Prostate cancer is the second leading cause of cancer-related death in men. An estimated 186,320 new cases will be diagnosed in 2009 in the United States (1). Androgen ablation therapy and bilateral orchidectomy have been the main therapeutic options for locally advanced and/or metastatic androgen-dependent prostate cancer (AIPC) cells. Furthermore, ectopic expression of Akt renders AIPC cells resistant to chemotherapy; however, psoralidin overcomes Akt-mediated resistance and induces apoptosis in AIPC cells. While dissecting the molecular events, both upstream and downstream of Akt, we found that psoralidin inhibits phosphatidylinositol 3-kinase (PI3K; 9), which plays an important role in the conversion of androgen-dependent prostate cancer to AIPC. Recently evidences suggest that Akt is the focal point of a number of signaling pathways that regulate cell growth, survival, proliferation, immune activation, apoptosis (5), and tumor progression in many cancer types (6), including AIPC (7, 8). Akt is activated by the lipid kinase, phosphatidylinositol 3-kinase (PI3K; 9), which plays an important role in the prosurvival mechanism in a number of cell types and in a majority of cancers. PI3K is a heterodimeric protein composed of a catalytic subunit (p110α/β/γ/δ) and a regulatory subunit (p85α/β/γ) that is involved in cell growth, transformation, and differentiation. Concurrent phosphatase and tensin homologue (PTEN) inactivation negatively regulates Akt activation; however, PTEN is frequently mutated or deleted in many cancers including prostate cancer (10).

Three isoforms of Akt (Akt-1, Akt-2, and Akt-3) have been identified, and all three share a similar domain structure, (3). Most patients with AIPC show resistance to current chemotherapeutics, and even docetaxel-based combination therapy yield only a limited improvement in patient survival (4), suggesting that novel targeted therapies are much needed. Recent evidences suggest that Akt plays an important role in the conversion of androgen-dependent prostate cancer to AIPC.

Accumulating evidence suggests that Akt is the focal point of a number of signaling pathways that regulate cell growth, survival, proliferation, immune activation, apoptosis (5), and tumor progression in many cancer types (6), including AIPC (7, 8). Akt is activated by the lipid kinase, phosphatidylinositol 3-kinase (PI3K; 9), which plays an important role in the prosurvival mechanism in a number of cell types and in a majority of cancers. PI3K is a heterodimeric protein composed of a catalytic subunit (p110α/β/γ/δ) and a regulatory subunit (p85α/β) that is involved in cell growth, transformation, and differentiation. Concurrent phosphatase and tensin homologue (PTEN) inactivation negatively regulates Akt activation; however, PTEN is frequently mutated or deleted in many cancers including prostate cancer (10).

Three isoforms of Akt (Akt-1, Akt-2, and Akt-3) have been identified, and all three share a similar domain structure,
which is widely expressed in most organs and tissues. Akt-1 and Akt-3 is also expressed to some extent in prostate cancer cell lines and human prostate cancer samples (11). Evidence suggests that overexpression of Akt decreases the cellular levels of p27, thereby causing cell proliferation (12). Conversely, activation Akt increases expression of cyclin D1, which is an established driving force for cell cycle progression, through the phosphorylation and resultant activation of GSK-3β (13). Down-regulation of constitutively active Akt (by PI3K inhibitors, wortmannin, or PD98059) reverses both cell survival and resistance to chemotherapeutic agents. In a transgenic mouse model, a constitutively activated Akt has been shown to promote prostate intraepithelial neoplasia (14). Similarly, in vitro studies in prostate cancer suggest that overexpression of Akt inhibits the apoptotic response and releases the cells from cell cycle arrest, ultimately rendering these tumor cells resistant to current treatments (15). Phosphorylation of Akt (Ser473) has been shown to correlate with higher Gleason score, which is a prognostic marker for poor clinical outcome in prostate cancer patients (16). These studies indicate that activation of this key survival kinase plays an important role in tumor development by activating nuclear factor-κB (NF-κB)/Bcl-2 prosurvival signaling pathway.

Chemoprevention as well as chemotherapeutic intervention by phytochemicals provides new dimensions for the management of AIPC. Psoralidin is one of the active ingredients (Fig. 1) in *Psoralea corylifolia*, a herbal plant that is extensively used in traditional medicine against gynecologic bleeding, vitiligo, and psoriasis (17). Moreover, it possesses hepatoprotective properties (18), osteoblastic proliferation-stimulating activity (19), and antibacterial activity (20). The molecular components of *P. corylifolia* include coumarins and flavones, such as psoralen, isopsoralen, bavachalcone, etc. (21). Sporadic reports detail the cytotoxic effect of psoralidin on MCF-7 (breast), HT29 (colon; ref. 22), SNU-1, and SNU-16 (stomach carcinoma) cell lines (23), with no significant effects observed in A549 (lung) and HepG2 (liver hepatoma) cell lines (22). This study provides the first evidence of the anticancer effect of psoralidin and enables mechanistic insights into antitumor action of this compound against AIPC cells. Our data suggest that psoralidin exhibits a potent anticancer effect on AIPC cells; also, in combination, psoralidin may enhance the anticancer activity of the current chemotherapeutic agents by inhibiting the PI3K-mediated prosurvival signaling and inducing apoptosis in AIPC cells.

