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SELECTIVE GROWTH REGULATORY AND PRO-APOPTOTIC EFFECTS OF DIM IS MEDIATED BY AKT AND NF-kappaB PATHWAYS IN PROSTATE CANCER CELLS

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1. ABSTRACT

Prostate cancer is the second leading cause of cancer related deaths in men in the United States. I3C and its *in vivo* dimeric product, DIM, have been found to inhibit the growth of prostate cancer cells. However, the molecular mechanism(s) by which DIM elicits its effects on prostate cancer cells has not been fully elucidated. We have previously shown that I3C induces apoptosis and inhibits the activation of NF-kappaB pathway, which could be mediated via Akt signaling pathway. In this study, we investigated whether there is any cross-talk between Akt and NF-kappaB during DIM-induced apoptosis in PC-3 prostate cancer cells. We found that DIM inhibited cell growth and induced apoptosis in PC-3 prostate cancer cells but not in non-tumorigenic CRL2221 human prostate epithelial cells. DIM also inhibited EGFR expression, PI3K kinase activity, and Akt activation, and abrogated the EGF-induced activation of PI3K in prostate cancer cells. NF-kappaB DNA-binding analysis and transfection studies with Akt cDNA constructs revealed that Akt transfection resulted in the induction of NF-kappaB activity and this was inhibited by DIM treatment. DIM treatment also showed significant induction of apoptosis in non-transfected cells compared to Akt and Akt-Myr transfected prostate cancer cells. From these results, we conclude that the inhibition of Akt and NF-kappaB activity and their cross-talk is a novel mechanism by which DIM inhibits cell growth and induces apoptotic processes in prostate cancer cells but not in non-tumorigenic prostate epithelial cells.

2. INTRODUCTION

Prostate cancer is one of the most common cancers in men and the second leading cause of male cancer death in the United States (1). However, Asians have relatively low incidence of prostate cancers. Dietary and epidemiological studies have shown an association between high dietary intake of vegetables and decreased prostate cancer risk (2, 3). Among vegetables with anticarcinogenic properties, the cruciferous vegetable family including broccoli, cabbage, brussels sprouts, and cauliflower appears to be most effective at reducing the risk of cancers. Indole-3-carbinol (I3C), a common phytochemical in the human diet, is present in almost all members of the cruciferous vegetable family, and it is readily converted to its dimeric product, 3,3'-diinolylmethane (DIM) (4). There are growing evidences showing that I3C and DIM have the potential to inhibit a number of common cancers, especially those that are hormone-related (5-8).

It has been demonstrated that I3C and DIM possess anti-carcinogenic effects in experimental animals and inhibits the growth of human cancer cells *in vitro* (5, 7-10). DIM has been found to induce cell cycle arrest at G1 phase with up-regulation of $p21^{WAF1}$ and down-regulation of CDK6 (6, 11). It has been reported that DIM increases the expression of Bax, decreases the expression of Bcl-2, and induces apoptosis (12). Because of these effects, the interest in I3C and DIM as cancer chemopreventive and/or

therapeutic agents has significantly increased in the past years. We have previously shown that I3C up-regulates $p21^{WAF1}$, Bax, and $p27^{KIP1}$, and down-regulates Bcl_{XL}, EGFR, and Akt kinase activity, leading to the induction of apoptosis in prostate cancer cells (13, 14). We have also reported the gene expression profiles of prostate cancer cells exposed to I3C and DIM, showing that I3C and DIM induce the expression of genes related to the Phase I and Phase II enzymes and regulate the expression of genes involved in the control of cell growth, cell cycle, apoptosis, signal transduction, and oncogenesis (15). However, the precise molecular mechanism by which DIM exerts its effect on the induction of apoptosis and the cell signaling pathways, has not been fully elucidated.

