ANTIOXIDANTS & REDOX SIGNALING
Volume 19, Number 2, 2013

DOI: 10.1089/ars.2013.5233

Therapeutics

FORUM REVIEW ARTICLE

Antioxidant Function of Isoflavone and 3,3′-Diindolylmethane: Are They Important for Cancer Prevention and Therapy?

Yiwei Li,[1] Dejuan Kong,[1] Aamir Ahmad,[1] Bin Bao,[1] and Fazlul H. Sarkar[1,2]

Abstract

Significance: Oxidative stress has been mechanistically linked with aging and chronic diseases, including cancer. In fact, oxidative stress status, chronic disease-related inflammation, and cancer occurred in the aging population are tightly correlated. It is well known that the activation of nuclear factor kappa B (NF-κB) plays important roles in oxidative stress, inflammation, and carcinogenesis. Therefore, targeting NF-κB is an important preventive or therapeutic strategy against oxidative stress, inflammation, and cancer. ***Recent Advances:*** A variety of natural compounds has been found to reduce oxidative stress through their antioxidant activity. Among them, isoflavone, indole-3-carbinol (I3C), and its *in vivo* dimeric compound 3,3′-diindolylmethane (DIM) have shown their promising effects on the inhibition of NF-κB with corresponding reduction of oxidative stress. ***Critical Issues:*** It has been found that isoflavone, I3C, and DIM could inhibit cancer development and progression by regulating multiple cellular signaling pathways that are related to oxidative stress and significantly deregulated in cancer. ***Future Directions:*** The antioxidative and anticancer effects of these natural agents make them strong candidates for chemoprevention and/or therapy against human malignancies. However, more clinical trials are needed to evaluate the effects of isoflavone and DIM for the prevention of cancer development and also for the treatment of cancer either alone or in combination with conventional cancer therapeutics. *Antioxid. Redox Signal*. 19, 139–150.

Introduction

SINCE HUMANS ARE LIVING in an aerobic environment, it is obvious that they are continuously and unavoidably exposed to reactive oxygen species (ROS). To overcome ROS, the biological system in the human body interacts with the external environment to maintain a healthy internal environment that maintains homeostasis of cell survival, growth, differentiation, and reproduction. The defense systems in human body have evolved to reduce the accumulation of ROS; however, these defense systems are not always sufficient to decrease the production of ROS, resulting in the accumulation of ROS, causing systemic oxidative stress, especially in older age (40, 126). It is known that oxidative stress is linked to aging and various chronic diseases such as atherosclerosis, neurodegenerative diseases, diabetes, pulmonary fibrosis, and arthritis, which are commonly accompanied by inflammation (12, 35, 102). More importantly, ROS could induce severe DNA damage, which plays an important role in carcinogenesis; therefore, oxidative stress from ROS accumulation could be one of the factors responsible for the development and progression of cancer (43, 47, 97, 98). Once DNA damage occurs, the defense systems in the human body try to repair DNA to prevent mutagenesis. However, under sustained oxidative stress, the repair of DNA damage can be suppressed by several redox-dependent metals, resulting in mutagenesis and carcinogenesis (43, 64) (Fig. 1). Therefore, ROS could induce both inflammation and cancer. Examination of the inflammatory microenvironment in tumor tissues has supported the hypothesis that inflammation is a cofactor in oncogenesis for a variety of cancers (46). Moreover, it is known that ROS under oxidative stress activate nuclear factor kappa B (NF-κB), which also contributes to the inhibition of DNA repair (91). The activation of NF-κB together with deregulation of multiple signaling pathways plays important roles in carcinogenesis. Therefore, antioxidative agents could be effective compounds for the prevention and/or treatment of cancer and inflammation, both of which are associated with oxidative stress.

To overcome the oxygenic threat, natural antioxidants have evolved in parallel with the natural oxygenic atmosphere. It is

Departments of [1]Pathology and [2]Oncology, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan.

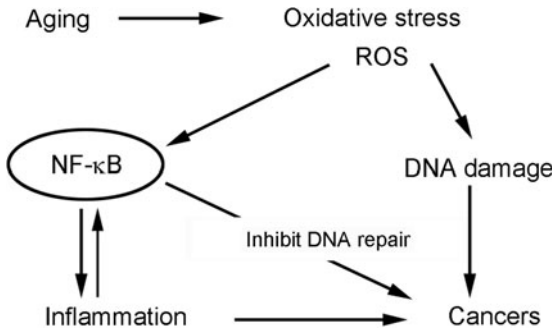

FIG. 1. The relationship between oxidative stress, aging, inflammation, and cancer.

known that plants have their own defense system to protect their structures against ROS produced during photosynthesis (14). Therefore, with evolution, plants produce a variety of antioxidant components, which could be beneficial for human health. Indeed, *in vitro* and *in vivo* experimental studies have shown that various plant-derived components possess their ability to reduce oxidative stress. Among them, isoflavones, indole-3-carbinol (I3C), and its *in vivo* dimeric product 3,3′-diindolylmethane (DIM) exhibit a promising effect on the inhibition of ROS accumulation (13, 38, 39, 95, 96). The main sources of isoflavones are soy and other plants in the Legume family. The isoflavones include genistein, daidzein, glycitein, formononetin, biochanin A, desmethylangolensin, and equol. The Brassica family is the main source of I3C and DIM. The isoflavones, I3C and DIM, have shown their beneficial effects on human health. Importantly, these natural compounds inhibit NF-κB activation stimulated by ROS (23, 30), suggesting their potent ability as antioxidants. Moreover, these antioxidants have shown their inhibitory effects on inflammation, oncogenesis, tumor growth, and progression, suggesting that they could be useful as chemopreventive and/or therapeutic agents for the protection against inflammation and cancer.

