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Reviews: Current topics

Targets for indole-3-carbinol in cancer prevention

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Abstract

Mounting preclinical and clinical evidence indicate that indole-3-carbinol (I3C), a key bioactive food component in cruciferous vegetables, has multiple anticarcinogenic and antitumorigenic properties. Evidence that *p21*, *p27*, cyclin-dependent kinases, retinoblastoma, Bax/Bcl-2, cytochrome *P*-450 1A1 and GADD153 are targets for I3C already exists. Modification of nuclear transcription factors including Sp1, estrogen receptor, nuclear factor κB and aryl hydrocarbon receptor may represent a common site of action to help explain downstream cellular responses to dietary I3C and, ultimately, to its anticancer properties. While the current information is intriguing, future I3C research needs to focus on why these changes in nuclear transcription factors occur and how they relate to phenotypic responses and the quantity and duration of exposure to I3C and its dimer 3,3′-diindolylmethane.

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Keywords: Indole-3-carbinol; Estrogen receptor; Aryl hydrocarbon receptor; Sp1

1. Introduction

Increased vegetable intake is linked to a reduction in the risk of acquiring several types of cancers [1,2]. Within this food group, enhanced consumption of cruciferous vegetables (e.g., broccoli, cabbage, cauliflower, bok choy and Brussels spouts) surfaces as a factor associated with a reduction in cancers particularly in the colon, lung, prostate, cervix and breast [3–6], although admittedly, considerable controversy exists [5]. While the reason for these inconsistencies remains unresolved, variation in the content of one or more bioactive food components and consumer gene profiles may be important determinants.

Molecular studies suggest that variations in detoxification enzymes, particularly glutathione S-transferase (GST) M1 and GSTT1, may influence cancer risk in response to cruciferous vegetables. Genetic polymorphisms in receptors and transcription factors that interact with bioactive food components in cruciferous vegetables may contribute to a variation in response to their intake. Thus, it is likely that at least part of the variation arises from differences in individual gene profiles that are associated with altered sensitivity to cruciferous vegetables and their metabolic parameters.

One of the most extensively examined bioactive food components within crucifers is indole-3-carbinol (I3C; Cas No. 700-06-1). This compound arises from indolyl–methyl glucosinolate when crucifers are crushed or cooked [7,8]. Ingested I3C can be converted into a biologically active dimer, 3,3′-diindolylmethane (DIM), within the gastrointestinal tract. Since DIM accumulates in the nucleus, it likely contributes to nuclear events that have been ascribed to I3C.

Several mechanisms may account for the anticancer properties of I3C/DIM including changes in cell cycle progression, apoptosis, carcinogen bioactivation and DNA repair (Fig. 1). It remains uncertain as to which of these is most important to bring about the anticancer properties attributed to crucifers and if a common cellular mechanism may account for the observed diverse phenotypic changes. This review focuses on the relationships among these anticancer properties and nuclear events as a function of the quantity and duration of I3C/DIM exposure. Finally, this review highlights the current limitations in knowledge about the sites of action of I3C and its dimer.

2. Nuclear factors modulated by I3C

A host of preclinical studies provide evidence that I3C/DIM can influence several nuclear transcription factors that are involved with regulating cellular apoptosis and/or proliferation. Four nuclear transcription factors [estrogen receptor

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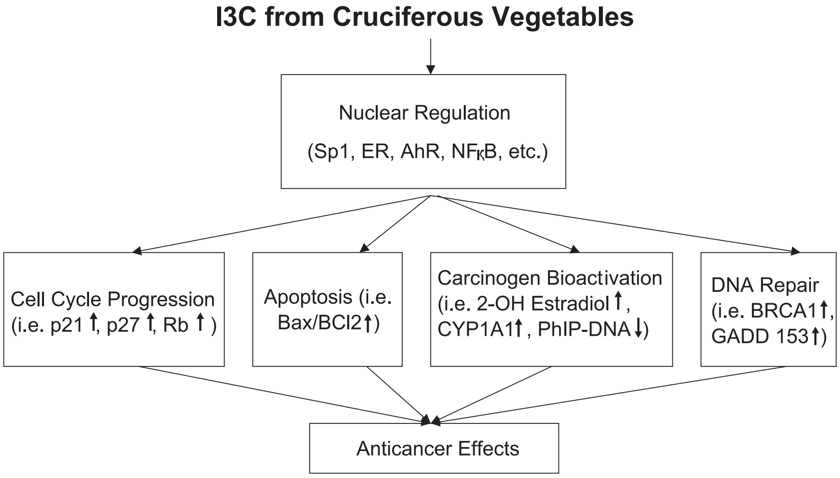