**Materials and Methods**

**Cell lines, plasmid constructs, and natural compounds**

PC-3, DU-145, LNCaP, 22Rv1, and PzHPv-7 cells were purchased from the American Type Culture Collection. The cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 1% glutamine, and antibiotics. The plasmids pcDNA, pcDNA3 flag HA Akt1, and pcDNA KD-Akt were kindly given by Dr. Naoya Fujita (24). Psoralidin was isolated in Jurgen Rohr’s laboratory (University of Kentucky); high-performance liquid chromatography, nuclear magnetic resonance, and elemental analysis were done to determine the purity of the compound.

**Cell viability and apoptotic assays**

Cells were treated with psoralidin (indicated concentrations) or vehicle (DMSO) for 24 h to determine cell viability as described earlier (25). Another deoxyxynucleotidyl transferase dUTP nick end labeling (TUNEL) and Annexin V-FITC staining assays were done following treatment of PC-3, DU-145, 22Rv1, LNCaP, PC-3/Neo, and PC-3/PzHPv-7 with psoralidin as described earlier (25).

**Transfection and luciferase assays**

For NF-κB promoter assays and ectopic expression of Akt, prostate cancer cells (PC-3 and DU-145 at 50-60% confluency) were transiently transfected using the Lipofectamine reagent (Life Technologies) with 2.5 μg of plasmid DNA. The plasmids included the pcDNA3 flag HA Akt1 vector, which contains the same hemagglutinin-tagged myr-Akt or a kinase-inactive mutant of Akt pcDNA (KD-Akt) or pCB6+ (control vector). Each construct was cotransfected with NF-κB luciferase reporter plasmid (containing two tandem NF-κB-responsive sites) in the presence of pRL-TK (renilla plasmid control) to normalize transfection efficiency and promoter activation studies were done as described earlier (26).

**Binding studies for NF-κB activity**

PC-3 and DU-145 cells were treated with psoralidin for different time intervals and different doses as indicated, and ELISA was done for the activity of NF-κB using universal EZ-TFA transcription factor assay kit (Millipore) as per the manufacturer’s instructions.

**IκBα activation studies**

PC-3 and DU-145 cells were treated with psoralidin for indicated time intervals and IκBα activity was quantified using IκBα-ELISA kit (Calbiochem) as per the manufacturer’s instructions. Briefly, standards and samples (cell lysates) were incubated in a streptavidin-coated plate for 2 h, then an anti-IκBα detector antibody was used to detect IκBα present in the sample that was then detected using a second antibody conjugated with horseradish peroxidase. Finally, a TMB substrate was added to detect the activity in each sample and the absorbance was read at 450 nm. The absorbance was directly proportional to IκBα activity in the samples.

**Western blot analysis**

PC-3 and DU-145 cells were treated with psoralidin for varying time intervals or doses; whole-cell lysates or nuclear and cytoplasmic extracts were obtained and subjected to Western blot analysis using the antibodies Akt, NF-κB p50, p65, IκBα, p-IκKα/β, p-IRKα/β, p-IKKβ, NIK, Bcl-2, cyclin-D1, GSK-3, and Bax from Santa Cruz Biotechnology; PI3K p85, p110, and pAkt from Cell Signaling Technology; and Bcl-xL from MBL International. β-Actin and h (Santa Cruz Biotechnology) were used as the internal loading controls.
Immunoprecipitation studies
PC-3 and DU-145 cells were treated with psoralidin or vehicle (DMSO) at different time intervals; cell lysates were immunoprecipitated using PI3K antibody from Upstate USA, Inc.; and the immunoprecipitated samples were subjected to Western blot analysis using PI3K p85 and p110 antibodies from Cell Signaling Technology.

Kinase assays
PI3K assay was done in psoralidin-treated and vehicle-treated PC-3 and DU-145 cells. Briefly, cells were treated with psoralidin or vehicle for up to 24 h, cell lysates were subjected to immunoprecipitation, and kinase assay was done as per the manufacturer’s instruction from Echelon using PI(4,5)P2 as a substrate and PI(3,4,5)P3 as a primary and (K-SEC1) as a secondary detector. The kinase reaction was done in streptavidin-coated detection plate and incubated with TMB solution. The reaction was stopped by adding 1 N H2SO4. Absorbance was read at 450 nm on a plate reader.