Oxidative Stress and NF-κB Activation in Cancer

It is well known that the activation of NF-κB is the most important consequence of inflammation associated with all types of cancer (58, 60). In fact, the oxidative stress status, chronic disease-related inflammation, and cancer occurred in the aging population are tightly associated with the activation of NF-κB signaling (58) (Fig. 1). Under the situation of oxidative stress, ROS induce DNA damage and activate the activity of NF-κB (19, 94). The laboratory experiments showed that direct addition of hydrogen peroxide (H_2O_2) to the culture medium activated the NF-κB DNA-binding activity in many types of cell lines (19). In addition, it was found that ROS accumulated in cells were increased in response to agents that also activated NF-κB (19, 37). These lines of evidence demonstrate that oxidative stress activates NF-κB activity in cells, including inflammatory and cancerous cells.

Once NF-κB is activated, it binds to the NF-κB-specific DNA-binding sites and regulates the transcription of target genes (53, 84). By regulating the transcription of its targets, NF-κB controls the expression of many genes that are involved in the stress response, inflammation, differentiation, cell growth, and apoptosis (61, 83, 84). The alteration in these biological processes has been critically linked with the development and progression of cancer. Since activated NF-κB promotes cell growth and inhibits apoptotic cell death, the uncontrolled cell proliferation leads to the development of cancer. Therefore, the activated NF-κB under oxidative stress has been described as a major culprit in cancers (59).

Indeed, clinical and experimental studies have shown that NF-κB is constitutively activated in Hodgkin's lymphoma (9), multiple myeloma (49), and solid tumors, including lung, pancreatic, ovarian, prostate, breast, and other cancers (20, 51, 68, 77, 88, 105, 114). The activated NF-κB activity is also correlated with drug resistance and poor treatment outcome (20). Therefore, targeting NF-κB signaling activation by specific inhibitors or natural agents with multitargets could be an effective therapeutic strategy for the treatment of cancer by increasing drug sensitivity and inhibiting cancer invasion and metastasis. Inhibition of ROS-mediated activation of NF-κB is now widely accepted as a valid therapeutic strategy for the treatment of inflammation (86, 123) and cancers (15, 44, 57, 93). Thus, plant-derived antioxidants that inhibit NF-κB activity may serve as potential agents for cancer prevention and therapy.

Signaling Pathways that Crosstalk with NF-κB in Cancer

Since cellular signaling is a complex signal network with positive or negative feedback loops, the deregulations of signaling pathways, which crosstalk with NF-κB, often exist in cancer cells. Protein kinase B (Akt) signaling plays important roles in mammalian cell survival, and it is activated in response to various stimuli. It has been shown that H_2O_2 treatment increased Akt activity in multiple cell lines (119), suggesting the activation of Akt under oxidative stress. Activated Akt promotes cell survival by inhibition of proapoptotic factors, including Bad, Forkhead transcription factors, and caspase-9 (87). It has also been found that NF-κB stimulates Akt activation, while Akt regulates the NF-κB pathway *via* activation of molecules in the NF-κB signaling pathway (89, 103), suggesting the crosstalk between NF-κB and Akt under oxidative stress (Fig. 2).

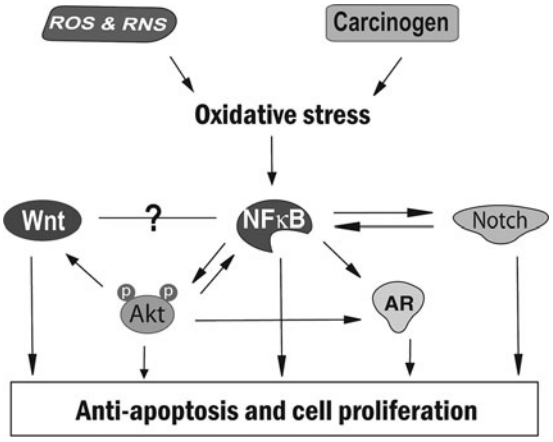

FIG. 2. Crosstalk between NF-κB, Akt, Wnt, Notch, and AR signaling that contributes to cell proliferation and antiapoptosis under oxidative stress. Akt, protein kinase B; AR, androgen receptor; NF-κB, nuclear factor-kappa-B.

Another signaling that crosstalk with NF-κB is Notch signaling. Notch signaling plays a critical role in the regulation and maintenance of stem cells; therefore, appropriate functioning of Notch signaling is required for normal development. It has been shown that Notch is an essential upstream regulator of NF-κB, and that NF-κB activates Notch signaling by inducing the Notch ligand Jagged1 (10, 108), suggesting the existence of an active crosstalk between Notch and NF-κB (Fig. 2). Importantly, activation of Notch receptors and their ligands has been found in various cancers, including lymphomas and cervical, lung, colon, head and neck, renal, and pancreatic cancer (120). In cancer cells, Notch signaling is abnormally activated, leading to the increased proliferation of cancer cells (Fig. 2); therefore, Notch is another important target in cancer therapy, which could indeed be achievable by natural antioxidants, as discussed later in this chapter.

