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REVIEW ARTICLE

Role of Nuclear Factor-kappa B Signaling in Anticancer Properties of Indole Compounds

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Indole compounds, obtained from cruciferous vegetables, are known to possess potent anticancer properties. Studies with indole-3-carbinol (I3C) and its dimeric product, 3,3' diindolylmethane (DIM), have indicated the efficacy of these compounds against a number of human cancers, which is related to their ability to interfere with and modulate multiple cellular signaling pathways. The nuclear factor-kappa B (NF-κB) pathway plays an important role in the control of cell proliferation, metastasis, angiogenesis, and drug resistance. Emerging evidence points to an effective inhibition of NF-κB signaling by I3C and DIM. This seems to be central to most of the observed anticancer properties of these compounds. Here, we summarize our current understanding of regulation of NF-κB signaling by I3C and DIM and the resulting biological effects. Breast, prostate, and pancreatic cancers are relatively better characterized in terms of modulation of NF-κB signaling by indoles, and it is our intent that this review incites similar studies in other human cancer models as well.

1. Introduction

Cancer is a major public health problem worldwide. In the United States, cancer-related deaths are second only to deaths caused by heart diseases and account for close to a quarter of all deaths.[1] It is now well accepted that cancer represents a highly heterogeneous disease and, accordingly, various therapeutic regimes are available for specific cancer subtypes, largely determined by the presence and absence of molecular targets. Chemotherapy, an important cancer therapy advocated by clinicians, has the advantage of targeting receptor-positive and receptor-negative cancers. In the area of scientific research, major efforts are underway to considerably improve the efficacy of chemotherapy through development of novel chemotherapeutic agents and through identification of novel molecular targets.

As important as the identification of novel therapeutic targets is, the ever evolving knowledge of molecular signaling pathways has taught us that despite the vast array of putative molecular targets, there are certain factors that are more important than the others. This primarily is because of their unique position in the cellular signaling where they function as a convergence point for multiple signaling pathways. Nuclear factor-kappa B (NF-κB) is one such molecular target that has gained recognition for its crucial role in the development and progression of human cancers as well as in the acquisition of drug-resistant phenotype in highly aggressive malignancies.[2–8] NF-κB pathway includes several important molecules, such as NF-κB, inhibitor of kappa B (IκB), ikappa B kinase (IKK), and others; however, NF-κB is the key protein in the pathway that has been described as a major culprit and a critical target for therapy in human cancers.[4,8,9]

With the identification of a valid target for therapy, such as NF-κB, the next challenge for researchers is to identify agents that can modulate the validated target, resulting in inhibition of growth of cancer cells. A major roadblock in this search is the relative toxicity of therapeutic agents, and many of these agents are associated with unacceptable dose-related toxicity. Recently, lot of attention has been given to natural dietary compounds in relation to their ability to inhibit cancer cell growth, invasion, and metastasis through their ability to target NF-κB signaling pathway.[7,10,11] Use of such natural compounds as anticancer agents provides the advantage that these compounds are well tolerated by patients and are not usually toxic. Several natural products found in fruits and vegetables are reported to possess antimutagenic and anticarcinogenic property.[12] Moreover, epidemiological studies have shown that the consumption of fruits, soybean, and vegetables is associated with reduced risk of several types of cancers.[13,14] In particular, indole-3-carbinol (I3C) and its *in vivo* dimeric product 3,3′-diindolylmethane (DIM) are known to be potent inducers of apoptosis in human cancer cells.[15] These compounds are known to modulate multiple signaling pathways leading to their observed biological effects. A plethora of information is available in literature documenting the effects of

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indole compounds, I3C, and DIM on several distinct signaling pathways.[8,14–25] Modulation of NF-κB pathway is considered central to the anticancer activity of indole compounds[15,26,27] because inhibition of NF-κB signaling pathway not only plays an important role in effective induction of apoptosis by these compounds but also leads to sensitization of aggressive cancer cells to conventional chemotherapeutics. Through this review article, we summarize our current understanding of mechanisms by which these compounds regulate NF-κB signaling in various human cancer models.

