Invited Review

Multiple therapeutic and preventive effects of 3,3′-diindolylmethane on cancers including prostate cancer and high grade prostatic intraepithelial neoplasia

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Abstract

Cruciferous vegetables belong to the plant family that has flowers with four equal-sized petals in the pattern of a crucifer cross. These vegetables are an abundant source of dietary phytochemicals, including glucosinolates and their hydrolysis products such as indole-3-carbinol (I3C) and 3,3′-diindolylmethane (DIM). By 2013, the total number of natural glucosinolates that have been documented is estimated to be 132. Recently, cruciferous vegetable intake has garnered great interest for its multiple health benefits such as anticancer, antiviral infections, human sex hormone regulation, and its therapeutic and preventive effects on prostate cancer and high grade prostatic intraepithelial neoplasia (HGPIN). DIM is a hydrolysis product of glucosinolates and has been used in various trials. This review is to provide an insight into the latest developments of DIM in treating or preventing both prostate cancer and HGPIN.

Keywords: cruciferous vegetables, 3,3′-diindolylmethane (DIM), indole-3-carbinol (I3C), prostate cancer, high grade prostatic intraepithelial neoplasia (HGPIN)

INTRODUCTION

Cruciferous vegetables

The consumption of fruits, soybean and vegetables has been associated with reduced risk of several types of cancers\cite{4-8}. Cruciferous, or \textit{Brassica} vegetables in Latin, is one category of these vegetables. Cruciferous vegetables arise from plant cells in the family known to botanists as cruciferae or \textit{Brassicaceae}. Plants in the cruciferae family have flowers with four equal-sized petals in the shape of a “crucifer” cross. Many commonly consumed cruciferous vegetables arise from the \textit{Brassica} genus, including broccoli, Brussels sprouts, cabbage, cauliflower, kale, turnips, collard greens, kohlrabi and mustard rutabaga \cite{1,2}. Like other vegetables, cruciferous vegetables contain a number of nutrients and phytochemicals with cancer chemopreventive properties, including folate, fiber, carotenoids and chlorophyll. However, cruciferous vegetables are unique in

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that they are rich sources of glucosinolates, sulfur-containing compounds that are responsible for their pungent aromas and spicy taste. The hydrolysis of glucosinolates by the plant enzyme myrosinase results in the formation of biologically active compounds, including indoles and isothiocyanates. Myrosinase and glucosinolates, which are normally compartmentalized separately in intact plant cells, are brought together during the processes of cutting, freezing, pressurizing or cooking the vegetables, thus facilitating the formation of indole-3-carbinol. By 2011, more than 132 glucosinolates with unique hydrolysis products have been identified in plants. For example, broccoli is a good source of glucoraphanin, the glucosinolate precursor of sulforaphane, and glucobrassicin, the glucosinolate precursor of indole-3-carbinol.

**Indole-3-carbinol (I3C)**

I3C is the initial compound released from cruciferous glucobrassicin by the action of myrosinase and this compound has proved to be highly instable both in vitro and in vivo. I3C given orally is almost immediately converted to a number of condensation products in the acidic environment of the stomach in varying proportions depending upon the exact pH, including a dimer (3,3'-diindolylmethane, DIM), trimers (linear trimer, LTR: cyclic trimer, CTR; indolocarbazole, ICZ), ascorbigen, and ascorbic acid although DIM predominates. In multiple studies, measuring plasma I3C after oral administration of I3C to mice showed only a transient blood level of I3C at 15 minutes. Other testing found no I3C in the plasma even at the earliest 1 hour time point tested after giving single one-time dose. Some chronic studies also showed that I3C can result in hepatocyte hypertrophy and P-450 enzyme induction, and CYP1A2 induction. Although studies have confirmed antitumor activity in I3C against cancers of breast, endometrium, colorectal and prostate, I3C instability and toxicity associated with enzyme induction have limited the use of I3C as a chemoprotective agent.

