Dietary Cancer Chemopreventive Agents – Targeting Inflammation and Nrf2 Signaling Pathway

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- Nrf2
- cancer chemoprevention

Abstract
Accumulating epidemiological and clinical evidence shows that chronic inflammation plays a critical role in neoplastic transformation and progression. Long-term users of selective cyclooxygenase-2 (Cox-2) inhibitors (coxibs) and non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to have a reduced risk of developing colorectal cancer. However, the adverse gastrointestinal and cardiovascular side effects associated with these drugs have limited their routine use for cancer chemoprevention. Basic leucine zipper (bZIP) protein Nrf2, a key transcription factor mediating the antioxidant response is an important modulator of tumor susceptibility in mouse models. Mice lacking Nrf2 are more susceptible to carcinogenesis induced by carcinogens. Moreover, induction of the Nrf2 signaling pathway is essential for many food phytochemicals to exert their cancer chemopreventive activity as demonstrated in many preclinical studies. It has been recently shown that the combination of coxibs or NSAIDs and natural phytochemicals can synergistically inhibit carcinogenesis in rodent models. This review will focus on the role of chronic inflammation and the Nrf2 signaling pathway in carcinogenesis and the feasibility of targeting these signaling pathways with dietary cancer chemopreventive agents and for cancer chemoprevention.

Introduction
Chronic inflammation was identified as one of the predisposing factors for neoplastic transformation by Rudolph Virchow over a century ago. Today, his hypothesis is supported by accumulating epidemiological and clinical evidence. It is estimated that 15% of malignancies are associated with chronic inflammation as a result of persistent pathogen infections [1]. Studies from our laboratory, as well as the findings of others, demonstrate that nuclear factor-erythroid 2-related factor 2 (Nrf2) plays a crucial role in modulating susceptibility to carcinogenesis in mouse models [2], [3], [4], [5], [6], [7], [8]. This review will focus on the role of chronic inflammation and the Nrf2 signaling pathway in carcinogenesis and the feasibility of targeting inflammation and Nrf2 for cancer chemoprevention. Isothiocyanates and curcumin will be used as examples of natural phytochemicals that showed significant anti-inflammatory and cancer chemopreventive effects in rodent and human trials.
One of the most obvious causal relationships between chronic inflammation and cancer is in colon carcinogenesis. Epidemiological studies have shown that individuals with long-standing inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn’s disease have an increased lifetime risk of developing colorectal cancer [12, 13, 14]. While the infectious organism responsible for IBDs is yet to be identified, many pathogenic infections have been linked with cancers. It is estimated that 90% of ulcers and 60% of gastric adenocarcinomas are attributed to H. pylori infection [15]. Approximately 85% of patients infected by H. pylori remain asymptomatic but in the remaining 15% infection is associated with gastritis, peptic ulcers and gastric adenocarcinoma [16, 17]. It has been shown that in patients with vacA cytotoxin-expressing H. pylori, infection is associated with a higher degree of inflammation and they are significantly more likely to develop peptic ulcers or gastric cancer [18]. In fact, antibiotic therapy against H. pylori has been found to effectively prevent gastritis, peptic ulcers and ultimately gastric cancer [19, 20, 21]. Accumulating epidemiological evidence links chronic HBV and HCV infection with hepatocellular carcinoma (HCC) in humans [22, 23, 24, 25, 26]. It is believed that liver cancer is the consequence of a chronic process of active hepatitis that produces a continuous stimulus of hepatic regeneration and cirrhosis [27]. Likewise, increased risk for bladder cancer is associated with schistosomiasis [28], [29], [30] and opisthorchiasis in humans [31]. It has been shown that in patients with Schistosoma haematobium infection is associated with increased urinary bladder carcinoma and hepatocarcinogenesis in vivo [32]. Findings from our laboratory and others have demonstrated that Nrf2 is an important modulator of susceptibility to carcinogen-induced carcinogenesis (Table 2). Using an azoxymethane-dextran sodium sulfate (AOM/DSS) colon carcinogenesis model, we demonstrated that tumor incidence, multiplicity, size, and stage of progression are increased in Nrf2 deficient mice [33]. Similarly, Osburn et al. in 2007 reported that Nrf2 ablation resulted in increased colonic inflammatory injury and formation of aberrant crypt foci upon DSS treatment [8]. We have also reported that Nrf2-deficient mice are more susceptible to 7,12-dimethylbenz(a)anthracene (DMBA) and 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced skin tumorigenesis [4]. In addition, accelerated DNA adduct formation and severe epithelial hyperplasia induced by diesel exhaust exposure, increased gastric neoplasia induced by benzopyrene. Increased incidences of N-nitrosobutyl(4-hydroxybutyl)amine (BBN)-induced urinary bladder carcinoma and hepatocarcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline were also reported in Nrf2-deficient mice compared to their wild-type counterparts [2, 3, 6, 7]. Taken together, it is obvious that Nrf2 functions as a suppressor of tumorigenesis, at least in preclinical animal models.

