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EXPERT REVIEW

Nrf2-Keap1 Signaling as a Potential Target for Chemoprevention of Inflammation-Associated Carcinogenesis

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ABSTRACT Persistent inflammatory tissue damage is causally associated with each stage of carcinogenesis. Inflammation-induced generation of reactive oxygen species, reactive nitrogen species, and other reactive species not only cause DNA damage and subsequently mutations, but also stimulate proliferation of initiated cells and even metastasis and angiogenesis. Induction of cellular cytoprotective enzymes (e.g., heme oxygenase-1, NAD(P)H:quinone oxidoreductase, superoxide dismutase, glutathione-*S*-transferase, etc.) has been shown to mitigate aforementioned events implicated in inflammation-induced carcinogenesis. A unique feature of genes encoding these cytoprotective enzymes is the presence of a *cis*-acting element, known as antioxidant response element (ARE) or electrophile response element (EpRE), in their promoter region. A stress-responsive transcription factor, nuclear factor erythroid-2-related factor-2 (Nrf2), initially recognized as a key transcriptional regulator of various cytoprotective enzymes, is known to play a pivotal role in cellular defense against inflammatory injuries. Activation of Nrf2 involves its release from the cytosolic repressor Kelch-like ECH-associated protein-1 (Keap1) and subsequent stabilization and nuclear localization for ARE/EpRE binding. Genetic or pharmacologic inactivation of Nrf2 has been shown to abolish cytoprotective capability and to aggravate experimentally induced inflammatory injuries. Thus, Nrf2-mediated cytoprotective gene induction is an effective strategy for the chemoprevention of inflammation-associated carcinogenesis.

KEY WORDS anti-inflammation · chemoprevention · chemopreventive agents · inflammation · Keap1 · Nrf2 · redox signaling

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CHEMOPREVENTION BY CURBING INFLAMMATION

Chemoprevention, the strategy to inhibit, retard or even reverse the specific stage of carcinogenesis, is one of the most rational approaches to reduce the global burden of cancer. The course of tumorigenesis involves three apparently distinct phases; initiation, promotion and progression. The tumor initiation is an irreversible process associated with the genotoxic damage of cellular DNA shortly upon exposure to endogenous or exogenous carcinogens. In contrast, tumor promotion is a long-lasting reversible process characterized by clonal expansion of initiated cells to form a benign tumor with rapidly proliferating potential. Progression is the terminal stage of neoplastic transformation that involves invasion and metastasis of transformed cells (1).

Chronic inflammation accounts for the substantial part of human cancers. Each stage of carcinogenesis is fueled by inflammation. In inflamed tissues, a wide variety of activated inflammatory-immune cells (e.g., mast cells, neutrophils, leukocytes, macrophages, monocytes, eosinophils, dendritic cells, phagocytes, and natural killer cells) generate reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), which can cause DNA damage and thereby initiate carcinogenesis by activating oncogenes and/or by inactivating tumor suppressor genes. For example, chronic exposure to ultraviolet (UV) B radiation leads to an inflammatory tissue

damage and ROS generation (2), which, by activating *ras* oncogene and inactivating p53 tumor suppressor gene, contributes to skin carcinogenesis (3). In fact, ROS induce strand breaks, base modifications, and cross-links in the DNA molecule that can result in replication errors and genomic instability, thereby initiating tumorigenesis (4,5). Nitric oxide (NO) also plays an important role in inflammation-associated carcinogenesis by direct structural modification of DNA and inactivation of DNA repair enzymes (6). NO radical reacts rapidly with superoxide anion to form a more powerful oxidant peroxynitrite capable of attacking DNA to produce 8-nitroguanine (8-NG) (7,8), which is considered as a hallmark of inflammation-associated cancers (9). In addition, ROS and RNS can induce lipid peroxidation to generate other reactive species, such as malondialdehyde and 4-hydroxynonenal (4-HNE), which by forming DNA adducts, trigger the process of cell transformation (10). One of the key enzymes involved in inflammatory signal propagation is prostaglandin H synthase (PGHS), which catalyzes the biosynthesis of prostaglandins. The hydroperoxidase component of PGHS activates certain carcinogens to generate highly reactive species, which can damage DNA, thereby initiating carcinogenesis (11).

Besides directly causing structural modifications in DNA or activating carcinogens, persistent inflammatory tissue damage alters the expression and functions of proteins engaged in fine tuning of regular intracellular signal transduction pathways. As a result, abnormal growth and proliferation of cells are favored. Events like perturbation of the DNA-protein interactions and post-translational modification of proteins are examples of such altered biochemical processes. Elevated expression and/or activity of a wide variety of pro-inflammatory signaling molecules constitute the molecular basis of inflammation-driven carcinogenesis (12,13). Important components of pro-inflammatory signaling pathways include, but are not limited to, cytokines, chemokines, cyclooxygenase-2 (COX-2), prostaglandins (PGs), inducible nitric oxide synthase (iNOS), and nitric oxide (NO) (12–14). The persuasive role of inflammation in grounding cancer has become evident from studies reporting that cancers of stomach, liver, gallbladder, prostate and pancreas are causally linked to gastritis, chronic hepatitis, cholecystitis, inflammatory atrophy of the prostate, and chronic pancreatitis, respectively (15–17). Most notably, inflammatory bowel disease increases the risk of colorectal cancer by 10-fold (14,18). Thus, colitis, a condition characterized by persistent colonic mucosal inflammation, often progresses to colorectal cancer (19). The management of colitis with anti-inflammatory therapy reduces the risk of colorectal cancer (20). Although an approximately 25% of all cancers have an etiologic background of chronic infection and/or inflammation (21), the expanding role of inflammation in changing cellular genetic and epigenetic events associated with malignant conversion suggests that this figure might be greater than estimated. Therefore, protecting cells or tissues from inflammatory stress would be of pragmatic importance for the chemoprevention of majority of human cancer.

ROLES OF ANTIOXIDANT AND CYTOPROTECTIVE ENZYMES IN CHEMOPREVENTION

The search for molecular targets to unfasten the ugly tie between inflammation and cancer has long been endeavored. Several distinct cellular signaling components, such as pro-inflammatory enzymes, cytokines, PGs, stress-activated kinases, and redox-sensitive transcription factors have been identified as potential molecular targets of diverse classes of chemopreventive agents. However, these molecules are more relevant in setting off the inflammation-cancer connection largely at the promotion and progression stages of carcinogenesis (13,22,23). Since oxidative stress is a smoldering issue in setting up an inflammation-cancer link at the early stage of carcinogenesis, reinforcing the body's antioxidant arsenal would be the foremost strategy to cut the inflammation-cancer loop.

