

Review Article

NF- κ B and Nrf2 as potential chemopreventive targets of some anti-inflammatory and antioxidative phytonutrients with anti-inflammatory and antioxidative activities

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Chemoprevention refers to the use of defined non-toxic chemical regimens to inhibit, reverse or retard the process of multi-stage carcinogenesis that involves multiple signal transduction events. A new horizon in chemoprevention research is the recent discovery of molecular links between inflammation and cancer. Components of the cell signaling network, especially those that converge on the ubiquitous eukaryotic redox-sensitive transcription factor, nuclear factor-kappa B (NF- κ B), have been implicated in the pathogenesis of many inflammation-associated disorders. A wide variety of chemopreventive and chemoprotective phytochemicals and phytonutrients can alter or correct undesired cellular functions caused by abnormal pro-inflammatory signal transmissions, mediated by NF- κ B. Modulation of cellular signaling involved in chronic inflammatory responses, induced by anti-inflammatory agents, hence provides a rational and pragmatic strategy in molecular target-based chemoprevention and cytoprotection. Induction of phase-2 detoxifying or antioxidant genes represents an important cellular defence in response to oxidative and electrophilic insults. Nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a crucial role in regulating phase-2 detoxifying/antioxidant gene induction. Many antioxidants derived from dietary and medicinal plants have been found to activate this particular redox-sensitive transcription factor, thereby potentiating the cellular antioxidant or detoxification capacity.

Key Words: chemo-prevention, NF- κ B, Nrf2, phytochemicals, signal transduction

CANCER CHEMOPREVENTION WITH EDIBLE PHYTOCHEMICALS

Chemoprevention, the term coined by Michael Sporn in the 1970s, is an attempt to utilize non-toxic chemical substances or their mixtures to interfere with neoplastic development.¹ There has been a substantial body of evidence that suggests that chemoprevention provides realistic promise of reducing the risk of human malignancies. Over the past few decades, there has been a growing body of interest in identifying naturally occurring chemopreventive agents, particularly those present in our diet. Dietary chemopreventives are present predominantly in fruits, vegetables, grains, spices and herbs and have diverse chemical structures. Chemical substances derived from plant-based diet are collectively called 'phytochemicals' ('phyto-' in Greek means plants).

The chemopreventive effects that most edible phytochemicals exert are likely to be the sum of several distinct mechanisms. These include 1) blockage of metabolic activation and DNA binding of carcinogens; 2) stimulation of detoxification; 3) repair of DNA damage; 4) suppression of cell proliferation and metastasis or angiogenesis; 5) induction of differentiation or apoptosis of precancerous or malignant cells, etc.² Recently, it has been shown that

common dietary phytochemicals act on the human genome, either directly or indirectly, to alter gene expression, thereby regulating the carcinogenic processes.

MULTISTAGE CARCINOGENESIS AND THE CLASSIFICATION OF CHEMOPREVENTIVE PHYTOCHEMICALS

Carcinogenesis is a complex process that involves a series of individual steps, each of which accompanies distinct biochemical and cellular alterations. Experimental carcinogenesis in rodents has indicated that tumor development consists of at least two stages, i.e., initiation and promotion. Some scientists prefer to include progression as a final stage leading to malignancies.

Initiation is an irreversible process which involves a series of events: the uptake of carcinogenic agents, their distribution and transport to organs and tissues where metabolic activation and/or detoxification occurs, the interaction

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of the reactive metabolite(s) with target cell DNA and subsequent base modifications, and finally fixation of the genotoxic damage. If such genetic mutations are not repaired, the initiated or altered cell may undergo neoplastic transformation via the promotion (and also progression) stage. Therefore, Chemopreventive phytochemicals can be subdivided into two main categories—blocking agents and suppressing agents.³ Blocking agents prevent carcinogens from reaching the target sites, from undergoing metabolic activation or from subsequently interacting with critical cellular macromolecules, such as DNA, RNA and proteins. Suppressing agents, on the other hand, inhibit the neoplastic transformation of initiated cells, in either the promotion or the progression stage.

THE INTRACELLULAR SIGNAL NETWORK AS A NOVEL TARGET FOR CHEMOPREVENTION WITH EDIBLE PHYTOCHEMICALS

Research directed toward elucidating molecular mechanisms underlying chemopreventive or chemoprotective actions of dietary phytochemicals has recognized components of signal transduction pathways as potential targets. Since the cellular signaling network often goes awry in carcinogenesis, it is fairly rational to target intracellular signaling cascades for achieving chemoprevention.² Numerous molecules and events are involved in relaying intracellular signals. Both external and endogenous stimuli turn on or switch off critical events of this relay, thereby transmitting the appropriate signals to diverse downstream target molecules in a highly sophisticated fashion for the fine-tuning of cellular homeostasis. Components of upstream or cytoplasmic signaling networks include protein kinases, such as the family of proline-directed serine/threonine kinases named mitogen-activated protein (MAP) kinases, protein kinase C, phosphatidylinositol-3-kinase, protein kinase B/Akt, glycogen synthase kinase, etc. Chemopreventive phytochemicals can work as modifiers of signal transduction pathways to elicit their beneficial effects.²