Fig. 1. I3C/DIM modulates several nuclear transcription factors. I3C exerts anticancer effects by modulating the expression of several genes that play a key role in various cancer processes. The responses of these genes to I3C including the inhibition of cell cycle progression/apoptosis and the enhancement of carcinogen metabolism and DNA repair are specifically regulated by a number of nuclear transcription factors such as Sp1, ER, NFκB and AhR.

(ER), Sp1, nuclear factor κB (NFκB), and aryl hydrocarbon receptor (AhR)] have been found to interact with I3C and/or DIM and thus may account for changes in downstream events in normal and neoplastic cells. As described below, the influence of I3C on these nuclear factors may occur directly or indirectly via cross talk among these factors.

2.1. Estrogen receptor

ERs are crucial for the mitogenic response to estrogens occurring in most malignant breast tumors. ERs consist of two subtypes, ERα and ERβ. The effects of I3C/DIM on the ERβ in breast cancer cells remain unclear. Fortunately, far more information exists about the interaction between I3C and ERα. Despite the low affinity of I3C for ERα, it leads to significant shifts in this receptor. Specifically, I3C can influence the functional domains of ERα phosphorylation [(activation function-1 (AF-1)], estrogen binding (AF-2) and DNA-binding regions. These I3C-induced modifications bring about a shift in estrogen-mediated cellular and biochemical effects in estrogen-responsive cells and tissues [9,10]. A dose-dependent depression in the estrogen-activated ERα signaling and transcriptional activity occurs when I3C is added to cultures at 10–125 μM [10]. The phosphorylation of ERα, which enables this receptor to bind to the 5′-regulatory estrogen response elements (EREs) in target gene promoters through its DNA-binding domain or to interact with other DNA-bound transcription factors such as Sp1, AhR and c-*jun* [11], is particularly sensitive to I3C since concentrations as low as 10 μM I3C can markedly modify [12]. Adding 50 μM of I3C can completely abrogate ER phosphorylation in MCF-7 cells [12] and presumably in other types of tumor cells. Full activity of AF-1 is known to be mediated by the mitogen-activated protein kinase that phosphorylates serine residues of ERα at the 118 position [13]. This phosphorylation can be stimulated by DIM, which activates not only ER but also the accompanied nuclear cofactors including steroid receptor coactivator-1 (SRC-1) and cyclic adenosine monophosphate response element-binding protein (CREB) [14]. Currently, it is unclear if phosphatase activity might contribute to the observed changes in ER phosphorylation caused by I3C or DIM.

I3C also influences the degree of estrogen binding to ERα either by competing as a ligand or by enhancing the estradiol metabolism to 2-hydroxyestrone, which then competes with estrogen for binding [15]. While I3C suppresses ERα activity through the ligand dependent mechanism, the response to DIM is both dependent and independent of estrogen binding to the receptor [16]. In culture, DIM exhibited potent estrogen-independent ER agonist activity at a concentration of 10 μM, and this was accompanied by a strong inhibition of endometrial tumor cell growth and the induction of transforming growth factor-α-responsive gene expression [17]. Therefore, I3C and DIM can influence the activity of ER either directly or indirectly by modulating its activity, which is likely related to the antitumorigenic effects of these bioactive food components.

2.2. Aryl hydrocarbon receptor

The AhR is a nuclear transcription factor that can be activated by binding to several different types of aromatic compounds including dioxins, flavonoids, I3C and DIM. Ligand-activated AhR heterodimerizes with AhR nuclear translocator (Arnt) proteins and activates the transcription of Ah-responsive genes such as *CYP1A1* through xenobiotic response elements (XREs). For example, DIM at concentrations >50 μM induces *CYP1A1* gene expression in MCF-7 human breast cancer cells [18]. DIM is reported to enhance the coimmunoprecipitation of these two receptors in both MCF-7 and ZR-75 human breast cancer cells [19]. This suggests that DIM may bring these two receptors in closer proximity. The transcriptional activation of both ER and AhR is recognized to require the recruitment of coactivator complexes including histone acetyltransferase complexes containing p300 and CREB-binding protein (CBP). It is unclear if these transcriptional cofactors are influenced by DIM.