A wide array of antioxidant and cytoprotective enzymes, such as NAD(P)H:quinone oxidoreductase-1 (NQO1), superoxide dismutase (SOD), glutathione *S*-transferase (GST), heme oxygenase-1 (HO-1), glutamate cysteine ligase (GCL), etc. guard against oxidative and electrophilic insults, thereby preventing the development of cancer (24,25). Table I represents chemopreventive potential of some of these cytoprotective enzymes. It has been reported that NQO1-null mice are more susceptible to benzo[*a*]pyrene (B[*a*]P)- or 7,12,-dimethylbenz[*a*]anthracene (DMBA)-induced skin carcinogenesis (26). A subsequent study has revealed that the electrophilic metabolite B[*a*]P-*trans*-7,8-dihydrodiol-9,10-epoxide contributes to increased production of skin tumors in NQO1-null mice treated with B[*a*]P. It has been shown that the untreated skin of NQO1-null mice, as compared with wild-type mice, develops an altered intracellular redox state due to accumulation of NADH and reduced levels of NAD/NADH. In comparison to NQO1 wild-type mice, NQO1-null mice failed to exhibit p53 upregulation and apoptosis in the skin following treatment with B[*a*]P (27). NQO1 also plays an important role in inhibiting initiation of carcinogen-induced aberrant crypt foci (ACF) formation. Thus, dietary administration of oltipraz, an NQO1 inducer, inhibited the formation of ACF in the colons of azoxymethane (AOM)- or methylnitrosourea-treated rats. Treatment with an NQO1 inhibitor dicoumarol reversed the protective effect of oltipraz in this model (28). Topical application of a

Table I Role of Cytoprotective Enzymes in the Chemoprevention of Experimental Carcinogenesis

Cytoprotective enzymes	Effect on tumorigenesis
NQO1	↑ B[*a*]P-induced skin papillomas in NQO1-null mice as compared to C57BL/6 wild-type mice (26)
	↑ DMBA-initiated and TPA-promoted skin tumor formation in NQO1-null mice as compared to their wild-type littermates (76)
	↑ Skin tumorigenesis in NQO1-null mice treated with B[*a*]P; ↓ p53 induction and decreased apoptosis (27)
	↓ Number of aberrant crypt foci in Sprague-Dawley rats treated with AOM or methylnitrosourea in the presence or absence of NQO1-inducing agent oltipraz (28)
	↓ UVB-induced multiplicity of papillomas and squamous cell carcinomas in SKH-1 hairless mice topically treated with oleanane dicyanotriterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile (TP-225), a potent NQO1 inducer, for 17 weeks (29)
MnSOD	↓ DMBA-initiated and TPA-promoted skin papillomagenesis in MnSOD-transgenic mice as compared to MnSOD nontransgenic mice; decreased TPA-induced lipid peroxidation and delayed AP-1 activation in MnSOD transgenic mouse skin as compared to their wild-type counterparts (32)
	↓ TPA-induced soft agar colony formation in JB6 cells transfected with MnSOD; ↓ TPA-induced AP-1 activity in MnSOD overexpressing cells (31)
CuZnSOD	↑ Spontaneous hepatocarcinogenesis in CuZn-SOD-null mice (33)
GSTP	↑ Skin tumorigenesis in GSTP1/2-null mice treated with DMBA and TPA (34)

synthetic triterpenoid, oleanane dicyanotriterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile (TP-225), to SKH-1 hairless mice increased the levels of NQO1 and HO-1 and protected against UV-radiation-induced skin inflammation and tumor promotion (29). Likewise, induction of HO-1 in the colonic tissue by administering cobalt protoporphyrin (CoPP) attenuated acute dextran sulfate sodium (DSS)-induced mouse colitis with a concomitant reduction of interferon (IFN)-gamma production. However, induction of HO-1 after the onset of colitis in this model failed to attenuate colonic inflammation (30).

Stable transfection with MnSOD significantly reduced the anchorage-independent growth of mouse epidermal JB6 cells treated with the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) (31). Likewise, overexpression of MnSOD attenuated chemically induced skin tumor formation in mice (32). Genetic ablation of CuZnSOD showed increased hepatic nodule formation and a higher incidence of hepatocellular carcinoma (HCC) as compared to the wild-type littermates (33). Similarly, deletion of murine pi-class GST (GST-*P)* gene cluster increased the susceptibility to papilloma formation in a two-stage mouse skin tumor model (34). Interestingly, a series of synthetic triterpenoid analogs of oleanolic acid were found to inhibit IFN-γ-induced expression of iNOS and COX-2, while these compounds markedly induced the expression and activities of NQO1 and HO-1 in murine macrophages. A closely linear co-relation was observed between the anti-inflammatory activity and cytoprotective enzyme-inducing potency of these triterpenoids (35). A subsequent structure-activity relationship study with the same triterpenoids revealed that suppression of inflammation is a consistent property of these cytoprotective enzyme inducers (36). The above findings inflated one hypothesis that induction of antioxidant and phase 2 detoxifying enzymes may constitute the first line strategy for the chemoprevention of inflammation-associated carcinogenesis.

ACTIVATION OF Nrf2 SIGNALING AS A RATIONAL STRATEGY TO BREAK UP INFLAMMATION-CANCER CONNECTION

Initial analysis of genes encoding antioxidant and phase 2 detoxification enzymes has revealed the presence of a *cis*-acting element, known as antioxidant response element (ARE) or electrophile response element (EpRE), located in their promoter region. The transcriptional activation of these genes is predominantly under the control of a stress-responsive transcription factor, nuclear factor erythroid-2-related factor-2 (Nrf2) (25). A distinct set of Nrf2-regulated proteins have been shown to maintain cellular-reducing equivalents and to potentiate antioxidant capacity, thereby thwarting the noxious effects of electrophiles and ROS (37,38).

The molecular details of Nrf2 activation in response to stress signals have been reviewed elsewhere (39,40). In principle, Nrf2 remains sequestered in the cytoplasm as an inactive complex by binding to its cytosolic repressor Kelch-like ECH-associated protein 1 (Keap1), which is anchored to the actin cytoskeleton. In association with Cullin 3 (Cul3) and Rbx1, Keap1 forms a functional E3 ubiquitin ligase complex that ubiquitinates multiple lysine residues located

in the N-terminal Neh2 domain of Nrf2, thereby facilitating degradation of Nrf2. Keap1, by acting as a substrate adaptor protein for Cul3-dependent E3 ubiquitin ligase, maintains a steady-state level of Nrf2 (41,42). The activation of Nrf2 signaling involves cytosolic stabilization and subsequent nuclear translocation of Nrf2 via oxidation or covalent modification of distinct Keap1 cysteine residues, enhanced degradation of Keap1, and phosphorylation of Nrf2. Dissociation of Nrf2 from its cytosolic repressor Keap1 is a prerequisite for nuclear translocation of this stress-responsive transcription factor. After translocation to nucleus, Nrf2 forms a heterodimer with small Maf protein and binds to the *cis*-acting ARE/EpRE sequence located in the promoter region of aforementioned cytoprotective genes, and subsequently regulates their transactivation (39,40). Recently, it has been recognized that Nrf2 and its target genes not only confer protection against oxidative and electrophilic stresses but also suppress inflammatory responses (43–45). Fig. 1 highlights activation of Nrf2 signaling as a strategy to prevent inflammation-associated carcinogenesis. How Nrf2 and ARE-regulated genes preclude inflammation will be discussed in the following sections.