Similarly, Akt kinase assays were done using kinase assay kits from Cell Signaling Technology. As per the manufacturer’s protocol, cell lysates were immunoprecipitated with immobilized Akt. The kinase assay was done using purified proteins that were incubated with GSK-3 fusion protein in the presence of ATP for 30 min at 30°C. The reaction was stopped using 3× SDS sample buffer and the samples were subjected to Western blot analysis using p-GSK-3α/β (Ser21/9) antibody.

Xenograft studies
We tested the effect of psoralidin on tumors derived from PC-3 cells in 5- to 6-wk male nude (nu/nu) mice from Harlan, in accordance with the University of Kentucky Animal Care and Use Committee guidelines. About 5 × 10⁶ cells were injected s.c. and tumors were allowed to grow until they reached a volume of 50 mm³. At this time, the animals were randomized into three groups, with 9 mice in each group, and psoralidin (15 and 25 mg/kg body weight), dissolved in corn oil, or same amount of corn oil was administrated intratumorally 5 d/wk for up to 4 wk. Tumor volume was measured daily over the observation period.

Following completion of the study, regressed tumors were obtained and TUNEL assay was done to determine whether the tumor regression observed in the animals was a result of the induction of apoptosis (25).

Statistical analysis
All experiments were done thrice to ascertain the reproducibility of the results. The Student’s t test was used to calculate statistical significance.

Results
Psoralidin targets Akt signaling in prostate cancer
To investigate whether psoralidin targets Akt signaling in AIPC cells, we treated PC-3 and DU-145 cells with psoralidin in a dose- and time-dependent manner and the activity of Akt was assessed using phosphorylation site-specific antibodies. Psoralidin inhibits the phosphorylation of Akt at Ser⁴⁷³ without altering total Akt levels in PC-3 (IC⁵₀ 60 μmol/L) and DU-145 (IC⁵₀ 45 μmol/L) cells (Fig. 2A). We used LY294002 (20 μmol/L) as the positive control for determining the phosphorylation status of Akt at Ser⁴⁷³ in PC-3 and DU-145 cells and treatment with
LY294002 resulted to a decrease in Akt phosphorylation in both the cell lines, which was comparable with psoralidin treatment (Fig. 2A). Similarly, dose-dependent inhibition of pAkt was seen in both PC-3 and DU-145 cells (Fig. 2B). Additionally, cyclin D1, one of the major downstream targets of Akt, was also down-regulated in a dose-dependent fashion in both the cell lines following treatment with psoralidin (Fig. 2B).

GSK-3 is a direct substrate of Akt kinase; thus, we assessed the kinase activity of Akt based on the expression of phospho-GSK-3 (Ser21/9) in AIPC cells treated with psoralidin. As expected, in PC-3 and DU-145 cells, the phospho-GSK-3 expression was down-regulated and total GSK-3 levels remained constant following treatment with psoralidin (Fig. 2B). We subsequently investigated whether overexpression of Akt could overcome the anticancer effect of psoralidin on AIPC cells, for which we transiently overexpressed myr-Akt, KD-Akt, or pCB6+ followed by treatment with psoralidin, and the treated cells were subjected to Western blot or apoptotic assays. The results revealed that KD-Akt and pCB6+ transfected AIPC cells were sensitized to psoralidin due to the inhibition of pAkt; however, the myr-Akt overexpressed PC-3 cells renders resistance to psoralidin, in which a complete inhibition of pAkt and a significant level of apoptosis was seen only at higher concentrations (100 μmol/L; Fig. 2D and E). Similar results were observed in DU-145 cells, suggesting that psoralidin overcomes Akt-mediated resistance in AIPC cells (data not shown).

**Effect of psoralidin on PI3K activation in AIPC cells**

To investigate the role of PI3K-mediated Akt activation, we treated PC-3 and DU-145 cells with both psoralidin and pharmacologic inhibitors. Pretreatment with psoralidin rapidly inhibited PI3K activation in a dose-dependent (Fig. 3A) and time-dependent manner (Fig. 3B) in both cell lines; these results are similar to the inhibition effected by the commercially available PI3K inhibitor, wortmannin (data not shown). PC-3 and DU-145 cells were treated with psoralidin and whole-cell lysates were immunoprecipitated with PI3K antibody and subjected to Western blot analysis. Psoralidin treatment resulted in a decrease in the expression of PI3K p110 and p85 in time-dependent fashion for up to 24 hours (Fig. 3A and B). To confirm PI3K activity levels, we used commercially available PI3K kits, which revealed that psoralidin inhibits PI3K activation in a time-dependent (3-24 hours) fashion (Fig. 3A and B, bottom). Statistical analysis of the data in the control and psoralidin-treated population yielded a P value of 0.0001, indicating a significant difference between the groups.