Akt and NF-kappaB pathways are important cell signaling pathways involved in the processes of apoptosis, carcinogenesis, and tumor progression (16-19). NF-kappaB is a cell survival factor and can be activated by many types of stimuli including TNF-α, EGF, UV radiation, etc. There is growing evidence to suggest the role of NF-kappaB in the protection against apoptosis (18, 20). An *in vivo* study showed that mice lacking NF-kappaB p65/RelA died embryonically from extensive apoptosis in the liver (21), suggesting anti-apoptotic role of NF-kappaB. Akt can be activated by various growth factors including EGF through activation of phosphatidylinositol-3 kinase (PI3K) (17). Activated Akt functions to promote cell survival by inhibiting apoptosis through its ability to phosphorylate and inactivate downstream targets (16, 17). Several reports have showed that Akt also regulates the NF-kappaB pathway via phosphorylation and activation of molecules in the NF-kappaB pathway (22-24). Because both Akt and NF-kappaB have been critically involved in the cell survival and apoptotic process, in this study, we investigated whether DIM could inhibit Akt and NF-kappaB activation leading to apoptosis, and whether Akt and NF-kappaB pathways could cross-talk during apoptotic process induced by DIM. Finally we also investigated whether there is any differential effect of DIM between PC-3 prostate cancer cells and non-tumorigenic CRL2221 prostate epithelial cells.

3. MATERIALS AND METHODS

3.1. Cell culture and reagents

PC-3 human prostate cancer cells (ATCC, Manassas, VA) were cultured in RPMI-1640 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin in a 5% CO_2 atmosphere at 37°C. CRL-2221 human non-tumorigenic prostate epithelial cells (ATCC, Manassas, VA) were cultured in keratinocyte-SFM media (Invitrogen, Carlsbad, CA) supplemented with EGF (0.2 microgram/L), bovine pituitary extract (30 milligram/L), and 1% penicillin and streptomycin. DIM (LKT, St. Paul, Minnesota) was dissolved in DMSO to make 60 millimole/L stock solution. Wherever indicated, EGF (Invitrogen, Carlsbad, CA) was added to the media at a final concentration of 100 microgram/L.

3.2. Cell growth inhibition by MTT assay

The PC-3 and CRL-2221 cells were seeded at a density of 1×10^3/well in 96 well culture dishes. After 24 hours, the cells were treated with 15, 30, and 60 micromole/L DIM for one to three days. Control PC-3 cells received 0.1% DMSO for same time points. The cells were then incubated with MTT (0.5 gram/L, Sigma, St. Louis, MO) at 37°C for 4h and with DMSO at room temperature for 1 h. The spectrophotometric absorbance of the samples was measured by using ULTRA Multifunctional Microplate Reader (TECAN, Durham, NC) at 595 nm. The experiment was repeated three times and t test was performed to verify the significance of cell growth inhibition after treatment.

3.3. Histone/DNA ELISA for detecting apoptosis

Cell Apoptosis ELISA Detection Kit (Roche, Palo Alto, CA, USA) was used to detect apoptosis in PC-3 and CRL-2221 cells with different treatments according to manufacturer's protocol. Briefly, the cytoplasmic histone/DNA fragments from PC-3 and CRL-2221 cells treated with 15, 30, and 60 micromole/L DIM or 0.1% DMSO (vehicle control) for 24, 48, 72 hours, were extracted and bound to immobilized anti-histone antibody. Subsequently, the peroxidase-conjugated anti-DNA antibody was used for the detection of immobilized histone/DNA fragments. After addition of substrate for peroxidase, the spectrophotometric absorbance of the samples was determined by using ULTRA Multifunctional Microplate Reader (TECAN, Durham, NC) at 405 nm.

3.4. Western blot analysis

The PC-3 and CRL-2221 cells were plated on culture dishes and allowed to attach for 24 hours followed by the treatment with 15, 30, or 60 micromole/L DIM for 48 hours. Control cells were incubated in the medium with 0.1% DMSO using same time points. After incubation, the cells were lysed in 62.5 millimole/L Tris-HCl and 2% SDS. Protein concentration was then measured using BCA protein assay (PIERCE, Rockford, IL). Cell extracts were subjected to 10% SDS-PAGE, and electrophoretically transferred to nitrocellulose membrane. Membranes were incubated with anti-Akt (Santa Cruz Biotech, Santa Cruz, CA), anti-phospho-Akt Ser473 (Cell signaling, Beverly, MA), anti-EGFR (Santa Cruz Biotech, Santa Cruz, CA), anti-Bcl_{XL} (Santa Cruz Biotech, Santa Cruz, CA), and anti-β-actin (Sigma, St. Louis, MO) antibodies, washed with TTBS and incubated with secondary antibody conjugated with peroxidase. The signal was then detected using the chemiluminescent detection system (PIERCE, Rockford, IL).

