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REVIEW ARTICLE

Molecular Targets, Anti-cancer Properties and Potency of Synthetic Indole-3-carbinol Derivatives

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Abstract: The indole-3-carbinol (I3C) displays anti-cancer/proliferative activities against human cancer cells. Cellular proliferation is an event associated with the progress and its continuation. This manifest is described by variation in expression and/or functions of genes that are related with cell cycle relevant proteins. The constitutive activation of several signal transduction pathways stimulates cells proliferation as well. The immediate stages in cancer development are accompanied by a fibrogenic response and the progression of the hypoxic environment is in favor of survival and proliferatory functions of cancer stem cells. A main part for prevention of in cancer cells death may manifest through altering cell metabolism. Cellular proliferation and metastasis are reported to be supported with increased generation of responsible hormones (in hormone dependent malignancies), and further promotion the angiogenesis, with epithelial to mesenchymal transition. This may be facilitated by progression of autophagy phenomenon, as well as *via* taking cues from neighboring stromal cells. Several signaling pathways in association with various factors specific for cellular viability, including hypoxia inducible factor 1, NF-κB, insulin-like growth factor 1 (IGF-1) receptor, Human foreskin fibroblasts (HFF-1), phosphoinositide 3 kinase/Akt, Wnt, cell cycle related protein, with androgen and estrogen receptor signaling are reported to be inhibited by I3C. These evidences, in association with bioinformatics data represent very important information for describing signaling pathways in parallel with molecular targets that may serve as markers for early diagnosis and/or critical targets for designing and development of novel therapeutic regimes alone or combined with drugs, to prevent tumor formation and further progression. In particular, I3C and DIM have been extensively investigated for their importance against numbers human cancers both *in vitro* and *in vivo*. We aimed the present manuscript, current study, to review anticancer properties and the miscellaneous mechanisms underlying the antitumorigenicity in an in-depth study for broadening the I3C treating marvel.

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1. INTRODUCTION

Cancers are defined as combined uncontrollable growth and expansion of abnormal cells which without effective and timely therapeutic intervention cause death. Several therapeutic procedures including surgery, radiation, and chemotherapy are employed for cancer treatment, to date. The conclusive goal of these remedies is either treatment or prolong and improvement of the patient's quality of life, in addition to and duration of survival. However chemotherapy has led to progression in this trend, drug resistance and related toxicities have remained as major limitations for improving the overall response and survival from cancer in patients [1-4]. Drug resistance is divided into two categories as intrinsic (which is also known as de novo type) and acquired form [5]. The intrinsic resistance causes ineffective therapy from initial point, due to intra tumor tissue the presence of resistant phenotypes of tumor cells. In other words, the acquired form of drug resistance develops during treatment whenever tumor cells exhibit initial responsiveness to the anticancer

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drugs which in turn further leads to ineffective therapy and thus results is tumor growth and development [6, 7]. Indole fits in aromatic heterocyclic organic derivatives and waving a bicyclic structure, composing a six-membered ring which is fused into the five-membered nitrogen-containing pyrrole ring [8]. The epidemiological reviews and animal based investigations reported the beneficial effects of I3C and its analogs against tumorigenesis (Fig. **1**) [9]. Studies have also provided evidences that consumption of high dietary cruciferous vegetables enables consumers to attenuate cancer risk [10-14]. Accommodating evidences attribute these chemo-preventive effects to the antitumor activity of I3C as well as its related metabolic side products. The relative derivatives of indole were reported to limit proliferatory activities of several tumor cells (at the concentrations ranged from 50 to 100 μM), such as breast [15-18], colon [19, 20], prostate [21, 22], and endometrium [23], *via* targeting a broad variety of signals which and able regulate hormonal homeostasis, development of cell cycle, in addition to cells growth and survival [24-28]. Additionally, I3C has inhibited the spontaneous or chemically-induced tumorigenesis in several mammalian organs including, mammary glands, liver, cervix, lung, along with gastrointestinal tract in animal models [29-31]. Current pre-clinical findings are in favor of the translational advantage of I3C in cancer development as a therapeutic target [32], in vulvar intraepithelial neoplasia [33] cervical dysplasia [34], revolving respiratory papillomatosis [35], vulvar intraepithelial neoplasia [33], and breast cancer [36]. In mechanistic perspective, the I3C efficacy is raised by the interaction among their metabolic disposition and pleiotropic fashion *in vivo*, which would pharmacologically be exploited to foster novel strategies or development of newly chemo-preventive/therapeutic reagents [14, 24]. Hence, it is likely that the I3C is perhaps effective in the tumor therapy. Regardingly, in the current review article we aimed to focus on the paramount roles played by I3C in both tumor therapy and pathogenesis. Accordingly, in present review article we tried our best to address the latest reviews discussing relevance between I3C and pathogenesis of tumors. In addition, to the above fact current study was also aimed to address the multifactorial aspects of the chemical biology of I3C with giving a narrative overview within next coming parts of this manuscript:

2. INDOLIC COMPOUNDS

All chemical structure containing indole rings are nominated as indolic compound. Indole, compounds are heterocyclic organic with aromatic features, composing a bicyclic structure, including a six-membered ring which is fused to a five-membered nitrogen-containing pyrrole ring. A previous review which has assessed 206 epidemiological studies in parallel with 22 animal based investigations reported the beneficiary effects of I3C against tumorigenesis of glucosinolates with an indole side chain responsible for further forming of indoles [9]. Another study has evidenced that in taking of cruciferous vegetables is significantly associated with attenuated risk of bladder tumor [37]. Cruciferous vegetables are of the main sources of a number of phytochemicals, including indole derivatives, dithiolthiones, and isothiocyanates. Primarily, indoles economically were considered as substantial dye stuffs (*e.g*., indigo) as similar as, vinca alkaloids (*e.g*., vincristin, vinblastin) which were initially separated from Catharanthus roseus (Madagascar periwinkle) fitting within the active antitumor reagents that target tubulin [38]. Staurosporin, which was initially isolated from Streptomyces staurosporeus, is a lead compound and blueprint for the progress of kinase inhibitors. Its purified form is employed as a promising candidate (nominated as sunitinib and enzastaurin) and both are presently undergoing progresses of clinical trials for tumor therapy [39-41]. The duocarmycins feature of DNA-targeting 5,6,7-trimethoxyindole moieties is well linked to alkylating spirocyclopropylhexadienones which binds to the minor groove of Deoxyribonucleic acid DNA by sequence-selective N3-adenine alkylation. The two well examined indoles exhibiting anticancer properties. Further being consumed (from Brassica vegetable sources), I3C is immediately converted into DIM in stomach where by exposed by gastric acid. These indole derivatives exert pleiotropic anticancer activities through apoptosis induction as well as modulating of various signaling pathways [42].

