Cancer chemotherapy with indole-3-carbinol, bis(3′-indolyl) methane and synthetic analogs

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Abstract

Indole-3-carbinol (I3C) conjugates are phytochemicals expressed in brassica vegetables and have been associated with the anticancer activities of vegetable consumption. I3C and its metabolite bis (3′-indolyl)methane (DIM) induce overlapping and unique responses in multiple cancer cell lines and tumors, and these include growth inhibition, apoptosis and antiangiogenic activities. The mechanisms of these responses are complex and dependent on cell context. I3C and/or DIM activate or inactivate multiple nuclear receptors, induce endoplasmic reticulum stress, decrease mitochondrial membrane potential, and modulate multiple signaling pathways including kinases. DIM has been used as a template to synthesize a series of 1,1-bis(3′indolyl)-1-(substituted aromatic)methanes (i.e. C-DIMs) which are also cytotoxic to cancer cells and tumors. Some of the effects of C-DIMs resemble those reported for DIM analogs; however, structure–activity studies with the aromatic ring has resulted in generation of highly unique receptor agonists. For example, *p*-trifluoromethylphenyl, *p*-*t*-butylphenyl and *p*-biphenyl analogs activate peroxisome proliferator-activated receptor γ (PPARγ), and *p*-methoxyphenyl and *p*-phenyl compounds activate nerve growth factor-induced-Bα (NGFI-Bα, Nur77) orphan nuclear receptor. The effects of C-DIMs on PPARγ and Nur77 coupled with their receptor-independent activities has resulted in the development of a novel group of multi-targeted anticancer drugs with excellent potential for clinical treatment of cancer.

Keywords

1. Introduction

The development of cancer is a complex process that is initiated by some form of DNA damage which ultimately can lead to tumor formation. The conversion of a damaged cell into a tumor requires a series of steps associated with tumor promotion and progression and, for many cancers, the initiation–promotion–progression pathways are accompanied by activating specific protooncogenes and inactivating mutations of tumor suppressor genes [1,2]. Not surprisingly, many of these critical genes are involved in DNA repair and maintenance of DNA integrity, regulation of cell proliferation, and maintenance of cell survival and cell death pathways. Inherited genetic factors play an important role and can explain enhanced cancer

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susceptibility in only 5–15% of most cancers, whereas "environmental/lifestyle" or dietary factors are major determinants in cancer formation. With few exceptions, the precise contributions of environmental, lifestyle, dietary or other risk factors for cancer are not well-defined, although, it is generally considered that diets high in vegetables, fruit and fish products and low in red meats are generally protective. Nevertheless, the precise contributions of various food products as protective against or as risk factors for development of specific cancers is not well-defined.

2. Cruciferous vegetables and cancer

Epidemiology studies on various population groups have been used to investigate the association of various food-types with development of specific cancers, and results of these studies are highly variable and sometimes conflicting. Large prospective studies in North America and Europe have examined the association between consumption of different foods and cancer incidence, and this approach provides important insights on cancer chemoprevention. Examination of participants in the Nurses' Health and Health Professionals follow-up studies (US cohort) showed that high fruit and vegetable consumption was associated with a decreased risk for cardiovascular disease [relative risk (RR) = 0.88] but had no effect on cancer (RR = 1.0) [3]. Similar results were observed for two large prospective studies on breast cancer [4,5]; however, in the US cohort, it was reported that higher dietary intakes of fruits and vegetables were associated with lower risks of lung cancer in men, but not in women [6]. Several other prospective studies have demonstrated a protective effect of high intakes of fruit and/or vegetables or specific sub-types of vegetable consumption for urothelial cancer, non-Hodgkin's lymphoma, colorectal cancer, pancreatic cancer, bladder cancer, and renal cell carcinoma [7–12]. Moreover, in many of these studies, the vegetable sub-type frequently associated with decreased cancer risk was cruciferous vegetables including broccoli, cauliflower and Brussel's sprouts. For example, in the bladder cancer study, total fruit and vegetable intake was not significantly associated with decreased bladder cancer; however, intake of cruciferous vegetables was inversely associated with risk with a relative risk of 0.49 in the highest consumption group [11]. Although prostate cancer has been inversely associated with high consumption of cruciferous vegetables in case-control studies [13,14], results of a prospective study did not confirm the protective effects [15]. The predictive nature of prospective and case-control epidemiology studies depends on several factors including recall of dietary intakes which has several problems. Nevertheless, there is evidence that dietary consumption of cruciferous vegetables may provide protection from some chronic diseases and specific cancers.