3.5. Reporter gene constructs and transfection

pLNCX-Akt (normal Akt), pLNCX-Myr-Akt (constitutively activated Akt), pLNCX-Akt-K179M (dominant negative), and pLNCX (control vector) were generously provided by Dr. Sellers (Dana-Farber Cancer Institute, Boston, MA). NF-kappaB-LUC (Stratagene, La Jolla, CA) contains six repeated copies of the NF-kappaB DNA-binding site and a luciferase reporter gene. CMV-beta-gal reporter construct transfection was used for normalization of transfection efficiency. The pLNCX-Akt,

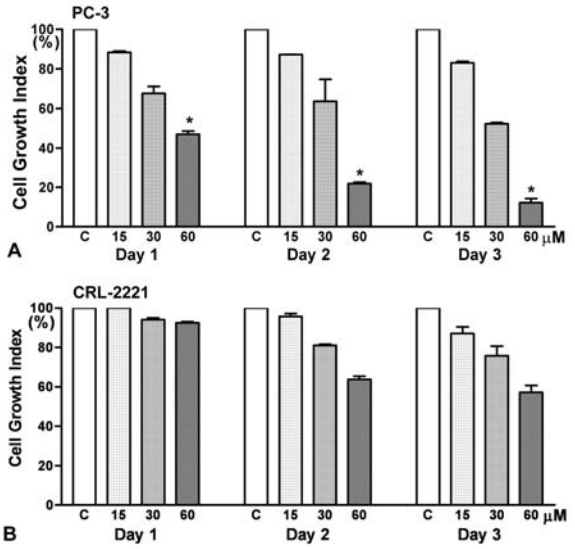

Figure 1. Effects of DIM on the growth of PC-3 (A) and CRL-2221 cells (B) tested by MTT assay. (*: $p<0.05$; n=3).

pLNCX-Myr-Akt, pLNCX-Akt-K179M, or pLNCX was transiently co-transfected with NF-kappaB-LUC and CMV-beta-gal into PC-3 cells when they were at ~70% confluent using the LipofectAMINE (Invitrogen, Carlsbad, CA). After incubation for 5 hours, the transfected cells were washed and incubated with RPMI-1640 media (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum overnight followed by treatment with 60 micromole/L DIM for 48 hours. Subsequently, the luciferase activities in the samples were measured by using Steady-Glo™ Luciferase assay system (Promega, Madison, WI) and ULTRA Multifunctional Microplate Reader (TECAN, Durham, NC). To detect the NF-kappaB activity in transfected PC-3 cells, the samples were subjected to NF-kappaB DNA-binding activity measurement using EMSA method as described below. Cell Apoptosis ELISA Detection Kit was also used to detect apoptosis in transfected and parental PC-3 cells.

3.6. NF-kappaB DNA-binding activity measurement

PC-3 and CRL-2221 cells were treated with 15, 30, and 60 micromole/L DIM for 72 hours. Following treatment, the nuclear proteins from cells were extracted. Ten microgram of nuclear proteins was subjected to electrophoretic mobility shift assay (EMSA) as described previously (25). Competition assay using unlabeled specific competitor (NF-kappaB oligo) was conducted to confirm the specificity of NF-kappaB DNA-binding activity.

3.7. Immunoprecipitation and PI3K kinase assay

The PI3K kinase activity of PC-3 cells treated with 60 micromole/L DIM for 24 and 48 hours, 60 micromole/L DIM for 24 and 48 hours followed by EGF treatment for 20 minutes, EGF only, or 0.1% DMSO was measured by using PI3K kinase assay kit (Echelon, Salt Lake, UT) according to manufacturer's protocol. Briefly, the cells after treatments were lysed in ice-cold cell lysis buffer (137 millimole/L NaCl, 20 millimole/L Tris-HCl, 1 millimole/L $CaCl_2$, 1 millimole/L $MgCl_2$, 0.1 millimole/L sodium orthovanadate, 1% NP-40, and 1 millimole/L PMSF) on ice for 20 minutes. After centrifugation, the protein concentration of supernatant was measured by BCA protein assay (PIERCE, Rockford, IL). 600 micrograms of proteins from each sample were used for immunoprecipitation with PI3K antibody (Upstate, Charlottesville, VA) overnight and protein G-agarose for one hour at 4°C. Then, the samples were collected by centrifugation, washed with kinase buffer, and subjected to PI3K kinase assay in kinase buffer (20 millimole/L Tris pH 7.4, 4 millimole/L $MgCl_2$, 10 millimole/L NaCl), 25 micromole/L ATP, and 2.4 micrograms of $PI(4,5)P_2$ as kinase substrate. PI(3,4,5)P3 was measured by competitive ELISA.