2.3. Sp1

Sp1 is a ubiquitously expressed transcription factor that binds its specific GC-rich binding sites in multiple promoters and thereby regulates the expression of selected genes. Recently, transfection and electrophoretic mobility-shift analysis revealed that I3C regulates the expression of cyclin-dependent kinase 6 (*Cdk6*) and *p21* through changes in their Sp1-binding sites in their promoter regions. The Sp1-binding site in the *Cdk6* promoter forms a specific I3C-responsive DNA-protein complex that contains the Sp1 transcription factor. I3C selectively disrupts the interactions of Sp1 with a composite DNA-binding site within the *Cdk6* promoter, which may account for the suppressive effects of I3C on *Cdk6* expression [20].

While I3C stimulates the interaction between Sp1 and the Sp1 binding sites of both *Cdk6* and *p21,* DIM has an effect on *p21* but not on *Cdk6* promoter activity, at least in MCF-7 cells in culture. When MCF-7 cells are treated with 50 μM of DIM for 48 h, *P21* expression is increased. This increase may reflect the binding of Sp1 to the consensus Sp1 responsive elements in the promoter region of *p21* [21]. This up-regulation of *P21* is evident after short-term exposures (6 h) yet reaches a maximum in about 48 h. A similar, but less pronounced, cell cycle blockade has been observed in ER-negative MDA-MB-231 breast cancer cells, suggesting that ER may indirectly contribute to the interaction between I3C and the promoter Sp1 of *p21*. The increased *p21* resulted in the inhibition of *Cdk2*-mediated phosphorylation of retinoblastoma (*Rb*) and in a concomitant induction of G1 cell cycle arrest [21]. The mechanism by which I3C modulates Sp1-mediated expression of *Cdk6* and *p21* in opposite directions is not clear. Neither the level of total functional Sp1 nor the expression of transfected reporter plasmid driven by three consensus Sp1 sites was altered in response to the I3C treatment [22]. This may suggest that the chromatin structure of the target genes such as *p21* and *Cdk6* may have a role in I3C responsiveness to Sp1.

2.4. Nuclear factor κB

NFκB is a transcription factor that has an important role in regulating the expression of genes involved with the apoptotic process. Recent studies suggest that I3C modulates the apoptosis by inhibiting the activation of NFκB, which brings about the significant down-regulation of antiapoptotic Bcl-2 and its related gene Bcl-X_L [23]. As little as 60 μM of I3C has been found to induce apoptosis in MCF10CAIa tumor cells within 2 days and by 24 h of cells that were treated with 100 μM of I3C [23]. It remains unclear if this I3C modification of NFκB relates to its own functional alteration or through modulating IkappaB kinase complex or related kinases such as Akt kinase.

2.5. Cross-talks

While the modulating effects of I3C/DIM on the function of ER, AhR and Sp1 are intriguing, dietary indoles have a relatively low affinity for these transcription factors; thus, one assumes that other mechanisms account for their biological effects. Furthermore, some of the effects are independent of estrogen in hormone-responsive cancer cells. These findings suggest that the dynamics among I3C/DIM and promoter regions of ER, AhR and Sp1 may arise from the interactive regulation of transcription factors. Some potential sites where I3C/DIM may modulate the expression of a variety of cancer-related genes are discussed in the succeeding sections.

2.5.1. Estrogen receptor and aryl hydrocarbon receptor

The expression of several estrogen-induced genes including cathepsin D and c-*fos* is inhibited by AhR agonists [24,25]. DIM, a ligand of AhR, was found to inhibit estrogen-induced responses in ER-positive MCF-7 cells at concentrations ranging from 10 to 50 μM [18,26]. Recent studies demonstrate that ligand activation of the AhR coordinately recruits unliganded ERα, ERβ, the coactivator p300 and CBP to estrogen-responsive gene promoters [27,28]. The activated AhR was found to inhibit the ERE-mediated transcription in the presence of estrogen but to stimulate the expression of ERE-dependent genes in the absence of estrogen [27]. The mechanisms responsible for these actions remain unresolved. It has been proposed that ligands for AhR such as DIM may compete with estrogen for the recruitment of the same transcriptional cofactors including histone acetyltransferase, which thereby decreases estrogen-induced transactivation [19]. Fig. 2 summarizes the possible mechanism by which DIM may influence AhR interactions with ER and thus modulate the expression of ERE-mediated genes.