2.1. Anticancer activities of cruciferous vegetables and indole-3-carbinol (I3C)

Natural products from vegetable sources have been widely used in traditional medicine as potions for treatment of a variety of human ailments, and phytochemicals and their derivatives are major sources and building blocks for development of new drugs [16–18]. Cruciferous vegetables contain a wide spectrum of active phytochemicals; however, most studies have focused on isothiocyanates and indole-3-carbinol (I3C) as the major chemopreventive and chemotherapeutic phytochemicals associated with the anticancer activities of cruciferous vegetables [19–23]. Glucobrassican, a major component of cruciferous vegetables, is a 3-indolylmethyl glucosinolate which is readily hydrolyzed in the acidic conditions of the gut to give I3C. This compound is highly unstable in acidic conditions (pH 2–4) and undergoes a series of condensation reactions to give a broad spectrum of products [23–25]. At higher pHs (pH 5–7), such as those encountered under cell culture conditions, I3C is primarily converted into the dimeric product bis(3-indolyl)methane (DIM) [26] (Fig. 1). Thus, many of the responses observed for I3C *in vivo* are due to a complex mixture of acid-catalyzed indole derivatives, whereas in cell culture, the effects may be due to a combination of I3C/DIM.

Early *in vivo* reports demonstrated that I3C inhibited carcinogen-induced mammary tumor growth, and subsequent studies in other animal models have confirmed the antitumorigenic activity of I3C [27–37]. In some animal models, the effects of I3C have been related to its activity as an inducer of phase I (CYPs) and phase II (glutathione *S*-transferase) drug-metabolizing enzymes. This may be particularly relevant for hormone-dependent cancers where I3C induces changes in the 2-hydroxylation (increases) and 16α-hydroxylation (increases) of 17β-estradiol [24,28,32,38–41].

The mechanism of action of I3C has been extensively investigated in cancer cell culture models, and a recent review article has summarized these responses [41]. Since the effects are probably due to a combination of I3C and DIM, it is difficult to dissect out those responses that are due to I3C alone. It is also important to note that direct comparisons between studies are difficult since only selected responses are observed, and there may be important cell context-dependent differences. Fig. 2 summarizes major pathways associated with the effects of I3C on cancer cells, namely inhibition of cell proliferation and cell cycle progression and induction of cell death or decreased cell survival. These effects undoubtedly contribute to the cytotoxicity of I3C in most cancer cell lines over concentrations that range from the low μM to 500 μM. For example, several studies show that I3C modulates expression of several genes and proteins that lead to inhibition of cell growth, and these include decreased cyclin D1, cyclin-dependent kinases (cdks) 2 and 6, phospho-retinoblastoma protein, and increased expression of several cyclin-dependent kinase inhibitors [42–49]. Many of these responses are highly dependent on cell context, suggesting that the underlying mechanisms of growth inhibition are different. For example, treatment of MCF-7 breast cancer cells with I3C blocks G_1 to S phase progression, and this is associated with conversion of the active 90 kDa cdk2 complex to an inactive higher molecular weight (200 kDa) complex [44]. Moreover, in this cell line, I3C did not affect expression of p21 or p27 cdk inhibitors, and the effects on cdk2 were not observed for DIM. Interestingly, a previous study by the same group showed that I3C decreased cdk6, and this response was linked to decreased Sp1 binding to the cdk6 promoter in MCF-7 cells [45,47].