3. PHARMACOLOGICAL UTILIZATION OF I3C AND DIM FOR DEVELOPMENT OF NOVEL ANTITUMOR REAGENTS

Based on the view point of the mechanistic perspective, the capability of I3C/DIM is targeting of wide range of signals underlies their antitumor effects against different cancer cells with various genetic and cellular abnormalities. Although, I3C and DIM display low to moderate tendencies suppression of tumor cells proliferation, *in vitro*. The metabolic instability and/or unpredictable pharmacokinetic properties are also reported for I3C, DIM *in vivo* [43]. The intrinsic nature of the I3C in acidic milieu is due to the vinyl hemiaminal half of the indole ring [44]. This unique structural feature of I3C potentiates this compound to be acid-catalyzed dehydraed and further condensed to produce several oligomeric products, such as DIM, CTr , CTet, ICZ, and LTr1, *in vivo* [45-48]. The chemo-preventative effect which reported for I3C *in vivo* might be, at least partially, attributable to these types of acid condensation yields. Amongst these, indolic compounds DIM stimulates apoptosis as well as cell cycle arrest pathways in tumor cell lines *via* signaling mechanisms as similar as I3C [22, 49, 50], whilst the functional activities of CTr, ICZ, and LTr1 are related to the nuclear receptors, like AhR and/or ER [51, 52]. Notably tetrameric produced form of CTet has able to suppress breast cancer and other cancer cells growth by preventing CDK 6 along with other cell-cycle related proteins expression, fivefolds more potently than I3C [14, 53, 54].

4. PLEIOTROPIC EFFECTS OF I3C ON SIGNALING TARGETS

The I3C has incredible capacity for both cancer prevention and treatment. I3C exerts its beneficial effects *via* numerous mechanisms (Fig. **2**). Several evidences were observed in favor of the reality that the anticancer properties of I3C are as a results of its potential to target an array of signaling which are governing many biological aspects, including apoptosis, cell cycle improvement, hormonal homeostasis, DNA repair, angiogenesis, and multiple drug resistance

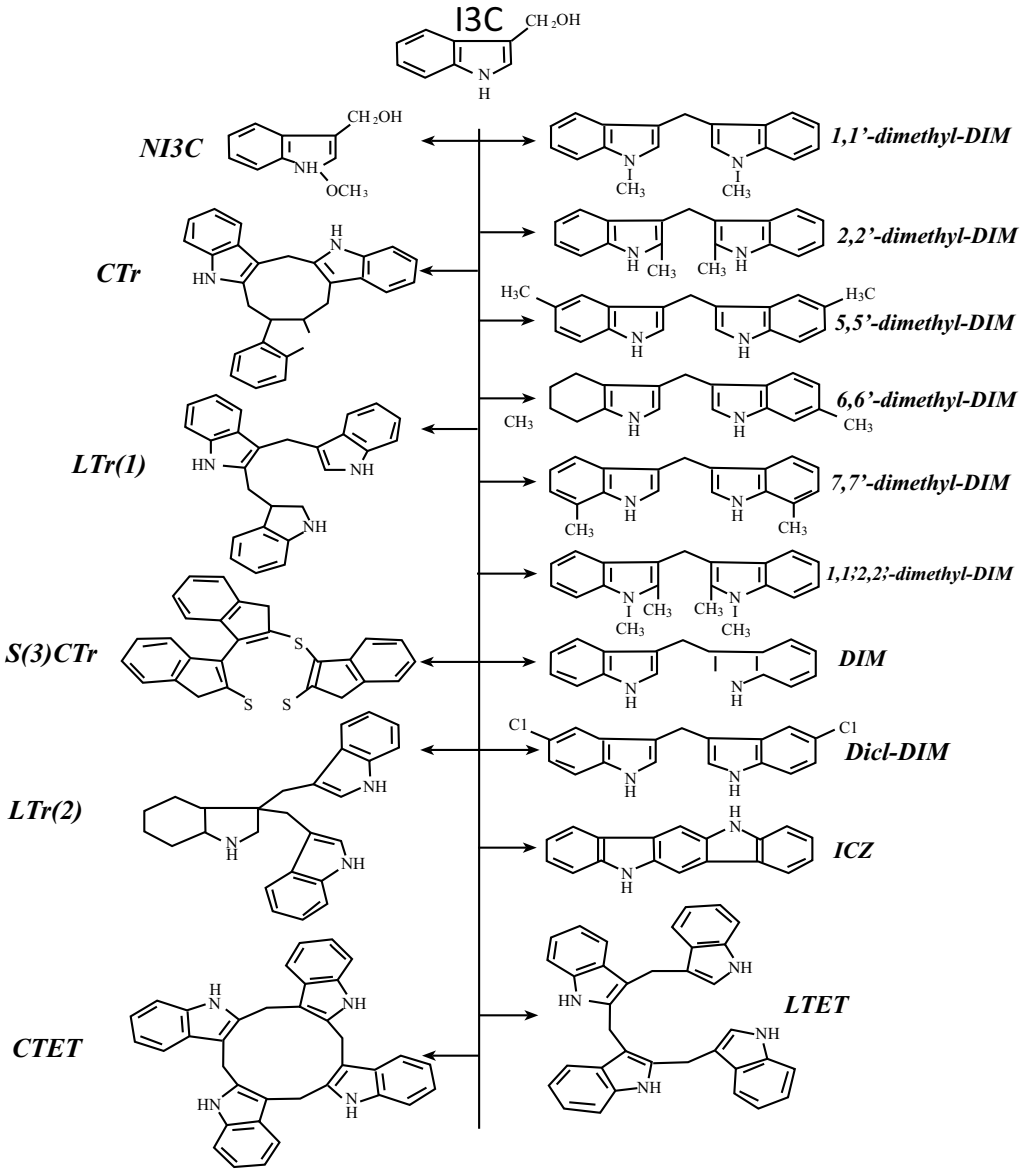

Fig. (1). Structure of I3C and its analogues.

[27, 28]. There with, evidences are provided that I3C functions as a protective agent against breast, cervical and other cancers [55-58]. The pharmacological properties of I3C in various cellular events is well approved, at least in part, with its major metabolite, DIM as the two indole compounds display high degrees of similarity in their fashion of action. Therefore, the present section of this reviews was aimed at providing an insight to of the rational relationship between individual pathway targets, in addition to various I3C activated cellular responses (Fig. **3**) [14].

4.1. Induction of Apoptosis

Several evidences revealed that blocking of Akt and its relative downstream target, the nuclear factor factor-κB (NF-κB) transcription factor, play remarkable in in favors of pro-apoptotic action of I3C/DIM in tumor cells [16, 59, 60]. The Akt also promotes cell survival *via* activating of NF-κB pathway by inhibiting kappa B (IκBα) kinase degradation with phosphorylation activities of a number pro-apoptotic proteins such as GSK3β, Bad, Forehead transcription factors in addition to, caspase-9, playing fundamental parts in cancer therapy [61, 62]. Additionally NF-κB serves as a paramount survival factor for different cancer cells by transcriptional regulation of some antiapoptotic genes, including: Bcl-2 (B-cell lymphoma 2), Bcl-xL, survivin, p53, and p21 [63]. Furthermost it has been demonstrated that I3C is able to activate several pathways such as stress-induced mitogen-activated protein kinases (MAPKs), p38 and cJNK in tumor cells [64], as well as *via* constitutively active signal transducer. The activator of transcription 3 (STAT3) that serves as a unique transcription factor for the pancreatic cancer cells is also in activated by I3C [65]. The effects of indole derivatives on these pro-apoptotic constituents underlie the potency of I3C/DIM to enhance mitochondria independent of tumor cells apoptosis (Fig. **4**) [14].

