Potential of Spice-Derived Phytochemicals for Cancer Prevention

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Abstract

Although spices have been used for thousands of years and are known for their flavor, taste and color in the food, they are not usually recognized for their medicinal value. Extensive research within the last two decades from our laboratory and others has indicated that there are phytochemicals present in spices that may prevent various chronic illnesses including cancerous, diabetic, cardiovascular, pulmonary, neurological and autoimmune diseases. For instance, the potential of turmeric (curcumin), red chilli (capsaicin), cloves (eugenol), ginger (zerumbone), fennel (anethole), kokum (gambogic acid), fenugreek (diosgenin), and black cumin (thymoquinone) in cancer prevention has been established. Additionally, the mechanism by which these agents mediate anticancer effects is also becoming increasingly evident. The current review describes the active components of some of the major spices, their mechanisms of action and their potential in cancer prevention.

Abbreviations

ADT:	anethole ditholethione
Bcl-3:	B cell lymphoma protein-3
CDK7:	cyclin-dependent kinase 7
cIAP:	inhibitor of apoptosis
COX-2:	cyclooxygenase-2
DNA:	deoxyribonucleic acid
EGF:	epidermal growth factor
ELAM-1:	endothelial leukocyte adhesion molecule-1
ERK or Erk:	extracellular signal-regulated kinases
GA:	gambogic acid
GSH:	glutathione
GST:	glutathione S-transferase
H_2O_2:	hydrogen peroxide
hTERT:	human telomerase reverse transcriptase
ICAM-1:	intercellular adhesion molecule-1
IKK:	IκBα kinase
IL:	interleukins
JAK:	Janus kinases
5-LOX:	5-lipoxygenase
MM:	multiple myeloma
MMP:	matrix metalloproteinase
NADH:	nicotinamide adenine dinucleotide hydride
NF-κB:	nuclear factor kappa B
NIK:	NF-κB inducing kinase
NNK:	nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
RANKL:	receptor activator for nuclear factor κB ligand
RHD:	Rel homology domain
RTX:	resiniferatoxin
STAT:	signal transducer and activator of transcription
TNF:	tumor necrosis factor
TNFR:	TNF receptor
TQ:	thymoquinone
TRAF:	TNF receptor associated factor
uPA:	urokinase plasminogen activator
VCAM:	vascular cell adhesion molecule
VEGF:	vascular endothelial growth factor
VEGFR2:	VEGF receptor 2

Introduction

It is generally believed that prevention is better than treatment. It is also an accepted reality that more than 90% of the cancers are preventable. Cigarette smoke, alcohol, environmental pollution, sunlight and diet have been shown to play a major role in causing cancer. How these agents

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cause cancer is also becoming evident. One of the major mediators of cancer that has emerged within last five years is chronic inflammation [1]. In contrast to acute inflammation, chronic inflammation is a low level inflammation that can persist over 20 – 30 years; thus eventually leading to cancer. Perhaps the best-known markers of chronic inflammation include inflammatory cytokines [such as tumor necrosis factor (TNF), receptor activator for nuclear factor κ B ligand (RANKL), interleukins (IL-1, IL-6, IL-8)] and chemokines, inflammatory enzymes [cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), matrix metalloproteinase (MMP) and urokinase plasminogen activator (uPA)], adhesion molecules [intercellular adhesion molecule-1 (ICAM-1)], vascular cell adhesion molecule (VCAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), and certain growth factors such as epidermal growth factor (EGF). Interestingly, all the mediators of inflammation are primarily regulated by two different transcription factors, nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription-3 (STAT-3).

The process of tumorigenesis requires cellular transformation, hyper-proliferation, invasion, angiogenesis, and metastasis. Several genes that mediate these processes are regulated by the transcription factor NF-κB. The latter is activated by various carcinogens, inflammatory agents, and tumor promoters. Thus, agents which can suppress NF-κB activation have the potential to suppress carcinogenesis. NF-κB is a ubiquitous transcription factor that binds to a specific deoxyribonucleic acid (DNA) sequence as a dimeric complex composed of various combinations of members of the Rel/NF-κB family of polypeptides [2]. Family members of this transcription factor are 35 to 61 % homologous to each other and have a Rel homology domain (RHD) of about 300 amino acids. NF-κB proteins are present in the cytoplasm of all cells, where they are kept in an inactive state by a family of anchorin domain-containing proteins, which includes IκBα, IκBβ, IκBγ, IκBε, B cell lymphoma protein-3 (Bcl-3), p105, and p100. Under resting conditions, NF-κB consists of a heterotrimer of p50, p65, and IκBα in the cytoplasm; only upon activation, do the p50 and p65 subunits translocate to the nucleus leading to the sequence of events. Most carcinogens, inflammatory agents, and tumor promoters, including cigarette smoke, phorbol ester, okadaic acid, hydrogen peroxide (H_2O_2), and TNF, have been shown to activate NF-κB. The activation of NF-κB involves the phosphorylation, ubiquitination and degradation of IκBα and phosphorylation of p65, which, in turn, leads to the translocation of NF-κB to the nucleus where it binds to specific response elements in the DNA. The phosphorylation of IκBα is catalyzed by IκBα kinase (IKK), which is essential for NF-κB activation by most agents. NF-κB has been shown to regulate the expression of several genes whose products are involved in tumorigenesis. These include antiapoptotic genes [e. g., inhibitor of apoptosis (cIAP), survivin, TNF receptor associated factor (TRAF), Bcl-2, and Bcl-xL], genes encoding adhesion molecules (ICAM, VCAM), chemokines, inflammatory cytokines and cell cycle regulatory genes (e. g., cyclin D1 and c-myc).