I3C also causes G_1 cell cycle arrest in prostate cancer cells; however, the specific targets of I3C exhibit both similar and overlapping effects in LNCaP and PC3 prostate cancer cells compared to MCF-7 breast cancer cells. In androgen-responsive LNCaP prostate cancer cells, treatment with I3C decreased expression of cdk6, decreased cdk2-dependent enzymatic activity, and induced the cdk inhibitors p16, p21 and p27 [42]. The effects of I3C in androgen-insensitive PC3 cells were similar to those observed in LNCaP cells, and expression of both p21 and p27 were increased and cdk6 protein and cdk6-dependent activity were decreased [48]. Growth inhibitory responses in breast and prostate cancer cells required concentrations of I3C as high as 100 μM, whereas the characteristic I3C-induced G_1 arrest and decreased proliferation of human immortalized keratinocyte HaCaT cells was only observed at concentrations between 200 and 500 μM [43]. Moreover, in this cell line, the cdk inhibitor p15 was induced but expression of p21, p27, p19 and cdk6 was unchanged at all concentrations of I3C [43].

The cytotoxicity of I3C in cancer cell lines is also associated with the induction of cell death and the modulation of multiple genes and proteins associated with this response [48,50–61]. Rahman and coworkers reported that I3C induced apoptosis and caspase-dependent PARP cleavage in ER-negative MDA-MB-453 breast cancer cells, and this was accompanied by caspase-3 activation, mitochondrial uptake of bax, decreased bcl-2, and an overall increase of bax/bcl-2 ratios. In contrast, another report showed that induction of apoptosis in MCF-7 cells was bax- and p53-dependent [56]. These results suggest that I3C may activate the intrinsic mitochondrial pathway; however, the proapoptotic effects of I3C in cancer cell lines need further investigation.

The mechanisms of I3C-induced cytotoxicity in cancer cells may be related, in part, to a host of other responses that can effect cell proliferation and cell death (Fig. 2). For example, I3C inhibits both androgenic responses in prostate and estrogenic responses in cervical and breast cancer cells [61–67], and induction of CYP-dependent estradiol metabolism also decreases the mitogenic effects of this hormone [28,32,38–41]. In addition, I3C modulates expression or activation of many other genes including p53 (increase), interferon γ, NFκB (decrease), non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1, increase), breast cancer susceptibility gene (BRCA1, increase), and the phosphatidylinositol-3-kinase (PI3-K, decrease) survival pathways [59,60,66–73]. I3C is also an aryl hydrocarbon receptor (AhR) agonist which can activate growth inhibitory pathways in breast and other cancer cell lines [74,75]. A recent paper showed that I3C induces BRCA1 and BRCA2 in breast (MCF-7 and T47D) and prostate (LNCaP and DU145) cancer cells [67]. Using RNA interference studies, they showed that I3C-mediated antiestrogenic/antiandrogenic activity, and cytotoxicity was due to BRCA1 (and/or BRCA2). Moreover, the results suggest that induction of BRCA1 and BRCA2 by I3C is due to activation of ER stress and these results, coupled with studies on DIM and synthetic DIM analogs, may be one of the important underlying mechanisms of action of I3C (see below).

2.2. Anticarcinogenic activity of DIM

DIM is the major dimeric condensation product of I3C (Fig. 1) and, although this compound is photolabile, it is relatively stable in cell culture media. In MCF-7 cells treated with radiolabeled I3C, a significant amount of the radiolabel is found as DIM in the nuclear fraction [26]. Like I3C, DIM is cytotoxic to cancer cells and inhibits growth of multiple tumor types [32,50,53,55,72,76], and these responses are accompanied by activation of growth inhibitory and proapoptotic pathways [32,50,53,55,72,73,75–95] (Fig. 2). DIM induces some of the same responses observed for I3C, and this is consistent with the conversion of I3C into DIM in cell culture media [26]. However, the mechanisms of action of DIM are more well-defined than those for I3C, and DIM induces several unique pathways that are not observed for I3C. In this section of the review, a more detailed examination of some tumor type-specific effects and mechanisms of action of DIM will be discussed and, where possible, compared to those observed for I3C.