In addition to Notch signaling, Wnt (wingless-type MMTV integration-site family) signaling also plays important roles in the embryonic developmental processes. It is well known that Akt signaling regulates Wnt signaling through glycogen synthase kinase-3-beta (GSK-3β), which phosphorylates β-catenin in Wnt signaling. Wnt and NF-κB could also crosstalk with each other; however, the detailed regulation between these two signaling is still unclear (18, 36) (Fig. 2). The inappropriate expression of Wnt molecules and the inappropriate activation of Wnt signaling have been found in various human tumors. Activation of Wnt signaling promotes β-catenin translocation to the nucleus, resulting in the consequent transcriptional activation of specific target genes and the uncontrolled cell proliferation (Fig. 2).

Androgen receptor (AR) signaling is another cellular signaling that interacts with NF-κB. It is known that AR signaling plays important roles in the normal prostate development as well as in prostate cancer development and progression through the regulation of transcription of androgen-responsive genes (82). Importantly, prostate-specific antigen (PSA), one of the AR-target genes, is a clinically critical marker used to monitor diagnosis, progression, and prognosis of patients with prostate cancer, suggesting the importance of AR signaling, especially in castrate-resistant prostate cancer. It has been shown that Akt could activate AR in a ligand-independent manner in hormone-refractory prostate cancer. Moreover, NF-κB could also upregulate AR, leading to the AR transactivation, PSA expression, and prostate cancer cell growth (33, 125). These findings suggest a complex signal interaction between NF-κB, Akt, and AR under oxidative stress (Fig. 2). Therefore, targeting multiple signaling by natural antioxidants could open a new avenue for cancer therapy.

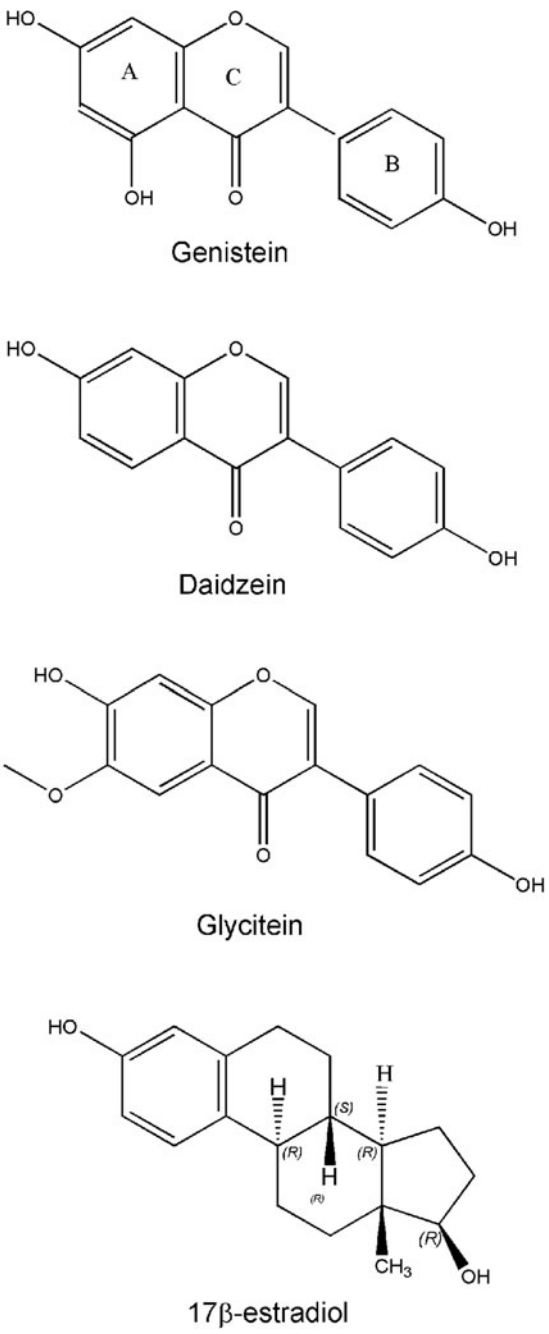

FIG. 3. Molecular structures of isoflavones and estradiol.

Isoflavones as Antioxidants Inhibiting Oxidative Stress and Regulating Cellular Signaling in Cancer

Isoflavones are a subclass of flavonoids and mainly found in soybeans. Genistein, daidzein, and glycitein are three main isoflavones found in soybeans (Fig. 3). Genistein and daidzein are present at a relatively high concentration in soybeans and most soy products, while much lower amount of glycitein exists in soybeans. The basic molecular structure of isoflavones is the flavone nucleus, which consists of 2 benzene rings (A and B) linked through a heterocyclic pyrane C ring (Fig. 3). In the molecular structures, genistein, daidzein, and glycitein have the same flavone nucleus with a some other different side chain. Isoflavones have a close similarity in structure to estradiol (Fig. 3). Because of the similarity, isoflavone can bind to the estrogen receptor (ER). It has been reported that isoflavones exert weak estrogenic activity because of their binding to the ER. Therefore, isoflavone has also been known as phytoestrogens. Since isoflavones bind to the ER and show weak estrogenic activity, the binding of isoflavone to ER prevents more-potent estrogen binding to the ER and thereby inhibits ER signaling, suggesting the estrogen-antagonistic activity of isoflavones. However, it is important to note that under a low-estrogenic environment, isoflavones could function as estrogenic.