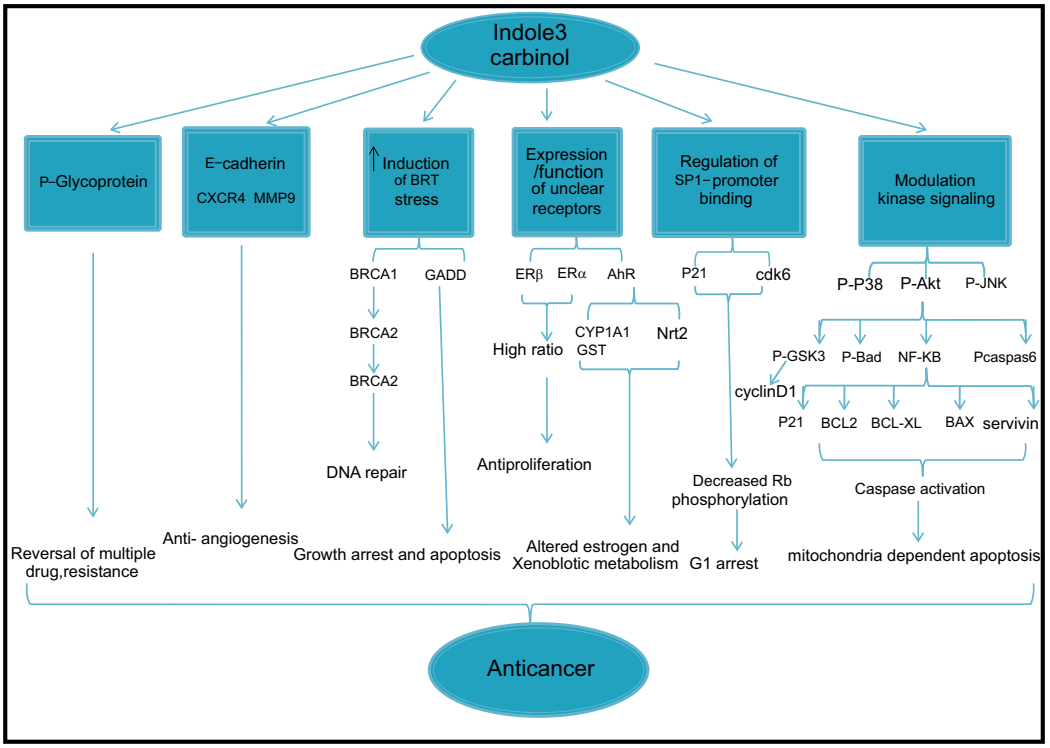

Fig. (2). An overview of the signaling pathways targeted by indole-3-carbinol and DIM.

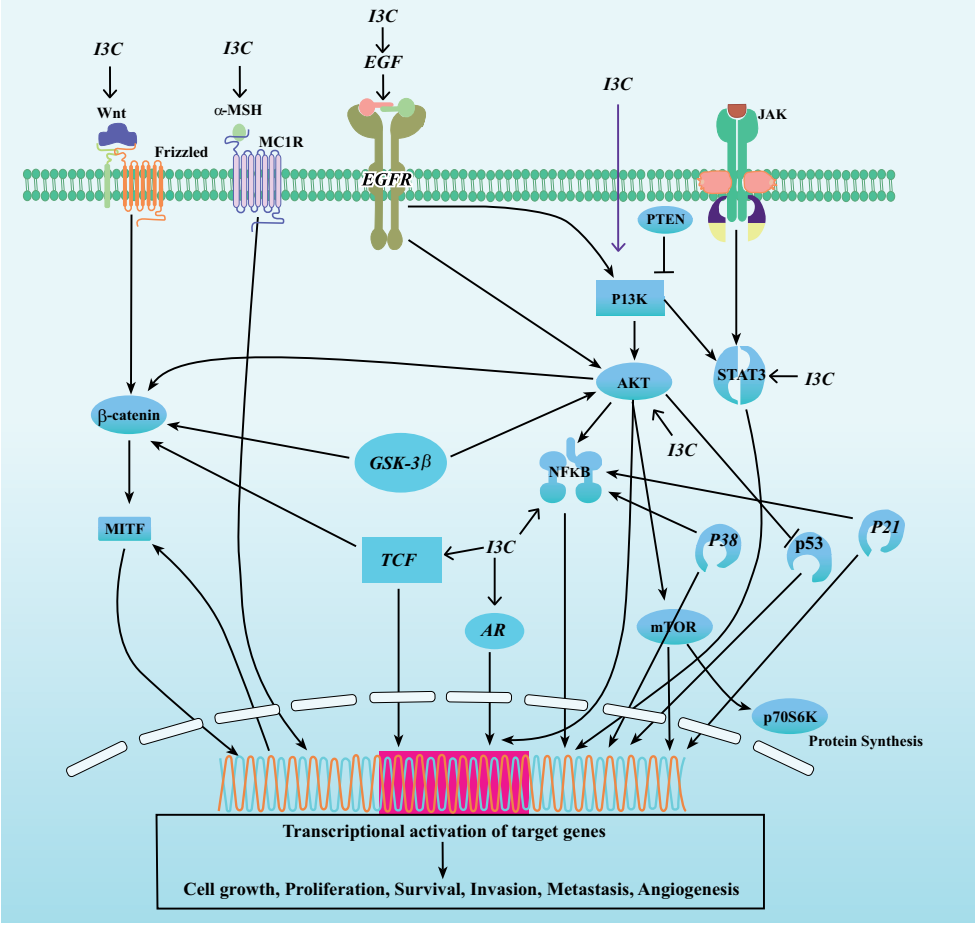

Fig. (3). Summarizing the intracellular pathway by I3C.