DIM inhibits breast cancer cell and tumor growth and the mechanisms of action are complex and cell context-dependent (Fig. 2). Studies in this laboratory have shown that DIM inhibits carcinogen- induced rat mammary tumor growth at a dose of 5 mg/kg/d and, in MCF-7 cells, DIM acts as a selective AhR modulator (SAhRM) and activates inhibitory AhR-ERα crosstalk [96]. This mechanism has been extensively investigated in breast cancer cells for many other AhR agonists and undoubtedly contributes to the activity of DIM in breast cancer cells [97, 98]. The importance of the AhR in MCF-7 cells has been investigated by RNA interference, and knockdown of this receptor increased the percentage of cells progressing through G_0/G_1 to S phase, indicating a constitutive (ligand-independent) role for this receptor as an inhibitor of MCF-7 cell proliferation [99]. However, there are also reports that DIM is estrogenic and activates genomic and non-genomic ER-dependent pathways in breast cancer cell lines and rainbow trout [100,101]. This seemingly contradicts the antiestrogenic effects of DIM through activation of inhibitory AhR-ERα crosstalk in breast cancer cells. However, recent studies have demonstrated that many AhR agonists, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and DIM, directly activate ERα; however, these effects are decreased by competitive AhR binding and do not necessarily block inhibitory AhR-ERα crosstalk [102–104]. Despite the evidence that activation of the AhR by DIM plays a role in blocking estrogen-dependent breast cancer and tumor growth, there is evidence that DIM also activates AhR-independent growth inhibitory/proapoptotic breast cancer cells [76,78–88]. However, there are some inconsistencies in some of the proposed mechanisms of growth inhibition by DIM and I3C in

breast cancer cells. For example, DIM induces p21 expression in MCF-7 and MDA-MB-231 cells, and this is associated with enhanced Sp1 binding to the p21 promoter [85], whereas I3C does not activate p21 through enhanced Sp1 binding [45]. Another report shows that DIM induces p21 in MCF-7 but not MDA-MB-231 cells and does not affect Sp1, Sp3 or Sp4 protein expression [81]. These differences between I3C and DIM and between studies have not been resolved and may be due to multiple factors including the type of serum used and the cell passage number since Sp protein expression can also change with passage number [105].

DIM also modulates kinase activities and other proapoptotic and growth inhibitory genes and proteins in breast cancer cells, and there is also evidence that DIM decreases mitochondrial membrane potential (MMP) and induces ER stress in breast cancer cells [80,81,86]. Both of these responses may be important underlying mechanisms of action for DIM in breast and other cancer cell lines. For example, in MCF-7 cells, DIM induces several markers of ER stress including CHOP and JNK phosphorylation [80], and studies in pancreatic cancer cells show that induction of these responses by DIM leads to constitutive activation of death receptor 5 (DR5) and the extrinsic apoptosis pathway [82]. Based on these results, it is possible that many of the proapoptotic and growth inhibitory effects of DIM may be due to direct targeting of mitochondria and the ER, and it is possible that perturbation of these organelles may be related.

The cytotoxicity of DIM has also been observed in many other cancer cell lines; however, there has been an extensive focus on both androgen- sensitive and androgen-insensitive prostate cancer [89–93]. DIM binds directly to the AR [106] and is an AR antagonist in AR-positive LNCaP prostate cancer cells. However, it is evident from studies in AR-positive and AR-negative prostate cancer cells that DIM induces growth inhibitory and proapoptotic responses that are AR-independent. Savino and coworkers investigated the role of DIM on ER stress and intracellular calcium levels in mediating the cytotoxicity of DIM in AR-negative DU145 prostate and C33A cervical cancer cell lines and found cell context- dependent differences [92]. There is also evidence that DIM induces apoptosis through the mitochondrial pathway in AR-negative PC3 cells [93], demonstrating that in prostate cancer cells, both the ER and mitochondria can also be targets for DIM (Fig. 2). Future studies in different cancer cell lines are required to confirm the role of the ER and mitochondria or their interactions on the activity of DIM and also identify other critical cellular sites responsible for the remarkable anticancer activity of DIM.