**Table 1** Chronic inflammation-associated non-malignant diseases and malignancy

<table>
<thead>
<tr>
<th>Etiological agents</th>
<th>Non-malignant disease</th>
<th>Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental/Chemical irritants</td>
<td>Asbestos, siliosis (Grinder’s disease)</td>
<td>Mesothelioma, lung carcinoma</td>
</tr>
<tr>
<td>Asbestos fibres, silica dusts</td>
<td>Bronchitis</td>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>Cigarettes smoking</td>
<td>Pancreatitis, liver cirrhosis</td>
<td>Pancreatic carcinoma, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Skin inflammation</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Ultraviolet light</td>
<td>Reflux esophagitis, Barrett’s esophagus</td>
<td>Esophageal carcinoma</td>
</tr>
<tr>
<td>Gastric acids</td>
<td>Genetic warts/condyloma</td>
<td>Cervical cancer</td>
</tr>
<tr>
<td>Pathogen infections</td>
<td>Infectious mononucleosis</td>
<td>Nasopharyngeal carcinoma, Burkitt’s lymphoma, Hodgkin’s lymphoma, Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Human papilloma virus 16 and 18 (HPV)</td>
<td>Hepatitis, cirrhosis</td>
<td></td>
</tr>
<tr>
<td>Epstein-Barr virus (EBV)</td>
<td>AIDS</td>
<td>Kaposi’s sarcoma, squamous cell carcinoma, non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>Hepatitis B and C virus (HBV, HCV)</td>
<td>Opisthorchis, cholangitis</td>
<td>Cholangiosarcoma, colon carcinoma</td>
</tr>
<tr>
<td>Human herpes 8 (H8H), human immuno-</td>
<td>Gastritis, peptic ulcers</td>
<td>Gastric carcinoma</td>
</tr>
<tr>
<td>deficiency virus (HIV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver flukes (Opisthorchis viverini)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistosoma haematobium</td>
<td>Schistosomiasis</td>
<td></td>
</tr>
<tr>
<td>Unidentified factor</td>
<td></td>
<td></td>
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<tr>
<td>Inflammatory bowel diseases (IBDs),</td>
<td>Colon cancer</td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease, chronic ulcerative colitis</td>
<td></td>
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</tr>
</tbody>
</table>

Modified from refs. [78], [79], [80].