It is known that isoflavones exert antioxidant effects on human cells. Genistein has been found to protect cells against ROS by reducing free radicals and downregulating the expression of the stress-response related genes (104, 128). Isoflavones also upregulated gene expression of antioxidant proteins in Caco-2 cells (55). It has been found that isoflavone supplementation reduced H_2O_2-induced DNA damage in sperm (109), suggesting the antioxidant effects of isoflavone. Importantly, isoflavone inhibited H_2O_2 production induced by a tumor promoter (12-*O*-tetradecanoylphorbol-13-acetate) in human polymorphonuclear leukocytes and HL-60 cells, suggesting the inhibitory effect of isoflavone on carcinogenesis mediated through antioxidant activity (122).

The inhibitory effects of isoflavone on cancer

The effects of isoflavone on cancer cells have been widely studied in a variety of cancer cells. The results from our laboratory and other investigators showed that isoflavone inhibited the growth of various cancer cells, including leukemia, lymphoma, neuroblastoma, breast, prostate, lung, gastric, ovarian, and head and neck cancer cells (1, 21, 29, 32, 45, 70, 78, 81, 127). The inhibition of cancer growth by isoflavone could be mediated by the induction of apoptosis and the modulation of expression of the genes related to the cell growth and apoptotic processes (1, 70, 78, 81, 128). By microarray gene expression analysis and mechanistic experiments, we and other investigators have found that isoflavone regulates the molecules in multiple cellular signaling pathways, including NF-κB, Akt, Wnt, Notch, and AR signaling pathways. All of these cellular signaling pathways are critically involved in the control of cell growth, apoptosis, angiogenesis, tumor cell invasion, and metastasis (45, 75–77), suggesting the pleiotropic effects of isoflavone (multitargeting effect) on cancer cells.

Importantly, isoflavones have been found to sensitize cancer cells to conventional therapies, including chemotherapy and radiotherapy (8, 69, 73, 111). Pretreatment of cancer cells with isoflavone before treatment with lower doses of chemotherapeutic agents or radiotherapy caused a significantly greater degree of growth inhibition and apoptosis of cancer cells, suggesting enhanced therapeutic effects of cancer therapy with isoflavone. Moreover, phenoxodiol, one of the isoflavone analogs, showed its promising anticancer effect by sensitizing cancer cells to conventional chemotherapeutics (5). In ovarian cancer cells that became resistant to the conventional chemotherapeutics, treatment with phenoxodiol removed drug resistance and, therefore, made cancer cells susceptible once again to conventional chemotherapeutics, including cisplatin, carboplatin, taxanes, and gemcitabine (56, 106). Phenoxodiol is currently undergoing phase II/III clinical trials to elucidate its effects combined with carboplatin, docetaxel, cisplatin, or paclitaxel in patients with ovarian, fallopian tube, or primary peritoneal cavity cancers (28). Recently, more clinical trials are being conducted using the isoflavone genistein or isoflavone analogs in combination with IL-2, docetaxel, cisplatin, paclitaxel, or other natural agents such as lycopene and vitamin D in the treatment of melanoma, kidney, and ovarian cancers (27, 28). In addition, several clinical trials are being conducted to test the effects of isoflavone treatment combined with radiotherapy in patients with prostate cancer. All of these clinical trials are based on *in vitro* mechanistic experiments showing that isoflavone inhibits cancer cell growth through the regulation of NF-κB and other signaling pathways.

Inhibition of oxidative stress and NF-κB activation by isoflavone

To investigate the effects of the isoflavone genistein on oxidative stress-induced NF-κB activation, we have conducted a series of *in vitro* and *in vivo* experiments to measure the NF-κB DNA-binding activity in lymphocytes from human subjects and in various cancer cells. We found that isoflavone genistein treatment significantly inhibited the NF-κB DNA-binding activity and blocked NF-κB induction stimulated by oxidative stress inducers, H_2O_2 and tumor necrosis factor (TNF)-α, in cancer cell lines (32). We further investigated the effect of isoflavone supplementation on NF-κB activation *in vivo* in human volunteers (30). The lymphocytes from healthy volunteers were isolated from peripheral blood and cultured for 24 h in the absence and presence of the isoflavone genistein. The results showed that isoflavone genistein treatment inhibited the NF-κB DNA-binding activity and abrogated TNF-α-induced NF-κB activity (30). Moreover, when human volunteers received soy isoflavone supplements (Novasoy™), TNF-α failed to activate NF-κB activity in lymphocytes harvested from these volunteers (30), suggesting that isoflavone could also inhibit NF-κB activation *in vivo*.

To test the effects of isoflavone on oxidative stress, we further tested the levels of oxidative DNA damage in the blood of the subjects before and after supplementation with isoflavones. We found that the mean value of 5-OHmdU, a modified DNA base that represents the endogenous status of cellular oxidative stress, was significantly downregulated after 3 weeks of isoflavone supplementation (30), which has also been reported in our published review articles and book chapters. Collectively, these observations clearly demonstrate that isoflavone supplementation reduces the level of 5-OhmdU, decreases oxidative damage, and inhibits NF-κB activation in humans *in vivo*. Taken together, these *in vitro* and *in vivo* studies provide strong evidence showing that isoflavone could function as an antioxidant. Moreover, the antioxidative effects of isoflavone could be responsible for its chemopreventive and chemotherapeutic activity.