2.3. Synthetic analogs of DIM as anticancer agents

2.3.1. Ring substituted DIMs—Initial studies in our laboratory focused on the AhR agonist activity of DIM in breast cancer cells and activation of inhibitory AhR-ER crosstalk in which DIM inhibits estrogen-induced responses in breast cancer cells and mammary tumors [77]. Our results characterized the antiestrogenic activity of DIM and the inhibition of carcinogen-induced mammary tumor growth in female Sprague–Dawley rats at a dose of 5 mg/kg/d [77]. A series of symmetrical ring-substituted DIM analogs were readily synthesized by the condensation of commercially available substituted indoles with formaldehyde. These compounds were initially screened in the carcinogen- induced rat mammary tumor model at a dose of 1 mg/kg/d to identify compounds that were at least 5 times more active than DIM [107,108]. Fig. 3 illustrates the anticarcinogenic activity of several active ring-substituted DIM analogs that inhibited tumor growth at a dose of 1 mg/kg/d, whereas in this same model, DIM was not active at this dose. Subsequent *in vivo* studies at lower doses have not been carried out; however, it was evident that the anticarcinogenic activity of some ring-substituted DIMs was >5-fold higher than DIM [77]. 5,5′-DibromoDIM has been used as a prototype in two studies comparing the activity of the ring-substituted compound with DIM. Both 5,5′-dibromoDIM and DIM inhibited pancreatic cancer cell survival and activated ER stress pathways which enhanced DR5 and the extrinsic apoptotic pathway [81,82].

In this study, 5,5′-dibromoDIM was clearly more potent that DIM (≥2-fold); however, both compounds induced the same ER stress-dependent responses in pancreatic cancer cells. In contrast, in ER-negative MDA-MB-231 and ER-positive MCF-7 breast cancer cells, the anticarcinogenic activity of 5,5′-dibromoDIM was not only >2-fold higher than DIM but there was also evidence for differences in their mechanisms of action [81]. DIM significantly induced p21 expression in MCF-7 cells; 5,5′-dibromoDIM decreased p21 in the same cell line, and neither compound affected levels of p21 protein in MDA-MB-231 cells. Similar differences were observed for the mitochondriotoxic effects of these compounds where 5,5′-dibromoDIM significantly decreased MMP in MCF-7 and MDAMB- 231 cells, whereas minimal effects were observed for DIM. These data, coupled with results of ongoing studies, demonstrate differences in the potency and mechanism of action between DIM and ring-substituted DIMs. Moreover, there are also similar differences among ring-substituted DIMs which are dependent on the substituent and its position on indole ring.

2.3.2. PPARγ-active C-DIMs—A second class of DIM derivatives was developed by condensing indole or substituted indoles with substituted benzaldehyde derivatives to give 1,1-bis(3′-indolyl)-1-(p-substituted phenyl)methanes (C-DIMs) [81,82,109–122]. These compounds are triarylmethane derivatives which differ from DIM and ring-substituted DIMs which are diarylmethanes. These compounds did not bind or activate the AhR, ER or AR; however, initial studies showed that some C-DIMs also inhibited carcinogen- induced rat mammary tumor growth and growth of various cancer cell lines [109–118]. Initial studies surveyed the activation of several orphan nuclear receptors by a series of C-DIMs containing various p-substituted phenyl groups, and the results showed that some analogs activated peroxisome proliferator-activated receptor γ (PPARγ) in breast cancer cells [109]. Subsequent studies showed that one or more of the three most active compounds, namely the p-trifluoromethyl (DIM-C-pPhCF$_3$), p-t- butyl (DIM-C-pPhtBu), and p-phenyl (DIM-CpPhC$_6$H$_5$) analogs (Fig. 4) also activated PPARγ in colon, pancreatic, prostate, bladder, breast, endometrial and kidney cancer cell lines [109–113,121]. The PPARγ-active C-DIMs exhibit highly tissue-specific receptor-dependent activation of responses and genes. PPARγ-active C-DIMs induced differentiation in 3T3-L1 adipocyte cells characterized by an increase in lipid droplets and Oil red-O staining [109]. In addition, these compounds induced p21 gene expression in Panc28 pancreatic cancer cells and caveolin-1 in colon and bladder cancer cells that was PPARγ-dependent [110,111,113]. However, for most other responses, the C-DIM-induced proapoptotic and growth inhibitory effects were PPARγ independent. A direct comparison of the effects of DIM, 5,5′-dibromoDIM and DIM-C-pPhtBu was carried out in Panc1 and Panc28 pancreatic cancers, and all three compounds activated ER stress pathways leading to induction of CHOP and DR5 and the extrinsic apoptosis pathway [82]. PPARγ-active C-DIMs activated ER stress in other cancer cell lines [82,117,122], but not in breast cancer cells [81]. In contrast, DIM activated ER stress pathways in breast cancer cells. This demonstrates that DIM and C-DIMs exhibit cell context-dependent differences in their activation of ER stress in cancer cell lines.