The interactions between AhR and ER may be attenuated transcriptionally or posttranslationally. Recent findings reveal that treatment of MCF-7 cells in culture with DIM (1 μM) down-regulated ERα mRNA about threefold compared with untreated cells [9,29]. Gel mobility shift and DNA footprinting assays in MCF-7 cells indicate that ligand-stimulated heterodimerization of AhR/Arnt fosters binding to a specific pentanucleotide (GCGTG) that is required for ER action in the promoter region of estrogen-responsive genes (i.e., cathepsin D and c-*fos*) and thus diminishes ER–AhR cross-talk [30]. In addition to the decreased ER transcripts and activity, the protein levels of ER and AhR are rapidly reduced following the exposure to AhR ligands in breast cancer cells. These decreases are accompanied by an enhanced formation of ubiquitinated forms of these receptors and their degradation by proteasomes [28]. These findings suggest that ER–AhR cross talk likely has a role in explaining the inhibitory effects of I3C/DIM on estrogen-stimulated cancer gene expression.

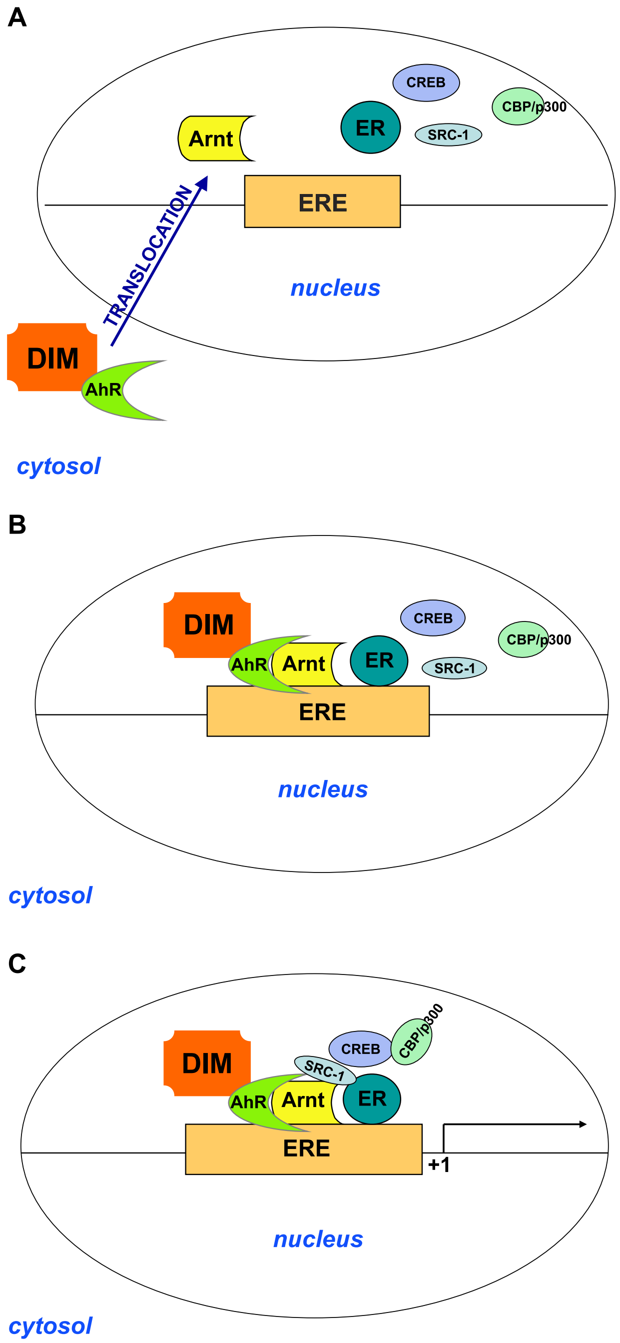

Fig. 2. DIM activation of estrogen-responsive genes. (A) The AhR compartmentalizes within the cell depending on its ligand-binding status. Whereas the unliganded AhR resides predominantly in the cytosol, the binding of ligands such as DIM induces a translocation process to the nucleus. (B) Once in the nucleus, the AhR heterodimerizes with the partner protein Arnt. The DIM-activated AhR/Arnt heterodimer directly associates with ERs and binds to the EREs. (C) This association results in the recruitment of unliganded ER and a set of coactivators including SRC-1, CREB and CBP/p300 to estrogen-responsive gene promoters, leading to activation of transcription and estrogenic effects. In the presence of estrogen, the DIM-induced nuclear complex of AhR/Arnt suppresses the expression of estrogen-responsive genes, possibly by competing with E2 for binding sites in ERE or for common cofactors that are required for the transcription.