**3,3’-diindolylmethane (DIM)**

Under acidic conditions, I3C is converted to an estimated 15 or more other oligomeric compounds thought to be responsible for its biological effects in vivo and DIM is the unique dimer form from I3C among these oligomeric products. Approximately 10–20% of I3C is estimated to be metabolized to DIM and the number may fluctuate due to possible variability of conversion of I3C to DIM. To date, DIM has been studied more extensively than any other I3C metabolites.

Studies in vitro and in vivo prove that DIM provides a more predictable response than I3C. DIM should be regarded as the chemoprotective compound of choice. Because obtaining DIM from the consumption of cruciferous vegetables is not a practical way to obtain the daily amounts of DIM, semisynthetic or synthetic sources of cruciferous indole compounds can now be obtained commercially.

**DIM’s CRITICAL HEALTH BENEFITS FOR HUMANS**

**DIM shows anti-proliferative property in cancers**

The consumption of fruits and vegetables has been associated with reduced risk of some types of cancers. In vitro and in vivo studies have demonstrated that the dietary components from cruciferous vegetables have inhibitory effects on human cancers through multiple mechanisms, suggesting that they may serve as chemopreventive agents. One challenge is separating the benefits of diets that are non-specifically rich in vegetables from those that are specifically rich in cruciferous vegetables including DIM and indole-3-carbinol.

DIM has recently received much attention amid the public and the medical society. In vitro studies have found that DIM has anti-proliferative and anti-cancer activities in various cancer cells including prostate, breast, endometrial, colorectal and pancreatic cancers, and leukemic cells. DIM inhibits the proliferative and anti-metastatic, PSA relapse.

In a phase I study using DIM, all 12 prostate cancer patients (castrate-resistant, non-metastatic, PSA relapse) had 50% PSA decline (1 patient), stabilization (1 patient), or PSA deceleration of their PSA rise (10 patients). DIM treatment inhibited growth of cancer stem cells in a phase II clinical trial through downregulation of EZH2 expression in prostate cancer tissue.
The anti-proliferative action from DIM may be mediated through its abilities to induce G1/S arrest of the cell cycle\(^{[24,28-30]}\), induce apoptosis\(^{[31-35]}\), inhibit angiogenesis\(^{[30-36]}\), regulate sex hormones and their receptors\(^{[39-45]}\), anti-inflammatory activity\(^{[46-48]}\), and other potential anti-carcinogenic properties\(^{[11]}\). uPA (urokinase-tyle plasminogen activator) and its receptor uPAR is a tumor-associated serine protease which is linked to tumor progression and metastasis. DIM has been reported to inhibit cell growth and migration by downregulating uPA and its receptor uPAR in prostate cells\(^{[49]}\) or inhibit cancer stem cell growth\(^{[27]}\).

**DIM causes G1/S arrest in cell cycle**

In studies on G1/S cell cycle arrest caused by DIM, researchers found that DIM inhibited cells progressing from G1 to S phase by downregulating cyclin D1\(^{[31,50]}\) and cyclin E\(^{[24,50]}\), cyclin-dependent kinase CDK2\(^{[20,30]}\), CDK4\(^{[20,29,50]}\) and CDK6\(^{[24,90]}\), but upregulating cell cycle inhibitor p15\(^{[24,90]}\), p21\(^{[24,51]}\) and p27\(^{[24,28-30]}\). In a study using LNCaP cell line, DIM inhibited the increase of cyclin D1 and CDK4 mediated by R1881, a synthetic androgen steroid. In in vitro breast cancer lines, DIM modulates p27 through transcription, prolongation of protein half-life, and nuclear localization; these effects are independent of Akt and estrogen receptor status\(^{[52]}\). In a study using both androgen-dependent LNCaP and androgen receptor negative DU145 prostate cancer cells, DIM-mediated G1 cell cycle arrest involves activation of the p38 MAPK and Sp1 pathways in the DU145 cells regardless of their androgen-dependence and p53 status\(^{[39]}\). DIM-induced cell arrest can be also mediated by inhibiting mitochondrial ROS (mitochondrial reactive oxygen species) release\(^{[51]}\). Several studies found that induction of p21 expression by DIM was independent of p53\(^{[21]}\). In oral squamous cell carcinoma cell lines, DIM induced G2/M cell cycle arrest via DIM’s suppressive effect on the expression of cyclin B1 and cdc25c\(^{[54]}\). Except for its cell cycle effects in prostate cancer cell lines, DIM has also been found to downregulate cell cycle regulators or upregulate cell cycle inhibitors in other cancer cell lines including esophageal cancer\(^{[50]}\), colorectal cancer\(^{[54]}\), breast cancer\(^{[24]}\), endometrial cancer\(^{[24]}\), oral cancer\(^{[53]}\), and leukemic cells\(^{[24]}\).