PPARγ-active C-DIMs also induce other proapoptotic responses that are both receptor- and ER stress-independent. For example, in SW480 colon cancer cells, DIM-C-pPhCF$_3$ and DIM-C-pPhC$_6$H$_5$ do not induce ER stress and, in both SW480 and HCT116 cells, the C-DIM compounds induce expression of NAG-1 and activating transcription factor 3 (ATF3) which are proapoptotic genes and proteins [115,116]. DIM also induces NAG-1 and ATF3 in HCT116 cells; however, the mechanism of this response has not been reported. Induction of NAG-1 in HCT116 cells by PPARγ-active C-DIMs is dependent on PI3-K-dependent activation of early growth response-1 (Egr-1) gene which in turn activates NAG-1 through interactions with a proximal Egr-1 element in the NAG-1 promoter [115]. In contrast, induction of NAG-1 by DIM-C- pPhCF$_3$ in LNCaP cells is MAPK-dependent[118] and suggests that induction of some

proapoptotic genes such as NAG-1 are dependent on activation of kinase pathways by C-DIMs. However, the mechanisms of kinase activation have not been determined.

Both C-DIMs and DIM induce apoptosis in prostate cancer cells and, in LNCaP cells, DIM is an antiandrogen and blocks hormone-induced responses [118]. In contrast, C-DIM-dependent modifications of androgen responsiveness in LNCaP cells is completely different from the antiandrogenic activity of DIM. PPARγ-active C-DIMs decrease AR mRNA and protein levels and AR-promoter activity and these responses are PPARγ-independent. The effects on AR mRNA are not affected by cycloheximide, suggesting that C-DIM decreases AR mRNA stability or decreases promoter activity in a transcription-independent manner. C-DIMs also decrease prostate specific antigen (PSA) protein and mRNA and promoter activity; however, cycloheximide inhibits the effects of C-DIMs on PSA mRNA and this suggests induction of an inhibitory *trans*-acting factor. These C-DIM-induced effects on AR clearly distinguish between these compounds and DIM in androgen-sensitive prostate cancer cells. Current studies on C-DIMs are focused on determining their cell context- dependent mechanisms of action and on development of clinical applications for cancer chemotherapy.

2.3.3. Nur77-active D-CIMs—Another sub-class of C-DIMs containing *para*-methoxy (DIM-C-pPhOCH$_3$) or hydrogen (DIMC-Ph) groups (Fig. 5) activate the orphan nuclear receptor nerve growth factor-induced Bα (NGFI-Bα) [125,126] often referred to as Nur77 [4]. The NGFI-B subfamily contains three members (Nur77, Nurr1 and Nor1) which were initially identified as immediate early genes induced by nerve growth factor in neuronal PC-12 cells [123].

Nur77 plays an important role in thymocyte-negative selection and in TCR-mediated apoptosis in thymocytes [124,125], and overexpression of Nur77 in transgenic mice resulted in high levels of apoptosis in thymocytes [126,127]. Several studies suggest that Nur77 plays a role in cell death pathways activated by apoptosis-inducing agents [128–134]. Li and coworkers [128] reported that treatment of LNCaP prostate cancer cells with several apoptosis-inducing agents, such as retinoids, TPA and TNF-α, resulted in induction of Nur77 gene expression. Surprisingly, induction of apoptosis and cytochrome c release from the mitochondria was independent of the DBD of Nur77. Treatment with leptomycin B (a blocker of nuclear export) inhibited induction of Nur77-dependent apoptosis. Using a series of wild-type and deletion GFP-Nur77 constructs, it was reported that induction of apoptosis was accompanied by translocation of Nur77 from the nucleus to the mitochondria. Moreover, Nur77 specifically interacted with Bcl-2 to form a proapoptotic complex in HEK293T and HCT116 cells [129]. Nur77 translocation from the nucleus has been observed in several cell lines after treatment with apoptosis inducers [128–130]. However, a study in colon cancer cells reported that butyrate-induced apoptosis was associated with nuclear to cytoplasmic translocation of Nur77 which was not accompanied by subsequent mitochondrial interactions [133]. Results with C-DIM compounds showed that DIM-C-pPhOCH$_3$ and DIM-C-Ph activated nuclear Nur77 in pancreatic and colon cancer cells lines [119,120]. It was also reported that these same compounds activated a construct (pNuRE) containing a Nur responsive element (NuRE), and also activated a Gal4-Nur77(EF) construct containing the ligand binding domain of Nur77. We also demonstrated that these Nur77 agonists induced growth inhibition and apoptosis in pancreatic and colon cancer cells [119,120], and this was associated with induction of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mRNA and protein. Moreover, these responses were partially reversed either by the Nur77 antagonist DIM-C- pPhOH or by transfecting small inhibitory RNA for Nur77 (iNur77). Microarray studies show that DIM-C-pPhOCH$_3$ activates several proapoptotic genes in colon, pancreatic, prostate and bladder cancer cells through both Nur77-dependent and Nur77-independent pathways, and these are currently being investigated.