Members of the STAT family of transcription factors regulate the expression of gene products involved in cell survival, proliferation, chemoresistance, and angiogenesis [3]. The activation of STATs involves the phosphorylation of a critical tyrosine residue by Janus kinases (JAK), or the Src family kinases, leading to dimerization of STAT monomers, nuclear translocation, and binding to specific DNA response elements in the promoters of target genes. Among the STATs, STAT3 is perhaps most intimately linked to tumorigenesis. Although STAT3 is activated by IL-6, EGF, and other growth factors; constitutive activation of STAT3 has been discovered in a wide variety of tumors.

Dietary agents have been linked with prevention and therapy of cancer through a mechanism that is not well understood. We postulated that inflammation plays a major role in tumorigenesis through the activation of NF-κB and STAT3 [1]. We also postulated that dietary agents mediate their effect through modulation of NF-κB and STAT-3 activation [4]. This factor regulates the expression of various genes that control apoptosis, viral replication, tumorigenesis, various autoimmune diseases, and inflammation. NF-κB has been linked to the development of carcinogenesis for several reasons. Firstly, various carcinogens and tumor promoters have been shown to activate NF-κB. Secondly, activation of NF-κB has been shown to block apoptosis and promote proliferation. Thirdly, the tumor microenvironment can induce NF-κB activation. Fourthly, constitutive expression of NF-κB is frequently found in tumor cells. Fifthly, NF-κB activation induces resistance to chemotherapeutic agents. Sixthly, several genes involved in tumor initiation, promotion, and metastasis are regulated by NF-κB. Seventhly, various chemopreventive agents have been found to down-regulate the NF-κB activation. All these observation suggest that NF-κB could mediate tumorigenesis and thus can be used as a target for chemoprevention and for the treatment of cancer. Besides NF-κB, we have also targeted STAT3, another transcription factor that mediates tumorienesis. The evidence below shows that phytochemicals derived from spices are important inhibitors of NF-κB and STAT3 activation, and can suppress the expression of genes involved in carcinogenesis and tumorigenesis *in vivo*.

Evidence That Spice-Derived Phytochemicals can Mediate Cancer Prevention

Phytochemicals derived from numerous spices have been linked with cancer prevention. This review, however, will focus on some of the major spice-derived phytochemicals as chemopreventive agents (**Fig. 1**).

Capsaicin (red chilli)

Capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) is a principal pungent ingredient of hot and red chili peppers that belong to the plant genus *Capsicum* (Solanaceae). In addition to alleviating neuropathic pain and itching in humans, capsaicin has exhibited anticancer effects in animal models, suppressing carcinogenesis of the skin, colon, lung, tongue, and prostate. The mechanism by which this vanilloid mediates its anticarcinogenic effects is not understood but it has been shown to alter the metabolism of carcinogens such as aflatoxin B1 and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). In culture, capsaicin has been found to selectively suppress the growth of various human tumor cells [5], [6] including leukemic [7], [8], [9], gastric [10], hepatic [11], glioma [12], and prostate [13]. The roles of nicotinamide adenine dinucleotide hydride (NADH) oxidase activity, proteasome, COX, JNK, NF-κB, peroxisome proliferators-activated receptor gamma, peroxynitrite and mitochondrial respiration have been implicated. Its immunosuppressive effects have been linked to its ability to suppress NF-κB activation. We examined the effect of capsaicin and its analogue, resiniferatoxin (RTX), on the activation of NF-κB induced by different agents including TNF [14]. The pretreatment of human myeloid cells with capsaicin blocked TNF-medi-