The inhibitory effect of isoflavone on NF-κB activation was also observed in different types of cancer cells. The isoflavone genistein reduced NF-κB activity in T-lymphoma cells *via* a caspase-mediated cleavage of IκBα (11). The isoflavone genistein also inhibited constitutive and inducible NF-κB activation and decreased IL-8 production in human cystic fibrosis bronchial gland cells (115). It is also reported that isoflavone inhibited NF-κB activation in various solid cancers, including neuroblastoma, breast, prostate, lung, gastric, ovarian, and head and neck cancer cells (1, 32, 45, 78, 81, 127), suggesting that downregulation of NF-κB signaling is an important mechanism by which isoflavone functions as an antioxidant, and thereby inhibits cancer development and progression (Fig. 4).

Inhibition of other important signaling pathways by isoflavone

As we have discussed in previous sections, oxidative stress regulates Akt and NF-κB signaling, and there is an active

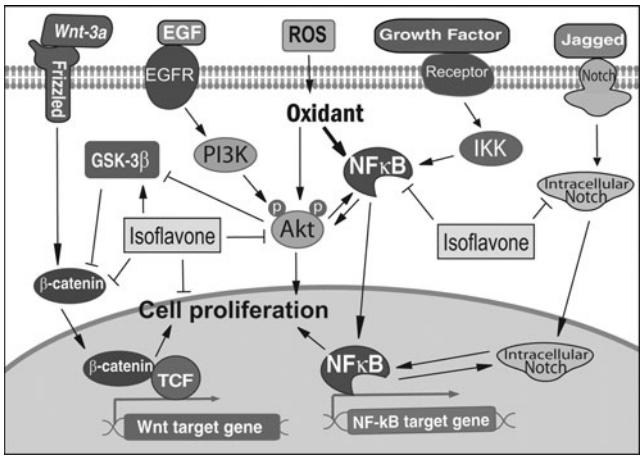

FIG. 4. Isoflavone regulates major cellular signaling pathways.

crosstalk between NF-κB and other signaling, including Akt, Wnt, Notch, and AR. In cancer cells, the alterations in multiple signaling have been observed. Among, multiple signaling such as NF-κB, Akt, Wnt, Notch, and AR crosstalk with each other and appears to play important roles in cancer development and progression. Importantly, isoflavone could inhibit the activation of these signaling pathways, suggesting the multitarget effects of isoflavone.

It has been found that there is a crosstalk between NF-κB and Akt signaling as stated earlier (Fig. 4). We found that isoflavone could inhibit phosphorylation of Akt and thereby downregulate Akt kinase activity in prostate cancer cells. Isoflavone pretreatment also abrogated the activation of Akt stimulated by EGF (77). Moreover, we found that isoflavone could exert its inhibitory effects on NF-κB signaling through the downregulation of Akt signaling, resulting in the inhibition of cell growth and induction of apoptosis (77). Isoflavone could also increase the expression of GSK-3β and decrease the phosphorylation of Akt and FOXO3a, leading to the increased apoptosis and decreased cell growth (79). Similar reports by other investigators also showed that isoflavone induced apoptosis by upregulation of $p21^{WAF1}$ and downregulation of Akt and NF-κB (85, 99).

Wnt signaling has been found to crosstalk with Akt signaling, leading to cancer cell proliferation and cancer progression. We found that isoflavone could induce the expression of GSK-3β, promote GSK-3β binding to β-catenin, and consequently increase the phosphorylation of β-catenin, suggesting the inhibitory effects of isoflavone on both Akt and Wnt signaling (79). Isoflavone also inhibited Wnt-induced cancer cell growth and downregulated the expression of two important Wnt targets, c-Myc and Cyclin D1 (112). Microarray gene expression analysis together with *in vivo* animal studies have shown that isoflavone could downregulate the expression of upstream and downstream Wnt-signaling molecules, including Wnt-5a (113), Wnt-7a (118), and Cyclin D1 (113), suggesting the inhibitory effects of isoflavone on the Wnt signaling pathway (Fig. 4).

It is well known that NF-κB and Notch form a positive signaling loop that plays important roles in cancer development and progression. The inhibitory effects of isoflavone on both NF-κB and Notch signaling have been observed. We found that isoflavone could suppress Notch signaling, resulting in the subsequent downregulation of NF-κB activity. This downregulation of both Notch and NF-κB signaling by isoflavone increased apoptotic cell death and inhibited cell proliferation of pancreatic cancer cells (121). Isoflavone could also suppress the expression of Notch-2, suggesting that isoflavone inhibits cancer cell proliferation and induces apoptotic cell death through interrupting the crosstalk between Notch and NF-κB signaling (Fig. 4).