2. Indole Compounds

Indole compounds are naturally occurring compounds commonly found in cruciferous vegetables, such as broccoli, cauliflower, cabbage, and Brussels sprouts. All compounds that contain an indole ring are called indoles. Chemically, indole is an aromatic heterocyclic organic compound. It has a bicyclic structure, consisting of a six-membered ring fused to a five-membered nitrogen-containing pyrrole ring. A review of 206 epidemiological studies and 22 animal studies reported a tumorigenesis-inhibiting effect of I3C,[28] and another study showed that the consumption of cruciferous vegetables associates strongly with reduced bladder cancer risk.[29] Cruciferous vegetables are a good source of many phytochemicals, such as indole derivatives, dithiolthiones, and isothiocyanates. I3C and its dimer, DIM, are representative indoles with well-characterized anticancer properties. To keep our review focused, we will use these two indole compounds as the representative indoles that exert their anticancer effects through modulation of NF-κB signaling.

I3C is found in some fruits and vegetables, including members of the cruciferous family, and, in particular, members of the genus *Brassica*. Its anticarcinogenic effects in experimental animals[30–33] and humans[34,35] are well established. In cruciferous vegetables, I3C is produced by the hydrolysis of glucobrassicin, a glucosinolate. Glucosinolates with an indole side chain form indoles. Glucobrassicin, the most prevalent glucosinolate with an indole side chain, is predominant in *brassica* vegetables, including broccoli, brussels sprouts, cabbage, cauliflower, collard greens, kale, kohlrabi, mustard greens, radish, rutabaga, and turnip. I3C is made from indole-3-glucosinolate by the action of enzyme myrosinase, which is physically separated from its substrate glucosinolate in intact plant cells.[36] The amount of I3C in the diet can vary, ranging from 20 mg and 120 mg daily, depending on the dietary intake of cruciferous vegetables and the metabolic events leading to I3C production within plants.[15,37]

Though I3C is an effective anticancer agent,[27] it is very unstable and, under the acidic environment of stomach, it self-combines leading to the formation of a complex mixture of biologically active compounds, known collectively as acid condensation products.[38] Among several known acid condensation products of I3C, DIM is the most prominent one that is readily detectable in the liver and feces of I3C-fed rodents.[39] DIM accounts for about 10–20% of the breakdown products of I3C and, therefore, a typical daily ingestion of I3C from the diet can provide roughly 2–24 mg of DIM. I3C, itself, can be absorbed from the gut and distributed systemically into a number of well-perfused tissues, which raises the possibility of pharmacological activity of the parent compound *in vivo*.[40] DIM is the most active and effective metabolite of I3C that persists in the liver even 24 hours post-I3C administration.[41]

3. NF-κB Signaling

NF-κB is a transcription factor that is very important for the processes of cell growth, invasion, and metastasis.[3,42–46] It exists in a latent state in the cytoplasm bound to specific inhibitory proteins, IκBs. Many prosurvival stimuli cause IKK-dependent phosphorylation and subsequent proteasome-mediated degradation of IκB proteins. Such degradation of inhibitory IκB proteins results in the release of NF-κB. This activated NF-κB migrates into the nucleus to regulate the transcription of multiple target genes, which, in turn, influence the various stages of carcinogenesis and cancer progression.[6,47] In addition to the inhibition of apoptosis, the expression of several angiogenic factors is also regulated by NF-κB. Cancer cells are well known to produce vascular endothelial growth factor (VEGF) under the control of NF-κB activation, and VEGF promotes the proliferation and migration of endothelial cells. NF-κB activation also regulates the expression and activity of matrix metalloproteinases (MMPs), which play an important role in the degradation of extracellular matrix and basement membranes, thus felicitating tumor invasion. Constitutively active NF-κB is now believed to be a hallmark of many tumors and, therefore, NF-κB is recognized as a therapeutic target in human cancers.[6,48–50]

Chemical inhibitors that block NF-κB activation by means of a direct action on IKK or on the proteasome machinery have shown antitumor and proapoptotic activity both in preclinical and clinical studies[51]; however, further research is needed to fully understand the mechanism of such chemical inhibitors before they can be clinically useful. In particular, the toxicity associated with such chemical inhibitors needs to be evaluated. Numerous studies with indole compounds, on the other hand, have suggested a very potent action of these compounds against NF-κB activation, which seems to be responsible for their tumor suppressive effect.[8,16,52–65] These *in vitro* and *in vivo* studies demonstrate the efficacy of indole compounds against the activation of NF-κB in multiple cancer models. In the next few sections, we will review the available data describing the modulation of NF-κB signaling pathway by I3C and DIM in various cancers, with special mention of three cancer types, namely, breast, prostate, and pancreatic cancer, where such activity of these compounds is relatively much more characterized.