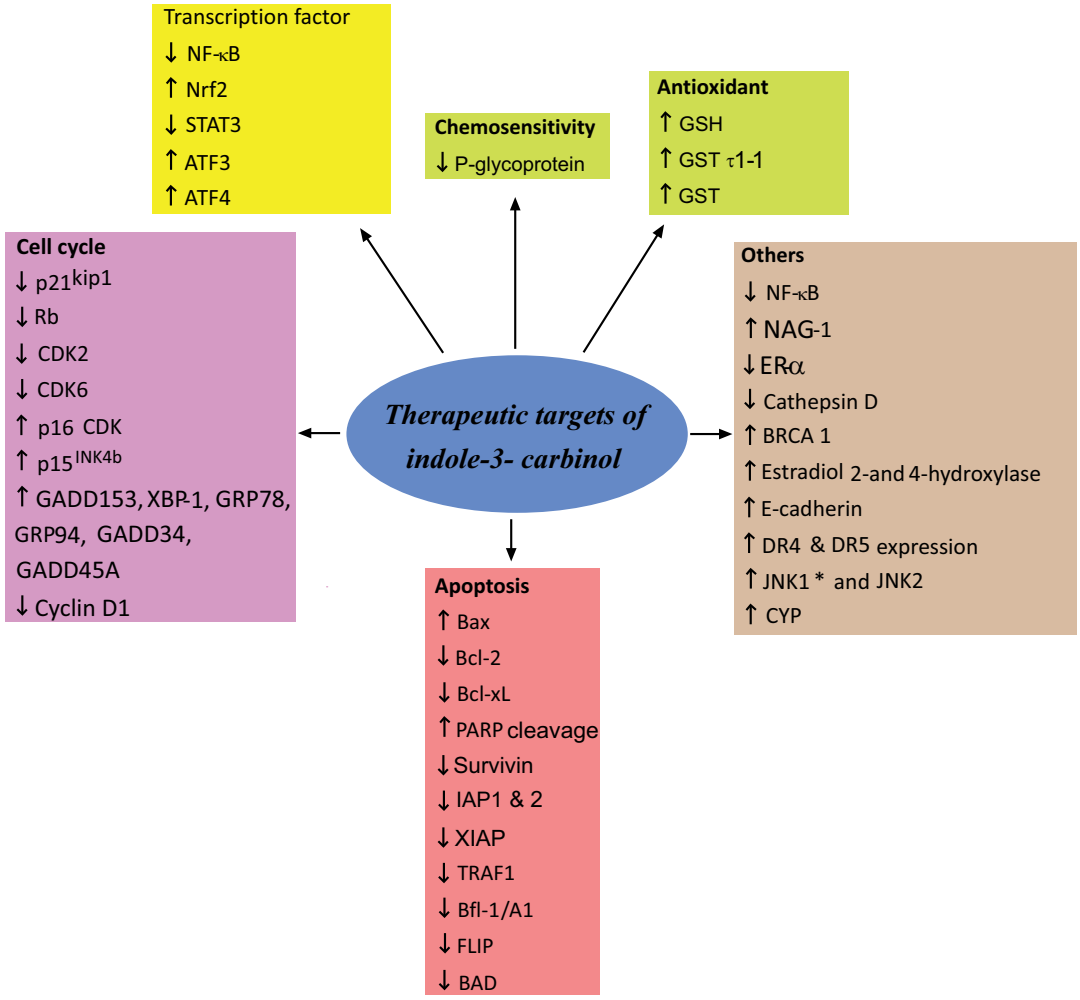

Fig. (4). Therapeutic Targets of I3C and its analogues. Superscript indicates reference number.

4.2. Induction of the Cell Cycle Arrest

Both I3C and DIM exhibit regulatory effects on G1 arrest in cancer cells [66, 67]. Arrest of cell cycle causes enhancement of the namely CDK inhibitors p21WAF1 and p27kip1, which is coincides with down regulation of cyclin D1 and E, and CDKs 2, 4, and 6, that all of them are partially attributable to the effects of I3C/DIM axis on modulating Sp1-promoter binding function [60, 68, 69]. Moreover, I3C and derivatives were revealed to prevent CDK2 kinase activity by breast cancer cells *via* selective changes in cyclin E composition, size distribution, and subcellular CDK2 proteins complex localization [14, 70]. The of CDK4/6/cyclin D1 inhibition in parallel with CDK2/cyclin E functions caused reduced retinoblastoma Rb, and in turn led to Rb protein attachment to the E2 factor (E2F) transcription factor. The sequestration of E2F inhibits the S phase genes transcription, resulting in G1 arrest. Multiple studies have evidenced modulatory effects of I3C on the expression gene and protein (which serve as cell growth inhibitors), such as cdks 2 and 6 reduction, phospho-retinoblastoma protein, cyclin D1 and enhanced expression of various cyclin-dependent kinase inhibitors is modulated by I3C [71-73]. Cancer cells have been reported to inhibit the development of G1 to S phase (which is in association with alteration of the active 90 kDa cdk2 complex to a 200 KDa inactive further I3C treatment [74]. Furthermore I3C has not affected the expression of p27 or p21cdk inhibitors, while these effects on cdk2 yet to be observed for DIM (Fig. **4**) [42].

4.3. I3C and Molecular Mechanisms of Action

It has been well evidenced that I3C regulates the expression of multiple genes, including transcription factors. As figured out in (Fig. **4**), Li and research team have employed laboratory has utilized complementary DNA (cDNA) microarray to study the impacts of I3C and DIM on the expression profile of some genes in PC3 cells [75] and found that about 738 had been changed by more than twofold further 24 h treatment with DIM. of the detected genes 677 were down expressed while 61 exhibited over expression. Likeness, 727 genes were altered by more than twofold and down regulation were observed for 685 genes while 42 genes were upregulated. Expression changes of genes were seen within 6 h and were more considerable when cells were treated in a longer period. Li and group reported that I3C and DIM have upregulated the expression of some of these genes which are related Phase I and Phase II enzymes, applying cluster analysis proposing their enhanced capacity for detoxification of carcinogens and chemicals-detoxification. Researchers stated

that both I3C and DIM have down regulated expression of genes pivotally active in the regulation of several aspects such as cell proliferation, apoptosis, cell cycle, signal transduction through activation of Pol II transcription factor and further oncogenesis. The cDNA microarray method was employed by Carter and colleagues to investigate the early changes in gene expression profile in response to 100 μM concentration of DIM in C33A and epidermoid cervical carcinoma cells (CaSki), in an immortalized HaCat cell line, and normal HFK cell line [76]. Analyses were undertaken further periods of 4h and 12h treatment of C33A and following 6h treatment of CaSki, HaCat and HFK cell lines. DIM has altered the expression of more than 100 genes with at least by twofold. These mostly encode transcription factors and protein molecules that are involved in signaling, stress response and growth. Eight genes encoding Basic Leucine Zipper Domain (bZIP domain) proteins were consistently and robustly upregulated. Induction of stress-associated immediate early DNA damage-inducible gene 153 (GADD153) (over 50 folds in C33A) and the nuclear factor/interleukin 6 (NF-IL6) which also is known as CCAAT-enhancer-binding proteins (or C/EBPs) (over 5 folds in C33A) which are involved in down regulating of HPV oncogenes was also observed. Induction of GADD153, NF-IL6 and Activating Transcription Factor 3 (ATF3) was corroborated with western blot method when functionally analysis. Further functional analyses, DIM not only has repressed the transcription of a luciferase gene driven with Human papillomavirus type 11 (HPV-11) the URR in C33A, CaSki, HaCat and HFK cell lines from two-fold to 37-fold (varied from cell to cell type), but it has also decreased endogenous levels of transcription of HPV16 oncogenes to undetectable levels in CaSki cells. The exogenous expression level of GADD153 or NF-IL6 was repressed transcription driven with the HPV11 URR in a dose-dependent fashion in C33A, CaSki, HaCat and HFK cells. The Sun's group has performed DNA microarray studies on transformed keratinocytes and cancer cells treated with DIM [77]. The enhancement of the stress response genes (including GADD153, GADD34 and GADD45A, the X-box binding protein 1 (XBP1), glucose-regulated protein 78 (GRP78), GRP94, and asparagine synthase) were confirmed at protein and mRNA levels in C33A cervical cancer cells with western blotting and Real time-PCR techniques, respectively. Moreover a Seri of these events was considered in tumor cells such as MCF-7 and DU145. This finding was consistent by function of more than one signal of stress response in C33A cells treated with the 75 μM concentration of DIM. The phosphorylation of elF2α was immediately and transiently induced and continued with enhanced levels of ATF4 protein. It was demonstrated that activation of inositol-requiring enzyme 1α (IRE1α) has rapidly increased stress-specific splices form of the XBP-1 mRNA and in turn persistent activation of JNK and JNK2. The caspase 12 cleavage was reported in DIM-exposed and DIM non-exposed C33A cells which was not correlated with cytotoxicity. The caspase 7 cleavage was achieved during later times, coinciding with the consequence of apoptosis. Meng and colleagues claimed that invasive breast tumor suppression and migration is achieved by I3C is accompanied by upregulation of breast cancer type 1 susceptibility protein (BRCA1 (as well as E-cadherin/catenin complexes [78]. I3C has remarkably induced a dose-dependent E-cadherin cleavage three major catenin's (α, β and γ-catenin) along with increased BRCA1 expression. The elevated QR expression was also correlated with anticancer processes. Chen and colleagues tested the impacts of I3C and ICZ on expression of QR in murine Hepa-1 and human HepG2 hepatoma cell lines [79] and demonstrated that ICZ has elevated QR function in both cell types. Furthermore, ICZ-related QR function was higher in Hepa-1 cell lines. Analysis of QR mRNA expression with Real time-PCR showed a similar pattern to that was observed for the enzyme activity [80]. NAG-1 is a member of the TGF-β superfamily gene which possesses pro-apoptotic and anti-tumorigenic properties. Lee and co-workers claimed enhanced expression of NAG-1 that I3C and DIM [81]. DIM has also activated transcription factor 3 (ATF3), earlier than NAG-1. The DIM was also demonstrated to increase the luciferase function of NAG-1 in HCT-116 cells that were transfected with a NAG-1 promoter construct. This proposes that it suppresses cell growth *via* upregulation of NAG-1 and ATF3. The DIM-induced NAG-1 expression may play pivotal roles in human colorectal cancer cells as well [82].