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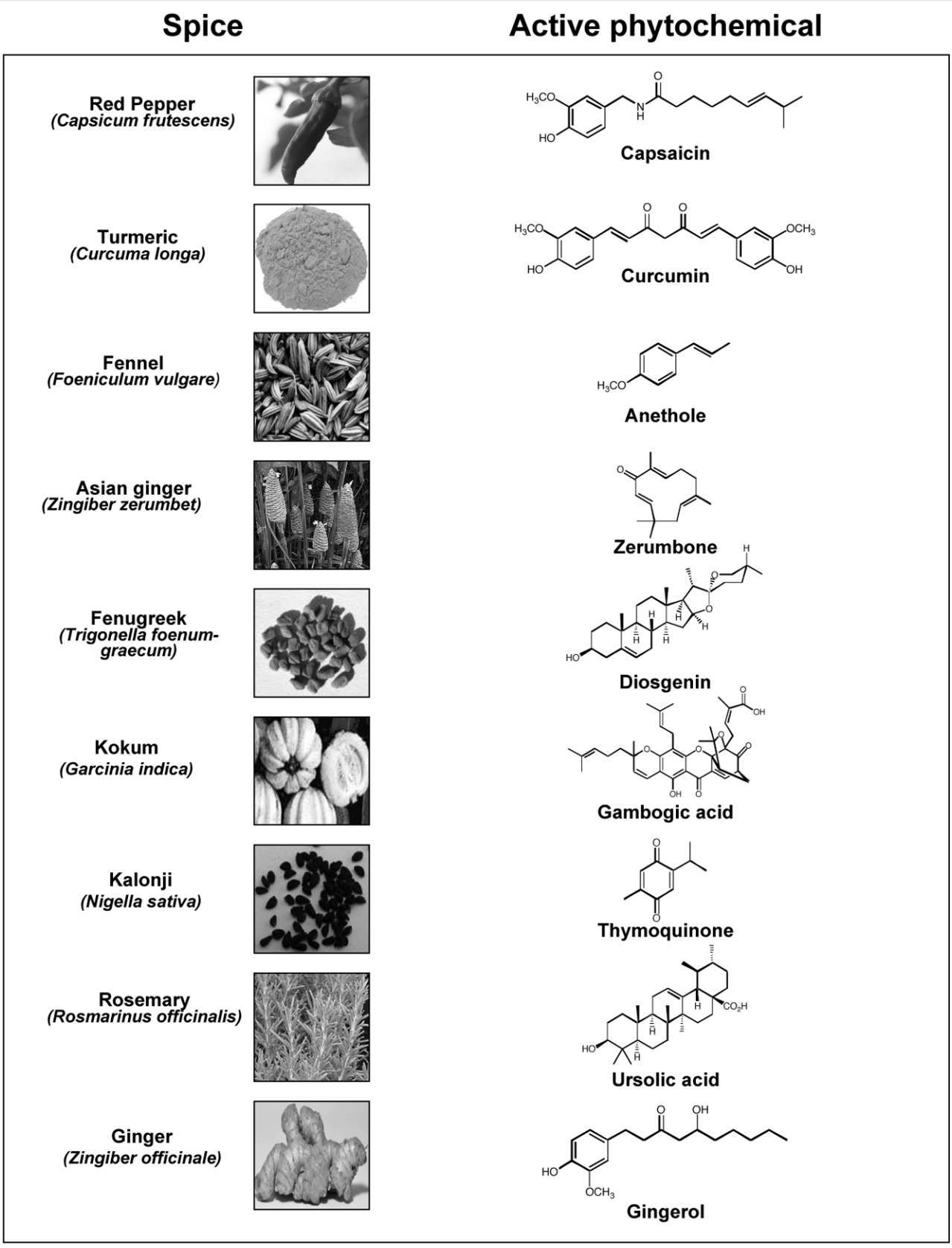

Fig. 1 Major spices and their active phytochemicals.

ated activation of NF-κB. RTX was at least 8 times more potent than capsaicin in inhibiting NF-κB activation. Neither agent by itself activated NF-κB or affected the DNA-binding ability of NF-κB. Capsaicin also blocked phorbol ester-mediated NF-κB activa-

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tion but that mediated through okadaic acid was less effective, suggesting that there is a difference in the mechanism of activation of NF-κB by different agents. Capsaicin treatment of cells also blocked the degradation of IκBα and thus the nuclear translocation of the p65 subunit of NF-κB, which is essential for NF-κB activation. TNF-dependent promoter activity of IκBα, which contains NF-κB binding sites, was also inhibited by capsaicin.