**NF-κB Signaling and the Inflammatory Pathways**

A key player in inflammatory processes is the transcription factor NF-kB (nuclear factor-kappa-B) [34], which consists of a number of closely related protein dimers that bind a common sequence motif [35]. Consisting of homo- and heterodimeric complexes formed from the Rel family of proteins, Rel/NF-κB nuclear transcription factors have been found to play important roles in inflammatory processes.
by exposing the nuclear localization signals on NF-KO mice [37]. It has been shown that Nrf2 protects against pro-inflammatory mediators like inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2), was observed in Nrf2 knockout mice [38], whereas genetic ablation of Nrf2 enhances the susceptibility to cigarette smoke-induced emphysema and to severe airway inflammation and asthma in mice [40], [41]. In addition, Nrf2 was found to be a crucial regulator of the innate immune response and survival during experimental sepsis [42]. It is postulated that attenuation of inflammation through induction of anti-oxidative enzymes and suppression of pro-inflammatory mediators in an Nrf2-dependent manner, as demonstrated in these acute inflammation animal models, results in decreased sensitivity of wild-type mice towards inflammatory oxidative damage. These studies show that the Nrf2 signaling pathway is essential for protection of the host against inflammation and inflammatory damage. Despite all the findings in preclinical models suggesting that there is a possible crosstalk between Nrf2 and inflammation, the underlying mechanisms are still elusive. Liu et al. have recently provided an important insight, elucidating how pro-inflammatory signaling can negatively regulate the Nrf2/ARE signaling pathway [43]. They found that NF-xB/p65 could antagonize this pathway through deprivation of the coactivator, C/EBP binding protein (CBP) from Nrf2. They also hypothesized that disruption of Nrf2-CBP interaction may restore the ability of CBP to recruit the corepressor, histone deacetylases (HDACs) to ARE resulting in ARE repression [43]. Since in vivo data indicated that ablation of Nrf2 resulted in increased expression of pro-inflammatory mediators like IL-1β, IL-6 and COX-2, which are modulated by NF-xB, it will be interesting to know if overexpression of Nrf2 can reverse the suppression effect of p65 leading to suppression of the pro-inflammatory NF-xB signaling pathway. A previous study showed that Nrf1 and Nrf2 can differentially modulate the expression of NF-xB and activator protein-1 (AP-1) family members [44]. The question whether modulation of these pro-inflammatory mediators by Nrf2 is a direct transcriptional regulation or indirect through its transactivated target genes such as HO-1 and NQO-1 requires further investigation.

Cross-Talk between Inflammation and Nrf2 Signaling Pathway

There is a growing body of evidence that the Nrf2 signaling pathway is closely involved with the regulation of inflammation. We have recently shown that Nrf2-deficient mice have increased susceptibility to DSS-induced colitis. In comparison with wild-type mice, the colonic colitis observed in Nrf2 KO mice appeared to be more severe as demonstrated by loss of colonic crypts, massive infiltration of inflammatory cells and anal bleeding. In addition, immunocytochemical staining of nitrotyrosine, a biomarker of inflammation, was more intense in Nrf2 KO mice. These observations in KO mice were associated with a lower induction of phase II detoxifying enzymes including HO-1, NAD(P)H-quinone reductase-1 (NQO1), UDP-glucosyltransferase 1A1 (UGT1A1), and glutathione S-transferase Mu-1 (GSTM1). Concomitantly, more intense induction of pro-inflammatory biomarkers, such as interleukin (IL)-1β, IL-6 and TNFα, as well as pro-inflammatory mediators like inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2), was observed in Nrf2 KO mice [37]. It has been shown that Nrf2 protects against chemical-induced pulmonary injury and inflammation [38], [39], whereas genetic ablation of Nrf2 enhances the susceptibility to cigarette smoke-induced emphysema and to severe airway inflammation and asthma in mice [40], [41]. In addition, Nrf2 was found to be a crucial regulator of the innate immune response and survival during experimental sepsis [42]. It is postulated that attenuation of inflammation through induction of anti-oxidative enzymes and suppression of pro-inflammatory mediators in an Nrf2-dependent manner, as demonstrated in these acute inflammation animal models, results in decreased sensitivity of wild-type mice towards inflammatory oxidative damage. These studies show that the Nrf2 signaling pathway is essential for protection of the host against inflammation and inflammatory damage.