4.4. I3C, Tumor Invasion and its Relation with Angiogenesis

The migration and further invasion of tumor cells were limited with I3C *via* regulating of the expression of several pathway proteins related to these events, such as E-cadherin, α, β, as well as γ catenin, BRCA1 [78, 83], receptor of CXCL12 (the CXCR4), and MMP-9 [78]. Additionally, I3C has been confirmed to restrict the phorbol ester-activated tube formation as well as endothelial cells proliferation, accompanied with reduced VEGF, along with increasing the secretion of IL-8 and down regulation of MMP-2 and MMP-9 [14].

4.5. I3C Inversely Affects MDR

The I3C has been reported to convert the MDR AML as well as murine melanoma cells to both doxorubicin and Vinca alkaloids by down expression of the MDR-1 and its gene product (the p-glycoprotein) [84-86]. Furthermore, the ability of dietary administrated of I3C has sensitized MDR tumors to chemotherapy drugs in *in vivo* [86]. Together, these remarks are in favour of the notion that I3C might possibly be effective as a dietary remedy for the therapy of MDR tumors [14].

5. I3C/DIM REGULATES VARIOUS RESPONSES AND GENES EXPRESSION BY TUMOR CELLS *VIA* ACTIVATION OF MANY SIGNALS

I3C mediates G1 cell cycle arrest in tumor cells nonetheless I3C peculiar targets display identical and overlapping impacts on LNCaP and PC3 tumor cell lines compared to MCF-7 as androgen-responsive. I3C has attenuated. LNCaP tumor cell lines, the expression of cdk6, reduced the cdk2-dependent enzymatic activity, and induced the cdk inhibitors p16, p21 and p27 [65]. Interestingly, the I3C impacts on androgen non-responsive PC3 cell lines has demonstrated homology to what was claimed in LNCaP cell lines, and ex-

pression of both p27 and p21 was elevated while cdk6 protein and cdk6-dependent function was considerably reduced [87]. The proliferation inhibition of breast tumor and other tumor cells have required lower measures of I3C (100 μM). In contrast, I3C decreased proliferation and caused G1 arrest of human immortalized keratinocyte HaCaT cells with higher concentrations (200, 500 Mm) [88]. Furthermore, in Hacat cells, the cdk inhibitor p15 was increased while p19, p21, p27 and cdk6 expression was not altered at all concentrations of I3C which were used [88]. The cytotoxic effects, of I3C in tumor cells were in concert by increased cells death and regulation of several genes and proteins is accompanied by this response [18, 22, 59, 87, 89]. Rahman and colleagues have demonstrated that I3C caused apoptosis and caspase-dependent Poly ADP ribose polymerase (PARP (cleavage in ER-negative MDA-MB-453 cell types of breast cancer. This was in combination with activation of caspase-3, mitochondrial uptake of Bax, reduced Bcl-2, and on the other hand an overall increased Bax/Bcl-2 ratio. Contrastly, the enhanced apoptosis of Breast cancer cells were both Bax and p53-dependent [90]. These types of finding propose that I3C perhaps activates the innate mitochondrial signaling, although, the pro-apoptotic impacts of I3C in tumor cells deserved to be investigated in further studies. The mechanisms of I3C-induced cytotoxicity in tumor cells is possibly at least, partially, be associated with other responses influencing cell proliferation and death. As an instance, I3C is able to prevent both androgenic and estrogenic responses in cervical and MCF-7 cells [91-93] and enhances CYP-dependent estradiol metabolism and thus reduces the mitogenic impacts of this hormone [93-96]. Additionally, I3C regulates the expression or activation of several genes varying from p53 (increased), interferon γ, NFκB (decreased), NAG-1 (increased), BRCA1 (increased), to PI3-K, (decreased) [59, 78, 97]. The I3C also serves as AhR agonist, activating the proliferation inhibitory signals in breast and also other tumor cells [98, 99]. More recently it has been confirmed that I3C has induced BRCA1 and BRCA2 in tumoral breast cells including MCF-7, T47D, LNCaP and DU145 [93]. Employing RNA interference technology revealed that I3C-mediated anti estrogenic/anti androgenic activity, and its related cytotoxicity is due to BRCA1 and BRCA2. The induction of BRCA1 and BRCA2 by I3C is caused by activation of ER stress. Moreover, DIM and the synthesized analysis of DIM analogs possibly are related to important underlying mechanisms of the I3C function [100, 101].

6. EPIGENETIC MODULATION CONCERNS

DNA is in association with a variety of basic proteins called histones within the nucleus. Generally, histones acetylation with histone acetyltransferases (HATs) making the nuclear DNA content more available for transcription factors and in turn binding DNA and relatively gene transcription. Deacetylation of histones by histone deacetylases (HDACs) limits binding of transcription factors to DNA. The acetylation/ deacetylation processes of nucleus histones were pivotally important cellular mechanisms in modulating of gene transcription [102], although, HAT/HDAC activities balance which exist in normal human cells can be disrupted in tumor cells. Generally, derivatives that prevent HDACs possess the tendency for upregulation of the transcription of tumor suppressing proteins promoting either differentiation or apoptosis in cancer transformed cells [103]. Both AITC and Sulforaphane (SFN) metabolites prevent HDAC activity in cultured tumor cell lines and in animal models, and SFN-rich broccoli sprouts prevented HDAC function in human PBMNC [104-106]. The trans-placental tumor preventative impacts I3C and other dietary regulators, involving alterations in DNA promoter methylation, chromatin remodeling and expression of imprinted genes have been evidenced [31, 107, 108].