Because STAT-3 has been closely linked with tumorigenesis, we also investigated the effect of this vanilloid on the STAT3 pathway in human multiple myeloma (MM) cells [15]. We found that capsaicin inhibited constitutive activation of STAT3 in MM cells, with a minimal effect on STAT5. Capsaicin also inhibited IL-6-induced STAT3 activation. The activation of JAK1 and c-Src, implicated in STAT3 activation, were also inhibited by this vanilloid, with no effect on extracellular signal-regulated kinases (Erk1/2) activation. Pervanadate reversed the capsaicin-induced down-regulation of STAT3, suggesting the involvement of a protein tyrosine phosphatase. Capsaicin down-regulated the expression of the STAT3-regulated gene products, such as cyclin D1, Bcl-2, Bcl-xL, survivin, and VEGF. Finally, capsaicin induced the accumulation of cells in G1 phase, inhibited proliferation, and induced apoptosis, as indicated by caspase activation. Capsaicin also significantly potentiated the apoptotic effects of velcade and thalidomide in multiple myeloma cells. When administered intraperitoneally, capsaicin inhibited the growth of human multiple myeloma xenograft tumors in male athymic *nu/nu* mice**.** These results suggest that capsaicin is a novel blocker of the STAT3 activation pathway, with a potential role in the prevention and treatment of multiple myeloma and other cancers.

Curcumin (turmeric)

Curcumin is a component of the culinary spice turmeric, which is also often used in curry powder. Its active ingredient was first isolated in 1842 by Vogel. In 1910, Milobedzka determined that the structure was diferuloylmethane, and this compound was first synthesized in 1918 by Lampe and cocrystallized with 5-LOX in 2003 by Skrzypczak-Jankun [16]. Extensive research over the last 50 years has indicated that this polyphenol can both prevent and treat cancer. It has also been demonstrated that curcumin can suppress tumor initiation, promotion, and metastasis. The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of tumor cells, to down-regulate transcription factors, to down-regulate the expression of COX-2, LOX, inducible nitric oxide synthase (iNOS), MMP-9, uPA, TNF, chemokines, cell surface adhesion molecules, and cyclin D1, to down-regulate growth factor receptors [such as epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER2)], and to inhibit the activity of c-Jun N-terminal kinase, protein tyrosine kinases, and protein serine/threonine kinases [17]. In several systems, curcumin has been described as a potent antioxidant and anti-inflammatory agent. The compound has been found to be pharmacologically safe: human clinical trials indicated no dose-limiting toxicity when administered at doses up to 10 g/day [18]. All of these studies suggest that curcumin has an enormous potential in prevention of and therapy for cancer. However, a better understanding of the mechanism would enhance the therapeutic potential of curcumin either alone or in combination with chemotherapy.

We showed that curcumin could suppress NF-κB activation induced by TNF, phorbol ester, and H_2O_2 through suppression of IκBα degradation [19]. How curcumin suppresses NF-κB activation has also been investigated in detail. Curcumin inhibited TNF-induced NF-κB-dependent reporter gene expression in a dose-dependent manner. Curcumin also suppressed NF-κB reporter activity induced by TNF receptor 1 (TNFR1), TNF receptor2 (TNFR2), NF-κB inducing kinase (NIK), IKK, and the p65 subunit of NF-κB. TNF-induced NF-κB-regulated gene products involved in cellular proliferation (COX-2, cyclin D1, c-myc), antiapoptosis (IAP1, IAP2, XIAP, Bcl-2, Bcl-xL, Bf1 – 1/A1, TRAF1, cFLIP) and metastasis (VEGF, MMP-9, ICAM-1) were also downregulated by curcumin. COX-2 promoter activity induced by TNF was abrogated by curcumin. We found that curcumin suppressed TNF-induced nuclear translocation of p65, which corresponded with the sequential suppression of IκBα kinase activity, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, p65 nuclear translocation, and p65 acetylation. Curcumin also inhibited TNF-induced AKT activation and its association with IKK. Glutathione and dithiothreitol reversed the effect of curcumin on TNF-induced NF-κB activation. Thus, it is likely that suppression of NF-κB by curcumin plays a major role in its ability to prevent cancer.

Because curcumin has been shown to down-regulate the expression of Bcl-xL and cyclin D1 which are also regulated by activation of STAT3, whether curcumin can suppress constitutive or inducible activation of STAT3 was also investigated by us. We found that curcumin inhibited IL-6-induced STAT3 phosphorylation and consequent STAT3 nuclear translocation [20]. Curcumin had no effect on STAT5 phosphorylation but inhibited interferon-α-induced STAT1 phosphorylation. The constitutive phosphorylation of STAT3 found in certain MM cells was also abrogated by treatment with curcumin [20]. Curcumin-induced inhibition of STAT3 phosphorylation was reversible. Compared with AG490, a well-characterized JAK2 inhibitor, the action of curcumin was more rapid (30 min vs. 8 h) and it was a more potent (10 μM vs. 100 μM) inhibitor of STAT3 phosphorylation. Similarily, curcumin completely suppressed proliferation of MM cells whereas the same dose of AG490 had no effect. In addition, dexamethasone-resistant MM cells were found to be sensitive to curcumin.

