DNA adducts by rosemary extract. *Cancer Lett* 60: 169–175, 1991.
115. Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, and Sonenshein GE. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. *J Clin Invest* 100: 2952–2960, 1997.
116. Suk K, Lee H, Kang SS, Cho GJ, and Choi WS. Flavonoid baicalein attenuates activation-induced cell death of brain microglia. *J Pharmacol Exp Ther* 305: 638–645, 2003.
117. Surh YJ. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 3: 768–780, 2003.
118. Surh Y-J, Kim SG, Liem A, Lee JW, and Miller JA. Inhibitory effects of isopropyl-2-(1,3-dithietane-2-ylidene)-2-[N-(4-methylthiazol-2-yl)carbamoyl]-acetate (YH439) on benzo[a]pyrene-induced skin carcinogenesis and micronucleated reticulocyte formation in mice. *Mutat Res* 423: 149–153, 1999.
119. Surh YJ, Han SS, Keum YS, Seo HJ, and Lee SS. Inhibitory effects of curcumin and capsaicin on phorbol ester-induced activation of eukaryotic transcription factors, NF-kappaB and AP-1. *Biofactors* 12: 107–112, 2000.
120. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, and Biswal S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res* 62: 5196–5203, 2002.
121. Tominaga S and Kuroishi T. An ecological study on diet/nutrition and cancer in Japan. *Int J Cancer* 10 (Suppl): 2–6, 1997.
122. Vayalil PK, Elmets CA, and Katiyar SK. Treatment of green tea polyphenols in hydrophilic cream prevents UVB-induced oxidation of lipids and proteins, depletion of antioxidant enzymes and phosphorylation of MAPK proteins in SKH-1 hairless mouse skin. *Carcinogenesis* 24: 927–936, 2003.
123. Vermeulen L, De Wilde G, Notebaert S, Vanden Berghe W, and Haegeman G. Regulation of the transcriptional activity of the nuclear factor-kappaB p65 subunit. *Biochem Pharmacol* 64: 963–970, 2002.
124. Wadsworth TL, McDonald TL, and Koop DR. Effects of *Ginkgo biloba* extract (EGb 761) and quercetin on lipopolysaccharide-induced signaling pathways involved in the release of tumor necrosis factor-alpha. *Biochem Pharmacol* 62: 963–974, 2001.
125. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, and Talalay P. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* 101: 2040–2045, 2004.
126. Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, and Chiao PJ. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin Cancer Res* 5: 119–127, 1999.
127. Wang ZQ, Grigoriadis AE, Mohle-Steinlein U, and Wagner EF. A novel target cell for c-fos-induced oncogenesis: development of chondrogenic tumours in embryonic stem cell chimeras. *EMBO J* 10: 2437–2450, 1991.
128. Whitmarsh AJ and Davis RJ. Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med* 74: 589–607, 1996.

129. Wild AC, Moinova HR, and Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 274: 33627–33636, 1999.
130. Woo JH, Lim JH, Kim YH, Suh SI, Min do S, Chang JS, Lee YH, Park JW, and Kwon TK. Resveratrol inhibits phorbol myristate acetate-induced matrix metalloproteinase-9 expression by inhibiting JNK and PKC delta signal transduction. *Oncogene* 23: 1845–1853, 2004.
131. Yang CS and Wang ZY. Tea and cancer. *J Natl Cancer Inst* 85: 1038–1049, 1993.
132. Yang CX, Kuroishi T, Huang XE, Inoue M, and Tajima K. Correlation between food consumption and colorectal cancer: an ecological analysis in Japan. *Asian Pac J Cancer Prev* 3: 77–83, 2002.
133. Yang GY, Liao J, Li C, Chung J, Yurkow EJ, Ho CT, and Yang CS. Effect of black and green tea polyphenols on c-jun phosphorylation and H_2O_2 production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction. *Carcinogenesis* 21: 2035–2039, 2000.
134. Young MR, Li JJ, Rincon M, Flavell RA, Sathyanarayana BK, Hunziker R, and Colburn N. Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. *Proc Natl Acad Sci U S A* 96: 9827–9832, 1999.
135. Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, Tan TH, and Kong AN. Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. *J Biol Chem* 275: 39907–39913, 2000.
136. Yu R, Hebbar V, Kim DW, Mandlekar S, Pezzuto JM, and Kong AN. Resveratrol inhibits phorbol ester and UV-induced activator protein 1 activation by interfering with mitogen-activated protein kinase pathways. *Mol Pharmacol* 60: 217–224, 2001.
137. Zhang DD and Hannink M. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol Cell Biol* 23: 8137–8151, 2003.
138. Zhong H, Voll RE, and Ghosh S. Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* 1: 661–671, 1998.
139. Zhu M and Fahl WE. Functional characterization of transcription regulators that interact with the electrophile response element. *Biochem Biophys Res Commun* 289: 212–219, 2001.
140. Zipper LM and Mulcahy RT. Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. *Toxicol Sci* 73: 124–134, 2003.