Exacerbation of Inflammation by Depletion or Functional Inactivation of Nrf2

Multiple lines of evidence suggest that mice lacking Nrf2 are more vulnerable than wild-type animals to the cytotoxic and genotoxic effects of electrophilic toxicants and oxidants. Moreover, disruption of Nrf2 aggravates the inflammatory injury as well as electrophilic and oxidative stresses (44) (Table II). Certain inflammatory conditions of the airway epithelium, such as acute lung injury, emphysema, and chronic obstructive pulmonary diseases (COPD), can predispose a condition of inflammation in the airways, which eventually progresses to pulmonary carcinogenesis (46). A primary cause of COPD is the exposure to cigarette smoke that causes oxidative alveolar tissue damage through depletion of antioxidants. As an adaptive response to cigarette smoke-induced oxidative tissue damage, the Nrf2 signaling is activated in the lung epithelial cells, thereby inducing the expression and activities of ARE/EpRE-driven antioxidant and detoxification enzymes. Silencing Keap1 by genetic ablation or by RNA interference in Clara cells, which are predominantly present in upper airways epithelium, increases the activation of Nrf2 and elevates the expression of NQO1 and the modifier subunit of GCL (GCLM). The activation of Nrf2 increased the intracellular glutathione (GSH) level, which alleviated CS-induced oxidative stress and inflammation in lungs (47). Nrf2-deficient mice exposed to sublethal hyperoxia have been shown to readily succumb to death during recovery from acute lung injury. A short exposure to hyperoxia caused persistent cellular injury, impaired alveolar and endothelial cell regeneration, and persistent cellular infiltration by macrophages and lymphocytes in the pulmonary tissue of Nrf2-deficient mice. Hyperoxia-induced oxidative and inflammatory injuries in Nrf2-deficient mice were recovered by supplementation with GSH immediately after hyperoxia challenge (48).

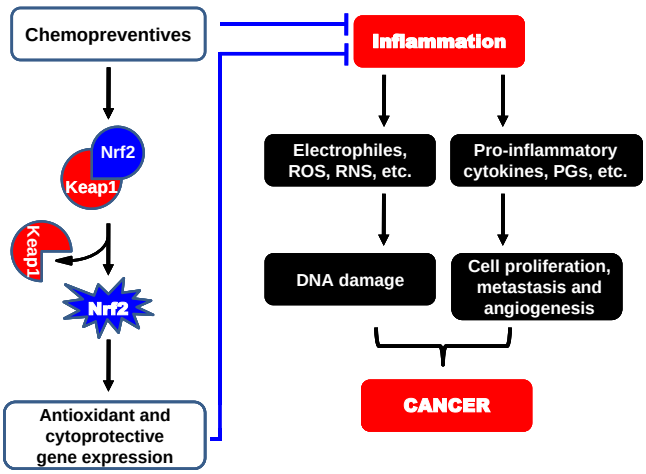

Fig. 1 Suppression of inflammation-associated carcinogenesis via Nrf2 activation by chemopreventive agents. Inflammation can cause production of ROS and other reactive species (e.g., RNS and electrophiles such as 4-HNE) capable of directly damaging DNA, thereby initiating carcinogenesis. Alternatively, pro-inflammatory cytokines and PGs can stimulate cell proliferation to promote neoplastic transformation and can also mediate metastasis and angiogenesis. Some chemopreventive agents can inhibit inflammation by suppressing the pro-inflammatory signaling, while others activate Nrf2 and induce expression of antioxidative/cytoprotective proteins, which in turn abrogate the inflammatory response.

In a carrageenan-induced pleurisy model of acute lung injury, persistent accumulation of neutrophils and delayed recruitment of macrophages in Nrf2-null mice suggest that the presence of functional Nrf2 is essential for cellular defense against inflammation (49). In another study, carrageenan-induced acute lung injury was markedly aggravated in Nrf2-knockout mice as evidenced by the magnitude and the duration of acute inflammation and the number of surviving neutrophils in bronchoalveolar lavage (50). Interestingly, treatment of Nrf2 wild-type mice with a selective COX-2 inhibitor significantly exacerbated acute lung injury to the extent observed in Nrf2-knockout mice. Since a COX-2 product, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), mediates resolution of acute inflammation by inducing apoptosis of neutrophils and leukocytes at the site of inflammation (51), the inhibition of 15d-PGJ_2 production in wild-type mice by the COX-2 inhibitor may have blocked induction of Nrf2-mediated expression of cytoprotective genes. When these animals were given exogenous 15d-PGJ_2, COX-2 inhibitor-induced acute lung injury subsided. As one of the major terminal products of COX-

Table II Role of Nrf2 in the Modulation of Inflammatory Responses

Animal model	Inflammation model	Inflammatory responses
Keap1-conditional knockout mouse, siRNA-directed Keap1 knockdown in lung epithelial cells	Lung inflammation	Increased mRNA expression of Nrf2 and cytoprotective enzymes and decreased H_2O_2- induced ROS generation; decreased cigarette smoke-induced accumulation of inflammatory cells in lung or bronchoalveolar fluid and decreased MCP-1 expression (47)
Nrf2-deficient mice exposed to hyperoxia	Acute liver injury	Persistent cellular injury, impaired regeneration of alveolar and epithelial cells, increased infiltration of macrophages, lymphocytes, and inhibition of inflammatory responses by GSH supplementation (48)
Nrf2+/+ and Nrf2−/− mice	Lung inflammation	Reduced expression of antioxidant enzymes; increased lymphocyte and eosinophil counts; Elevated IL-12 and IL-13 level in bronchoalveolar lavage of Nrf2−/− mice (53)
Nrf2+/+ and Nrf2−/− mice; Keap1 Knockout mice; treatment with Nrf2 inducer CDDO-Im	Liver injury	Reduced signs of hepatic inflammation in Keap1 knockout mice; CDDO-Im-treated wild-type mice were protected from ConA-mediated hepatotoxicity (55)
Nrf2+/+ and Nrf2−/− mice	Colitis	Increased severity of DSS-induced colitis in Nrf2−/− mice; Decreased colonic expression of HO-1 and GSTμ1 and increased expression of COX-2, iNOS, IL-1β and TNFα in Nrf2−/− as compared to Nrf2+/+ mice when treated with DSS (64)
Nrf2+/+ and Nrf2−/− mice	Colitis	Increased incidence and multiplicity of AOM plus DSS-induced colorectal tumors in Nrf2−/− mice; Increased expression of COX-2, 5-LOX, leukotriene B4, and nitrotyrosine and increased production of PGE_2 in Nrf2−/− as compared to Nrf2+/+ mouse treated with AOM plus DSS (43)
Nrf2+/+ and Nrf2−/− mice	Colitis	Increased incidence and multiplicity of AOM plus DSS-induced ACF in Nrf2−/− mice; Increased mucosal damage, mRNA expression of pro-inflammatory cytokines, myeloperoxidase activity and nitrotyrosine expression in Nrf2−/− mice as compared to Nrf2+/+ mice treated with AOM plus DSS (65)

2-mediated reactions, 15d-PGJ_2 is likely to exert anti-inflammatory effects by inducing Nrf2-regulated cytoprotective gene transcription (50,51). Itoh and colleagues demonstrated that 15d-PGJ_2 activated Nrf2 through removal of its cytosolic repressor Keap1 (49).