7. CANCER INHIBITION IS ACHIEVED BY I3C AND DIM

The DIM effects on dextran sodium sulfate (DSS)-induced experimental model of colitis and colitis-associated colon carcinogenesis which was caused with throughout azoxymethane (AOM)/DSS in rodents was explored and indicated that DIM inhibited the processes of colon tumor genesis in AOM/DSS mice [109]. The DIM also significantly diminished severe clinical symptoms in the colitis model through decreasing prostaglandin E2, nitric oxide as well as pro-inflammatory cytokines [109]. Regardingly, it could be proposed that DIM suppresses inflammatory response which in turn its imaging outcome is inhibiting of tumor-genesis. Treatment with I3C was also shown to inhibit clonogenic cancer cell growth and inducing higher levels of basal caspase-3 and 7 activities [110]. Several research teams have also claimed that I3C and DIM could both prevent oncogenesis proliferation of tumor cells and cause apoptosis in different tumor cells [18, 19, 59, 87, 111, 112], making I3C and DIM as potent promising compounds either for the inhibition or cancer therapy. Additionally, DIM was evidenced to facilitate its potent effects *via* enhancing the antitumor potencies of chemotherapy and radiotherapy therapeutic methods. Studies demonstrated that DIM has significantly increased the cancer cells sensitivity to erlotinib and cisplatin by down expression of epidermal growth factor receptor and degradation of NF-κB signal pathway in pancreatic tumor and squamous cells cancer cells [113, 114]. Although, ET-743 is considered as an experimental antitumor drug with potent antitumor activity as shown in phase II trials it has hepatotoxic side effects. Supplementation diet with I3C in mice prior ET-743 administration has abolished the hepatotoxicity without any alterations in its antitumor efficacy [115], suggesting that I3C may attenuate the unwanted adverse effects of ET-743, and regardingly I3C could serve as antitumor in combination with ET-743. DIM could also induce the efficacy of radiotherapy, proposing that DIM and radiation together are able to significantly limit primary tumor growth in parallel with reducing metastasis to lymph nodes in cancer [100, 116]. Collectively, these evidences propose that DIM has the capability to be regarded as a promising compound in cancer therapy in combination with ordinary therapeutic reagents. Currently, more clinical trials are continuously going on for determination of the effects of DIM therapy either as a dietary supplement or in combination with conventional cancer therapy protocols in patients in several cancer types such as prostate, breast, cervical and

laryngeal tumors. Results of these clinical trials may delineate the values of chemically inhibited liver, mammary gland, colon, and tumorigenesis [29, 117, 118]. DIM was also revealed to increase CYP1A1, 1A2, 2B, and 3A activities in rodent liver [119, 120]. Another study showed that I3C has up-regulated the level and function of GST [119-121] and hence the anti-oncogenic activity of the administration of I3C prior or simultaneously by a tumorigenic appears to be mediated *via* changing in both levels and functions of Phase I (*e.g*., p450 or CYP) and phase II (*e.g*., GST) isozymes intra/ extrahepatic tissues, leading to elevation of their capacity for carcinogens detoxification [121]. I3C also reported to inhibit effects of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone plus benzo(a)pyrene-induced lung carcinogenesis in A/J rodent and regulated tumorigen-induced alterations at proteins levels [122]; while other group claimed cancer promoting function for I3C [123], which propose I3C may impose harmful impacts in this selected cancer model. This also be worth noting that however DIM has not genotoxic side impacts that was typically observed with I3C [124], proposing future clinical trials which seems to be essentially performed employing DIM alone but not I3C. DIM also has limited formation of new vessels and the proliferation of transplantable breast cancer in athymic rodents through enhancement of p27Kip1 and reduction cdk2 and cdk6 expression [125]. DIM also inhibited proliferation of cancer cells in mice *via* reduction of uPA, MMP-9, mammalian target of rapamycin complex 1) mTOR, (Platelet-derived growth factor D) PDGF-D), and angiogenesis [126, 127]. A research group found that DIM is able to induce anti-tumor functions of erlotinib and further lead to remarkable restriction of pancreatic cancer cell proliferation *in vitro* and limit cancer growth in an *in vivo* model of severe combined immunodeficiency (SCID) mice [113]. Collectively, evidences showed that DIM significantly inhibited growing of transgenic line of C57BL/6 mice (transgenic adenocarcinoma mouse prostate) TRAMP-C2 mouse tumor cells proliferation in C57BL/6 rodents *via* increasing apoptosis and simultaneous decreasing cellular growth [128]. Overall all of these evidences suggesting that both I3C and DIM are able to restrict tumor growth and development in experimental models and propose that DIM could be considered as a beneficial therapeutic compound for the tumor growth inhibition in clinic as well [129].

8. CLINICAL TRIALS WITH I3C OR DIM

Multiple clinical trial investigations were undertaken to determine both tolerance and impacts of I3C and DIM in mankind. In a phase I trial using I3C, a number of 17 females at high risk of breast tumor cohort were enrolled. All of studied subjects have tolerated a chronic dose of daily I3C(400 mg) for 4 weeks which was followed by a 4-week duration with 800 mg of daily I3C [36], proposing the safety of I3C at these doses. The I3C (400 mg daily) has elicited a maximum protective impact if the ratio of hydroxylated estrogen metabolites are observed as biomarkers of chemoprevention [36]. In a phase II clinical trial was performed for exploring the potential therapeutic advantages of I3C in therapy of VIN therapy. Studies also reported a marked development in symptomatology and vulvoscopic appearance of VIN further I3C treatment [75]. Applying of I3C for the of RRP therapy demonstrated that patients 33% entered remission from papillomatous and exhibited requirement for surgery following I3C intervention while 30% of patients displayed reduction in papillomatous growth that may minimize the essence of frequent surgery, proposing I3C as a therapy line for RRP [35]. Some other clinical trials are performing for investigating clinical benefits of I3C/DIM in tumor suffering patients and the focus of them is determination of the impacts of I3C or DIM for therapy of tumor or cervical dysplasia. As stated in previous reports, I3C and DIM have the tendency to be used as appropriate and potent anticancer compounds either alone or combined by other conventional therapeutics for cancers in future. The safe and free of toxicity nature of DIM in healthy subjects was also confirmed in a phase-1 trial and [130] the formulated DIM (which is B-DIM) shown to be biologically active in pre-clinical and *in vitro* as well as *in vivo* animal models [131, 132]. Heath EI and coworkers indicated that formulated B-DIM has been well tolerated. Pharmacokinetics investigations showed that exposure to drug was dose proportional to the plasma B-DIM [129, 133].

9. I3C FOR TREATING CANCER: EPIDEMIOLOGIC EVIDENCES

Similarly as other compounds, cruciferous vegetables are good sources for nutrients and phytochemicals including I3C which may serve synergistically to aid cancer prevention [5, 103]. Epidemiological evidences showed that high intake of Brassica vegetables protects versus carcinogenesis. Brassica vegetables are enriching sources of glucosinolates which are hydrolyzed during digestion to different products, such as including I3C. Several reviews with epidemiologic subjects suggested that relationship between I3C related to and risk of multiple cancers are more complicated than that was previously thought [103]. Findings related to lung, colorectal, liver, breast, leukemia and prostate cancers as six major causes of death cancer, are summarized next parts of this article.