**DIM causes cell apoptosis**

Results from various research groups have revealed that DIM induces cell apoptosis through two mechanisms, either by downregulating anti-apoptotic gene products including Bcl-2, Bcl-xL, survivin, inhibitor-of-apoptosis protein (IAP), X chromosome-linked IAX (XIAP), and Fas-associated death domain protein-like interleukin-1-beta-converting enzyme inhibitory protein (FLIP), or by upregulating proapoptotic protein Bax, release of mitochondrial cytochrome C, p53, N-myc downstream regulated gene-1, activation of caspase-9 and caspase-3\(^{[24,31-34,36-38]}\).

Survivin is an inhibitor of apoptosis and is associated with both prostate cancer progression and drug resistance. Targeting survivin could enhance therapeutic efficacy in prostate cancer. In a study using DIM and Taxotere, DIM enhanced Taxotere-induced apoptotic death in LNCaP and C4-2B prostate cells and bone tumor cells by decreasing survivin expression, AR expression and nuclear factor-kappaB (NF-κB) DNA-binding activity\(^{[33]}\). In an *in vitro* study, DIM inhibited cell proliferation and induced apoptosis via inhibition of Akt activation, NF-κB, AR phosphorylation and expression PSA, and p38 mitogen-activated protein kinase (p38 MAPK)\(^{[32,53]}\). DIM could activate the AMPK signaling pathway and downregulation of androgen receptor expression, and induction of apoptosis\(^{[51]}\). In an *in vitro* study using physiologic concentrations, DIM caused cell growth inhibition and this effect is only associated with apoptosis but not cell cycle arrest\(^{[50]}\).

p75 (NTR, neurotrophin receptor) functions as a tumor suppressor in prostate epithelial cells and its expression declines when malignant cancer progresses. In a study comparing anti-inflammatory drugs and chemopreventive agents including ketorolac, etodolac, indomethacin, 5-methylindole-3-acetic acid and I3C, DIM exhibits the greatest activity of induction of p75 (NTR) levels and inhibition of cell survival in prostate PC-3 and DU-145 cancer cells and this p75 (NTR)-dependent apoptosis is via the p38 MAPK pathway\(^{[50]}\).

**DIM causes anti-angiogenesis**

Many articles also report anti-angiogenic effect from DIM\(^{[30,36-38,50-61]}\). DIM can inhibit angiogenesis via different pathways. In PDGF-D (platelet-derived growth factor-D)-overexpressing PC-3 cells, DIM inhibits invasion and angiogenesis in this cell line by reducing the tube formation of human umbilical vein endothelial cells (HUVECs) via inactivation of both mTOR (mammalian target of rapamycin) and Akt activity\(^{[37]}\). DIM can also effect modulations of downstream transcription factors NF-kB signaling\(^{[52]}\). In a separate article from the same research group, DIM inhibited angiogenesis and cell invasion by reducing the bioavailability of vascular endothelial growth factor via repressing extracellular matrix-degrading proteases, such as matrix metalloproteinase-9 and urokinase-type plasminogen activator\(^{[50]}\).