It is clear from studies on C-DIM compounds that DIM is an excellent scaffold from which new chemotherapeutic agents can be derived. A recent paper reported the synthesis and antitumorigenic activities of a series of substituted benzenesulfonyl or benzoyl I3C derivatives in which the indole nitrogen was derivatized [135]. Like I3C, these compounds were antitumorigenic and proapoptotic; however, they were up to 100 times more potent than I3C and were stable under acidic conditions. These I3C derivatives and the C-DIMs demonstrate that the chemoprotective and chemotherapeutic properties of I3C and DIM can be retained and enhanced in their synthetic analogs which are a promising class of anticancer drugs that activate multiple pathways in cancer cells and tumors but not in non-tumor tissues or organs.

Acknowledgements

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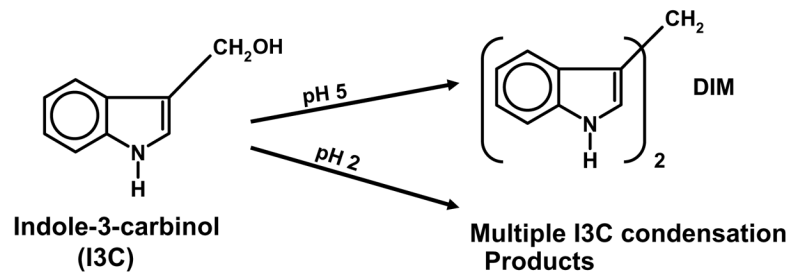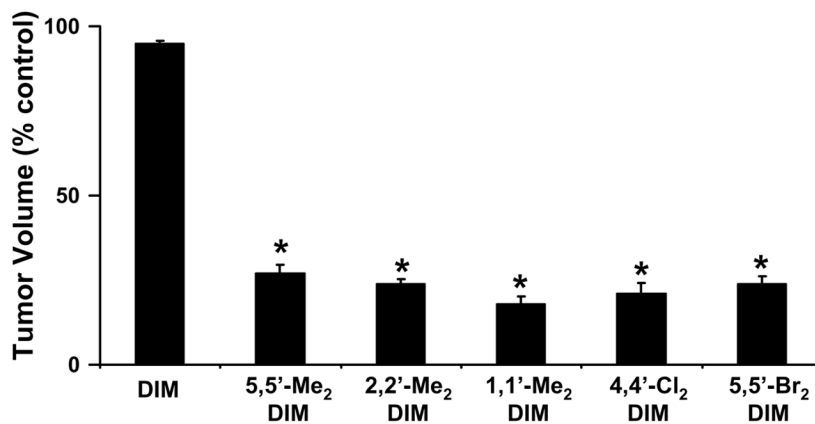
Fig. 1.
I3C undergoes acid-catalyzed rearrangement into multiple condensation products including DIM.
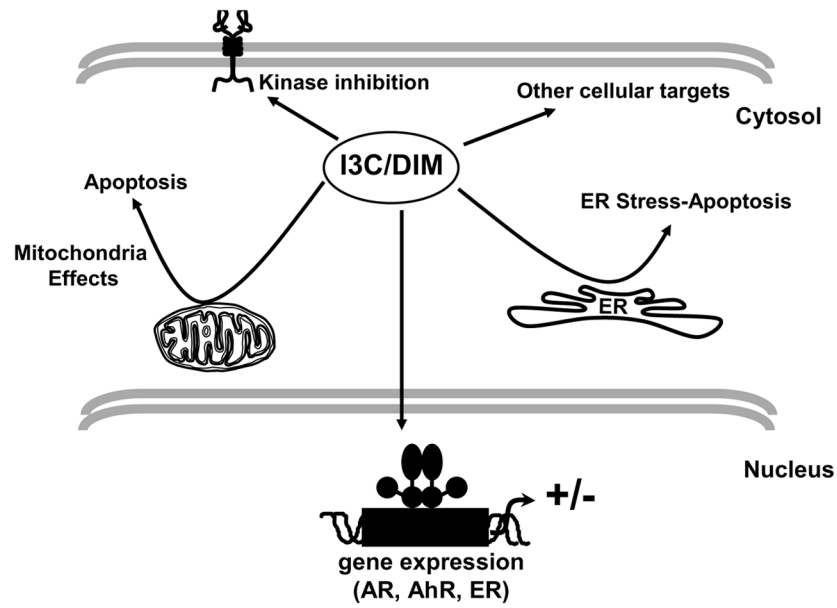
Fig. 2.
I3C and DIM modulate multiple responses and genes in cancer cell lines through several pathways.