4. Inhibition of NF-κB Signaling by Indoles in Breast Cancer

In one of the earlier studies demonstrating a beneficial effect of I3C against breast cancer, it was shown that oral administration of I3C for 7 days to men and women (6–7 mg/kg/d) significantly increased the extent of estradiol 2-hydroxylation.[66,67] Because increased estradiol 2-hydroxylation is linked with reduced incidence of breast cancer,[68] it was hypothesized that the incidence of mammary tumors in mice and/or human females might be inversely related to a direct stimulation of estradiol 2-hyroxylation by dietary or pharmacological means. Increased extent of 2-hydroxylation,[66,67] therefore, was supportive of an anticancer effect of I3C against breast cancer. In another study by the same group, the effect of dietary I3C intake was tested on female C3H/OuJ mice.[69] In a long-term feeding experiment of 8 months, the mice were administered diets containing I3C at 500 ppm or 2000 ppm. It was observed that the incidence of mammary tumors and multiplicity was significantly lower at both doses, compared with that of control diet without I3C. Also, tumor latency was observed to be prolonged in the mice receiving 2000-ppm diet. As a mechanism, it was proposed that I3C-induced P450-dependent estrogen metabolism was responsible for the chemopreventive action of I3C.

In a study to compare the mechanisms of the action of I3C in estrogen-responsive MCF-7 breast cancer cells and the estrogen-nonresponsive MDA-MB-231 breast cancer cells, it was reported that I3C was able to inhibit the growth of only estrogen-responsive cells with little effect on estrogen-nonresponsive cells.[70] It was reasoned that the inhibitory effects of I3C may involve selective

induction of estradiol metabolism and the related cytochrome P450 system that may be limited to estrogen-sensitive cells. However, it was later shown that I3C can suppress the growth of breast cancer cells independent of estrogen receptor signaling.[71] Here, it was shown that a combination of I3C and tamoxifen inhibited MCF-7 cell growth more stringently than either agent alone. I3C signaling was observed to induce the G1 cell cycle arrest of MCF-7 cells. Furthermore, I3C-mediated cell cycle arrest was also observed in estrogen receptor-negative MDA-MB-231 cells under conditions in which the antiestrogen tamoxifen had no effect on cell growth, thus demonstrating a more versatile effect of I3C, independent of estrogen receptor signaling. This study implicated cyclin-dependent kinase 6 as a target for cell cycle control in human breast cancer cells. In a follow-up study, the potent inhibitory action of I3C—tamoxifen combination against MCF-7 cells was attributed to decrease in cyclin-dependent kinase 2—specific enzymatic activity.[72]

With the establishment of an anticancer effect of I3C against breast cancer cells, irrespective of receptor status, more detailed studies on the effect of I3C on cellular signaling pathways, especially NF-κB pathway, followed. The first clue for the involvement of NF-κB pathway in I3C action came from a study where the effect of I3C was compared using estrogen receptor-α-negative MDA-MB-468 cells versus immortalized nontumorigenic HBL100 cells.[73] In this study, phosphatidylinositol 3′-kinase (PI3-K) and protein kinase B (PKB)/Akt were identified as targets of I3C. I3C was found to inhibit phosphorylation and activation of PKB in MDA-MB-468 cells but not in the nontumorigenic HBL100 cells. Because PKB can regulate NF-κB by the activation of IKK, resulting in increased phosphorylation of IκB and consequent release of NF-κB from the inhibitory complex, the effect of I3C was tested on NF-κB and IKK. Despite inhibition of PKB, no decrease in IKK activity was observed in response to I3C treatment. In support of this, nuclear levels of NF-κB (p65) were found to be unaltered. However, I3C decreased NF-κB—DNA binding, as determined using electrophoretic mobility shift assay (EMSA). These results led the authors to suggest that I3C affected DNA binding of NF-κB protein family members, including p65 and p50, by a mechanism that does not involve the inhibition of IKK activity.