9.1. Lung Cancer

Song JM and colleagues studied the efficacy of I3C against mouse lung tumors and their findings were paralleled with remarkable decrease in the level of pro-inflammatory and pro-tumorigenic proteins (such as pSTAT3, pIκBα and COX-2) and cell proliferation regulatory proteins (including pAkt, cyclin D1, CDKs 2, 4, 6 and pRB). Investigations in premalignant bronchial cells demonstrated that anticancer impacts of I3C combined with were higher than individual derivatives and these impacts were mediated with targeting cyclin D1, CDKs 2, 4 and 6 and pRB. Combination of I3C with Sil has suppressed cyclin D1 with decreasing its mRNA level and with increasing its proteasomes degradation. They reported that the chemo-preventive effects of I3C and Sil in smokers and former smokers lung cancer [134]. Li and coworkers explored the anti-metastasis impacts related and molecular mechanisms of I3C and DIM on hepatocellular carcinoma (HCC) *in vitro* and *in vivo*. They have selected two cell lines of SMMC-7721 and MHCC-97H that show high potential of invasion. I3C and DIM has prevented the growth, migration and invasion of these two cells *in vitro*. Moreover, investigators demonstrated that I3C and DIM

have remarkably reduced the volumes of SMMC-7721 orthotropic hepatic cancer and repressed lung metastasis in a model of nude rodent and also this outcome showed that I3C and DIM inhibited HCC cells lines metastasis with suppressing cancer cells lines invasion and migration. The anti-metastatic properties of I3C and DIM may at least, partially be described the drugs and function of MMP2/9 with reduction of Phosphatase and tensing homolog PTEN and prevention of phospho-FAK (Tyr397) [135]. In a study Song and colleagues study, they increased a liposomal formulation of I3C and evaluated its delivery by lung in mice and demonstrated that intranasal administration of liposomal I3C has potential to remarkably progress the efficacy of I3C for lung tumor chemoprevention [136].

9.2. Colorectal Cancer

Lee and colleagues elucidated the molecular mechanism of ATF3 induction with I3C/DIM in Colorectal Cancer Cells (CRC) and demonstrated that I3C/DIM has stimulated the ATF3 expression *via* ATF4-mediated signaling and further induced apoptosis of these cells [137]. In study Kim and colleagues have investigated the anti-inflammatory impacts of I3C/DIM on experiential colitis and colitis-associated colorectal tumorigenesis. These showed that therapy by I3C/DIM has remarkably decreased loss of body weight, shorted the colon, and severe lessened clinical symptoms in a colitis model. They also were observed markedly amelioration of the disruption of the colonic architecture and remarkable attenuation in colonic myeloperoxidase function and production of prostaglandin and some pro-inflammatory cytokines. In addition, when administrated, I3C/DIM dramatically reduced the number of colon cancers in AOM/DSS rodents [109]. In an investigation Faust and colleagues studied, a protective impact of I3C on colon tumor. In another study Suzui reported that I3C has inhibited proliferation of colon cancer cells and their results suggested that I3C prevented the proliferation of human colon carcinoma cells, throughout upregulation of p27KIP1 and p21CIP1-mediated G1 cell-cycle arrest but dietary I3C promotes AOM-induced rat colon tumorigenesis with preventing the apoptosis of colon cancers [138]. Pappa and co-workers have also investigated the impact of I3C/DIM and their results indicated that the I3C/DIM compounds showed antagonistic interactions regarding cell growth inhibition [139].

9.3. Liver

Wang and colleagues reviewed the I3C/derivatives pharmacokinetics *in vitro* and *in vivo* in chronic liver diseases and reported that indole derivatives not only control transcription factors and their respective signaling, they but also relieved oxidative stress and prevented the synthesis of DNA to affect the function, growth and apoptosis of target cells. Furthermore, these derivatives regulate enzymes those are related to viral hepatitis replication, lipogenesis, as well as the metabolism of ethanol and hepatotoxic reagents to protect against hepatic injury. Indoles act as inhibitors for pro-inflammatory cyto/chemokines network decrease microbial induced liver injures. Indole and its derivatives, specifically I3C and DIM as phytochemicals, exert anti-fibrosis, anti-cancer, anti-oxidant, immunomodulatory, detoxification and anti-inflammation impacts on hepatic protection *via* pleiotropic mechanism [140]. In other study Wang and colleagues found that I3C therapy of cancer cells with I3C repressed the AKT signaling with enhancing the expression of phosphatase and tensing homolog PTEN in HCC xenograft cancer and HCC cells [141].

9.4. Breast

De Santi and colleague studied the synergistic activity of I3C, cis-platin and dox-orubicin in triple-negative breast tumor cells and indicated that this combination synergistically inhibited cell survival as well as induced autophagy. The namely MAP1LC3B gene was synergistically overexpressed in MDA-MB-231 cells which were treated with combination of I3C and -cisplatin. Furthermore, the cytotoxic functions of the I3C were progressed in cells pretreated by cisplatin and doxorubicin. It preliminary *in vitro* in ventilation has provided evidences for the potential of I3C as a chemo-preventive compound alone or in combined with standard protocols for triple-negative breast tumor [142]. In an investigation Caruso showed anticancer impacts of I3C in ERα-positive Breast cancer cells [143] and Tin and colleagues confirmed essential role I3C anti-proliferative role of I3C in human breast cancer cells. I3C has enhanced nucleostemin to sequester MDM2 in a nucleolus compartment, thereby freeing p53 to mediate induction of apoptosis. The siRNA knockdown of nucleostemin evidenced that nucleostemin is deserved for I3C for initiation of its activities, including cellular anti-cancer responses, prevent tumor sphere formation, and disrupt MDM2-p53 protein-protein interactions. The expression of an I3C-resistant form of elastase, which as the unique target protein of I3C inhibited the I3C anti-cancer responses in cells and in cancer xenografts *in vivo* [144]. Cevatemre and colleagues examined the cytotoxic function of a novel combination of fenretinide, a synthetic retinoid, with I3C, a natural product especially broccoli and cabbage, against MCF-7 and MDA-MB-231 cell lines. They have reported that this combined compound has eventuated in more powerful cytotoxic activity, compared to alone substance, to induce apoptosis [145].

9.5. Leukemia

Studies by Bai and co-workers revealed elevated cytotoxicity of primary cells and (HL-60 and THP-1 cells) in response to OSU-A9, as an I3C compound by improving the antitumor potency, in a dose-dependent fashion. This I3C derivative (OSU-A9) activates caspase PARP cleavage, and not in processes of autophagy cell death. Pretreatment of both AML cell lines and primary AML cells with N-acetylcysteine and glutathione has increased their survival of apoptosis (due to concomitant PARP cleavage). Akt hypophosphorylation, implicating a prominent function of ROS in OSU-A9 related cytotoxicity. Aantitumor application of OSU-A9 is increased *in vivo* and its, intraperitoneally, administrator suppressed the proliferation of THP-1 xenograft cancers in euthymic nude mice without apparent toxicity. This ROS-mediated cell death lead to the antitumor function of OSU-A9 in leukemia cells and in primary AML cells, and therefore could be observed in the further assessment of its translational values in leukemia therapy [146]. Perez-Chacon

and colleagues claimed that I3C in accompanying to F-ara-A shows highly cytotoxic effects against CLL cell lines from refractory patients and others with p53 deficiency. I3C also possesses anti-leukemic properties in B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL), NALM-6 cells. It also reported to abolish doxorubicin-induced NF-κB function as demonstrated with reduced nuclear accumulation of p65, inhibition of IκBα phosphorylation and its degradation, and reduced NF-κB DNA binding function. Western blotting indicated that doxorubicin-induced Bcl-2 proteins expressions were prevented with I3C [147].