Anethole (fennel)

Anethole, [1-methoxy-4-(1-propenyl)benzene], a chief constituent of fennel, anise, and camphor, has been shown to block both inflammation and carcinogenesis. This compound and related ones have striking metabolic effects. For example, anethole and its derivative, anethole dithiolethione (ADT), have been shown to increase the intracellular levels of glutathione (GSH) and glutathione S-transferase (GST) [21], [22], [23]. The structurally related compounds eugenol and isoeugenol, which are found in clove-oil, also modulate GSH metabolism [24]. These compounds act like antioxidants [25], [26], inhibit lipid peroxidation [24], [27], [28], and act as hydroxyl radical scavengers [29]. Because eugenol and isoeugenol inhibit arachidonic acid-induced thromboxane B2, they are extensively used as anti-inflammatory compounds [30], [31]. Besides their anti-inflammatory property, anethole and its analogues exhibit chemopreventive activities as indicated by suppression of the incidence and multiplicity of both invasive and non-invasive adenocarcinomas [32], [33], [34], [35], [36].

Since anethole exhibits anticarcinogenic, and anti-inflammatory properties, we proposed that the effects of anethole are mediated through modulation of TNF-induced cellular responses [37]. Our study showed that anethole inhibited TNF-induced activation of NF-κB, IκBα and degradation, and NF-κB reporter

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gene expression. Suppression of IκBα phosphorylation and NF-κB reporter gene expression induced by TRAF2 and NIK suggests that anethole acts on IκBα kinase. Anethole also blocked NF-κB activation induced by a variety of other inflammatory agents. Anethole analogues eugenol and isoeugenol also blocked TNF signalling. Thus, the inhibitory effects of anethole on TNF induced cellular responses may explain its role in suppression of inflammation and carcinogenesis.

Zerumbone (Asian ginger)

Zerumbone [2,6,9,9-tetramethyl-(2*E*,6*E*,10*E*)-cycloundeca-2,6,10-trien-1-one] was first isolated in 1956 from the essential oil of rhizomes of a wild ginger, *Zingiber zerumbet* Smith, which is widespread in Southeast Asia [38]. Over the years, a wide variety of activities have been assigned to this compound [39], [40], [41], [42], [43], [44], [45]. For instance, zerumbone has been found to suppress the proliferation of colon cancer [41], [45] and breast cancer [45], with minimal effects on normal cells [41]. Zerumbone has also been shown to suppress inflammation [39], suppress the initiation and promotion of skin tumors in mice [43], and prevent azoxymethane-induced aberrant crypt foci formation in rats [44]. This terpenoid has also been shown to suppress dextran sodium sulfate-induced colitis in mice [42] and inhibit the activation of the phorbol ester-induced Epstein–Barr virus [40]. Additional activities assigned to zerumbone are the suppression of superoxide and nitric oxide generation [46] and the down-regulation of COX-2 [47], IL-1 [42], and TNF [41], [42].

Several of these activities could be explained if zerumbone down-regulated NF-κB activation, since zerumbone has proven effects on related activities. We found that zerumbone suppressed NF-κB activation induced by TNF, okadaic acid, cigarette smoke condensate, phorbol ester, and H_2O_2 and that the suppression was not cell type specific [48]. Interestingly, α-humulene, a structural analogue of zerumbone lacking the carbonyl group, was completely inactive. Besides being inducible, constitutively active NF-κB was also inhibited. NF-κB inhibition by zerumbone correlated with sequential suppression of the IκBα kinase activity, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, p65 nuclear translocation, and p65 acylation. Zerumbone also inhibited the NF-κB-dependent reporter gene expression activated by TNF, TNFR1, TRADD, TRAF2, NIK, and IKK but not that activated by the p65 subunit of NF-κB. NF-κB-regulated gene products, such as cyclin D1, COX-2, MMP-9, ICAM-1, c-myc, survivin, IAP1, IAP2, XIAP, Bcl-2, Bcl-xL, Bfl-1/A1, TRAF1 and FLIP, were all down-regulated by zerumbone. This down-regulation led to the potentiation of apoptosis induced by cytokines and chemotherapeutic agents. Zerumbone's inhibition of expression of these NF-κB-regulated genes also correlated with the suppression of TNF-induced invasion activity. These results indicated that zerumbone inhibits the activation of NF-κB and NF-κB-regulated gene expression induced by carcinogens and that this inhibition may provide a molecular basis for the prevention and treatment of cancer by zerumbone.