3.8. Signal quantification and statistical analysis

The EMSA gel was scanned, and the signals in the gel were quantified and analyzed with Odyssey software (LI-COR, Lincoln, NE). Signal in the Western blots was also scanned and quantified with Molecular Analyst software (Bio-Rad, Hercules, CA). Comparisons were made between control and treatments. Statistical analysis was performed using t test between treated and untreated samples. P values less than 0.05 were used to indicate statistical significance.

4. RESULTS

4.1. DIM selectively inhibits growth of prostate cancer cells

PC-3 prostate cancer cells and CRL-2221 non-tumorigenic human prostate epithelial cells were treated with 0-60 micromole/L DIM over 3 days and the cell viability was determined by MTT assay. The treatment of PC-3 prostate cancer cells with DIM resulted in a dose and time-dependent inhibition of cell proliferation (Figure 1A). However, only 37.8% growth inhibition was observed in the CRL-2221 non-tumorigenic prostate epithelial cells treated with 60 micromole/L DIM for 3 days compared to 87.8% in PC-3 cells (Figure 1B), suggesting the selective growth inhibition of prostate cancer cells by DIM. Inhibition of cell proliferation observed by MTT could be partly due to the induction of apoptosis in prostate cancer cells. We, therefore, investigated whether DIM could selectively induce apoptosis in PC-3 prostate cancer cells.

4.2. DIM selectively induces apoptosis in prostate cancer cells

By ELISA analysis of cytoplamic histone/DNA fragments, we observed an induction of apoptosis in prostate cancer cells treated with 15-60 micromole/L DIM (Figure 2A). The induction of apoptosis was time- and dose-dependent, and was directly correlated with the inhibition of cell growth, suggesting that DIM treatment may result in the inhibition of cell proliferation through apoptotic cell death. More importantly, non-tumorigenic CRL-2221 prostate epithelial cells were much less responsive to DIM treatment than PC-3 cells (Figure 2B), suggesting that DIM selectively induced apoptosis in prostate cancer cells.

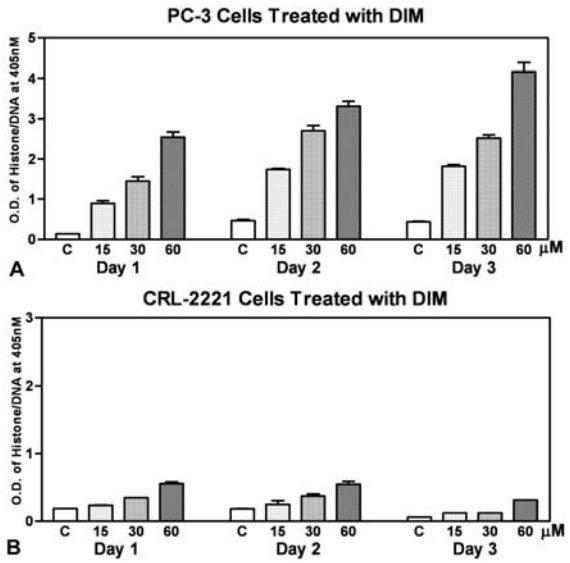

Figure 2. Induction of apoptosis in PC-3 (A) and CRL-2221 (B) cells tested by ELISA. (n=2).

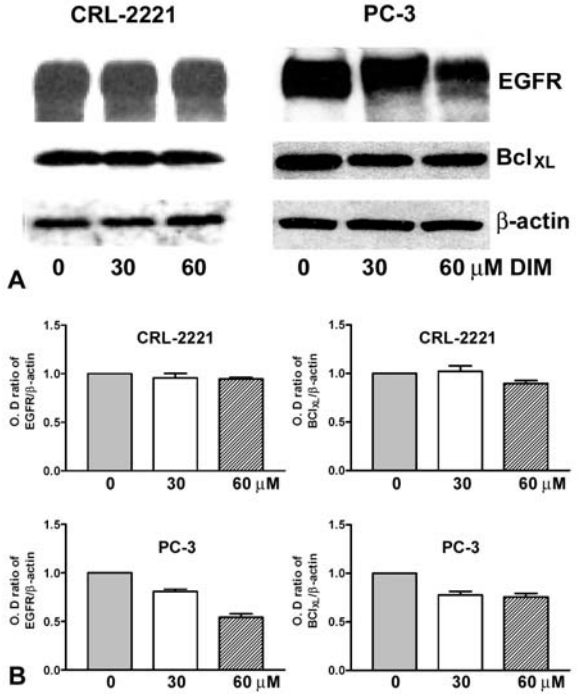

Figure 3. Western blot (A) and densitometric analysis (B) of Bcl_{XL} and EGFR in PC-3 and CRL-2221 cells treated with DIM.