In *in vitro* and in *in vivo* studies, DIM inhibits angiogenesis via inactivation of Akt and, at least in part, of
ERK1/2. In separated studies, Chang et al. found that DIM inhibits vascular endothelial growth factor (VEGF)-induced cell proliferation and DNA synthesis in HUVECs via Ras signaling\textsuperscript{60,61}. In multiple thyroid cancer cells, this anti-angiogenesis may be through inhibition of VEGF by anti-estrogen effect of DIM\textsuperscript{61}.

**DIM has anti-inflammatory activity**

Recurrent or chronic inflammation has been implicated in the development of a variety of human cancers\textsuperscript{60,61}. Inflammation promotes cellular proliferation, angiogenesis, inhibits apoptosis, and induces DNA damage, increasing the risk of developing cancer\textsuperscript{61-64}. An estimated 20% of adult cancers are attributable to chronic inflammatory conditions caused by infectious agents, chronic non-infectious inflammatory diseases and/or other environmental factors\textsuperscript{63}.

In a mice in vivo study, DIM exhibited a significant therapeutic effect in the experimental colitis model and decreased the number of colitis-associated colon tumors\textsuperscript{60}. This anti-inflammatory activity was associated with reduction of proinflammatory cytokines\textsuperscript{60}. In a study for nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1), DIM increased the expression of NAG-1 as well as activating transcription factor 3 (ATF3)\textsuperscript{60}. The anti-inflammatory effects of DIM may also be through the suppression of NF-κB activities\textsuperscript{60}.

Using mouse skin models, DIM pretreatment effectively inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ear edema formation via the downregulation of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), C-X-C motif chemokine 5 (CXCCL5) and interleukin-6 (IL-6) expression which may be mediated by reductions in NF-κB activation\textsuperscript{16,61}. These results show that DIM exerts anti-inflammatory and chemopreventive effects, which may be related to its anti-tumor property.

**DIM stimulates antioxidant response**

DIM has demonstrated excellent chemopreventive properties against carcinogenesis by regulating the redox status of the cells during oxidative stress\textsuperscript{62} through the antioxidant transcription factor NFE2L2 (Nrf2) and antioxidant response element\textsuperscript{60-63}. Nrf is a transcription factor and the antioxidant response element (ARE) is a critical regulatory element for the expression of antioxidant enzymes. DIM induces Nrf2-ARE-mediated gene expression that could potentially enhance cancer chemopreventive activity\textsuperscript{60}. In multiple animal models, DIM upregulates BRCA1 expression, which has antioxidant activity, and this induces Nrf2-ARE-mediated gene expression via BRCA1-dependent man-

**DIM has other anti-carcinogenic properties**

A few articles also have reported other anti-carcinogenic properties of DIM, including epigenetic modulation, anti-viral, anti-bacterial effects, and DNA methylation. In a tumor cell culture model, DIM decreases invasive properties of the tumor cells and these properties were associated with reduction of MMP-1 and MMP-12 (matrix metalloproteinases)\textsuperscript{61,62}. DIM can also alter gene expression and reduce cell growth by inhibiting DNA methylation\textsuperscript{63}. More trials are needed for further investigation\textsuperscript{11}.

**DIM REGULATES SEX HORMONES, THEIR RECEPTORS AND TARGET GENE EXPRESSIONS**

Many publications have identified effects from DIM on sex hormones, hormone receptors, including estrogen receptor and androgen receptor, and their signaling\textsuperscript{24,39-43,73,78-82}, therefore preventing prostate cancers in men. DIM is a unique bifunctional hormone disrupter for estrogen and androgen\textsuperscript{50,74}.