Cancer Chemoprevention by Targeting Inflammation and Nrf2 Signaling Pathway

Some of the best evidence for a causal relationship between inflammation and neoplastic transformation and progression comes from a study of cancer risk among long-term users of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). Epidemiological studies conducted on different populations with roles in cell proliferation, anti-apoptosis, and cancer development. The Rel protein family consists of five proteins, named c-Rel, Rel A (p65), Rel B, NF-kB1 (p50/p105), and NF-xB2 (p52/p100). NF-xB is activated by a wide variety of stimuli such as tumor necrosis factor (TNF)-alpha, interleukin-1 (IL-1), LPS, UV light, and oxidative stress. In most cell types, NF-xB is present in the cytosol in an inactive form and is associated with its inhibitor proteins, called inhibitor of NF-xB (I-xB). Of the many functional domains in NF-xB proteins – the Rel homology domain (RHD), DNA binding domains, the dimerization domain, and the nuclear localization signal (NLS) – I-xB binds to the RHD. Activation of NF-xB by extracellular stimuli leads to rapid phosphorylation, ubiquitination, and proteolytic degradation of I-xB, thereby exposing the nuclear localization signals on NF-xB and resulting in the nuclear translocation of the NF-xB complex and phosphorylation of p65. The binding of NF-xB to a specific sequence in the promoter region of a gene triggers the transcriptional activation of NF-xB-regulated genes, including cyclin D1, vascular endothelial growth factor (VEGF), Bcl-XL, COX-2, and MMP-9, which are involved in a variety of cellular events, including tumor cell proliferation, angiogenesis and metastasis [36]. Hence, NF-xB and the signaling pathways that mediate its activation have become attractive targets for development of new chemopreventive and chemotherapeutic approaches [34].
more than 13,000 cases, indicate that regular aspirin use is associated with a reduction in the risk of colorectal cancer. The pooled relative risk (RR) estimation was 0.59 (95% CI: 0.54 – 0.64) from 11 case-control studies, 0.85 (95% CI: 0.78 – 0.92) from seven cohort ones, and 0.71 (95% CI: 0.67 – 0.75) from all studies combined [45]. In addition, epidemiological studies also indicate that aspirin has a favorable effect on cancers of the esophagus (RR = 0.72, 95% CI: 0.62 – 0.84), stomach (RR = 0.84, 95% CI: 0.76 – 0.93), breast (RR = 0.91, 95% CI: 0.88 – 0.95), ovary (RR = 0.89, 95% CI: 0.78 – 1.02) and lung (RR = 0.94, 95% CI: 0.89 – 1.00) [45]. From the perspective of all these promising findings, the development of effective chemoprevention with these drugs appears to be a real possibility. However, several obstacles and challenges remain to be overcome. While the optimum doses and the duration of treatment needed to achieve an effective chemopreventive effect remain unclear, long-term consumption of these drugs is associated with adverse side effects. Therefore, cancer chemoprevention using coxibs and NSAIDs may only be suitable for patients with higher cancer risk.

**Natural Phytochemicals as Promising Anti-Inflammatory and Anti-Neoplastic Agents**

Naturally occurring compounds with potent anti-inflammatory properties have been noted as a plausible approach for clinical cancer prevention trials. In fact, dietary vegetables and fruits have been regarded as rich sources of chemopreventive agents. Food phytochemicals such as curcumin, EGCG, resveratrol, genistein and isothiocyanates, with strong anti-inflammatory activity, have been shown to inhibit carcinogenesis in preclinical animal models. Epidemiological studies have clearly documented that the frequent consumption of a diet high in fruits and vegetables has been linked to a lower risk of many types of cancers including prostate, colon, oral cavity, stomach, lung and esophagus [46], [47]. For example, several case-control and large prospective studies focusing on dietary assessment suggest that the intake of isothiocyanates, tomatoes and tomato-based food products may be associated with a lower risk of prostate cancer [48], [49], [50], [51], [52]. Furthermore, several studies reported that broccoli consumption could be linked to a lowered risk of colon cancer and that watercress consumption can inhibit oxidative metabolism of lung carcinogen NNK in humans, as reported in rodents [53]. In addition, intakes of yellow-orange and cruciferous vegetables were also inversely related to prostate cancer, especially for advanced cases, with findings that were generally consistent across ethnic groups and these results suggest that legumes (not limited to soy products) and cruciferous vegetables may protect against prostate cancer. Other nutritional epidemiological studies also provide support for the hypothesis that high intakes of *Brassica* vegetables reduce prostate cancer risk [54], [55].