2.5.2. Estrogen receptor and Sp1

Cellular extracts from breast cancer cells also contain Sp1, which is capable of activating ERα. ERα has been found to preferentially bind to the C-terminal DNA-binding domain of Sp1, and the resultant ERα/Sp1 complex can activate transcription from a consensus GC-rich Sp1 binding site of various genes in MCF-7 human breast cancer cells [31,32]. A number of 17β-estradiol (E2)-responsive genes can be regulated by ERα/Sp1 in breast cancer cells, suggesting that this pathway has a significant role in the regulation of ERα-dependent genes. Activation of the ERα/Sp1 complex does not require the DNA-binding domain but appears to be dependent on the AF-1 response within ERα [33], suggesting that ERα needs to be phosphorylated to bind to Sp1. Recently, the importance of the ERα and its Sp1-mediated transactivation in ER-positive breast cancer cells has been documented using inhibitory Sp1 RNA (iSp1 RNA). As expected, transfecting MCF-7 cells with iSp1 RNA decreased ER transactivation activity and inhibited estrogen-induced cell cycle progression from G(0)/G1 to the S phase [34]. At least part of the anticancer effects of I3C/DIM appears to involve Sp1-induced expression of cell cycle control proteins in human breast cancer cells [21,35]. It remains to be determined if these effects are involved with the suppressed capability of ERα transactivation.

3. Cancer processes influenced by I3C/DIM

Increasing evidence that I3C may alter cancer risk and tumor cell behavior by influencing various cancer processes exists. The expression of these targets is likely to be regulated by the nuclear transcription factors that interact with and are modified by I3C.

3.1. Cell cycle

Loss of cell cycle control is classically recognized as a deterrent to tumor development and proliferation. I3C has been demonstrated to cause G1 cell cycle arrest in human breast and prostate cancer cells [35,36]. The IC_{50} for I3C for breast cancer cells is 55 μM [37]. Several prostate and cervical cancer cell lines also respond to this quantity of I3C by suppression in cell division [38,39]. Since the transition from G1 to the S phase is controlled by the activation of CDKs and phosphorylation of Rb protein [40], these molecules have been examined as potential I3C targets. I3C has been reported to up-regulate CDK inhibitors including *p21WAF1/CIP1* (*p21*) and *p27*(*Kip1*) and to down-regulate CDK6 levels and activity. I3C also inhibited the hyperphosphorylation of the Rb protein [36]. These responses have been reported in both ER-negative (BT20, MDA-MB-231 and BT539) and ER-positive (MCF-7, 734B and BT474) human breast cancer cells, as well as in prostate cancer cells (PC-3) [21,22,35,36,41]. A dose- and time-dependent response to I3C has also been noted [35,36,41].

I3C-induced changes in Sp1 may be responsible for the induced G1 cell cycle arrest that is independent of ER status. I3C can selectively disrupt the interaction of Sp1 with a composite DNA-binding site within the CDK6 promoter [20]. In addition, DIM induces the binding of Sp1 to the consensus Sp1-responsive elements of *p21* in its promoter region, thereby increasing *p21* expression in breast cancer cells regardless of their ER status [21]. A synthetic I3C tetramer was about five times more active than monomeric I3C in producing these changes in ER-negative cells, suggesting opportunities for drugs based on I3C targets [41].

I3C is also reported to retard the development of multidrug resistance (MDR) in cancer cells. This response may relate to suppression in the expression of MDR-1 gene transcript P-glycoprotein (P-gp) [42,43]. Dietary I3C (300–500 mg/kg/day) significantly alleviated the resistance to the anticancer drugs such as doxorubicin or vinblastine [43]. The response to I3C that reduced by about 80% of the P-gp levels caused by MDR-1 gene inducers was about 20% greater than that of verapamil, a well-known MDR reversing agent [42]. The overexpression of the P-gp has been demonstrated during cell growth arrest in human and mouse tumor cell lines [44]. It remains unclear whether I3C inhibition of P-gp expression is correlated with specific cell cycle phases in tumor lesions [45,46]. Since the P-gp enhances cellular efflux of anticancer drugs and thus diminishes their effects on the target sites, the ability of I3C to reverse the expression levels of P-gp in drug resistance could have significance in cancer treatment.