Our laboratory has long been interested in studying the effects of I3C in breast cancer cells. With the report on the involvement of Akt/NF-κB pathway in anticancer effect of I3C,[73] we set out to study this in detail.[52] We found that I3C was capable of inducing apoptotic cell death in MCF10A-derived cell lines with premalignant and malignant phenotypes but not in nontumorigenic parental MCF10A cells. Our investigation showed that I3C specifically inhibits Akt kinase activity and abrogates the epidermal growth factor (EGF)-induced activation of Akt in breast cancer cells. Transfection of Akt gene was found to activate NF-κB directly, and such activation of NF-κB was completely abrogated by I3C treatment. Furthermore, we also showed an abrogation of EGF-induced, Akt-mediated activation of NF-κB. Our results further established the direct cross-talk between Akt and NF-κB pathways that we proposed to be important in mediating I3C-induced anticancer effects in breast cancer cells.

NF-κB is known to regulate chemokine receptor CXCR4[74] as well as MMPs,[75] and all of these factors play an important role in the metastasis of human cancers, including breast cancer. In addition to its inhibitory effect on DNA-binding activity of NF-κB, we studied the effect of I3C on CXCR4 expression *in vitro* and *in vivo*.[54] CXCR4 is now believed to be particularly involved in bone metastasis of breast cancer,[76] and our study was the first to demonstrate an inhibitory effect of I3C on bone metastasis of breast cancer through the inhibition of CXCR4 and MMP-9 expression. Inhibition of NF-κB signaling pathway was determined to be a critical event for this action of I3C. In the severe combined immunodeficient human—mouse model of experimental bone metastasis, I3C was observed to significantly inhibit MDA-MB-231 bone tumor growth by means of downregulation of NF-κB. In a more recent report on the mechanism of action of I3C, it has been shown that I3C directly inhibits the elastase-mediated proteolytic processing of CD40, which alters downstream signaling to disrupt NF-κB-induced cell survival and proliferative responses.[64] This study has further demonstrated the central role of NF-κB in the mediation of the anticancer effects of I3C.

Because I3C is metabolically converted to DIM, we included DIM in our studies to see if this physiologically relevant indole had similar anticancer effects and whether NF-κB signaling played a role in its biological activity. In our studies, it was confirmed that DIM has similar activity against breast cancer cells as I3C.[53] DIM was also able to induce apoptosis processes in MCF10A-derived malignant cell lines but not in nontumorigenic parental cells. DIM also specifically inhibited Akt kinase activity and abrogated the EGF-induced activation of Akt in breast cancer cells, similar to those observed for I3C. As a further mechanism, we found that DIM was able to reduce the phosphorylation of IκBα, an inhibitor of NF-κB. Confocal studies revealed that DIM blocks the translocation of p65 subunit of NF-κB to the nucleus. We were also able to show that the activation of NF-κB involves IκB kinase—mediated IκBα phosphorylation, which can be completely abrogated by DIM treatment. Our *in vitro* and *in vivo* data clearly showed, for the first time, that the inactivation of Akt and NF-κB plays a very crucial role in apoptosis induced by indole compounds in breast cancer cells.

Akt—NF-κB nexus plays a role in resistance of cancer cells to chemotherapy[15]; therefore, we tested whether the inactivation of Akt—NF-κB pathways could lead to chemosensitization of breast cancer cells to the chemotherapeutic agent taxotere.[57] We found that combinational treatment with DIM and taxotere caused significantly greater inhibition of cell growth and apoptosis induction *in vitro*, compared with either agent alone. Mechanistically, we tied these events to decreased activity of NF-κB in cells treated with DIM—taxotere combination. The results were verified *in vivo*, where DIM was shown to sensitize breast tumors to taxotere through potent inhibition of Akt and NF-κB. In view of the metabolic stability of I3C, a derivative of I3C, OSU-A9, was synthesized to improve chemical stability and antitumor potency.[77] In a study involving breast cancer cell lines, this agent was shown to exhibit significantly increased apoptosis-inducing effect.[78] Interestingly, this superior anticancer activity of OSU-A9 was linked to its efficient inhibition of Akt—NF-κB pathway. The compound was also shown to be highly effective *in vivo* without any signs of toxicity, thus underlining the potential of indole compounds as novel anticancer agents.

Many reports, as discussed earlier, have identified the inhibition of NF-κB signaling as an important step in the killing of cancer cells by indole compounds. However, this notion has been cautioned recently with the report that the regulation of constitutive NF-κB by I3C in breast cancer cells is cell specific.[79] It was observed that I3C-mediated regulation of NF-κB pathway is not related to apoptosis. This study is a reminder that although I3C, and probably other indole compounds, have been reported to possess several anticancer activities mainly through their modulation of NF-κB signaling, more detailed studies are still needed to fully elucidate the mechanism of action of these compounds.