When Nrf2-null and wild-type mice were exposed to diesel exhaust particles, Nrf2-null mice showed significantly more pronounced airway inflammation, which was characterized by increased counts of lymphocytes and eosinophils and production of cytokines in bronchoalveolar lavage fluid, than did the Nrf2-wild-type mice (53). Disruption of the Nrf2 gene led to earlier-onset and more extensive cigarette smoke-induced emphysema than those elicited in wild-type mice. Emphysema in Nrf2-deficient mice exposed to cigarette smoke for 6 months was associated with more pronounced bronchoalveolar inflammation (54).

Inflammatory liver injury is one of the etiologic factors for hepatic carcinogenesis. Osburn *et al.* (55) utilized genetic and pharmacologic approaches to assess whether activation of Nrf2 signaling could protect against inflammatory liver injury. Intravenous injection of concanavalin A (ConA) induced T-cell-mediated acute inflammatory liver injury and eventually caused cell death in both Nrf2-wild-type and -knockout mice. Treatment of hepatocyte-specific conditional Keap1-null [Alb-Cre:Keap1(flox/-), cKeap1-KO] mice expressing constitutive Nrf2 as well as Nrf2 wild-type mice, but not Nrf2-deficient mice, with a Nrf2 inducing triterpenoid cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl-imidazolide (CDDO-Im), showed resistance to ConA-mediated inflammatory liver injury. Moreover, enhanced hepatic expression of Nrf2-regulated antioxidative genes ameliorated inflammation-mediated oxidative stress, thereby preventing hepatocyte necrosis. Attenuation of hepatocyte death in cKeap1-KO mice and CDDO-Im-pretreated Nrf2-wild-type mice was associated with decreased late-phase pro-inflammatory gene expression in the liver after ConA challenge (55). Likewise, repair of carbon tetrachloride (CCl_4)-induced liver injury was severely damaged in Nrf2-deficient mice. Moreover, long-term treatment with CCl_4 aggravated the signs of fibrosis and inflammation in Nrf2 knockout mice (56). The development of non-alcoholic steatohepatitis, an oxidative stress-induced non-alcoholic fatty liver disease, was greatly accelerated in Nrf2 knockout mice receiving a methionine- and choline-deficient diet. Livers from Nrf2 knockout mice on this diet showed a massive increase in the number of neutrophils, significant depletion of GSH, increased oxidized glutathione (GSSG) and malondialdehyde, about a 10-fold increase in p65 protein and approximately 5-fold increases in the levels of mRNA for interleukin (IL)-1β, tumor necrosis

factor-α (TNFα), COX-2, and iNOS as compared to livers from similarly treated wild-type mice (57). Sustained Nrf2 activation in Keap1 gene-knockdown (Keap1-KD) mice led to an increase in antioxidant and detoxifying enzymes and reduced parenchymal necrosis caused by bile duct ligation, suggesting that activation of Nrf2 plays a protective role against cholestatic liver injury (58). The Keap1-KD mice exhibited a 55% decrease in Keap1 mRNA and a 200% increase in Nrf2 protein in liver (59). Comparison of hepatic mRNA expression of cytoprotective genes among Nrf2 wild-type, Nrf2-null, and Keap1-KD mice revealed that the expression of genes mainly responsible for the detoxification and elimination of electrophiles, such as NQO 1 and GST, and multidrug resistance-associated proteins, was lower in Nrf2-null mice, but considerably higher in Keap1-KD mice than wild-type mice (59).

Increased inflammatory response was also observed in wounded skin of Nrf2-null mice (60). By contrast, transgenic overexpression of dominant negative Nrf2 mutant in the epidermis of Nrf2 transgenic mice did not influence the wound healing, but the onset, the incidence and the multiplicity of chemically induced papillomagenesis were increased (61). While exposure of mouse hepatoma, mouse keratinocytes and human skin fibroblast cells to a low-dose (7.5 J/m^2) UVB led to the nuclear accumulation of Nrf2 and up-regulation of ARE-mediated gene expression, a high dose (20 J/m^2) exposure induced the Fyn kinase-mediated tyrosine (568 residue) phosphorylation and subsequent nuclear exclusion of Nrf2 (62). However, a subsequent study reported that UVB-induced skin papillomas remained unaltered in both Nrf2 knockout and wild-type mice, though the extent of UVB-induced acute inflammation was markedly increased in Nrf2-null mice (63). These findings suggest that Nrf2 may not play a protective role against chronic and excessive UVB radiation-induced carcinogenesis, but it is activated in response to mild and acute UVB exposure as an adaptive response to guard against oxidative and inflammatory tissue damage.

Nrf2-deficient mice appeared to be more susceptible to DSS-induced colitis, which was associated with decreased expression of antioxidant and phase 2 detoxifying enzymes HO-1, NQO1, UDP-glucurosyltransferase 1A1, and GSTμ-1. In addition, levels of pro-inflammatory mediators, such as COX-2, iNOS, IL-1β, IL-6, and TNFα, were increased in the colonic tissues of Nrf2-null mice compared with those in their wild-type counterparts (64). In addition, Nrf2-deficient mice showed exacerbated signs of colonic inflammation, such as increased myeloperoidase activity, 3-nitrotyrosine immunoreactivity and inflammatory cytokine production, and developed the increased number of aberrant crypt foci upon treatment with AOM plus DSS (65). Thus, depletion of Nrf2 sensitizes animals to develop colitis-associated colon cancer.

Anti-inflammatory Properties of Selected Nrf2-Regulated Gene Products

HO-1

HO-1 is an important mediator of mucosal defense in the gastrointestinal tract. The HO-1 catalyzed heme degradation products, biliverdin/bllirubin and carbon monoxide, can inhibit oxidative stress and inflammation. Analysis of surgical specimens of colorectal cancers revealed that focal HO-1 expression was evident in 41.8% cases. Colonic HO-1 expression in these patients was positively correlated with the reduced rate of lymphatic tumor invasion and increased survival (66). Incubation of human prostate cancer (PC3) cells with a HO-1 inducer hemin, or stable overexpression of HO-1 in these cells, attenuated proliferation, migration and invasion (67). Accordingly, small interfering RNA-mediated silencing of HO-1 expression in human prostate cancer (MDA PCa2b) cells resulted in increased proliferation and invasion. In contrast, production and activity of matrix metalloproteinase (MMP)-9, a marker of tumor invasion and metastasis, were diminished by HO-1 overexpression in these cells. Furthermore, the tumor growth and MMP-9 expression in HO-1 overexpressing human prostate cancer xenograft were significantly reduced (67).