3.2. Apoptosis

I3C growth inhibition may also occur because of increased apoptosis (programmed cell death). The absence of changes in p53 and Bcl-2 gene expression following DIM treatment suggests their lack of involvement in the apoptotic process [47]. However, the effect of I3C on Bax expression remains more controversial. Recently, DIM was reported to increase the Bax/Bcl-2 ratio in both estrogen-dependent and estrogen-independent human breast cancer cells. Studies with several different breast cancer cell lines indicate that the relative amounts of Bcl-2 and Bax proteins are highly predictive of the sensitivity to apoptosis in mammary tumor cells [48]. It is possible that the proapoptotic property of Bax might be inhibited by enhanced formation of its heterodimer with Bcl-2 in response to I3C. Thus, a decrease in Bcl-2 or an increase in Bax may explain the results from Sarkar et al. [49] However, Hong et al. [50] reported that DIM decreased the proportion of total Bax that was bound to Bcl-2 but resulted in little change in the proportion of total Bcl-2 that was bound to Bax. This finding supports the significance of increased relative levels of free Bax in the induction of apoptosis. Consistent with this view, Bcl-2 overexpression attenuated the DIM apoptotic effect in MCF-7 cells by approximately 50%. The down-regulation of Bcl-2 in response to I3C-treatment was also observed in PC-3 prostate cancer cells, which may be mediated by NFκB [36]. Thus, induction of free Bax protein by DIM may account for the triggering of apoptosis.

The influence of I3C on the Bax/Bcl-2 ratio may arise from its ability to abolish the mitochondrial membrane potential [49]. Antiapoptotic Bcl-2 factors are known to reside mainly in the mitochondrial outer membrane, nuclear envelope and endoplasmic reticulum. The ratio of Bax/Bcl-2 in the mitochondria determines the mitochondrial release of apoptosis-associated factors such as apoptosis-protease-activated factor 1, apoptosis-inducing factor and cytochrome *c*. Bax translocation from the cytosol to the mitochondria results in mitochondrial depolarization and the release of apoptotic factors through outer membrane channels formed by Bax oligomers [51], and this process has been shown to be modulated by I3C [49].

While I3C does not appear to alter the *fasL* gene, which is involved in the death receptor pathway of apoptosis [38,52], it does markedly influence the activity of the cell death enzyme caspase-3. Zhang and Malejka-Giganti [53] found that the feeding of I3C (5 or 25 mg/kg of body weight) for 4 days to female Sprague–Dawley rats with 7,12-dimethylbenz[alpha]anthracene induced mammary tumors increased the mammary gland activity of caspase-3 up to approximately 3.6-fold. It is also worth noting that the apoptotic effects of I3C and DIM were observed even with Her-2/neu-overexpressed breast cancer cells [54]. Future studies need to delineate the molecular mechanisms of the observed apoptotic effects of indole and its derivatives.

3.3. Carcinogen bioactivation

The induction of several enzymes including cytochrome *P*-450 (CYP)-dependent monooxygenases, GSTs and epoxide hydrolases likely explains at least part of the preclinical anticancer properties of I3C. It is interesting that I3C is a bifunctional blocking agent that is capable of influencing both Phase I and Phase II metabolizing enzymes. Concerns that compounds that induce cytochromes may be undesirable because of unwanted activation of some carcinogens are often expressed. At least with I3C, the preponderance of evidence points to a reduction in cancer risk, although admittedly, there is evidence that it may induce tumors under some circumstances. The modulating effects of I3C/DIM on the CYP-mediated carcinogen bioactivation are described in the succeeding sections.