**DIM regulates estrogen and estrogen receptor in prostate cancer**

Researchers have identified that estrogens, including 17-beta-estradiol (E2), play an important role in the development and progression of prostate cancer\textsuperscript{[26,44,84]}. Their actions are mediated by estrogen receptors (ERs), particularly ERβ in the prostate epithelium\textsuperscript{[44]}. ERα is found primarily in stromal cells of the prostate and appears to regulate the growth of prostatic epithelial cells, but ERβ is the predominant ER subtype in the prostatic epithelium\textsuperscript{[44]}. A new discovery using newly generated isoform-specific antibodies has found that ERβ can be divided into isoforms ERβ1-5 in prostate cancer\textsuperscript{[44,45]}, exerting their estrogenic effects by binding to estrogen to form the estrogen-ER complex, then further combine with DNA sequences in genes known as estrogen response elements to enhance the transcription of estrogen-responsive genes\textsuperscript{[41]}. Evidence suggests that E2 contributes to the risk of prostate cancer\textsuperscript{[59]}. Using LNCaP cells, an E2 sensitive cell line, E2 induces cancer cell proliferation and stimulates PSA expression, which is possibly related to its effects on androgen receptor (AR) as this E2-induced stimulation could be inhibited by the anti-androgen drug caso-
ERβ2 is commonly found in the cytoplasm which is the most abundant isoform, but ERβ1 is predominantly located in the nucleus and ERβ3 primarily in the cytoplasm. The study further found that nuclear ERβ2 (nERbeta2) is an independent prognostic marker for PSA failure and postoperative metastasis (POM) and the combined expression of both ERβ2 and ERβ3 can identify patients with the shortest POM-free survival[40]. But in a study using MCF-7 cells, DIM significantly reduced ERα mRNA expression in breast cancer cells and this effect is likely through the binding of DIM as a ligand to the nuclear aryl hydrocarbon receptor (AhR)[46].

DIM has effects on estrogen in two ways: affecting estrogen metabolism and regulating ER activity. For its metabolic effects, DIM can increase the conversion of E2 into 2-hydroxyestrone (2-OHE), a “good” estrogen metabolite with anti-estrogenic and anti-proliferative properties[79,80] while decreasing the conversion of E2 into 4-hydroxyestrone (4-OHE) or 16-α-hydroxyestrone (16-α-OHE), both “bad” estrogen metabolites that can increase cell proliferation[42]. DIM also increases urinary 16-α-OHE levels in postmenopausal women[70]. For its effects on ER, DIM can inhibit the transcription of estrogen-responsive genes stimulated by E2, and increase ERα protein degradation[40,45,42].

A separate study shows that DIM’s estrogenic effects can be mediated through ERβ but not ERα activation of estrogen response element and DIM selectively activated multiple endogenous genes through ERβ[40]. The possible mechanisms by which DIM induces these effects do not involve binding to ERβ but may involve a ligand-independent mechanism by recruiting coactivators to target genes[41]. It was reported that induction of p21 expression by DIM was independent of estrogen-receptor signaling[21].

**DIM’s effects on androgen, androgen receptor (AR) and prostate-specific antigen (PSA)**

The male sex hormones, androgens mainly including testosterone and DHT, mediate their effects by binding to AR, forming androgen-receptor complex in cytoplasm and then translocating into the nucleus. The complex in the nucleus then binds to a DNA segment in androgen-responsive genes, the androgen response element, to regulate gene transcription. By turning the genes on or off, androgen and AR help direct the development of male sexual characteristics. In prostate cancer, androgen and AR are critical in oncogenesis and cancer growth[15,16,23,25].

It has been shown in vitro[29,31-33] and in vivo studies[26,57] that DIM has an anti-androgen effect which downregulates AR and PSA. In androgen-dependent LNCaP cells, DIM suppresses cell proliferation, inhibits DHT stimulation of DNA synthesis and endogenous PSA transcription and suppresses androgen-induced AR translocation into the nucleus[81]. Similar effects of DIM were observed in PC-3 cells only when these cells were co-transfected with a wild-type androgen receptor expression plasmid[26].