By Western Blot analysis, we also found that DIM inhibited the expression of Bcl_{XL}, an anti-apoptotic protein in PC-3 cells (Figure 3). However, there was no significant effect on Bcl_{XL} in DIM-treated CRL-2221 cells, and these results were correlated with minimal apoptosis in DIM-treated CRL-2221 cells. Next, we investigated whether PI3K/Akt and NF-kappaB signaling pathways are involved in the apoptotic processes induced by DIM in prostate cancer cells.

4.3. DIM inhibits PI3K and Akt activation and induces apoptoisis through Akt pathway

Since Akt signaling pathway is an important signal transduction pathway that plays a critical role in cell survival and apoptotic processes, we investigated the status of Akt in PC-3 and CRL-2221 cells treated with 0-60 micromole/L DIM by Western Blot analysis. We did not find any alterations in the protein expression of un-phosphorylated Akt in DIM-treated PC-3 and CRL-2221 cells (Figure 4). However, a significant decrease in the phosphorylated Akt protein at Ser473 was observed in DIM treated PC-3 cells compared to control cells, suggesting inactivation of Akt kinase after DIM treatment (Figure 4). Treatment of DIM showed dose dependent inhibition of Akt phosphorylation in PC-3 cells, consistent with the induction of apoptosis by DIM. However, the phosphorylated Akt Ser473 protein was undetectable in CRL-2221 cells treated or untreated with DIM, suggesting that the inactivation of Akt by DIM is specific in prostate cancer cells compared to non-tumorigenic prostate epithelial cells.

Since Akt is activated through the activation of PI3K, we investigated the PI3K kinase activity in the PC-3 cells treated with DIM or pre-treated with DIM followed by EGF stimulation. We found that DIM treatment inhibited the activity of PI3K (Figure 5), suggesting that DIM could inactivate Akt through the inhibition of PI3K activity. We also found that EGF treatment alone activated PI3K kinase activity as expected, and that DIM pretreatment abrogated the activation of PI3K stimulated by EGF (Figure 5). Because the activation of Akt and PI3K could be mediated through EGFR pathways, we measured the expression of EGFR by Western Blot analysis. We found that DIM inhibited EGFR expression in a dose-dependent manner in PC-3 cells only and showed no inhibition of EGFR in CRL-2221 cells (Figure 3), corresponding with the selective effect of DIM on Akt activation in PC-3 cells.

Furthermore, we transfected Akt cDNA into PC-3 cells and measured the degree of apoptosis in transfected cells with and without DIM treatment. We found that DIM not only induced apoptosis in PC-3 parental cells but also in Akt transfected PC-3 cells although to a lesser extent as expected (Figure 6). More importantly, we found that transfection of constitutively activated Akt (pLNCX-Myr-Akt) and wild-type Akt (pLNCX-Akt) inhibited apoptosis induced by DIM compared to mutant Akt and empty vector transfectants, suggesting that the induction of apoptosis by DIM is partly mediated by active Akt and that the overexpression of Akt leads to resistance to DIM-induced apoptosis.

4.4. DIM selectively inhibits NF-kappaB activation in PC-3 cells

Nuclear extracts from control and DIM-treated PC-3 cells were subjected to NF-kappaB DNA-binding activity as measured by EMSA. Autoradiography revealed that 30-60 micromole/L DIM significantly inhibited NF-kappaB DNA-binding activity in PC-3 cells compared to untreated control (Figure 7). However, no such effect was observed in DIM-treated CRL-2221 cells, suggesting the