HO-1 functions as a suppressor of TNFα signaling. Overexpression of HO-1 protected against TNFα-mediated airway inflammation by inhibiting the expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, generation of IL-6, translocation of p47(phox) and activation of nuclear factor-kappaB (NF-κB) in both cultured human tracheal smooth muscle cells and the airways of mice (68). In addition, HO-1 overexpression reduced the formation of a TNFR1/c-Src/p47(phox) complex in this model. Moreover, HO-1 overexpression attenuated TNFα-induced oxidative stress, which was abrogated in the presence of a HO-1 inhibitor or a carbon monoxide scavenger (68). Caballero *et al.* (69) have suggested that decreased expression of HO-1 is correlated with the progression of *p*-dimethylaminoazobenzene-induced hepatocarcinogenesis in CF1 mice. HO-1 expression was elevated in normal hepatocytes, kuffer cells and macrophages surrounding the hepatic lesion in dimethylaminoazobenzene-treated mice, but markedly diminished in altered hepatic foci, oval cells, early preneoplastic lesions, and adenomas. In addition, the expression of HO-1 was inversely correlated with the degree of tumor differentiation in hepatocellular carcinomas (69).

In response to nitrosative stress, the activation of Nrf2 signaling led to the induction of HO-1 as a protective mechanism against inflammatory damage (70). Stimulation with lipopolysaccharide (LPS) induced iNOS expression in both Nrf2+/+ and Nrf2−/− macrophages, while HO-1 expression was only evident in Nrf2+/+ cells. The LPS-induced iNOS expression was suppressed by pretreatment with HO-1 inducers in Nrf2+/+ macrophages (71). Cytokines, especially those retaining the pro-inflammatory properties, are the key players in inflammation-driven carcinogenesis. The anti-inflammatory effect of HO-1 has been attributed to its ability to inhibit cytokine production. The production of IL-3 and TNFα was significantly reduced in HO-1 cDNA-transfected rat basophilic leukemia (RBL-2H3-HO-1) cells treated with anti-ovalbumin (OA) serum/OA as compared to mock-transfected cells. Inhibition of HO-1 activity in RBL-2H3-HO-1 cells by zinc protoporphyrin IX prevented the suppression of TNFα production. In addition, the inhibition of cytokine synthesis by HO-1 was associated with suppression of the DNA-binding activity of activator protein (AP-1) (72).

HO-1 catalyzes heme degradation to produce billiverdin/bilirubin and CO, which exert anti-inflammatory as well as antioxidative effects. CO has been reported to protect against severe acute pancreatitis, a condition, if left unresolved, may result in development of pancreatic cancer. In a rat model of severe acute pancreatitis, intravenous administration of CO-releasing molecule-2 (CORM-2) reduced the serum levels of TNFα and IL-1β and inhibited their mRNA expression. CORM also attenuated myeloperoxidase activity and the NF-κB DNA binding activity in pancreatic tissue. In addition, the mRNA level of an anti-inflammatory cytokine IL-10 was increased upon treatment with CORM-2 (73). Topical application of CORM-2 in the form of a lotion protected against solar UV radiation-induced skin photoinflammation, erythema, hyperplasia and papilloma formation (74). Furthermore, pretreatment with CORM-2 diminished mRNA expression of iNOS, IL-8, IL-6 and MMP-7, phosphorylation of mitogen-activated protein (MAP) kinases, and the reporter gene activity of NF-κB, AP-1 and CCAAT/enhancer binding protein (C/EBP) in human colonic epithelial (Caco-2) cells stimulated with a mixture of TNFα, IL-1β and IFN-γ (75).

NQO1

NQO1 is a phase 2 detoxification enzyme which converts reactive quinones to hydroquinones via two-electron transfer mechanism. Induction of NQO1 in cells prevents one-electron transfer-mediated generation of free radicals, thereby reducing the risk of DNA damage. NQO1−/− mice have been reported to be more susceptible to chemically induced mouse skin carcinogenesis (26,76). Dietary administration of oltipraz, a potent inducer of NQO1, to Sprague-Dawley rats before (28) or after (77) carcinogen challenge protected against AOM- or methyl nitrosourea-induced aberrant crypt foci formation, which was abrogated by treatment with the NQO1 inhibitor dicoumarol. In LPS-stimulated human monocytes, Nrf2-dependent induction of NQO1 negatively regulated LPS-induced expression of TNFα and IL-1β, thereby blunting the inflammatory response. LPS-induced expression of TNFα and IL-1β was markedly increased by silencing NQO1 alone or in combination with siRNA knockdown of HO-1. In addition, overexpression of NQO1 and/or HO-1 significantly attenuated LPS-stimulated expression of TNFα and IL-1β (78).

In a recent study, stimulation of cholangiocarcinoma cells with a mixture of cytokines resulted in decreased expression of NQO1 (79). The expression and activity of NQO1 are negatively regulated by certain components of pro-inflammatory signaling pathways. The transcription factor AP-1, a homo- or hetero-dimer of Jun and Fos family proteins, functions as a component of inflammatory signaling. Inappropriate amplification of AP-1 activity has been shown to account for inflammation-driven neoplastic transformation of cells. AP-1, through its bZIP motif, can interact with ARE and can affect the induction of NQO1. Overexpression of c-Fos in transfected cells repressed ARE-mediated gene expression (80). Conversely, the expression and activities of NQO1 and GST were significantly increased in kidney, liver and skin of c-Fos$^{-/-}$ mice (81).

SOD

The inhibition of DMBA-initiated and TPA-promoted mouse skin tumor formation by protandim, a well-defined formulation of medicinal plant products, was associated with an increased expression and activity of SOD. Mice receiving protandim diet showed reduced oxidative and inflammatory responses as evidenced by decreased protein carbonyl levels as well as suppression of the PKC-JNK-Jun and NF-κB signaling (82). Administration of oxykine, an orally active SOD, to mice co-implanted with a weakly tumorigenic and non-metastatic fibrosarcoma-derived QR-32 cells and inflammation-inducing gelatin sponge, resulted in the reduced tumor incidence, increased SOD activity, and decreased deposition of formazan upon *in situ* perfusion of tumor tissue with nitroblue tetrazolium (an indicator of superoxide formation) (83). Fibrosarcoma-derived (QR-32) cells implanted with an inflammation inducer in this study acquired a reduced metastatic phenotype by scavenging inflammatory cell-derived superoxide anion (83).

In a model of Barrett's esophagus, rats undergoing esophagojejunal anastomosis developed extensive esophageal

ulceration and inflammation which progressed to intestinal metaplasia beyond the anastomotic area and esophageal adenocarcinoma. This sequel of inflammation-driven pathologic events was associated with generation of superoxide anion and peroxynitrite, an increase in oxidative DNA damage and a significant decrease in SOD levels in the afflicted mucosa. Exogenous administration of SOD reduced mucosal superoxide levels, and lowered the risk of developing intestinal metaplasia beyond the anastomotic area and decreased esophageal adenocarcinoma (84). In the context of chronic inflammation, activated leukocyte-generated oxidants play a critical role in damaging cellular DNA, RNA and cytosolic nucleotides, thereby making the foundation for neoplastic transformation of cells. Shen and colleagues (85) demonstrated that incubation of DNA with either isolated neutrophil-derived myeloperoxidase or eosinophil peroxidase, together with halides and a cell-free O_2^--generating system resulted in the formation of 8-hydroxyguanine (8-OHG) in the presence of transition metal chelators, which was inhibited by catalase, SOD, and scavengers of hypohalous acids. Likewise, the formation of 8-OHG upon exposure of DNA to either neutrophils or eosinophils activated in media containing metal ion chelators was abrogated by peroxidase inhibitors, hypohalous acid scavengers, and catalytically active catalase and SOD. Thus, the peroxidase-H_2O_2-halide system of activated leukocytes creates a potential link between chronic inflammation, DNA damage and the development of cancer (85).