DIM results in cell cycle arrest in androgen-sensitive LNCaP cells and androgen-insensitive C4-2B cells not only by affecting cell cycle regulatory molecules including cdks and their inhibitors but also by downregulating AR[32]. In the same cell lines, DIM was found to promote cell cycle arrest and cause apoptosis that may be associated with effects on Akt and AR signaling pathways by affecting Akt/FOXO3a/GSK-3β/catenin signaling[46]. In a separate study, the same research group found that B-DIM’s anti-androgenic effects were associated with inhibition of AR phosphorylation, AR expressions and AR nuclear translocation, leading to the down-regulation of AR target genes[42]. The anti-androgen effect from DIM is not associated with prostate cancer cell’s androgen status[29]. The results of structural modeling studies showed that DIM is remarkably similar in conformational geometry and surface charge distribution to an established synthetic AR antagonist[81]. These observations provide rationales for DIM in treating hormone-sensitive, but more importantly hormone-refractory prostate cancer by using DIM alone or combining with other therapeutics[32].

**DIM’S THERAPEUTIC AND PREVENTIVE EFFECTS ON PROSTATE CANCER AND ITS PRECURSOR**

**DIM’s use in prostate cancers**

Epidemiological studies in in vitro and in vivo animal models have shown that DIM has anticancer properties in treating multiple cancers including prostate cancer[17-49,54-69]. Using prostate cancer cells including LNCaP, PC-3, DU145 and C4-2B, studies have shown that DIM has anti-prostate cancer properties involving apoptosis[45,45,45,46,47,48,49,50], anti-proliferation[25-34], anti-angiogenesis[35,36], cell cycle arrest[24,26-30], or anti-inflammatory activity[29,44,78]. In mice injected with TRAMP-C2 mouse prostate cancer cells, DIM significantly reduced tumor development to 50% compared with controls and tumors in treated mice were significantly smaller (P < 0.001) than tumors in the control groups[25]. In a phase I study, all 12 patients (castrate-resistant, non-metastatic, PSA relapse prostate cancer) had an initial PSA deceleration of their PSA rise and 2/12
experienced a 50% PSA decline or stabilization\textsuperscript{[20]}\textsuperscript{6}. The precise molecular mechanisms for these effects may involve regulations of Akt, NR-\kappaB, VEGF, AR signaling pathway, uPA/uPAR, MAPK pathway, let-7/DZH2\textsuperscript{[26]}\textsuperscript{90} and p75(NTR).

In recent reports, novel uses of DIM have been designed to enhance the overall efficacy of its preventive and therapeutic effects in vaginal neoplasia\textsuperscript{[17-91]}\textsuperscript{,} cervical dysplasia\textsuperscript{[17,83,92]}\textsuperscript{,} mastalgia\textsuperscript{[13]}\textsuperscript{,} leiomyomas, respiratory syncytial virus infection and atherosclerosis\textsuperscript{[17]}\textsuperscript{.} Recent articles have demonstrated that DIM is a unique bifunctional hormone disruptor for both estrogen and androgen\textsuperscript{[162]}\textsuperscript{,} Together with its multiple effects on cancer cells, including anti-angiogenic properties, inductive effects on cell apoptosis and G1/S cell cycle arrest, DIM is an ideal agent that could be used to prevent the progression of prostate cancers.

**High grade prostatic intraepithelial neoplasia (HGPIN)**

HGPIN has been thought as the precursor of prostatic adenocarcinoma and is now accepted as the most likely preinvasive stage of the cancer. Most patients with HGPIN will develop carcinoma within 10 years\textsuperscript{[94]}\textsuperscript{1}. The results from different researches are controversial in that it is unsure whether or not HGPIN can result in an elevation of PSA\textsuperscript{[95-97]}\textsuperscript{.} Development of HGPIN may involve multiple mechanisms including chronic or recurrent inflammation\textsuperscript{[99,100]}\textsuperscript{,} loss of function of apoptotic regulators\textsuperscript{[99,100]}\textsuperscript{,} elevated expression of angiogenic proteins\textsuperscript{[100,101]}\textsuperscript{,} loss of cell cycle inhibitor p27\textsuperscript{[99,102]}\textsuperscript{,} virus infection\textsuperscript{[1]}\textsuperscript{,} or gene mutations\textsuperscript{[103]}\textsuperscript{.} DIM may be one of the most hopeful agents in preventing HGPIN from becoming prostate cancer.