Diosgenin (fenugreek)

Diosgenin, is a steroidal saponin found in a variety of plants [49], [50] including fenugreek (*Trigonella foenum graecum*), roots of wild yam (*Dioscorea villosa*), *Solanum incanum* Lloydia [51], *Costus speciosus* [52], and *Solanum xanthocarpum* [53]. Extracts from these plants have been traditionally used for the treatment of diabetes [54], [55], [56], hypercholestrolemia [57], [58], and gastrointestinal ailments [59], [60]. Research during the last decade has shown that diosgenin suppresses proliferation and induces apoptosis in cells of human colon carcinoma [60], [61], osteosarcoma [62], [63], leukemia [64], [65], [66], and human rheumatoid arthritis [67]. Antiproliferative effects of diosgenin are mediated through cell cycle arrest [62], disruption of Ca^{2+} homeostasis [65], [66], the activation of p53, release of apoptosis-inducing factor, and modulation of caspase-3 activity [68]. It also inhibits azoxymethane-induced aberrant colon crypt foci [60] and has been shown to inhibit intestinal inflammation [69] and modulate the activity of LOX [70] and COX-2 [62]. More recently, diosgenin has been shown to bind to the chemokine receptor CXCR3, which mediates inflammatory responses [71].

We reported that diosgenin inhibited RANKL-induced osteoclastogenesis, suppressed TNF-induced invasion, and blocked the proliferation of tumor cells, all activities known to be regulated by NF-κB [72]. Diosgenin suppressed TNF-induced NF-κB activation as determined by DNA binding, activation of IκBα kinase, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, and p65 nuclear translocation through inhibition of Akt activation. NF-κB-dependent reporter gene expression was also abrogated by diosgenin. TNF-induced expression of NF-κB-regulated gene products involved in cell proliferation (cyclin D1, COX-2, c-myc), antiapoptosis (IAP1, Bcl-2, Bcl-xL, Bfl-1/A1, TRAF1 and cFLIP), and invasion (MMP-9) were also down-regulated by this saponin. Diosgenin also potentiated the apoptosis induced by TNF and chemotherapeutic agents. Thus diosgenin can suppress proliferation, inhibits invasion, and suppresses osteoclastogenesis through inhibition of NF-κB-regulated gene expression and enhances apoptosis induced by cytokines and chemotherapeutic agents.

Gambogic acid (kokum)

Gambogic acid (GA) is a naturally occurring brownish-to-orange resin called gamboges (also called kokkum), which is derived from *Garcinia indica*. It has a long history of medicinal use in Southeast Asia, and it is used as a folk medicine and coloring agent in China. Recent studies showed that GA can inhibit the growth of a wide variety of tumor cells, including cells of human hepatoma [73], breast cancer [9], gastric carcinoma [74], [75], [76], [77], and lung carcinoma [78]. Using cell- and caspase-based high-throughput screening assays, Zhang et al. identified GA as a potent inducer of apoptosis [9]. Studies have also indicated that GA suppresses the growth of human tumors, e.g., lung carcinoma and hepatoma [73]. How GA mediates these effects is not fully understood, but it has been shown to inhibit telomerase and telomerase reverse transcriptase mRNA expression [73], [76], [77], inhibit human telomerase reverse transcriptase (hTERT) promoter [76], suppress cyclin-dependent kinase 7 (CDK7)-mediated phosphorylation of CDC2/p34 [77], down-regulate Bcl-2 [74], and interact with c-Myc [73]. A recent report suggests that GA mediates its apoptotic effects through its interaction with the transferrin receptor [79]. Because hTERT, c-Myc, and Bcl-2 gene expression modulated by GA is regulated by NF-κB activation, it is possible that GA mediates its effects by modulating the NF-κB pathway. We found that GA enhanced apoptosis induced by TNF and chemotherapeutic agents, inhibited the expression of gene products involved in antiapoptosis (IAP1 and 2, Bcl-2, Bcl-xL, and TRAF1), proliferation (cyclin D1 and c-Myc), invasion (COX-2 and MMP-9) and angiogenesis (VEGF), all of which are known to be regulated by NF-κB [80]. GA suppressed NF-κB activation induced by various inflammatory agents and carcinogens accompanied by the inhibition of TAK1/TAB1-medi-

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ated IKK activation, thus inhibiting IκBα phosphorylation and degradation, suppressing p65 phosphorylation and nuclear translocation, and finally abrogating NF-κB-dependent reporter gene expression. The NF-κB activation induced by TNFR1, TRADD, TRAF2, NIK, TAK1/TAB1 and IKKβ was also inhibited. The effect of GA was mediated through transferrin receptor as down-regulation of the receptor by RNA interference reversed its effects on NF-κB and apoptosis. These results demonstrated that GA inhibits NF-κB signalling pathway.