Tumor progression is accelerated by inflammation-induced ROS, if particularly accompanied with a diminished expression and activities of intracellular antioxidative enzymes (86). While subcutaneous administration of weakly tumorigenic fibrosarcoma cells failed to grow in C57BL/6 mice, aggressive tumor growth was noted upon co-implantation with an exogenous inflammatory stimuli gelatin sponge. The frequency of tumor formation by tumorigenic fibrosarcoma clones was inversely co-related with the levels of manganese SOD (MnSOD) and glutathione peroxidase-chi (GPchi) in respective tumor tissue. Cells retaining high levels of MnSOD showed greater ability to scavenge superoxides and were relatively protected against DNA mutation as compared to those containing a low level of MnSOD (86). Topical application of a SOD-mimetic phthalic acid mono-n-butyl ester cupric salt reduced the incidence and the multiplicity of DMBA-initiated and TPA-promoted skin papillomas in CD1 mice (87).

Pi-Class GST (GSTP)

Human GSTP plays an important role as a detoxifying enzyme in xenobiotic metabolism. Recent studies revealed that this Nrf2-regulated gene product can abrogate inflammatory signaling by several distinct mechanisms. Intracellular GSTP suppressed LPS-induced excessive production of pro-inflammatory mediators by blocking the activation of MAP kinases and NF-κB (88). Likewise, transfection of Raw264.7 cells with GSTP inhibited LPS-induced activation of MAP kinases and NF-κB, resulting in the decrease of TNFα and NO synthesis (89). Moreover, incubation of Raw264.7 cells with recombinant GSTP attenuated LPS-induced inflammatory signaling as revealed by reduced expression of iNOS and COX-2. Intraperitoneal administration of rGSTP also rescued mice from inflammatory responses induced by either LPS or thioglycolate (90).

The anti-inflammatory function of GSTP has partly been ascribed to its ability to inhibit JNK activity (91). GSTP has been identified as a binding partner of JNK, accounting for a low basal JNK activity in non-stressed cells. Embryo fibroblasts from GSTP-null mice showed a relatively high basal level of JNK activity, which was reduced by overexpression of GSTP cDNA. Exposure to UV radiation or H_2O_2 results in the oligomerization of GSTP and subsequent dissociation of the GSTP-JNK complex. Incubation of the immunoprecipitated Jun-JNK complex with purified GSTP caused a dose-dependent inhibition of JNK activity, which was abrogated by immunodepleting GSTP from protein extracts. The JNK activity was increased in the presence of a GSTP inhibitor. Moreover, forced overexpression of GSTP attenuated phosphorylation of MKK4 and JNK, increased c-Jun ubiquitination and decreased c-Jun-mediated transcription, suggesting that GSTP exerts anti-inflammatory effects by blocking the activation of JNK-AP-1 signaling in cells under inflammatory stress (91).

Miscellaneous

Several other Nrf2-regulated cytoprotective proteins have been reported to ameliorate various inflammatory responses. GCL, a Nrf2-regulated gene product, is involved in the biosynthesis of GSH, which by acting as a thiol buffer, maintains cellular redox status by scavenging ROS, detoxifying electrophiles and reducing peroxides and protein disulfides (92). Overexpression of GCL inhibited TNFα-induced activation of NF-κB, AP-1 and JNK in rat hepatoma cells (93). Depletion of GSH increases the susceptibility to oxidative stress and inflammatory damage. Colonic inflammation in rat colitis as well as *H. pylori*-induced inflammation was attenuated by elevating the cellular GSH level (94,95). Likewise, enhanced GSH synthesis inhibited LPS-induced activation of NF-κB and production of TNFα in rat lung epithelial cells (96), and the expression of COX-2 and iNOS in rat peritoneal macrophages (97), supporting the anti-inflammatory functions of GCL and GSH. Two important enzymes, GSH peroxidase (GPx) and GSH reductase, are involved in maintaining a constant pool of cellular GSH. GPx inactivates hydrogen

peroxide to protect cells from oxidative damage that can cause oxidation of GSH. The oxidized GSH is recycled back to its reduced state by GSH reductase. Lack of GPx accelerates inflammatory responses, while fortifying GPx activity can suppress inflammation. Reduced GPx activity was associated with gastrointestinal inflammation (98). Incubation with ebselen, a GPx mimetic agent, attenuated LPS-induced COX-2 expression in Raw264.7 macrophage cells (99). Another Nrf2-regulated gene product is thioredoxin (TRX), which can counteract the inflammatory responses. Overexpression of TRX-1 ameliorated chronic pancreatitis by suppressing oxidative stress and monocyte chemoattractant protein-1 (MCP-1)-mediated chronic inflammation (100). TRX-1 also reduced cigarette smoke-induced systemic inflammatory responses, such as emphysema (101), and colitis (102) in mice.

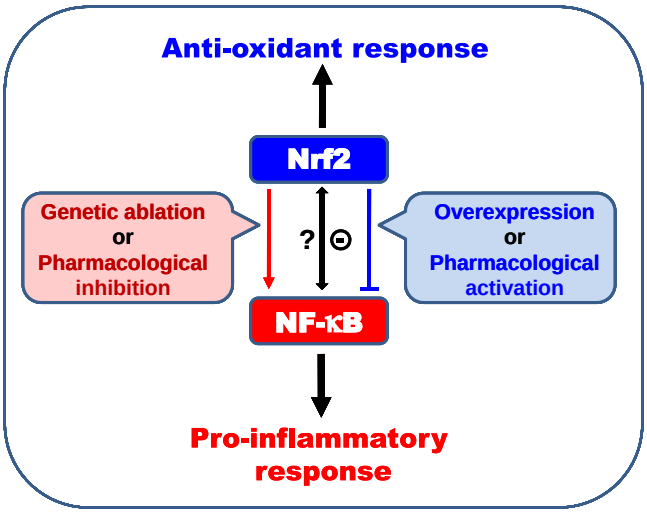

Fig. 2 Nrf2 as a modulator of inflammatory responses. There appears to be a negative cross-talk between Nrf2 and NF-κB. Nrf2 signal transduction may inhibit NF-κB signaling and *vice versa*. Although there is no evidence for the physical interaction between these redox-regulated transcription factors, Nrf2 deficiency/inactivation has been reported to aggravate inflammatory tissue damages (see Table II for further details).