3.3.1. CYP-mediated induction of 2-OH estradiol

It is possible that enhanced CYP-catalyzed estrogen metabolism accounts for the ability of I3C to suppress cervical carcinogenesis. The degradation of E2, a natural ligand for ER, by the liver results in increased 2 hydroxyestrone (2-OHE) or 16 α-hydroxyestrone (16 α-OHE) and, to a smaller degree, 4-hydroxyestrone. It is recognized that the 16 α-OHE is linked to proliferation of some tumor cell lines, especially those associated with the cervix. 2-OHE appears to promote antiestrogenic and antiproliferative effects by competing with E2 for ER binding. Enhanced formation of 2-OHE relative to16 α-OHE occurs when cervical cells are treated with I3C (100 mg/kg) [55]. These findings are consistent with the ability of 200–400 mg of I3C per day to markedly increase the proportion of 2-OHE relative to 16 α-OHE and to cause a regression in cervical intraepithelial neoplasia in humans [56].

Oral treatment of rats with 25, but not 5, mg of I3C per kilogram body weight increased the capacity of hepatic microsomes to convert E2 to various metabolites including 2-OHE. The low induction of *CYP1A1*, *CYP1A2* and *CYP2B1/2* activities caused by oral treatment with 5 mg of I3C per kilogram is likely insufficient to increase the capacity of hepatic microsomes to metabolize E2 [57]. Likewise, DIM treatment (5 mg/kg body weight) was more than 20-fold more effective than I3C in blocking DMBA-induced mammary tumor growth, but without a change in *CYP1A1* activity [18]. Overall, these observations suggest that the antiestrogenic effects of I3C may not involve altered CYP-dependent estrogen metabolism.

3.3.2. Modulating effects on CYP1A1 expression

CYP1A1 has long been recognized for its involvement in procarcinogen activation. The promoter region of the *CYP1A1* gene contains consensus dioxin response elements or XREs that are known to interact with the nuclear AhR complex. Cui et al. [58] examined the relative effectiveness of I3C to influence *CYP1A1* induction using a high-throughput reporter gene system that consisted of a stable transformation of H4IIE cells to incorporate the luciferase gene under control of the *CYP1A1* promoter. Using this model, they found I3C to be a rather weak inducer, causing only a sevenfold increase in activity at a concentration of 100 μM as compared with about a 40-fold increase caused by 30 μM of β-naphthoflavone. In addition, it was found that indoles bound to AhR with up to a concentration of 125 and 31 μM for I3C and DIM, respectively, did not induce *CYP1A1*-dependent detoxifying enzymes such as ethoxyresorufin *O*-deethylase (EROD) activity in T47D human breast cancer cells [59]. Therefore, physiological exposures to indoles are likely not to induce tumors due to their procarcinogen activation potential.

Horn et al. [57] demonstrated that I3C treatment (250 mg/kg body weight) for 4 or 10 days significantly increased rat liver and mammary mRNA for *CYP1A1* and *CYP2B1/2* compared with controls. An increase in EROD (*CYP1A1*) and methoxyresorufin *O*-demethylase (*CYP1A2*) activities was observed at I3C exposures of 5 mg/kg body weight, and benzyloxyresorufin *O*-dealkylase (*CYP2B1*) or pentoxyresorufin *O*-dealkylase (*CYP2B1/2*) increased when treated with 25 mg/kg or higher.

Inhibition by DIM of rat and human *CYP1A1*, human *CYP1A2* and rat *CYP2B1* in vitro using CYP-specific activity assays has been reported [60]. Likewise, I3C and DIM inhibition of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced EROD activity in AhR-responsive T47D human breast cancer cells has been ascribed to AhR antagonist effects. These suppressive effects of dietary indoles likely arise from the competition with TCDD for AhR binding [59]. Hence, I3C may act as both an AhR agonist and antagonist in vivo, which would affect the course of CYP-dependent metabolism of xenobiotics and endobiotics with the duration of treatment.

3.3.3. Inhibition of 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine metabolism

I3C can also retard adducts caused by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) [61], a heterocyclic amine that is capable of inducing lymphomas, intestinal