**Isothiocyanates**

Cruciferous vegetables, such as broccoli, Brussels sprouts, watercress, cabbage, kale, cauliflower, kohlrabi and turnip, are rich sources of sulfur-containing compounds called glucosinolates. Strong anticarcinogenic activities of cruciferous vegetables have been attributed to the high abundance of isothiocyanates (ITCs), hydrolysis products of glucosinolates. Isothiocyanates such as sulforaphane, phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC) and allyl isothiocyanate (AITC) have been repor-
Curcumin

Curcumin (CUR, a beta-diketone), a constituent of turmeric curry powder, possesses potent anti-inflammatory activity and prevents cancer in many animal models [67], [68], [69], [70], [71]. CUR is a potent inhibitor of TPA-induced ornithine decarboxylase activity and inflammation in mouse skin [69]. CUR is also a potent inhibitor of arachidonic acid-induced inflammation in vivo in mouse skin, and inhibits epidermal lipoxigenase and cyclooxygenase activity in vitro [69]. CUR inhibits COX2 induction by the tumor promoters, and TNFα. The induction of COX2 by inflammatory cytokines or hypoxia-induced oxidative stress can be mediated by NF-κB. Since CUR inhibits NF-κB activation, Plummer et al. examined whether its chemopreventive activity is related to modulation of the signaling pathway that regulates the stability of the NF-κB-sequestering protein, IκB [72]. Recently components of this pathway, NF-κB-inducing kinase and IκB kinases IKKα and beta, which phosphorylate IκB to release NF-κB, have been characterized. CUR prevents phosphorylation of IκB by inhibiting the activity of the IKKs. We have found that CUR potently inhibited phosphorylation of EGFR, PI3K, AKT, IKK-alpha and NF-κB activities in human prostate PC-3 cells [73], inhibition of NF-κB activities in human colon HT-29 cells [61] and that inhibition of Akt and NF-κB signaling pathways could contribute to the inhibition of cell proliferation and induction of apoptosis in the PC-3 prostate tumor xenografts in nude mice. Dietary administration of 2% CUR also caused a marked increase in apoptosis, and a significant decrease in angiogenesis in nude mice treated with LNCap prostate cancer cells [74]. Similarly, dietary administration of 2% CUR effectively inhibited azoxymethane (AOM)-induced colon tumors in male F344 rats and prevented tumors in C57BL/6J-Apc Min/+ mice. CUR was also reported to inhibit AOM-induced rat colon carcinogenesis by suppression of prostaglandin (PG) and thromboxane (Tx) formation [75]. Similar to isothiocyanates, CUR is a strong activator of Nrf2-mediated transcription of ARE-luciferase reporter gene, as well

Table 3  Synergistic inhibitory effect of coxibs and NSAIDs and food phytochemicals combination against carcinogenesis in rodent models