Fig. 3.
Comparative effects of DIM and ring-substituted DIMs as inhibitors of carcinogen-induced rat mammary tumor growth in female Sprague–Dawley rats. Animals were treated with 1.0 mg/kg/d DIM or ring-substituted DIMs, and corn oil (100%) served as a vehicle control [77,107, 108]. 5,5′-Dimethyl- DIM (5,5′-Me$_2$), 2,2′-dimethylDIM (2,2′-Me$_2$), 1,1′-dimethylDIM (1,1′-Me$_2$), 4,4-dichloroDIM (4,4′-Cl$_2$) and 5,5′-dibromoDIM (5,5′-Br$_2$) all significantly ($p < 0.05$) decreased tumor weights and volumes (*).
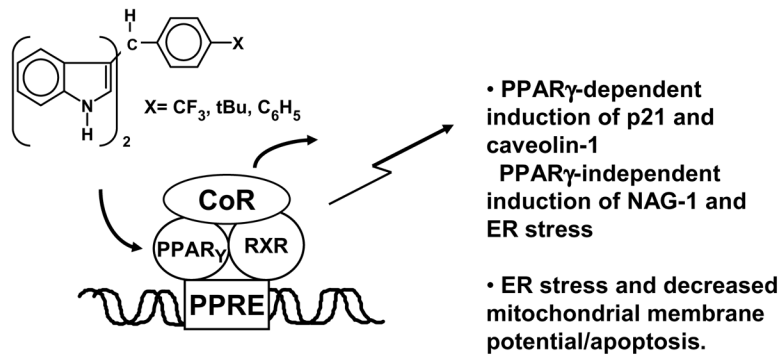
Fig. 4.
PPARγ-active C-DIMs and their effects on cancer cells and tumors [109–118].
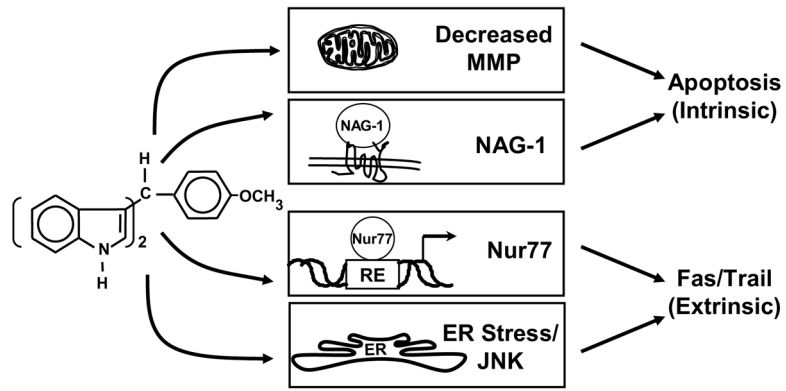
Fig. 5.
Nur77-active D-DIMs and their effects on cancer cells and tumors [119,120].