Address reprint requests to:
Ah-Ng Tony Kong
Department of Pharmaceutics
Ernest Mario School of Pharmacy
Rutgers, The State University of New Jersey
160 Frelinghuysen Road
Piscataway, NJ 08854

E-mail: KongT@cop.rutgers.edu

Received for publication May 17, 2005; accepted June 27, 2005.

This article has been cited by:

1. Anwar Anwar-Mohammed, Ayman O. S. El-KadiModulation of AHR Function by Heavy Metals and Disease States 343-372. [CrossRef]
2. Yoav Sharoni, Karin Linnewiel-Hermoni, Marina Khanin, Hagar Salman, Anna Veprik, Michael Danilenko, Joseph Levy. 2011. Carotenoids and apocarotenoids in cellular signaling related to cancer: A review. *Molecular Nutrition & Food Research* n/a-n/a. [CrossRef]
3. F. Di Domenico, C. Foppoli, R. Coccia, M. Perluigi. 2011. Antioxidants in cervical cancer: Chemopreventive and chemotherapeutic effects of polyphenols. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [CrossRef]
4. Jui-Hung Yen, Ching-Yi Weng, Shiming Li, Ya-Hsuan Lo, Min-Hsiung Pan, Shih-Hang Fu, Chi-Tang Ho, Ming-Jiuan Wu. 2011. Citrus flavonoid 5-demethylnobiletin suppresses scavenger receptor expression in THP-1 cells and alters lipid homeostasis in HepG2 liver cells. *Molecular Nutrition & Food Research* **55**:5, 733-748. [CrossRef]
5. Carcinogen Specific Expression Profiling: Prediction of Carcinogenic Potential? 160-271. [CrossRef]
6. Rong Hu , Constance Lay-Lay Saw , Rong Yu , Ah-Ng Tony Kong . 2010. Regulation of NF-E2-Related Factor 2 Signaling for Cancer Chemoprevention: Antioxidant Coupled with Antiinflammatory. *Antioxidants & Redox Signaling* **13**:11, 1679-1698. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Wen-Chin Chiu, Chun-Jung Chen, Tzong-Shyuan Lee, Zit-Jie Chen, Pei-Hsin Ke, An-Na Chiang. 2010. Oxidative stress enhances AP-1 and NF-#B-mediated regulation of #2-Glycoprotein I gene expression in hepatoma cells. *Journal of Cellular Biochemistry* **111**:4, 988-998. [CrossRef]
8. Ana Belén Granado-Serrano, María Angeles Martín, Guy Haegeman, Luis Goya, Laura Bravo, Sonia Ramos. 2010. Epicatechin induces NF-#B, activator protein-1 (AP-1) and nuclear transcription factor erythroid 2p45-related factor-2 (Nrf2) via phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) and extracellular regulated kinase (ERK) signalling in HepG2 cells. *British Journal of Nutrition* **103**:02, 168. [CrossRef]
9. Yiyi Sun, Keli Xun, Yitao Wang, Xiuping Chen. 2009. A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. *Anti-Cancer Drugs* **20**:9, 757-769. [CrossRef]
10. Guoxiang Shen, Ah-Ng Kong. 2009. Nrf2 plays an important role in coordinated regulation of Phase II drug metabolism enzymes and Phase III drug transporters. *Biopharmaceutics & Drug Disposition* **30**:7, 345-355. [CrossRef]
11. Claudine Manach, Jane Hubert, Rafael Llorach, Augustin Scalbert. 2009. The complex links between dietary phytochemicals and human health deciphered by metabolomics. *Molecular Nutrition & Food Research* **53**:10, 1303-1315. [CrossRef]
12. Young-Sam Keum, Tin Oo Khor, Wen Lin, Guoxiang Shen, Ki Han Kwon, Avantika Barve, Wenge Li, Ah-Ng Kong. 2009. Pharmacokinetics and Pharmacodynamics of Broccoli Sprouts on the Suppression of Prostate Cancer in Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) Mice: Implication of Induction of Nrf2, HO-1 and Apoptosis and the Suppression of Akt-dependent Kinase Pathway. *Pharmaceutical Research* **26**:10, 2324-2331. [CrossRef]
13. Courtney G. Woods, Jingqi Fu, Peng Xue, Yongyong Hou, Linda J. Pluta, Longlong Yang, Qiang Zhang, Russell S. Thomas, Melvin E. Andersen, Jingbo Pi. 2009. Dose-dependent transitions in Nrf2-mediated adaptive response and related stress responses to hypochlorous acid in mouse macrophages. *Toxicology and Applied Pharmacology* **238**:1, 27-36. [CrossRef]
14. Arun K. Mankan, Matthew W. Lawless, Steven G. Gray, Dermot Kelleher, Ross McManus. 2009. NF-#B regulation: the nuclear response. *Journal of Cellular and Molecular Medicine* **13**:4, 631-643. [CrossRef]
15. Kah Poh Tan, Kazuhiro Kosuge, Mingdong Yang, Shinya Ito. 2008. NRF2 as a determinant of cellular resistance in retinoic acid cytotoxicity. *Free Radical Biology and Medicine* **45**:12, 1663-1673. [CrossRef]
16. Y OMATA, Y SAITO, K FUJITA, Y OGAWA, K NISHIO, Y YOSHIDA, E NIKI. 2008. Induction of adaptive response and enhancement of PC12 cell tolerance by lipopolysaccharide primarily through the upregulation of glutathione S-transferase A3 via Nrf2 activation. *Free Radical Biology and Medicine* **45**:10, 1437-1445. [CrossRef]
17. Hesham M. Korashy, Ayman O. S. El-Kadi. 2008. NF-#B and AP-1 are key signaling pathways in the modulation of NAD(P)H:Quinone oxidoreductase 1 gene by mercury, lead, and copper. *Journal of Biochemical and Molecular Toxicology* **22**:4, 274-283. [CrossRef]
18. Ichiro Moriyama, Shunji Ishihara, M Azharul Karim Rumi, M D Monowar Aziz, Yoshiyuki Mishima, Naoki Oshima, Chikara Kadota, Yasunori Kadowaki, Yuji Amano, Yoshikazu Kinoshita. 2008. Decoy oligodeoxynucleotide targeting activator protein-1 (AP-1) attenuates intestinal inflammation in murine experimental colitis. *Laboratory Investigation* **88**:6, 652-663. [CrossRef]