Oxidative and inflammatory injuries are closely linked to each other in the process of multi-stage carcinogenesis. Thus, compounds with anti-inflammatory activities are anticipated to inhibit oxidative stress, and *vice versa*. We have investigated chemopreventive effects of some edible phytochemicals, with the focus on those with antioxidative and anti-inflammatory properties, and elucidated their underlying molecular mechanisms. Our research program has attempted to unravel common events mediated by two major redox-sensitive transcription factors, NF- κ B and Nrf2, which mediate inflammatory and antioxidant signaling.

Nuclear factor kappa B (NF- κ B)

A growing body of data indicates that NF- κ B, a ubiquitously expressed eukaryotic transcription factor, plays a central role in general inflammatory as well as immune responses. NF- κ B is regarded as a potential link between inflammation and cancer.^{4,5}

NF- κ B, predominantly a heterodimer of p65 and p50 proteins, is normally sequestered in the cytoplasm as an inactive complex with the inhibitory protein, I κ B. Upon stimulation with mitogens, pro-inflammatory cytokines,

UV radiation, viral infection, bacterial toxins, etc. it gets phosphorylated by activated I κ B kinases (IKKs) or MAP kinases. Phosphorylated I κ B, upon ubiquitination, is directed to proteasomes for degradation. The degradation of I κ B allows NF- κ B to translocate to the nucleus, and to bind to a - κ B element located in the promoter regions of *cox-2* and other proinflammatory genes, thereby controlling their expression.

The transcriptional activation of NF- κ B depends on the phosphorylation of its active subunit p65/RelA.⁶ The upstream IKK signalsome has been shown to phosphorylate both I κ B and p65.

Nuclear factor erythroid 2 p45 (NF-E2)-related factor (Nrf2)

The induction of phase II detoxifying or antioxidant enzymes represents one of the most important components of cellular defense mechanisms whereby a diverse array of electrophilic and oxidative toxicants can be eliminated from the cell before they damage genomic DNA.

Some representative phase II detoxifying enzymes include glutathione peroxidase (GPx), glutamate cysteine ligase (GCL), glutathione *S*-transferase (GST), and NAD(P)H:quinone oxidoreductase 1 (NQO1). The induction of these enzymes is primarily regulated by Nrf2 as evidenced by negation of their expression in the genetically engineered Nrf2-deficient mice.

Under normal physiological conditions, a cytoskeleton binding protein called Kelch-like erythroid CNC homologue (ECH)-associated protein 1 (Keap1) binds to Nrf2 thereby repressing its activation. Exposure to inducers of antioxidant/phase II detoxifying enzyme expression diminishes the affinity of Keap1 for Nrf2, which allows for the translocation of Nrf2 into the nucleus. Following nuclear translocation, Nrf2 binds to the specific consensus *cis*-element called the antioxidant-response element (ARE) or the electrophile-responsive element (EpRE) that are present in the promoter region of genes encoding many antioxidant enzymes. Therefore, Nrf2-Keap1 signaling has been recognized as an important target for chemoprevention and chemoprotection.⁷

While the molecular mechanisms involved in the Nrf2-derived transcriptional activation of antioxidant enzymes are not fully understood, it has been hypothesized that covalent modification or oxidation of critical cysteine residues contained in Keap1 may facilitate the dissociation of the Keap1-Nrf2 complex or increase the stability of Nrf2.^{8,9} It has been suggested that cysteine residues present in Keap1 could serve as a molecular sensor for the recognition of the altered intracellular redox status triggered by electrophiles or ROS.¹⁰

CHEMOPREVENTIVE PHYTOCHEMICALS MODULATING NF- κ B AND NRF2 ACTIVATION

Many redox-responsive transcription factors and their regulators have conserved cysteine residues that can sense changes in either the redox state of the cells or the levels of particular redox-related effector molecules. Recently, there has been a growing body of data demonstrating that modification of the cysteine moiety in p50 and Keap1 can influence transcriptional activity of NF- κ B and Nrf2, and their target gene expression. Some naturally occurring

cancer chemopreventive agents can suppress inappropriate overactivation of NF- κ B signalling and/or activate Nrf2.^{2,5,7} Some Examples of representative chemopreventive phytochemicals that modulate NF- κ B and Nrf2 signalling via thiol modification are given below:

Sulforaphane

This chemopreventive isocyanate derived from broccoli and other cruciferous vegetables, has been reported to inhibit NF- κ B activation in several cultured cell lines.¹¹⁻¹³ It selectively reduced DNA binding of NF- κ B without interfering with lipopolysaccharide (LPS)-induced degradation of I κ B nor with nuclear translocation of NF- κ B.¹⁴ The sulforaphane-mediated attenuation of NF- κ B DNA binding activity was prevented by the sulfhydryl-reducing agent mercaptoethanol, suggesting that sulforaphane could either directly inactivate NF- κ B subunits by binding to essential cysteine residues or interact with GSH or other redox regulators like thioredoxin and Ref-1 relevant for NF- κ B function.¹⁴

Sulforaphane up-regulated the expression of NQO1, GST and GCL in the small intestine of wild mice by inducing ARE-driven phase 2 enzyme expression through the activation of Nrf2,^{15,16} but their induction was abolished in *nrf2*-null mice.¹⁵ In support of this notion, sulforaphane failed to protect against chemically-induced neoplasia in the forestomach¹⁷ and skin¹⁸ of Nrf2 null mice. Sulforaphane is an electrophile that can react with cysteine thiols of Keap1.¹⁹

6-Methylsulfinylhexyl isothiocyanate, an analogue of sulforaphane, isolated from Japanese horseradish, wasabi (*Wasabia japonica*), induced the nuclear localization of Nrf2.²⁰

Curcumin

This yellow coloring agent contained in turmeric (*Curcuma longa* L., Zingiberaceae), has been reported to possess strong anti-inflammatory and antioxidant activities which contribute to its chemopreventive potential.²¹ Curcumin has two α,β -unsaturated carbonyl moieties and inhibits NF- κ B activation by interfering with I κ B α degradation and also by reacting with p50 in the NF- κ B complex through thiol modification.²²

Curcumin has been reported to induce heme oxygenase-1 (HO-1) expression through Nrf2 activation in several types of cultured cells,²³⁻²⁵ and rat livers, *in vivo*.²⁷ In contrast, tetrahydrocurcumin lacks the α,β -unsaturated carbonyl moiety, hence cannot act as a Michael-reaction acceptor, and failed to induce HO-1 expression and ARE activation.²⁴ Interestingly, demethoxy curcumin was more effective than curcumin in terms of ARE-driven Nrf2 activation and subsequent HO-1 expression in beta-cells of mice.²⁵ A recent study from my laboratory has also demonstrated that curcumin, but not tetrahydrocurcumin, protected against dimethylnitrosamine-induced hepatotoxicity and induced Nrf2 activation and HO-1 expression when given orally to rats.²⁶

Chalcone, an α,β -unsaturated flavonoid, was found to abrogate the activation of NF- κ B and STAT3 in IL-6- and LPS-treated endothelial cells.²⁷ Curcumin was also inhibitory. Other flavonoids, quercetin and cyanidin, which lack an α,β -unsaturated carbonyl group, showed weaker or no

inhibitory effect on both IL-6-induced STAT3 phosphorylation and LPS-induced p65 translocation. This electrophilic compound also induced the expression of thioredoxin reductase and HO-1, which appeared to be mediated by the activation of Nrf2.²⁷

Zerumbone

This sesquiterpene compound present in tropical ginger (*Zingiber zerumbet* Smith) has been reported to be protective against the formation of aberrant crypt foci in colons of rats²⁸ and papillomagenesis in the skin of mice.²⁹ Zerumbone inhibited LPS- or interferon-induced IKK β degradation in RAW264.7 macrophages.³⁰ It suppressed NF- κ B-IKK signaling induced by several proinflammatory stimuli, including TNF- α , okadaic acid, phorbol ester, cigarette smoke condensates, and hydrogen peroxide, in cultured human cancer cells, rendering these cells susceptible to apoptosis and less invasive.³¹

Zerumbone induces phase II enzymes including GCL, GPx, and HO-1 via the Nrf2-dependent pathway.³² Interestingly, α -humulene, a structural analog that lacks only the carbonyl group of zerumbone, was virtually inactive in the aforementioned events mediated by zerumbone, suggesting the critical role of the α,β -unsaturated carbonyl group in the chemopreventive and chemoprotective actions of zerumbone.^{31,32} As zerumbone contains such an electrophilic functional group, it is expected to react with nucleophiles such as thiols, leading to the formation of Michael reaction adducts with critical cysteine thiols present in NF- κ B and Keap1.

CONCLUDING REMARKS

Cysteine thiols, as presented in various transcription factors and their regulators, are recognized to function as redox sensors involved in the fine-tuning of transcriptional regulations of many genes essential for maintaining cellular homeostasis. Thus, oxidation or covalent modification of the thiol groups present in redox-sensitive transcription factors and their regulating molecules can provide a unique strategy for molecular target-based chemoprevention and cytoprotection that utilizes anti-inflammatory and antioxidant phytochemicals.

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AUTHOR DISCLOSURES

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