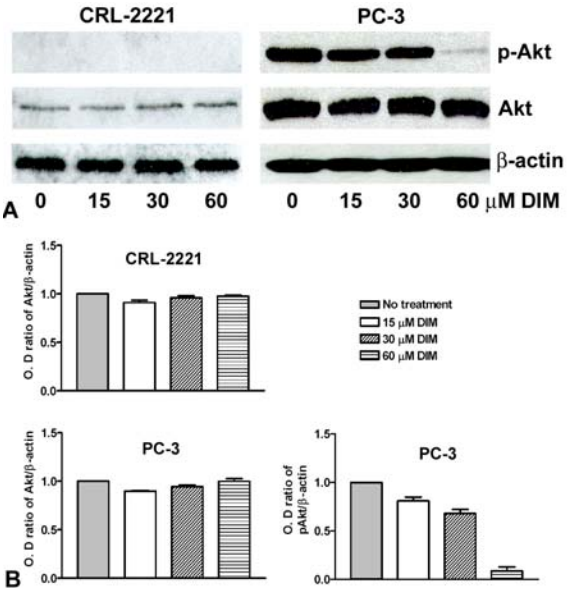

Figure 4. Western blot (A) and densitometric analysis (B) of total Akt and p-Akt in PC-3 and CRL-2221 cells treated with DIM.

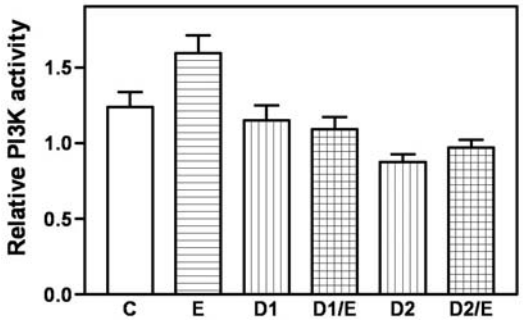

Figure 5. Relative PI3K activity in PC-3 cells treated with DIM. (C: control; E: treated with EGF; D1: treated with 60 micromole/L DIM for 24 hours; D1/E: treated with 60 micromole/L DIM for 24 hours followed by EGF treatment; D2: treated with 60 micromole/L DIM for 48 hours; D2/E: treated with 60 micromole/L DIM for 48 hours followed by EGF treatment).

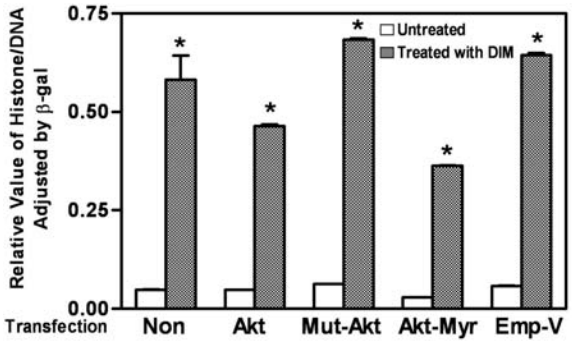

Figure 6. Induction of apoptosis in transfected and DIM treated PC-3 cells tested by ELISA (Non: no transfection; Akt: transfected with pLNCX-Akt; Mut-Akt: transfected with pLNCX-Akt-K179M; Akt-Myr: transfected with pLNCX-Myr-Akt; Emp-V: transfected with empty vector, pLNCX; *: $p<0.05$; n=2).

selective inhibitory effect of DIM on NF-kappaB DNA-binding activity in prostate cancer cells. In order to further explore the inhibitory effects of DIM on Akt and NF-kappaB pathways, we conducted transfection experiments as described under Materials and Methods. Luciferase assay showed a significant increase in luciferase activity in PC-3 cells co-transfected with pLNCX-Myr-Akt and NF-kappaB-Luc, and also in PC-3 cells co-transfected with pLNCX-Akt and NF-kappaB-Luc (Fig 8). Moreover, DIM significantly abrogated the induction of luciferase activity caused by pLNCX-Myr-Akt and pLNCX-Akt transfections (Figure 8).

To confirm these results, we also examined the NF-kappaB DNA-binding activity in PC-3 cells transfected with pLNCX-Myr-Akt or pLNCX. We observed an increase in NF-kappaB DNA-binding activity in PC-3 cells transfected with pLNCX-Myr-Akt (Figure 9). We also found that DIM abrogated the activation of NF-kappaB DNA-binding activity caused by pLNCX-Myr-Akt transfection (Figure 9). Collectively, these results provide evidence for a potential cross-talk between Akt and NF-kappaB pathways during DIM induced cell growth inhibition and apoptosis in prostate cancer cells.