Other cellular signaling could also contribute to the isoflavone-induced inhibition of cancer cell growth. It has been known that AR signaling could be activated by Akt or NF-κB signaling in a ligand-independent manner. Isoflavone could downregulate AR, inhibit AR translocation to the nucleus, and subsequently suppress the expression of the AR target PSA in prostate cancer cells (31, 116). Moreover, we found that isoflavone could inhibit cell proliferation and induce apoptotic cell death through the regulation of the Akt/FOXO3a/GSK-3β/AR signaling network (79). Further, we found that isoflavone could enhance the antitumor activity of chemotherapeutics in an experimental animal model of prostate cancer bone metastasis through the regulation of the OPG/RANK/RANKL/MMP-9 signaling network (73). Isoflavone also regulated RANKL/MITF, AR/PSA, and NKX3-1/Akt/p27, leading to the inhibition of osteoclast and osteoblast differentiation, which could suppress bone remodeling and prostate cancer bone metastasis. Therefore, isoflavone could function as an antioxidant affecting multiple targets, which could be responsible for its activity toward prevention and treatment of cancer.

I3C and Its *In Vivo* Dimeric Product DIM as Antioxidants Inhibiting Oxidative Stress and Regulating Cellular Signaling in Cancer

It is well known that diets rich in vegetables are beneficial for the prevention of cancer. In recent years, vegetables of the genus *Brassica* have received much attention in cancer prevention because of the glucosinolates present in the Brassica. The vegetables of the genus *Brassica* include all kinds of cabbages, broccoli, cauliflower, and Brussels sprouts. These vegetables contribute most to our intake of glucosinolates. It is known that the hydrolysis products of glucosinolates make a significant contribution to the health benefit of Brassica vegetables (54). Glucosinolates with an indole side chain form indole. The most prevalent glucosinolate with an indole side chain is glucobrassicin, which is predominant in Brassica vegetables. When hydrolysis occurs, glucobrassicin forms an unstable isothiocyanate that degrades to I3C (Fig. 5). Although I3C is biologically active, it is readily converted *in vivo* to its dimeric product DIM (Fig. 5). Under the acidic conditions of the stomach, I3C undergoes extensive and rapid self-condensation reactions to form several derivatives (117), but DIM is the major derivative and condensation product of I3C. DIM is also biologically active. It is believed that the production of DIM from I3C is a likely prerequisite for the anticarcinogenic activity of I3C.

I3C is capable of acting as a scavenger of free radicals. This scavenging activity of I3C has been implicated in the anticarcinogenic process (6). It has been reported that I3C, DIM, and DIM derivatives could reduce oxidative stress, stimulate

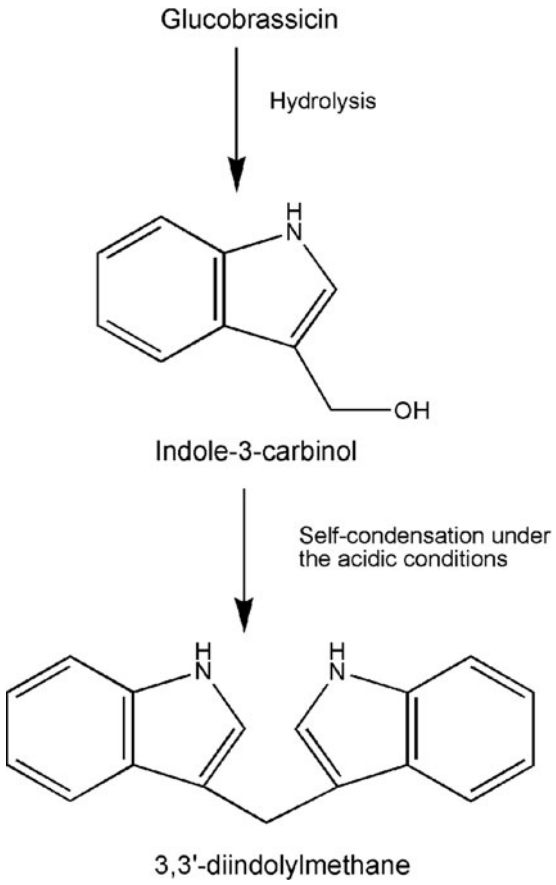

FIG. 5. The structures and the formation of I3C and DIM. DIM, 3,3′-diindolylmethane; I3C, indole-3-carbinol.

the expression of antioxidant response element-driven genes, and protect against DNA damage through its antioxidant activity (13, 17, 92). Moreover, BR-DIM, a formulated DIM with higher bioavailability, at physiologically relevant micromolar and submicromolar concentrations could protect against cell killing upon H_2O_2 and other oxidant treatment in breast cancer cells and normal cells (39). This protection against oxidative stress was found to be mediated by BR-DIM-induced upregulation of tumor suppressor protein BRCA1 and phosphorylation of BRCA1. BR-DIM also upregulated the antioxidant transcription factor NFE2L2 (NRF2) through the antioxidant-response element in a BRCA1-dependent manner (39). It is worth to note that other pathways such as the Keap1-Nrf2 signaling could also participate in the DIM-reduced oxidative stress. Further, the antioxidative effect of DIM mediated through deregulation of BRCA1 has also been investigated in inflammatory bowel disease and colitis, in which ROS play a key role. It has been shown that DIM could attenuate 2,4,6-trinitrobenzene sulfonic acid-induced colitis in an animal model. The inhibition of ROS-induced colitis by DIM was accompanied by increased expression of BRCA1, by reduced ROS generation, and by decreased expression of vascular cell adhesion molecule 1, which is typically induced by ROS (50). These findings suggest that I3C and DIM could reduce oxidative stress, and thus these agents function as antioxidants.