ANTIOXIDANT *VS.* PRO-INFLAMMATORY SIGNALING: A POSSIBLE CROSS-TALK BETWEEN Nrf2 AND NF-κB

The activation of Nrf2 and consequent upregulation of its target genes not only counteract oxidative and electrophilic assault but also limit the severity of inflammatory tissue damage, which represent a potential mechanism of cancer chemoprevention. Multiple lines of evidence suggest that genetic disruption or pharmacologic inhibition of Nrf2 signaling augments the expression and/or activity of pro-inflammatory mediators, such as COX-2, iNOS, TNFα, IL-1β, etc. and sustains inflammation. In general, transactivation of these pro-inflammatory mediators is under the control of NF-κB (13). Since this transcription factor has been recognized as an important molecular link between inflammation and cancer, it is possible that the anti-inflammatory and chemopreventive effects of Nrf2 inducers or Nrf2-regualted gene products may be mediated through downregulation of NF-κB (Fig. 2). Several recent studies have suggested the existence of a possible cross-talk between the Nrf2 and NF-κB signaling.

The coordinated regulation of Nrf2 and NF-κB has been shown to play a crucial role in translating cellular stress signal into an anti-inflammatory response. For example, genetic ablation of Nrf2 aggravated lung injury as revealed by increased serum levels and tissue expression of cytokines (e.g., TNFα, IL-1β, and IL-6) and adhesion molecules (e.g., ICAM1) by blocking the activation of NF-κB signaling in acute lung injury after traumatic brain injury (TBI) (103). Likewise, intestinal levels of NF-κB, pro-inflammatory cytokines and ICAM-1 in Nrf2-null mice were significantly higher than those in Nrf2 wild-type mice after challenge with TBI (104). Moreover, the absence of Nrf2 exacerbated TBI-induced cerebral inflammation via upregulation of NF-κB activity and pro-inflammatory cytokine production (105). In another study, cytokine stimulation of pancreatic RINm5F insulinoma cells caused beta-cell damage via the activation of NF-κB-mediated signaling. Treatment with an Nrf2 inducer, sulforaphane, or transient overexpression of Nrf2 inhibited NF-κB activation through suppression of H_2O_2 production (106). In contrast, overexpression of Nrf2 attenuated redox-sensitive inflammatory gene expression in TNFα-stimulated endothelial cells without affecting activation of NF-κB. According to this study, overexpression of Nrf2 suppressed TNFα-induced expression of MCP-1 and VCAM-1 at mRNA and protein levels and inhibited activation of p38 MAP kinase. Expression of a constitutively active form of MKK6 (an upstream kinase of p38 MAP kinase) partially reversed Nrf2-mediated inhibition of VCAM-1 expression, suggesting that p38 MAP kinase, at least in part, mediates the anti-inflammatory action of Nrf2 (107).

Other possible mechanisms underlying Nrf2-mediated anti-inflammatory response may involve an indirect modulation of NF-κB by a Nrf2-regulated protein, secretory leukocyte protease inhibitor (SLPI). Nrf2 has been shown to induce the expression of SLPI, a cationic serine protease inhibitor, in macrophages (108). SLPI has been reported to inhibit neutrophil elastase-induced pulmonary inflammation (109,110). Moreover, adenoviral delivery of SLPI inhibited NF-κB-mediated inflammatory response in human endothelial cells and macrophages (111).

Many chemopreventive phytochemicals exhibited simultaneous induction of Nrf2-regulated cytoprotective protein expression and inhibition of NF-κB-regulated pro-inflammatory signaling (112). However, direct evidence

linking Nrf2 activation to NF-κB downregulation is yet to be established.

CHEMOPREVENTION OF INFLAMMATION-ASSOCIATED CARCINOGENESIS WITH Nrf2 INDUCERS

Over the last several decades, chemoprevention has gained much attraction as one of the most realistic strategies to fight cancer. A wide variety of substances, both natural and synthetic, have been shown to retain chemopreventive potential (13,23). One of the approaches of cancer chemoprevention is the suppression of inflammatory tissue damage (13). The Keap1-Nrf2 signaling pathway is involved in transcriptional regulation of a battery of cytoprotective enzymes, many of which have been shown to possess anti-inflammatory properties. Thus, the activation of Nrf2 signaling and the induction of its target genes would be a rational strategy for cancer chemoprevention. In activating Nrf2 signaling, certain chemopreventive agents target Keap1 by oxidizing or directly modifying its critical sensor cysteine residues (39). Those chemopreventive agents that contain an α,β-unsaturated carbonyl moiety can act as a Michael reaction acceptor capable of directly modifying Keap1 cysteine thiols, while others can oxidize two highly reactive cysteine thiol groups of Keap1, resulting in disulfide bond formation (113). Oxidation of cystine residues or covalent modification of Keap1 cysteine thiols helps stabilize Nrf2, which then translocates to nucleus and upregulates transcription of genes harboring an ARE element. Besides Keap1 cysteine oxidation/modification, certain compounds activate Nrf2 through phosphorylation of its serine and/or threonine residues via activation of a series of upstream kinases, such as MAP kinases, phosphatidylionositol-3-kinase/Akt, protein kinase C, etc. Detailed mechanisms underlying the activation of Nrf2 signaling by structurally diverse chemopreventive agents have been addressed in several recent reviews (39,40,114). Since this article is intended to highlight the importance of activating Keap1-Nrf2 signaling as a strategy to prevent inflammation-associated carcinogenesis, discussion will be made in the following section with particular emphasis on the role of Nrf2-inducing agents in limiting inflammation and cancer.

One of the extensively investigated chemopreventive phytochemicals that targets the Keap1-Nrf2 signaling is sulforaphane, an isothiocyanate present in cruciferous vegetables such as broccoli and cabbage. Several studies have demonstrated that Nrf2 plays a critical role in exerting anti-inflammatory effects of sulforaphane. Effects of sulforaphane on LPS-induced activation of inflammatory mediators were investigated in peritoneal macrophages derived from Nrf2 wild-type and Nrf2 knockout mice. Expression of the representative Nrf2-regulated target gene HO-1 was upregulated by sulforaphane in LPS-treated Nrf2+/+ macrophages. Pretreatment with sulforaphane inhibited LPS-induced mRNA and protein expression of TNFα, IL-1β, COX-2 and iNOS as well as production of TNFα and IL-1β in Nrf2+/+ macrophages, but failed to provoke the same effects in LPS-stimulated Nrf2−/− macrophages (115). Likewise, sulforaphane induced nuclear translocation of Nrf2 in LPS-treated rat microglia cells, while it attenuated LPS-induced activation of NF-κB and AP-1, and production of nitrite and pro-inflammatory cytokines in these cells (116). Topical application of sulforaphane inhibited DMBA-initiated and TPA-promoted skin carcinogenesis in C57BL/6 mice harboring wild-type Nrf2, whilst no such chemopreventive effects were observed in the Nrf2-deficient mice (117). *H. Pylori*-induced gastric mucosal inflammation is linked to gastric carcinogenesis. Oral administration of sulforaphane-rich broccoli sprouts to C57BL/6 female mice infected with *H. pylori* Sydney strain 1 and maintained on a high-salt (7.5% NaCl) diet reduced gastric bacterial colonization, attenuated mucosal expression of TNFα and IL-1β, mitigated corpus inflammation, and prevented gastric atrophy. However, these anti-inflammatory effects were not observed in Nrf2−/− mice, corroborating the importance of Nrf2 activation in sulforaphane-mediated protection against gastric inflammation. Moreover, daily administration of sulforaphane-rich broccoli sprouts to mice as well as human volunteers showed decreased colonization of *H. pylori* as compared to placebo controls (45).