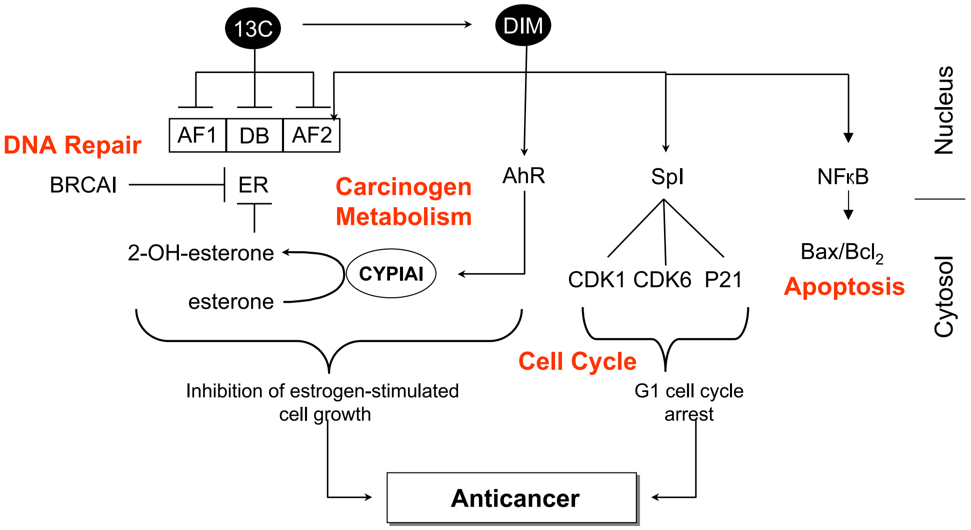

Fig. 3. Potential pathways by which I3C/DIM exerts anticancer effects. I3C/DIM is involved with various cancer processes including cell cycle progression, apoptosis, carcinogen metabolism and DNA repair. These downstream effects of I3C/DIM occurring in several subcellular compartments are likely regulated at the transcriptional level in the nucleus.

tumors, mammary adenocarcinomas and hepatocellular adenomas in rodents [62,63]. Supplementing the diet of female F344 rats with 0.1% of I3C reduced the formation of PhIP–DNA adducts by 40–100% compared with controls [64]. These findings are consistent with the protective role for I3C against PhIP-induced colon carcinogenesis [61]. It is unclear if enhanced repair mechanisms or altered PhIP detoxification account for these findings. Regardless, since this carcinogen has been identified in cooked meats [65], a depression in its bioactivation and cancer capabilities may have particular public health significance.

3.4. Induction of DNA repair by I3C

Extracts of cooked and autolyzed Brussels sprouts and some glucosinolates may also inhibit cancer by blocking DNA strand breaks. This reduction appears when diverse compounds such as benzopyrene and hydrogen peroxide are employed. Bonnesen et al. [66] demonstrated a 20% reduction in the number of single-strand DNA breaks by pretreatment of human colon adenocarcinoma LS-174 cells for 24 h with a mixture of indolo[3,2-b]carbazole and sulforaphane before treatment to benzopyrene compared with nontreated cells. This effect was only seen when both compounds were provided [66].

Recently, DIM has been found to induce DNA damage-inducible GADD153 gene expression in keratinocyte cells [67]. A tumor suppressor gene, *BRCA1*, that plays an important role in DNA repair process was also shown to be up-regulated by I3C/DIM treatment [68–70]. Fan et al. [71] reported that ability of theBRCA1 protein function, in part, to suppress estrogen-dependent mammary epithelial proliferation can be significantly modified by indoles. The *BRCA1* is also known to activate the *GADD45* promoter region mediated by its specific motifs, CAAT. The interrelationships among I3C/DIM, *BRCA1*, ERα and GADD proteins in response to dietary I3C deserve additional attention.

4. Summary and conclusions

I3C or DIM exposures appear to inhibit tumor cell growth via multiple cancer processes (Fig. 3). Evidence that the observed alterations in cell growth-related genes may arise from the interactions between I3C/DIM and the promoter activities of various transcription factors including ERα, Sp1, NFκB and AhR already exists. A variety of possible mechanisms including the involvement of Sp1 domains and cross talk between ERα and AhR have surfaced as potential mechanisms through which I3C may exert its antiproliferative effects. Currently, it is unclear if dietary indoles influence a single transcription factor or exhibit their effects through combined functional transcription machinery to bring about antitumorigenic responses. Regardless, ample evidence exist to justify additional attention to the mechanisms by which I3C/DIM alters cancer processes. Overall, the ability of I3C to alter various cancer processes with physiologically achievable concentrations makes it an intriguing dietary phytochemical. A better understanding of the targets for I3C/DIM in tumor cell proliferation and/or apoptosis, especially those related to nuclear regulatory factors, should assist in developing appropriate intervention strategies to promote cancer prevention.

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