The inhibitory effects of I3C and DIM on cancer

The effects of DIM in dextran sodium sulfate (DSS)-induced experimental colitis and colitis-associated colon carcinogenesis induced by azoxymethane (AOM)/DSS in animals have been investigated. It has been shown that DIM treatment significantly inhibited the formation of colon tumors in AOM/DSS mice (63). DIM treatment also significantly attenuated severe clinical signs in the colitis model with reduced prostaglandin E2, nitric oxide, and proinflammatory cytokines (63), suggesting that DIM suppresses inflammatory response and further inhibits oncogenesis. I3C treatment also inhibited clonogenic cancer cell growth and induced higher levels of basal caspase-3 and 7 activities (90). We and other investigators have also found that I3C and DIM could inhibit oncogenesis and cancer cell growth and induce apoptosis in various cancer cells (23, 24, 26, 41, 42, 100, 101, 107, 124), suggesting that I3C and DIM may serve as potent agents for the prevention and/or treatment of cancer.

DIM also showed its potent effects on enhancing the antitumor activity of chemotherapy and radiotherapy. We found that DIM significantly increased the sensitivity of cancer cells to erlotinib and cisplatin through the downregulation of epidermal growth factor receptor and NF-κB in pancreatic cancer and squamous cell carcinoma cells (2, 4). ET-743 is an experimental antitumor drug with promising antitumor activity as shown in phase II trials; however, it is hepatotoxic. In an animal study, addition of I3C to the diet before ET-743 administration almost completely abolished the hepatotoxicity without any alterations in the antitumor efficacy of ET-743 (34), suggesting that I3C may counteract the unwanted adverse effects of ET-743, and thus I3C could be used in combination with ET-743 therapy. DIM could also enhance the efficacy of radiotherapy as demonstrated by our recent report showing that DIM and radiation could significantly inhibit primary tumor growth and reduce metastasis to lymph nodes in prostate cancer (110). Collectively, these findings suggest that DIM is a promising agent for cancer therapy in combination with conventional therapy. Currently, more clinical trials are being conducted to test the effects of DIM treatment as a dietary supplement or combined with conventional cancer therapy in patients with prostate, breast, cervical, and laryngeal tumors. The results of these clinical trials will uncover the value of DIM in cancer clinic.

Inhibition of NF-κB activation by I3C and DIM

The anti-inflammatory effect of DIM through NF-κB signaling has been reported. It has been found that DIM inhibited lipopolysaccharide-induced NF-κB transcriptional activity, NF-κB DNA-binding activity, translocation of NF-κB p65 to the nucleus, and degradation of IκBα, leading to the decreased release of inflammatory factors, including nitric oxide, prostaglandin E2, IL-6, and IL-1β (25). Importantly, we and other investigators have shown that I3C and DIM could inhibit NF-κB signaling in a variety of cancers, including prostate, breast, pancreatic, lung, and skin cancer *in vitro* and *in vivo* (3, 7, 16, 22, 23, 52, 62, 66, 101). DIM significantly suppressed NF-κB activation and consequently reduced the expression of NF-κB target genes such as *VEGF*, *IL-8*, *uPA*, and *MMP-9*, leading to the induction of apoptosis and inhibition of cell proliferation, angiogenesis, invasion, and metastasis (16, 66). These reports clearly demonstrate that DIM

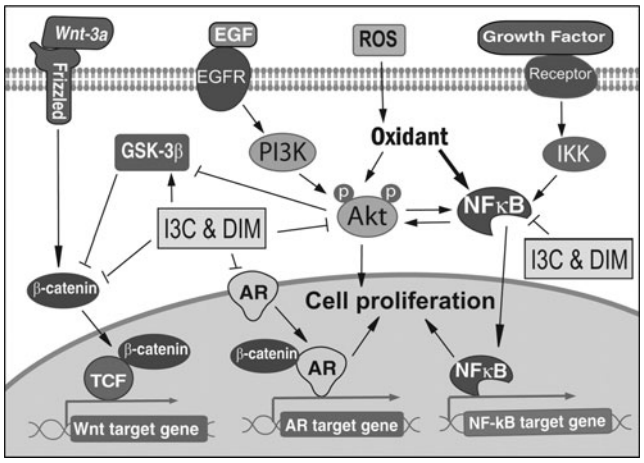

FIG. 6. I3C and DIM regulate the major cellular signaling pathways.

inhibits tumor growth and progression through down-regulation of NF-κB signaling (Fig. 6).

Inhibition of other important signaling pathways by I3C and DIM

Similar to isoflavone, I3C and DIM exhibit their multi-targeting activity in cancer cells. I3C and DIM function as an antioxidant; therefore, they suppress NF-κB and Akt signaling transduction. More importantly, because of the signal crosstalk between NF-κB, Akt, Wnt, and AR signaling, DIM also significantly inhibits AR and Wnt signaling by down-regulating NF-κB and Akt activities.