<table>
<thead>
<tr>
<th>Drugs/compounds (Dose)</th>
<th>Model</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin (0.6%), Celecoxib (0.16%)</td>
<td>2,3-Dimethylhydrazine (DMH)-induced colon cancer in rat</td>
<td>↓ 39%, ↓ 26.5% and ↓ 62% for curcumin, celecoxib, and combination, respectively.</td>
<td>[82]</td>
</tr>
<tr>
<td>Green tea polyphenol (GTP) (0.1%), celecoxib (10 mg/kg i.p. 5 days/week)</td>
<td>CWR22Rv1 xenografts in nude mice</td>
<td>↓ 42%, ↓ 57% and ↓ 81% for GTP, celecoxib alone and combination, respectively</td>
<td>[83]</td>
</tr>
<tr>
<td>alpha-Tocopherol ether-linked acetic acid analogue (α-TEA) (72 mg/day aerosol inhalation), celecoxib (500 ppm)</td>
<td>Ultraviolet-induced skin cancers in mice</td>
<td>↓ tumor numbers in all treatment group but continuous combination treatment resulted in the lowest total number of tumor</td>
<td>[84]</td>
</tr>
<tr>
<td>(72 mg/day aerosol inhalation), celecoxib (300 mg/kg and 1250 mg/kg diet)</td>
<td>MDA-MB-435-FL-GFP human cancer xenografts in nude mice</td>
<td>↓ mean tumor volume in all treatment group; inhibitory effect significantly stronger in combination of α-TEA + celecoxib (1250 group) than single compound/drug</td>
<td>[85]</td>
</tr>
<tr>
<td>Fructo-oligosaccharide (6%), celecoxib 1500 ppm</td>
<td>AOM-induced aberrant crypt foci (ACF) in rat</td>
<td>↓ 61% in combination; no effect with celecoxib alone</td>
<td>[86]</td>
</tr>
<tr>
<td>Anthocyanin-rich tart cherry extract (375, 750, 1500, 3000 mg/kg diet), sulindac (100 mg/kg diet)</td>
<td>APCmin/+ mice</td>
<td>Anthocyanin-rich extract + sulindac had fewer and smaller total tumor burden compared with sulindac alone</td>
<td>[87]</td>
</tr>
<tr>
<td>EGCG (0.01%), sulindac 10 mg/kg bw, p.o., 3 times/week</td>
<td>AOM-induced colon cancer in rat</td>
<td>↓ 54%, ↓ 58% and ↓ 78% for EGCG, sulindac alone and combination, respectively.</td>
<td>[88]</td>
</tr>
<tr>
<td>Green tea extract (0.1%), sulindac (0.03%)</td>
<td>APCmin/+</td>
<td>↓ 27%, ↓ 32% and ↓ 55.5% for Green tea extract, sulindac alone and combination, respectively.</td>
<td>[89]</td>
</tr>
</tbody>
</table>
as an inducer of endogenous Nrf2 protein and one of the Nrf2-mediated detoxifying/antioxidant enzyme HO-1 in human cells [76]. Using Affymetrix microarray analysis on the gene expression profiles induced by CUR in Nrf2(+/+), but not in Nrf2(−/−), our results indicated that, in addition to the classical Nrf2-mediated detoxifying and antioxidant cellular defense genes, other genes including transporters, kinases/phosphatases, those involved in ubiquitination and proteolysis, electron transport, apoptosis and cell cycle control, cell adhesion and transcription factors also require Nrf2 for their induction by dietary cancer chemopreventive compounds including CUR [76]. All these properties, together with a long history of consumption without adverse health effects, make CUR an important candidate for consideration in human cancer prevention.

Conclusion

Given that chronic inflammation has been linked with almost 20% of all human malignancies [77], Nrf2 signaling is important in the defense of acute inflammation, both inflammation and Nrf2 seem to be plausible targets for cancer chemoprevention. Fig. 1 summarizes the involvement and the possible cross-talk between Nrf2 and inflammatory signaling pathways. Concomitant induction of the Nrf2 signaling pathway and inhibition of inflammation is thought to be a crucial mechanism by which these phytochemicals exhibit their cancer chemopreventive effect. Combinations of sub-optimal doses of coxibs and NSAIDs such as celecoxib and sulindac with relatively non-toxic natural phytochemicals such as CUR, isothiocyanates and ECGC have been proven to be able to synergistically inhibit carcinogenesis in rodent models (Table 3). In addition to ongoing trials involving single phytochemicals with multiple activities, these combinational approaches certainly warrant further consideration in clinical human trials.

Acknowledgements

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