Since angiogenesis is crucial for cancer development and other human diseases, whether and how GA inhibits angiogenesis was also investigated [81]. We discovered that GA inhibited angiogenesis *in vitro* and *in vivo*, and identified GA as a novel inhibitor of VEGF receptor 2 (VEGFR2). We demonstrated that GA significantly inhibited human endothelial cell proliferation, migration, invasion, tube formation, and microvessel growth with all antiangiogenesis characters. The effects of GA on cell proliferation, migration, and apoptotic activation were more effective in human endothelial cells than cancer cells, providing additional clues for cancer therapy of GA with low chemotoxicity. Using the xenograft mouse model, we found that GA inhibited tumor angiogenesis and prevented tumor growth by dramatically inhibiting angiogenesis. Furthermore, we demonstrated that GA directly inhibited the activation of VEGFR2 and suppressed its downstream kinases, such as Src, FAK, and AKT. Thus GA inhibits angiogenesis through down-regulation of VEGFR2 and its signalling pathways, and that GA is a viable drug candidate in antiangiogenesis and anticancer therapies.

Thymoquinone (black cumin)

Thymoquinone (TQ), the most abundant component of black cumin (*Nigella sativa)* seed oil, has been reported to exhibit antioxidant [82], [83], [84], anti-inflammatory, and chemopreventive [85], [86], [87] effects. For instance, TQ has been shown to suppress the proliferation of various tumor cells, including colorectal carcinoma, breast adenocarcinoma, osteosarcoma, ovarian carcinoma, myeloblastic leukemia, and pancreatic carcinoma [85], [88], [89], [90], [91], [92] while it is minimally toxic to normal cells [93]. In animal models, TQ has been shown to suppress acetic acid-induced colitis in rats [94], inhibit TNF-α production in murine septic peritonitis [95], and reduce carrageenan-induced paw edema in rats [96]. TQ has also been reported to enhance the antitumor activity of ifosfamide in Ehrlich ascites carcinoma-bearing mice [86], prevent cisplatin-induced nephrotoxicity in mice and rats [97], ameliorate benzopyrene-induced forestomach carcinogenesis [98], inhibit COX-2 expression and prostaglandin production in a mouse model of allergic airway inflammation [99], and protect against doxorubicin-induced cardiotoxicity in mice [100]. How TQ manifests these activities is not fully understood, but it has been shown to down-regulate the expression of Bcl-xL [89], COX-2 [99], iNOS [101], 5-LOX [102], TNF [103], and cyclin D1 [104], all known to be regulated by NF-κB.

Because numerous effects modulated by TQ can be linked to interference with NF-κB signalling, we investigated in detail the effect of this quinone on the NF-κB pathway [105]. As examined by DNA binding, we found that TQ suppressed TNF-induced NF-κB activation in a dose- and time-dependent manner, and inhibited NF-κB activation induced by various carcinogens and inflammatory stimuli. The suppression of NF-κB activation correlated with sequential inhibition of the activation of IκBα kinase, IκBα phosphorylation, IκBα degradation, p65 phosphorylation, p65 nuclear translocation, and the NF-κB-dependent reporter gene expression. TQ specifically suppressed the direct binding of nuclear p65 and of the recombinant p65 to the DNA, and this binding was reversed by dithiothreitol. However, TQ did not inhibit p65 binding to DNA when cells were transfected with the p65 plasmid containing cysteine residue 38 mutated to serine. TQ also down-regulated the expression of NF-κB-regulated antiapoptotic (IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and survivin), proliferative (cyclin D1, COX-2, and c-myc), and angiogenic (MMP-9 and VEGF) gene products. This led to potentiation of apoptosis induced by TNF and chemotherapeutic agents. Our results indicate that the anticancer and anti-inflammatory activities previously assigned to TQ may be mediated in part through the suppression of the NF-κB activation pathway; and thus may have potential in the treatment of myeloid leukemia and other cancers.

We also recently reported that thymoquinone effectively inhibited human umbilical vein endothelial cell (HUVEC) migration, invasion, and tube formation [106]. Thymoquinone inhibited cell proliferation and suppressed the activation of AKT and ERK. Thymoquinone blocked angiogenesis *in vitro* and *in vivo*, prevented tumor angiogenesis in a xenograft human prostate cancer (PC3) model in mouse and inhibited human prostate tumor growth at low dosage with almost no chemotoxic side effects. Furthermore, we observed that endothelial cells were more sensitive to thymoquinone-induced cell apoptosis, cell proliferation and migration inhibition compared to PC3 cancer cells. Thymoquinone inhibited VEGF-induced ERK activation, but showed no inhibitory effects on VEGF receptor2 activation. Thus our results suggest that thymoquinone inhibits tumor angiogenesis and tumor growth, and could be used as a potential drug candidate for cancer therapy.