It has been shown that I3C and DIM could regulate Akt signaling in cancer cells. We found that the phosphorylated Akt protein was downregulated in I3C- or DIM-treated prostate cancer cells (24, 71). Moreover, Akt kinase activity was also decreased in I3C- or DIM-treated prostate cancer cells, suggesting the inactivation of Akt upon I3C or DIM treatment. Gene expression profiles also demonstrated the downregulation of PI3K expression in prostate cancer cells treated with I3C (74). Further, DIM suppressed phosphorylation of FOXO3a and abrogated the phosphorylation of Akt and FOXO3a stimulated by IGF-1 (80), suggesting the inhibitory effects of DIM on Akt activation in cancer cells.

DIM has a potent inhibitory effect on AR signaling, which also crosstalk with Akt and NF-κB signaling. DIM has been found to inhibit AR nuclear translocation, PSA expression, and cell proliferation induced by dihydrotestosterone in prostate cancer cells (67). We also found that DIM significantly inhibits NF-κB and Akt activation, AR phosphorylation and nuclear translocation, and the expression of AR and PSA, suggesting that DIM could interrupt the crosstalk between AR, Akt, and NF-κB signaling (16). Further, we found that DIM significantly decreased FOXO3a binding to the promoter of AR, resulting in the downregulation of AR expression, the inhibition of cell proliferation, and the induction of apoptosis in prostate cancer cells (80). These findings demonstrate that DIM inhibits cell proliferation and induces apoptosis through the regulation of Akt/FOXO3a/AR signaling.

DIM has been known to inhibit Wnt signaling through the suppression of Akt/FOXO3a signaling. We found that DIM significantly increased the phosphorylation of β-catenin and inhibited β-catenin nuclear translocation (80), suggesting that DIM could also downregulate the activation of Wnt signaling. Other cellular signaling could also contribute to the DIM-induced inhibition of cancer growth. It is known that more than 50% of human prostate cancer cells overexpress ERG due to the AR-regulated *TMPRSS2-ERG* fusion gene. Recently, we found that DIM could downregulate ERG and Wnt signaling through the suppression of AR/TMPRSS2-ERG/Wnt signaling, leading to the inhibition of cancer invasion (72). In addition, DIM also upregulated p27 and downregulated RANKL/MITF and AR/PSA signaling, resulting in the inhibition of osteoclast and osteoblast differentiation, which could suppress bone remodeling and prostate cancer bone metastasis. Similar to isoflavone, DIM exerts its pleiotropic (multitargeting) effects on multiple signaling pathways; therefore, it could be a promising agent for the prevention and/or treatment of cancer.

It is important to note that DIM (BR-DIM, which is manufactured by BioResponse, LLC.) has been tested in a phase I clinical trial in patients with prostate cancer by our laboratory, showing that BR-DIM at a oral dose of 225 mg twice daily was safe (48), and thus this dose was chosen for our ongoing phase II clinical trial in preradical prostatectomy patients with prostate cancer. It has been previously shown by our laboratory that DIM could inhibit AR through transcriptional inactivation and nuclear exclusion in cell culture studies (16); however, such observation has not been reported in patients with human prostate cancer. Therefore, we have done an interim analysis of prostate tissue specimens obtained from our currently ongoing phase II clinical trial. We found that the level of expression of AR was reduced after BR-DIM intervention. Most importantly, the nuclear exclusion of AR was quiet dramatic (65), and it appears to be in part due to induction in the expression of miR-34a. These findings are consistent with our previously reported *in vitro* finding (16). However, much more clinical trials are warranted to fully appreciate the beneficial role of I3C/DIM in cancer prevention and therapy.

Conclusion and Perspectives

Oxidative stress has been linked to aging, inflammation, and carcinogenesis. Moreover, NF-κB signaling plays critical roles in oxidative stress, inflammatory response, and carcinogenesis. Importantly, isoflavones, I3C and DIM, have been shown to reduce oxidative stress *via* inhibition of NF-κB activation among others. Moreover, these antioxidants also target multiple signaling pathways that are deregulated in cancer, leading to the inhibition of cancer development and progression. More clinical trials are being conducted to evaluate the effects of isoflavone and DIM for the prevention of cancer development and also for the treatment of cancer either alone or in combination with conventional cancer therapeutics. It is our perspectives that further mechanistic studies elucidating the antioxidative and multi-targeting effects of isoflavone and DIM will fully establish the beneficial effects of these important natural agents for the prevention and/or treatment of human malignancies.

Acknowledgments

This work was partly funded by the grants from the National Cancer Institute, NIH (5R01CA108535 and 5R01CA083695), to FHS.

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Address correspondence to:
Dr. Fazlul H. Sarkar
Department of Pathology
Barbara Ann Karmanos Cancer Institute
Wayne State University School of Medicine
740 Hudson Webber Cancer Research Center
4100 John R. Street
Detroit, MI 48201

E-mail: fsarkar@med.wayne.edu

Date of first submission to ARS Central, February 4, 2013; date of acceptance, February 7, 2013.

Abbreviations Used

Akt = protein kinase B
AOM = azoxymethane
AR = androgen receptor
DIM = 3,3′-diindolylmethane
DSS = dextran sodium sulfate
EGFR = epidermal growth factor receptor
ER = estrogen receptor
GSK-3β = glycogen synthase kinase 3 beta
I3C = indole-3-carbinol
MMP-9 = matrix metallopeptidase 9
NF-κB = nuclear factor-kappa-B
PSA = prostate-specific antigen
ROS = reactive oxygen species
TNF = tumor necrosis factor
Wnt = wingless-type MMTV integration site family