5. Inhibition of NF-κB Signaling by Indoles in Prostate Cancer

In one the earliest reports on anticancer activity of I3C against prostate cancer cells, it was shown that I3C induces G1 cell cycle arrest in PC-3 cells through the upregulation of inhibitory p21 (WAF1) and p27(Kip1).[80] I3C was also observed to induce apoptosis in these cells, and all these activities of I3C were found to be mediated by the downregulation of NF-κB signaling. Similar to the

results with breast cancer cells discussed earlier,[73] I3C has been reported to decrease phospho-Akt levels and induce apoptosis in the prostate cell line LNCaP,[73] which expresses very low levels of phosphatase and tensin homolog (PTEN) but expresses androgen receptor (AR). This suggests that I3C might have a therapeutic role in the treatment of AR−dependent prostate cancers. Interestingly, even OSU-A9, the synthetic derivative of I3C, has been shown to be highly effective against prostate cancer cells through the downregulation of phosphorylated Akt and RelA along with several other molecular targets.[77]

The preliminary reports mentioned earlier suggested an inhibition of Akt and NF-κB by the indole compound I3C. To tie these two events and to investigate a cross-talk between the two pathways, further studies were carried out in PC-3 cells using DIM.[81] It was found that DIM inhibited cell growth and induced apoptosis in PC-3 prostate cancer cells but not in nontumorigenic CRL2221 human prostate epithelial cells through inhibition of PI3-K kinase activity and Akt activation. NF-κB−DNA binding activity was also significantly reduced by DIM. These earlier studies laid the foundation for the function of indole compounds as inhibitors of Akt and NF-κB, which might be important for cell survival and chemoresistance of prostate cancer cells.[26] Before this study, it was reported that DIM was effective as an antiproliferative agent only against androgen-dependent LNCaP prostate cancer cells but not against androgen-independent PC-3 cells.[82] In a study comparing the efficacies of I3C and DIM directly, it was determined that DIM was a better antiproliferative agent than I3C in androgen-dependent LNCaP cells[83] as well as androgen-independent DU-145 cells,[84] and that it downregulated phosphorylated Akt and PI3-K, both of which are connected to NF-κB signaling.[85]

In a study to understand the molecular mechanism of action of indole DIM in AR-positive/hormone-responsive and AR-positive/hormone-nonresponsive prostate cancer cells, LNCaP and C4-2B prostate cancer cells were used as representative cells, respectively.[55] DIM was found to significantly inhibit Akt activation and NF-κB activity as well as the expression of AR and prostate-specific antigen, suggesting that it was capable of disrupting the cross-talk between these proliferative pathways. In yet another study,[56] DIM was reported to inhibit angiogenesis and invasion by reducing the bioavailability of VEGF by repressing extracellular matrix-degrading proteases, such as MMP-9 and urokinase-type plasminogen activator (uPA), in human prostate cancer cells. Also, DIM treatment led to a significant inhibition of DNA-binding activity of NF-κB. Because NF-κB is known to be upstream of VEGF, uPA, and MMP-9, all of which are involved in angiogenesis, invasion, and metastasis, this study revealed a central role of NF-κB inhibition in the anticancer action of indole compound DIM. As a further proof of antimetastatic action of DIM, it has been suggested that DIM effectively downregulates uPA as well as its receptor uPAR and that downregulation of uPA−uPAR leads to decreased sensitivity of prostate cancer cells to DIM.[60]

In our recent study to understand the role of survivin in hormone-refractory prostate cancer and bone metastatic disease, we observed that DIM enhanced taxotere-induced apoptotic death in both LNCaP and C4-2B prostate cancer cells.[62] These enhancing effects were related to decreased survivin expression and significantly reduced DNA-binding activity of NF-κB. The combination treatment also significantly inhibited C4-2B bone tumor growth, and the results correlated well with the downregulation of survivin and NF-κB activity. From the available data, it is clear that indole compounds, particularly I3C and DIM, are effective against human prostate cancer cells, irrespective of their hormone dependence. A consensus seems to be emerging that inhibition of NF-κB signaling pathway is central to many of the observed effects of indole compounds, including their ability to induce apoptosis *in vitro* and inhibit the tumor growth *in vivo*. Recently, in a Phase I dose escalation study, it has been determined that DIM is well tolerated and exhibits modest efficacy in nonmetastatic prostate cancer patients.[86]