Curcumin, the yellow pigment of turmeric, is another well-investigated chemopreventive phytochemical with strong anti-inflammatory properties (23). In addition to its pronounced inhibitory effects on pro-inflammatory signaling, the activation of Nrf2 signaling also contributes to the anti-inflammatory effects of curcumin. Dietary administration of curcumin enhanced the expression and nuclear translocation of Nrf2 in the liver and lung of mice treated with B[*a*]P as compared with controls. Moreover, increased ARE-binding of Nrf2 and induction of the activity as well as expression of GST and NQO1 and their mRNA transcripts in liver and lung of mice pretreated with dietary curcumin led to increased detoxification of B[*a*]P, thereby blocking the B[*a*]P-DNA adduct formation, oxidative stress and inflammation (118). Curcumin, given by gavage, attenuated dimethylnitrosoamine-induced hepatic injury in rats by activating Nrf2 and inducing the expression and activity of HO-1. Treatment with the HO-1 inhibitor zinc protoporphyrin abrogated this hepatoprotective effect of curcumin (119). Curcumin disrupted the Keap1-Nrf2 complex, increased Nrf2 binding to ARE and subsequently upregulated the expression and the activity of HO-1 in porcine renal epithelial proximal tubule (LLC-PK_1) cells and/or rat kidney

epithelial (NRK-52E) cells (120). Because of the presence of two α,β-unsaturated carbonyl moieties, curcumin acts as a Michael reaction acceptor and causes thiol modification of Keap1, thereby facilitating Nrf2 nuclear translocation. In contrast, tetrahydrocurcumin, which lacks an electrophilic carbon center, failed to induce the Nrf2-ARE binding as well as HO-1 induction when given orally to rats (119).

Dibenzoylmethane, a β-ketone analog of curcumin, induced the ARE-driven luciferase reporter activity and attenuated B[a]P-induced DNA adduct formation in the lung of A/J mice. These findings were in agreement with increased mRNA expression of NQO1, GSTA2, and GCLC in mouse hepatoma cells, which was negated by dominant-negative mutation of Nrf2 (121). A recent study demonstrated that treatment of Raw 264.7 cells with sulforaphane plus curcumin did not elicit a synergistic inhibitory effect on LPS-induced mRNA and protein expression of iNOS, COX-2 and TNFα, but exerted a synergistic effect on the induction of antioxidant enzyme HO-1 (122). In HO-1 knock-down cells, LPS stimulation increased the expression of COX-2 and iNOS, suggesting that combination of sulforaphane and curcumin may provide better anti-inflammatory effects by upregulating HO-1 expression (122).

A series of synthetic triterpenoids have been reported to induce cytoprotective gene expression through activation of Nrf2. For instance, a representative triterpenoid, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) induced the mRNA expression of GCLC, GCLM, HO-1 and NQO1 in peritoneal neutrophils derived from Nrf2 wild-type mice, but not in those isolated from Nrf2-deficient mice (123). Treatment of neutrophils derived from Nrf2+/+ mice with LPS showed significantly increased production of TNFα, IL-6, MCP1 and Mip2 as compared to Nrf2−/− neutrophils. Incubation with CDDO-Im significantly attenuated LPS-induced ROS generation and the production of aforementioned cytokines in Nrf2+/+, but not Nrf2−/−, neutrophils. These findings suggest that the activation of Nrf2-dependent compensatory antioxidative pathways by CDDO-Im protects against LPS-induced inflammatory response (123).

CONCLUSION AND FUTURE PERSPECTIVES

Living in an environment with constant unavoidable exposure to various toxicants (environmental pollutants, carcinogens, dietary mutagens, microorgasnisms, solar radiation, etc.), cells are constantly subjected to diverse oncogenic insults. Oxidative stress and inflammatory damages of cellular macromolecules play important roles in activating oncogenes and destroying tumor suppressor genes, thereby setting up a ground for transformed cells to develop tumors. Therefore, chemoprevention can be better achieved by blocking oxidative and inflammatory insults. The Keap1-Nrf2 system constitutes a stress response pathway that maintains normal cellular integrity by inducing the expression of a variety of cytoprotective proteins. Notably, overexpression of Nrf2-regulated gene products has been shown to suppress inflammatory events and inhibit various experimental carcinogenesis. Thus, translating the Keap1-Nrf2-mediated stress signal into an anti-inflammatory response would constitute the 'hitting the root' principle for chemoprevention of inflammation-associated carcinogenesis.

However, a paradoxical role of Nrf2 activation in progression of cancer has been reported. Several recent studies demonstrated that Nrf2 and its downstream genes are overactivated/overexpressed in some cancers, thereby providing survival and growth advantage to cancer cells. Constitutive activation of Nrf2 in transformed or cancerous cells and human cancer tissues often results from either somatic mutation Keap1 (124) or Nrf2 (125) or loss of Keap1 function due to epigenetic alterations (126). The mutation of Keap1 impaired its ability to repress Nrf2 in breast cancer (127). Keap1 expression was reduced in lung cancer cells as compared to normal bronchial epithelial cells as a consequence of promoter hypermethylation (126). Nrf2 mutation was detected in human esophageal, skin, lung and head and neck tumors. Mutations of Nrf2 occurred predominantly in its DLG and ETGE motif, which are important for its interaction with Keap1. As a consequence of mutation, Nrf2 acquires the ability to escape Keap1-mediated degradation (125). One outcome of such elevated Nrf2 levels in cancers is the induction of chemoresistance, which can be achieved by facilitated elimination of chemotherapy-induced cytotoxic ROS through elevated expression of Nrf2-regulated cytoprotective proteins. Aberrant activation of Nrf2 in cancerous cells also patronizes chemotherapy resistance by inducing drug transporters, commonly termed as multidrug resistant proteins (MRP), which by lowering cellular accumulation of cytotoxic drugs renders cancer cells resistant to chemotherapy. For example, 15d-PGJ_2, an endogenously produced cyclopentenone prostaglandin, induced Nrf2-dependent MRP1 expression in human breast cancer (MCF-7) cells (128). Treatment with 15d-PGJ_2 elevated the expression of HO-1 and MMP1 in an Nrf2-dependent manner in MCF-7 and MDA-MB 231 human breast cancer cells (129).

Although the timely induction of Nrf2 activation and subsequent expression of cytoprotective gene products protects normal cells from inflammatory stress, constitutive over-activation of Nrf2 in cancerous cells may alter the tumor microenvironment, thereby conferring survival advantages. Such controversial functions of Nrf2 in cancerous *vs.* normal cells merit further investigations.

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