5. DISCUSSION

DIM, the major *in vivo* product of dietary I3C, has been shown to inhibit cell growth and induce apoptosis in breast, cervical and prostate cancer cells (5-7, 11), suggesting its chemopreventive and/or therapeutic effects on cancer cells. However, the precise molecular mechanisms by which DIM inhibits cell growth and induces apoptosis have not been fully elucidated. Additionally, the effect of DIM on non-tumorigenic epithelial cells remains unknown. Here, we demonstrated that DIM significantly and selectively inhibited cell growth and induced apoptosis in PC-3 prostate cancer cells, while CRL-2221 non-tumorigenic cells showed much less response to DIM. These results provide evidence for selective effects of DIM on cell growth and apoptosis in cancer cells. To discover the molecular mechanisms responsible for the induction of apoptosis by DIM, we investigated the effects of DIM on Akt and NF-kappaB pathways, which have been known to play important roles in cell survival and apoptotic cell death processes.

It has been known that Akt signaling pathway can be activated by various growth and survival factors such as EGF, PDGF, insulin, etc, through activation of PI3K (16, 17). PI3K activation leads to the production of phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P_3), which interacts with Akt PH domain. This interaction subsequently causes conformational changes in Akt, resulting in the exposure of two main phosphorylation sites in Akt. Akt is then activated by phosphorylation at Thr308 by Phosphoinositide-dependent protein kinase 1 (PDK1) or at Ser473 by PDK2. Activated Akt functions to promote cell survival by inhibiting apoptosis through its ability to phosphorylate and inactivate several targets including Bad, Forkhead transcription factors, and caspase-9, all of which are involved in apoptotic pathway (26, 27). In the apoptotic

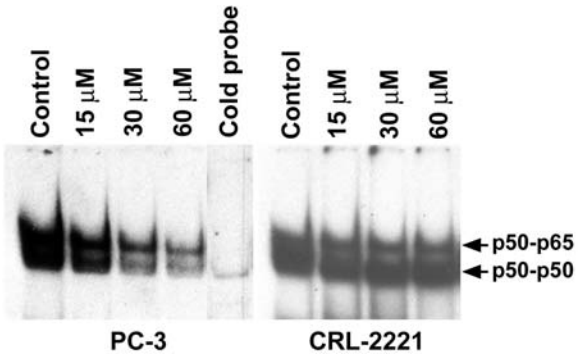

Figure 7. NF-kappaB DNA-binding activity in DIM treated PC-3 and CRL-2221cells tested by EMSA. (Cold probe: Unlabeled NF-kappaB oligonucleotide was used as specific competitor in DNA-binding reaction).

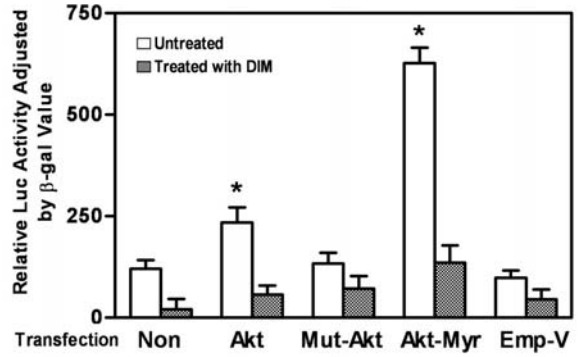

Figure 8. Luciferase activity in transfected PC-3 cells with or without DIM treatments (Non: no transfection; Akt: transfected with pLNCX-Akt; Mut-Akt: transfected with pLNCX-Akt-K179M; Akt-Myr: transfected with pLNCX-Myr-Akt; Emp-V: transfected with empty vector, pLNCX; *: $p<0.05$; n=2).

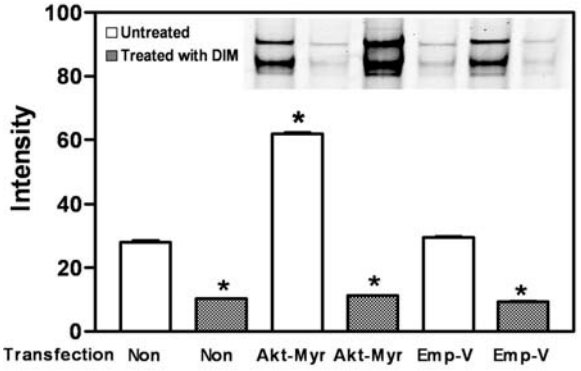

Figure 9. EMSA and densitometric analysis of NF-kappaB DNA-binding activity in transfected PC-3 cells with or without DIM treatments (Non: no transfection; Akt-Myr: transfected with pLNCX-Myr-Akt; Emp-V: transfected with empty vector, pLNCX; *: $p<0.05$; n=2).