6. Inhibition of NF-κB Signaling by Indoles in Pancreatic Cancer

Pancreatic cancer is a very aggressive human cancer. NF-κB signaling has been advocated as a factor responsible for chemoresistance of human pancreatic cancer cells and, as such, it was proposed that inhibition of basal NF-κB activity can serve to overcome the phenomenon of chemoresistance leading to a greatly improved therapeutic strategy.[87] As opposed to breast and prostate cancers where the anticancer role of indole compounds is relatively more established, reports on the anticancer effect of indole compounds against pancreatic cancer have emerged in the last couple of years only. In a study to evaluate a multitargeted approach, DIM was combined with erlotinib, and a significant inhibition of cell proliferation, viability and clonogenic potential was observed in the combinational treatment in BxPC-3 cells.[61] The DNA-binding activity of NF-κB was also found to be significantly reduced, which might explain the observed effects *in vitro* and *in vivo*. In a more recent study further supporting the idea of simultaneous inhibition of multiple survival pathways, such as NF-κB, cyclooxygenase-2, or epidermal growth factor receptor (EGFR) signaling, it was shown that DIM significantly inhibited the viability of pancreatic cancer cells that express high levels of cyclooxygenase-2, EGFR, and NF-κB.[88] *In vivo*, a combination of DIM with erlotinib and gemcitabine was reported to be significantly more effective than individual agents, thus further supporting the combinational approach.

In addition to DIM, other related compounds, such as 1,1-bis(3'-indoly)-1-(*p*-substituted phenyl) methanes; *p*-bromo (DIM-C-pPhBr), *p*-fluoro (DIM-C-pPhF), and structurally related analogs have also been shown to be effective against pancreatic cancer cells.[89,90] DIM-C-pPhBr and the 2,2'-dimethyl analog (2,2'-diMe-DIM-C-pPhBr) have been shown to inhibit proliferation and induce apoptosis in Panc28 pancreatic cancer cells through downregulation of surviving.[91] Although NF-κB signaling has not been directly implicated in the apoptosis-inducing action of these compounds, their mode of action resembles classical indoles I3C and DIM, which are known to act on similar molecular targets, such as survivin, with the essential involvement of NF-κB signaling and other cross-talking pathways.

In a direct evidence for modulation of NF-κB signaling by DIM in pancreatic cancer model, it has been shown that DIM potentiates the killing of pancreatic cancer cells by downregulation of constitutive as well as drug-induced activation of NF-κB and its downstream genes.[59] Such action of DIM was found to be relevant *in vivo* as well, with significantly reduced tumor burden. This study provided a functional model in support of the notion that NF-κB-inhibitory agents, such as DIM, can potentially chemosensitize pancreatic tumors to conventional therapeutics by abrogating chemotherapeutic drug (cisplatin, gemcitabine, and/or oxaliplatin)−induced activation of NF-κB. In a pioneering report detailing the role of microRNAs in DIM-mediated inhibition of pancreatic cancer cells, it has recently been shown that DIM treatment causes upregulation of miR-146a expression, which results in the downregulation of EGFR and NF-κB, thus resulting in the inhibition of pancreatic cancer cell aggressiveness.[92]

7. More Evidence of NF-κB Signaling Inhibition by Indoles

Although the modulation of NF-κB signaling by indole compounds I3C and DIM is much better understood in breast, prostate, and

Table 1 Molecular markers of NF-κB signaling pathway modulated by indoles in human cancers

Indole	Marker	Study
I3C	PI3-K/Akt	Howells et al, 2002[73]; Rahman et al, 2004[52]; Li et al, 2005[81]
I3C	p21/p27	Chinni et al, 2001[80]
I3C	NF-κB activity	Howells et al, 2002[73]; Li et al, 2005[81]
I3C	CXCR4	Rahman et al, 2006[54]
I3C	MMP-9	Rahman et al, 2006[54]
I3C	CD40 processing	Aronchik et al, 2010[64]
DIM	p27	Wang et al, 2008[96]
DIM	Akt	Rahman, 2005[53]; Rahman, 2007[57]; Bhuiyan, 2006[55]; Garcia, 2009[85]
DIM	IκB	Rahman and sarkar, 2005[53]; Kim et al, 2010[63]
DIM	NF-κB activity	Takada et al, 2005[93]; Bhuiyan et al, 2006[55]; Chen et al, 2006[94]; Kong et al, 2007[56]; Rahman et al, 2009[62]; Ali et al, 2008[61]; Banerjee et al, 2009[59]; Kim et al, 2010[63]
DIM	FoxM1	Aamir et al, 2010[25]; Rahman et al, 2009[62]
DIM	VEGF	Kong et al, 2007[56]
DIM	MMP-9	Kong et al, 2007[56]
DIM	uPA	Kong et al, 2007[56]; Ahmad et al, 2009[60]
DIM	uPAR	Ahmad et al, 2009[60]
DIM	Survivin	Rahman et al, 2009[62]; Rahman et al, 2006[97]; Sreevalsan et al, 2009[91]
DIM	miR-146a	Li et al, 2010[92]