19. Avanthika Gopalakrishnan, Ah-Ng Tony Kong. 2008. Anticarcinogenesis by dietary phytochemicals: Cytoprotection by Nrf2 in normal cells and cytotoxicity by modulation of transcription factors NF-#B and AP-1 in abnormal cancer cells. *Food and Chemical Toxicology* **46**:4, 1257-1270. [CrossRef]
20. Elizabeth H. Jeffery, Anna-Sigrid Keck. 2008. Translating knowledge generated by epidemiological andin vitrostudies into dietary cancer prevention. *Molecular Nutrition & Food Research* . [CrossRef]
21. Hesham M. Korashy, Ayman O.S. El-Kadi. 2008. The role of redox-sensitive transcription factors NF-#B and AP-1 in the modulation of the Cyp1a1 gene by mercury, lead, and copper. *Free Radical Biology and Medicine* **44**:5, 795-806. [CrossRef]
22. Peng LIU. 2008. Molecular cloning and preliminary function study of a novel human gene <I>AC3-33</I> related to suppress AP-1 activity. *Hereditas (Beijing)* **30**:5, 575. [CrossRef]
23. Wei Lu, Xujian Li, Jack P. Uetrecht. 2008. Changes in Gene Expression Induced by Carbamazepine and Phenytoin: Testing the Danger Hypothesis. *Journal of Immunotoxicology* **5**:2, 107-113. [CrossRef]
24. Yutaro Obara, Tomohiro Hoshino, Maria Carla Marcotullio, Rita Pagiotti, Norimichi Nakahata. 2007. A novel cyathane diterpene, cyrneine A, induces neurite outgrowth in a Rac1-dependent mechanism in PC12 cells. *Life Sciences* **80**:18, 1669-1677. [CrossRef]
25. Riham Zein Fardoun, Mohammad Asghar, Mustafa Lokhandwala. 2007. Role of nuclear factor kappa B (NF-#B) in oxidative stress-induced defective dopamine D1 receptor signaling in the renal proximal tubules of Sprague-Dawley rats. *Free Radical Biology and Medicine* **42**:6, 756-764. [CrossRef]