process, dephosphorylated Bad (activated form of Bad) translocates to mitochondria, where it heterodimerizes via its BH3 domain with anti-apoptotic BCL family members such as Bcl-2 and Bcl_{XL}, promoting the onset of apoptosis (28-31). In this study, we found that DIM treatment caused the down-regulation of EGFR, suggesting that DIM could inhibit the activation of PI3K through decrease in growth factor binding. By PI3K kinase assay, we found that DIM inactivated PI3K and abrogated the activation of PI3K caused by EGF, suggesting that DIM could inhibit Akt activity by inactivation of PI3K. Subsequently, we found no changes in the expression of un-phosphorylated Akt in DIM treated PC-3 cells. However, DIM decreased the level of phosphorylated Akt, which is the activated form of Akt. This could subsequently increase the activated Bad binding to Bcl-2 and Bcl_{XL}. We found that DIM inhibited the expression of Bcl_{XL}, suggesting an increase in the ratio of Bad/Bcl_{XL}, which could promote cancer cell to apoptotic cell death. Indeed, we observed significant induction of apoptosis in DIM treated PC-3 prostate cancer cells, and lesser apoptosis in Akt transfected PC-3 cells compared to parental PC-3 cells. These results suggest that the inhibition of PI3K/Akt signaling pathway by DIM is one of the mechanisms by which DIM induces apoptosis in prostate cancer cells.

It has been well known that NF-kappaB plays an important role in the apoptotic process (18, 20). Thus, DIM may induce apoptosis by modulating multiple components in the Akt and NF-kappaB pathways. In this study, we found that DIM selectively inhibited NF-kappaB DNA-binding activity in PC-3 prostate cancer cells. The inactivation of NF-kappaB DNA-binding activity may be another mechanism by which DIM induces apoptosis in PC-3 cells. It has been reported that the activity of NF-kappaB may be regulated by a variety of factors including Akt (22, 24). Akt has been shown to enhance the degradation of the IkappaB and induce NF-kappaB activation (32). The ability of Akt to regulate NF-kappaB activity may be through direct interaction with the IKK, supported by the observation that Akt is associated with the IKK complex *in vivo* (33). It has been demonstrated that Akt can phosphorylate and activate IKK at a critical regulatory site, Thr23, and subsequently activate NF-kappaB (24). In this study, we transfected Akt to PC-3 prostate cancer cells and tested the effect of Akt on NF-kappaB DNA-binding activity by EMSA and luciferase assay. Our results showed that Akt regulated NF-kappaB activation, and this observation is in accordance with our published data (25). Importantly, we found that DIM abrogated the NF-kappaB activation stimulated by Akt transfection, suggesting that the inhibition of NF-kappaB activity by DIM is partly mediated through Akt signaling pathway. Hence, the inactivation of DNA-binding activity of NF-kappaB by DIM appears to be responsible for DIM-induced apoptosis in PC-3 prostate cancer cells.

NF-kappaB has been described as a major culprit in cancer (34), and Akt has been known as a key molecule in cell survival (16, 17). Because of their importance in the control of cell survival and apoptotic cell death, both Akt and NF-kappaB have been believed to be very attractive therapeutic targets for cancer therapy (33, 35-37). Therefore, our results indicate that the inhibition of Akt and NF-kappaB activity could be easily achievable by DIM treatment, which inhibits cell growth and induces apoptosis in PC-3 prostate cancer cells, suggesting that DIM may be a useful agent for the prevention and/or treatment of

prostate cancer. More importantly, we found that DIM had no significant effects on cell growth, apoptosis, Akt and NF-kappaB activity in non-tumorigenic prostate epithelial cells, suggesting cancer cell specific effects of DIM. Similar cancer cell specific effects of I3C were also reported previously by our laboratory in breast epithelial cells (38). The fact that DIM selectively inhibits cell growth, Akt and NF-kappaB activation, and induces apoptosis in PC-3 prostate cancer cells, makes it a potent chemopreventive and/or therapeutic agent against prostate cancer. Our results warrant further animal and human investigations in order to fully appreciate the value of DIM in human health.

6. ACKNOWLEDGEMENT

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