DIM = 3,3' diindolylmethane; I3C = indole-3-carbinol; MMP = matrix metalloproteinase; NF-κB = nuclear factor-kappa B; pi3-k = phosphatidylinositol 3′-kinase; uPA = urokinase-type plasminogen activator; VEGF = vascular endothelial growth factor; CXCR4 = chemokine (C-X-C motif) receptor 4; IκB = inhibitor of kappa B; FoxM1 = forkhead box protein M1; uPAR = uPA receptor; miR-146a = microRNA 146a.

pancreatic cancers, such activity of these compounds in other human cancers is relatively less characterized. There, however, are reports in literature that provide a hint that indole compounds have similar activity in several unrelated cancers. One such activity of I3C has been reported against myeloid and leukemia cells, where I3C was shown to suppress constitutive and tumor necrosis factor—induced induction of NF-κB.[93] In this study, not only NF-κB, but all its downstream signaling molecules were found to be effectively downregulated, suggesting a potent inhibition of NF-κB signaling pathway as a mechanism of apoptosis induction by I3C. In a report on cholangiocarcinoma cells,[94] DIM has been shown to inhibit phosphorylation of Akt and associated inhibition of NF-κB, suggesting a potential role of DIM in therapy of cholangiocarcinoma. More recently, DIM has been shown to be protective against tumor progression in skin through inhibition of NF-κB signaling pathway.[63] DIM inhibited nuclear translocation of p65 and DNA-binding activity of NF-κB in addition to degradation of the inhibitor of IκB. All these events suggest an effective inhibition of NF-κB pathway leading to the observed antitumor effects.

8. Conclusions and Perspective

I3C and DIM, two representative indoles, have been linked with the ability to inhibit tumor growth. Modulation of a number of distinct signaling pathways by these agents has been proposed as their putative mechanism of action.[8,26,95] Inhibition of multiple signaling pathways by these indole compounds points to a very versatile action, which is consistent with the known pleiotropic effect of