9.6. Prostate

In an investigation Watson and colleagues reported that sulforaphane and I3C has inhibited prostate cancer by suppressing prostate tumor development both *in vitro* and *in vivo* [148]. Kim and colleagues also showed inhibitory effects for I3C and DIM on androgen-dependent pathways. I3C and DIM were both able in modulating the effect of androgen on CCL2-mediated signaling. I3C and DIM both have the prevented promotional effects of dihydrotestosterone on (C-C motif) ligand 2 (CCL2) and further migration. These finding may confirm that androgen can modulate CCL2 and develop an inflammatory microenvironment in prostate cancers and interestingly this process may be blocked with I3C [149]. Chen and co-workers proposed that I3C-DIM has potential to be administered as a promising antitumor reagent in the clinic for inhibition and therapy of prostate cancer [150]. Beaver and colleagues explored the effects of I3C and DIM on HDACs on male-derived prostate tumor cells and reported that the prevention of HDAC activity by DIM can limit proliferation of cells [151].

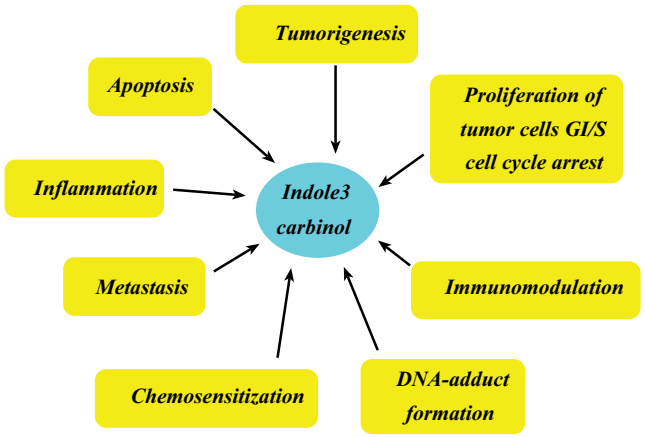

Fig. (5). Activities assigned to I3C.

CONCLUSION REMARKS FUTURE AND PERSPECTIVES

Indole derivatives are of the most important heterocyclic compounds in the drug discovery researchers which concerned as very important molecules, displaying fundamental parts in cell biology. Multiple investigators are attracted to employ indolic compounds as bioactive molecules against microbes, tumor cells, and different types of disorders in the human studies, worldwide. Recently, more plant-based substances such as isoflavone, I3C, DIM, curcumin and, lycopene are introduced as cancer preventative reagents, due to their anti-anticancer activities. I3C and DIM reported as more effective factors which serve in the limitation of carcinogenesis and tumor progression in *in vivo* models and this has been clearly supported by several *in vitro* experiments. These effects have been found to be mediated by regulation of several intracellular signals varying, from NF-κB, Akt, MAPK, to Wnt, Notch, and AR. Present observations which fruits and vegetables are able to lessen the risk of tumor and other diseases epidemiological facts for which the experimental evidences have been lacked. As described above all of the experimental studies proposing I3C as a constituent which presents in cruciferous vegetables has incredible potential for inhibition and therapy of tumor. I3C induces its beneficial impacts *via* various mechanisms that are represented in Fig. (**5**). Although, indicating cytoprotective effects in the normal cells, it accounts as an apoptotic compound against cancer cells. However, an in-depth mechanistic *in vitro* research programs, relevant *in vivo* animal model investigations, in parallel with rationally programmed novel clinical trials are highly deserved to fully appreciate the values of these "natural products" in the field of cancer therapy in mankind, in future.

LIST OF ABBREVIATIONS

AhR	=	Aryl Hydrocarbon Receptor
AOM	=	Azoxymethane
ATF3	=	Activating Transcription Factor 3
ATF3	=	Transcription Factor 3
BAD	=	Bcl-2-associated Death Promoter
Bcl-2	=	B-cell Lymphoma 2
Bcl-xL	=	B-cell Lymphoma-extra-large
BCP-ALL	=	B-Cell Precursor Acute Lymphoblastic Leukemia
BRCA1	=	Breast Cancer Type 1 Susceptibility Protein
bZIP domain	=	Basic Leucine Zipper Domain
C/EBPs	=	CCAAT-enhancer-binding Proteins
CaSki	=	Epidermoid Cervical Carcinoma Cells
CCL2	=	(C-C Motif) Ligand 2
CDK	=	Cyclin-dependent Kinase
cdks	=	Cyclin-dependent Kinases
cDNA	=	Complementary DNA
CRC	=	Colorectal Cancer Cells
CTet	=	Cyclic Tetramer
CTr	=	Cyclic Trimer
CXCR4	=	CXCL12 Chemokine Receptor

CYPs = Cytochromes P450
DIM = 3,3′-Diindolylmethane
DNA = Deoxyribonucleic Acid
DR5 = Death Receptor 5
DU145 = Prostate
E2F = E2 Factor
ER = Estrogen Receptor
GADD153 = DNA Damage-inducible Gene 153
GRP78 = Glucose-regulated Protein 78
GSH = Glutathione
GSK = Glycogen Synthase Kinase
GST = Glutathione S-transferase
GST = Glutathione S-transferases
HaCat = Human Epithelial Cell Line
HATs = Histone Acetyltransferases
HCC = Human Hepatocellular Carcinoma
HDACs = Histone Deacetylases
HDACs = Histone Deacetylases
HFF-1 = Human Foreskin Fibroblasts
HFK = Human Foreskin Keratinocytes
HPV-11 = Human Papillomavirus Type 11
I3C = Indole-3-carbinol
IAP1 = Inhibitor of apoptosis protein 1
ICZ = Indolo[3,2b]-carbazole
IGF-1 = Insulin-like Growth Factor 1
IL-8 = Increased Interleukin-8
IRE1α = Inositol-requiring Enzyme 1α
IκBα = Inhibitor of Kappa B
JAK = Janus Kinase
JNK = c-jun N-terminal Kinase
LTr_1 = A Linear Trimer
MAPKs = Mitogen-activated Protein Kinases
MCF-7 = Cancer Cell Lines From Breast
MCIR = Michigan Care Improvement Registry
MDR = Multiple Drug Resistance
MITF = Melanogenesis Associated Transcription Factor
MMP = Matrix Metalloproteinase
MSH = Melanocyte-stimulating Hormones
mTOR = Mammalian Target of Rapamycin Complex
NAG-1 = Nonsteroidal Anti-inflammatory Drug-activated Gene
NF-IL6 = Nuclear Factor-interleukin 6
NF-κB = Nuclear Factor-κB
PARP = Poly ADP Ribose Polymerase
PBMNC = Peripheral Blood Mononuclear Cells
PDGF-D = Platelet-derived Growth Factor D
PI3K = Phosphoinositide 3-kinase
PTEN = Phosphatase and Tensin Homolog
QR = Quinone Reductase
Rb = Retinoblastoma
RRP = Recurrent Respiratory Papillomatosis
SCID = Severe Combined Immunodeficiency
SFN = Sulforaphane
STAT3 = Signal Transducer and Activator of Transcription 3
TGF-β = Transforming Growth Factor Beta
TRAFs = TNF Receptor Associated Factors
TRAMP-C2 = transgenic Adenocarcinoma Mouse Prostate
URR = Upstream Regulatory Region
VEGF = Vascular Endothelial Growth Factor
VIN = Vulvar Intraepithelial Neoplasia
XBP1 = X-box Binding Protein 1
XIAP = X-linked Inhibitor of Apoptosis Protein

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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