The lack of treatment options for HGPIN remains an enormous obstacle in prostate cancer prevention and no cure has been offered for this stage of disease\textsuperscript{[95,102,104]}\textsuperscript{.} In the Prostate Cancer Prevention Trial (PCPT), HGPIN was treated with finasteride or placebo in 18,882 men. The investigators found that adenocarcinomas with high-grade appearance were more common in the finasteride group than the placebo group, casting doubt on the therapeutic effect of finasteride on HGPIN. Whether or not finasteride promotes high-grade prostate cancer remains a subject of intense ongoing debate\textsuperscript{[102,105]}\textsuperscript{.} A trial on men with HGPIN using toremifene showed a decrease of prostate cancer incidence at a lower dose but not in high doses, making it difficult to interpret the study, and larger studies will be necessary to substantiate these findings\textsuperscript{[102]}\textsuperscript{.}

Recent studies have provided an interesting trend in treating cervical intraepithelial neoplasia (CIN), which is believed to be associated with human papilloma virus (HPV) infection\textsuperscript{105}\textsuperscript{6}. CIN is the precursor of cervical cancer with a histologic similarity to HGPIN, and inflammation is likely an etiological factor for both CIN and HGPIN\textsuperscript{[102,106]}\textsuperscript{.} In a clinical study, DIM treatment has led to a high rate of clinically significant improvement in 64 patients with CIN\textsuperscript{[100]}\textsuperscript{.} In separated trials, DIM can inhibit or delay the progression from CIN or cervical dysplasia to cervical cancers\textsuperscript{[102,103]}\textsuperscript{.} To date, there have been no trials reported, focusing on HGPIN using DIM or I3C, yet.

Natural chemopreventive agents such as selenium and lycopene have been found with therapeutic effects in treating HGPIN\textsuperscript{[104,107]}\textsuperscript{.} New studies designed to use DIM in treating HGPIN are in a strong demand. DIM is well-tolerated and has not shown any side effects in preventive or therapeutic research studies\textsuperscript{[26,92]}\textsuperscript{.} In a phase I study in patients, 225 mg DIM twice daily was determined to be safe and did not affect body weight, kidney and liver functions, and was recommended for phase II testing\textsuperscript{[26]}\textsuperscript{.} Another clinical study showed that oral DIM at 2 mg/kg/day was well tolerated with no significant toxicity\textsuperscript{[92]}\textsuperscript{.} DIM has been reported with therapeutic effects to overcome drug-resistance from some forms of carcinoma to regain sensitization in patients diagnosed with drug-resistant tumors\textsuperscript{[108]}\textsuperscript{.}

**COMMENTARY**

Though risk factors for prostate cancer and HGPIN such as age, race, and family history cannot be modified, other factors can be modified including lifestyle in general and, specifically, nutritional, environmental, and hormonal factors. Thus diet-derived compounds have the potential to be safe and natural inhibitors of cancer\textsuperscript{[102]}\textsuperscript{.} The available data support the efficacy of DIM as an ideal supplement for prostate cancer and HGPIN. In in vitro or in vivo studies using cell lines and animal models, supplementation with DIM displays multiple effects of anticancer and anti-proliferation with the mechanisms related to cell cycle, angiogenesis, apoptosis, antioxidant, anti-inflammation, and regulation of sex hormones and receptors. More studies are needed to address the treatment of HGPIN. DIM, as a phytochemical from natural vegetables, has emerged as a strong contender in the arena of cancer chemoprevention, showing promise for treating prostate cancer and HGPIN. Its multiple anticancer properties make the agent one of the most hopeful phytochemicals in treating and preventing prostate cancer and HGPIN.
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