Ursolic acid (rosemary)

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) is a pentacyclic triterpenoid (a member of the cyclosqualenoid family) derived from rosemary (*Rosemarinus officinalis)* and other plants. Ursolic acid has been shown to suppress tumorigenesis [107], inhibit tumor promotion [108], [109], [110], and suppress angiogenesis [111]. Several of these effects of ursolic acid are mediated through suppression of the expression of LOX, COX-2, MMP-9, and iNOS [112], [113], [114], [115], [116], [117], all of which are genes regulated by NF-κB. In addition, ursolic acid and its derivatives have been shown to induce apoptosis in a wide variety of cancer cells including breast carcinoma, melanoma, hepatoma, prostate carcinoma and acute myelogenous leukemia [118], [119], [120], [121], [122], [123], [124], through inhibition of DNA replication [125], activation of caspases [121], [123], [124], inhibition of protein tyrosine kinases [122], and induction of Ca^{2+} release [126], [127]. Another mechanism by which ursolic acid induces apoptosis involves down-regulation of the cellular inhibitor of apoptosis gene [121], another gene regulated by NF-κB. We found that ursolic acid suppressed NF-κB activation induced by various carcinogens including TNF, phorbol ester, okadaic acid, H_2O_2 and cigarette smoke [128]. These effects were not cell type specific. Ursolic acid inhibited IκBα degradation, IκBα phosphorylation, IκBα kinase activation, p65 phosphorylation, p65 nuclear translocation, and NF-κB-dependent reporter gene expression. Ursolic acid also inhibited NF-κB-dependent reporter gene expression activated by TNF receptor, TRADD, TRAF2, NIK, IKK, and p65. The inhibition of NF-κB activation correlated with suppression of NF-κB-dependent cyclin D1, COX-2 and

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MMP-9 expression. These actions of ursolic acid may mediate its antitumorigenic and chemosensitizing effects.

We also found that ursolic acid inhibited both constitutive and IL-6-inducible STAT3 activation [129]. The suppression was mediated through the inhibition of activation of upstream kinases c-Src, JAK1, JAK2 and ERK1/2. Vanadate treatment reversed ursolic acid-induced down-regulation of STAT3, suggesting the involvement of a tyrosine phosphatase. Indeed, we found that ursolic acid induced the expression of tyrosine phosphatase SHP-1 protein and of mRNA. Moreover, knockdown of SHP-1 by siRNA suppressed the induction of SHP-1 and reversed the inhibition of STAT3 activation, thereby indicating the critical role of SHP-1 in the action of this triterpene. Ursolic acid down-regulated the expression of STAT3-regulated gene products such as, cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1, and VEGF. Ursolic acid inhibited proliferation, induced apoptosis and the accumulation of cells in G1/G0 phase of cell cycle. This triterpenoid also significantly potentiated the apoptotic effects of thalidomide and velcade in MM cells. Thus ursolic acid is a novel blocker of STAT3 activation that may have a potential in prevention and treatment of various cancers.

[6]-Gingerol (ginger)

[6]-Gingerol, the major active component of ginger (*Zingiber officinale*), has also been linked with prevention of cancer through numerous mechanisms. [6]-Gingerol has been shown to inhibit the proliferation of a variety of cancer cell lines including prostate [130], gastric [131], and breast [132]. It inhibits neoplastic transformation in mouse epidermal cells [133], blocks VEGF-induced capillary-like tube formation in the mouse cornea, and suppresses lung metastasis of B16F10-melanoma [134]. Kim et al. [135] reported that topical application of [6]-gingerol inhibited PMA-induced COX-2 expression in mouse skin by suppression of NF-κB. This phytochemical was found to suppress PMA-induced IκBα degradation and translocation of p65 to nuclear in mouse skin by blocking of upstream kinase p38 MAPK. Recently, Lee et al [136] reported that [6]-ginerol exhibits antitumorigenic effects in human colorectal cancer cells through up-regulation of NSAID-activated gene-1 (NAG-1). This accompanies G1 cell cycle arrest by down-regulation of cyclin D1 that was mediated through the degradation of β-catenin by gingerol.

Others

Besides the spice phytochemicals described above, there are numerous others including cumin (*Cuminum cyminum*), coriander (*Corriandrum sativum*), cinnamon (*Cinnamomum zeylanicum*) and black pepper (*Piper nigrum*), however, there is very little known about their chemistry or the chemopreventive activities of the compounds derived from them. Two flavonoid glycosides, apigenin [137] and luteolin [138] derived from cumin, have been shown to exhibit cancer chemopreventive activities.

Conclusions

From the description provided above it is clear that spice-derived phytochemicals have an enormous potential in the prevention and treatment of cancer. They can induce apoptosis, suppress proliferation of tumor cells, inhibit invasion and angiogenesis, and prevent even bone loss. These phytochemicals mediate their effects through multiple targets and yet pharmacologically they are highly safe. More animal studies and clinical trials are needed to prove the usefulness of these agents. Safety, inexpensive cost, years of intake by humans and their efficacy make them ideal agents. Therefore it is not too surprising to note that Vasco de Gama tried to look for these spices almost five centuries ago.

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