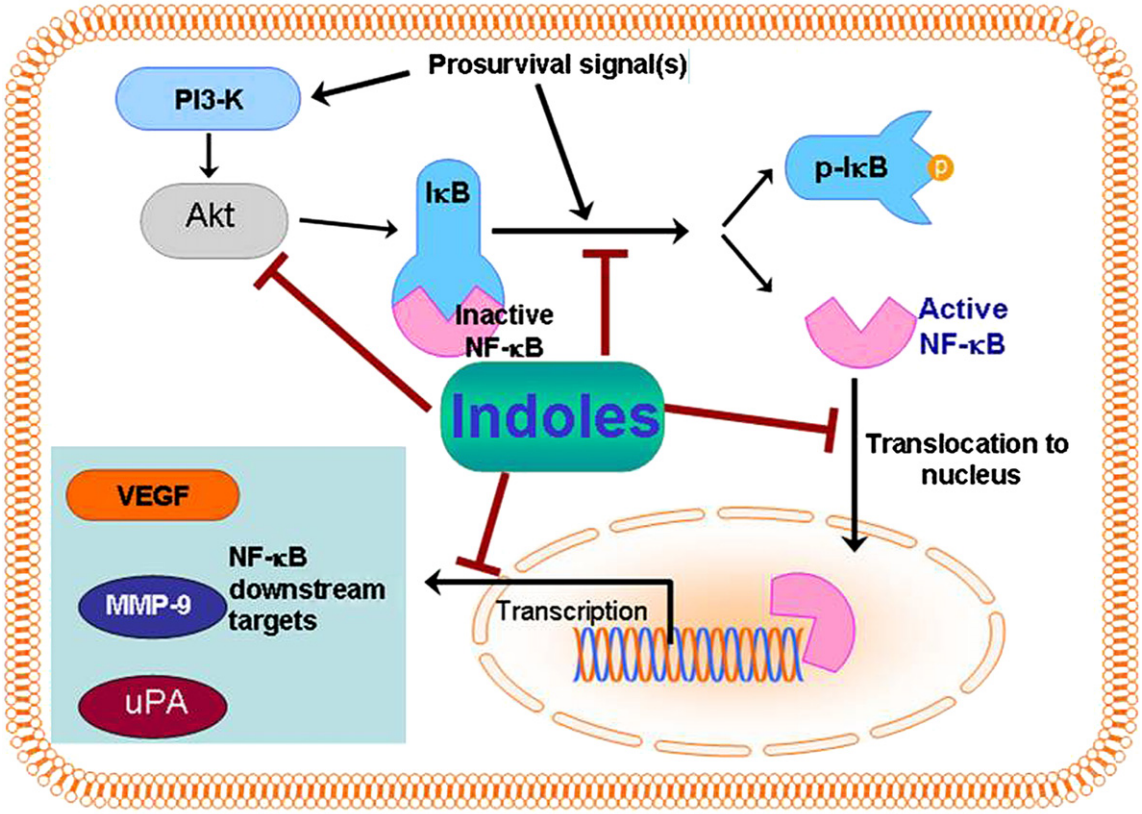

Figure 1 Pleiotropic effects of indoles through regulation of NF-κB signaling. Cancer progression involves upregulation of signaling pathways that favor proliferation, angiogenesis, and invasion. NF-κB is activated and translocated to nucleus leading to transcriptional upregulation of genes that play important roles in these processes. As efficient anticancer agents, indole compounds target an array of cellular pathways causing a reversal of prosurvival and invasion pathways and an efficient induction of apoptosis. As an example, indoles inhibit the upstream pathway (PI3-K-Akt) that regulates NF-κB signaling as well as block NF-κB activation and translocation to nucleus, thus preventing the generation of survival signals. Such a multistep regulation ensures a much increased efficacy and underlines the potential of these compounds as effective anticancer agents. MMP = matrix metalloproteinases; NF-κB = nuclear factor-kappa B; uPA = urokinase-type plasminogen activator; VEGF = vascular endothelial growth factor.

such natural compounds. In the recent years, it has been realized that modulation of NF-κB signaling pathway is, perhaps, much more central to the apoptosis-inducing and proliferation/invasion/metastasis-inhibiting property of these compounds[10,14,15,52,57] (Table 1).[25,52–57,61–64,73,80,81,85,91–94,96,97] NF-κB signaling pathway cross-talks with a number of survival pathways and its inhibition, therefore, has the potential to affect multiple signaling cascades that determine the cellular fate. In addition to an effect on growth, proliferation, clonogenicity, invasion, angiogenesis and metastasis, modulation of NF-κB signaling pathway by I3C and DIM also leads to sensitization of cancer cells to conventional chemotherapeutic agents.[59,61,62,98] This property of I3C and DIM is clinically very crucial because most of the advanced-stage cancers are characterized by resistance to available chemotherapies, leading to a very aggressive phenotype. As reviewed here, modulation of NF-κB signaling plays a very important role in such chemosensitizing effect of I3C and DIM.

The research on the anticancer effects of I3C and DIM has intensified in last decade or so with reports on the beneficial effects of these compounds against almost all different human cancer types. In last few years, many reports have emerged detailing the role of NF-κB signaling in anticancer activity of these compounds. Being true pleiotropic agents, indole compounds are now believed to inhibit each and every step, upstream and downstream of NF-κB activation, as illustrated in Figure 1. In particular, much more is known about such effect of indoles in breast, prostate, and pancreatic cancers. Because NF-κB signaling has been reported to be critical for the survival of other cancer types as well, it perhaps is just a matter of time before modulation of NF-κB pathway by indoles is elucidated in human cancers other than the three mentioned earlier. In addition to natural compounds, synthetic indole derivatives have also shown promise as anticancer agents.[19,89–91] This opens an entirely new field of cancer research where existing natural compounds can be modified to increase their efficacy and target specificity. Furthermore, with the more recent data describing the effect of DIM on the modulation of microRNAs,[92] it is apparent that indoles have enormous potential to be developed as potent anticancer agents.

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