**Psoralidin inhibits constitutive NF-κB activation in AIPC cells**

We subsequently analyzed the downstream effectors of Akt signaling specifically examining the effect of psoralidin on the constitutive activation of NF-κB in PC-3 and DU-145 cells. Our results showed that psoralidin significantly down-regulated (P < 0.003) the binding activity of NF-κB in both the cell lines in a time- and dose-dependent manner when compared with the control cells (Fig. 4A and B). Next, we determined the effect of psoralidin on nuclear translocation of the NF-κB subunits p65 and p50 in both cell lines. Immunoblot analysis with the nuclear and cytosolic extracts from psoralidin-treated cells revealed a down-regulation of p65 in both the cytosolic and nuclear fractions of PC-3 and DU-145 cells. Interestingly, a
complete down-regulation of the p50 expression in the cytosolic fraction in PC-3 and DU-145 cells was observed; however, the p50 levels in the nuclear fraction either up-regulated (PC-3) or down-regulated (DU-145) as seen in Fig. 4C and D. Similar results of NF-κB expression and localization were observed via confocal microscopy (data not shown).

To ascertain whether psoralidin regulates NF-κB at the promoter level, we transiently transfected PC-3 and DU-145 cells with NF-κB-Luc followed by treatment with psoralidin. Interestingly, NF-κB promoter was down-regulated by ~92% (P < 0.0003) from 3 hours onward in both PC-3 (Fig. 4E) and DU-145 (data not shown), suggesting that psoralidin regulates NF-κB at the promoter level in both the cell lines. To establish Akt-mediated NF-κB regulation, we overexpressed myr-Akt, KD-Akt, and pCB6+ in PC-3 cells and cotransfected them with NF-κB promoter luc followed by treatment with psoralidin. Overexpression of myr-Akt increased the constitutive activation of NF-κB, which in turn renders PC-3 cells resistant to psoralidin treatment by ~68% (P < 0.001; Fig. 4F). In contrast, psoralidin completely inhibited NF-κB promoter activation in KD-Akt and pCB6+ transfected PC-3 cells by ~90% (P < 0.0001; Fig. 4F). Similar observations were made in DU-145 cells (data not shown) and these results indicate that NF-κB signaling plays an important role in the treatment of AIPC.

Psoralidin-mediated NF-κB inhibition acts via the IκB-α pathway

To clarify whether psoralidin acts by blocking the phosphorylation of IκB-α, thereby sequestering the NF-κB dimers in the cytoplasm, we determined the expression profile of key regulatory proteins in the NF-κB signaling pathway. We determined the expression levels of NIK (NF-κB-inducing kinase), which phosphorylates IKKα and/or IKKβ, thereby initiating NF-κB activation in cells. Psoralidin caused a time-dependent decrease in NIK expression in both PC-3 and DU-145 cells, suggesting that NIK-inducible NF-κB activation is

---

**Fig. 4.** Psoralidin inhibits NF-κB signaling in prostate cancer cells. PC-3 (A) and DU-145 cells (B) were treated with varying doses and time intervals with psoralidin, and NF-κB transcription factor assay was done to determine DNA binding activity. PC-3 (C) and DU-145 (D) cells were treated with psoralidin for varying time points (0–48 h) or with DMSO (vehicle control); nuclear and cytoplasmic proteins were extracted and Western blot analysis was done to determine the localization of the NF-κB dimers p50 and p65. β-Actin and histone H3 was used as the loading control for cytoplasmic and nuclear extracts, respectively. E, PC-3 cells were transiently transfected with NF-κB reporter tagged with luciferase and either treated with psoralidin or DMSO (vehicle control) for 24 h, pRL-TK was used as the renilla control. F, PC-3 cells were transiently cotransfected with NF-κB Luc and pCB6+ or myr-Akt or KD-Akt, followed by treatment with the vehicle control (DMSO) or psoralidin. Luciferase reporter assay was done to determine NF-κB promoter activity using pRL-TK as the renilla control.
Psoralidin inhibits NF-κB signaling in prostate cancer cells via the IκB pathway. A, PC-3 and DU-145 cells were treated with psoralidin or vehicle control. Whole-cell lysates were subjected to Western blot analysis using NIK, IKKα, IKKβ, pIKKα/β (Thr23), IκBα, pIκBα, and β-actin. B, PC-3 and DU-145 cells were treated either with psoralidin or vehicle control for varying time points and the cell lysates were assayed to determine nonphosphorylated IκBα activity. C, PC-3 and DU-145 cells were treated with psoralidin or vehicle control. Whole-cell lysates were subjected to Western blot analysis using Bcl-2, Bcl-xL, Bax and survivin. β-Actin was used as the loading control. D, PC-3/Neo and PC-3/Bcl-2 cells were treated with psoralidin or vehicle control. Whole-cell lysates were subjected to Western blot analysis using Bcl-2 and β-actin. E, PC-3/Bcl-2 and PC-3/Neo were treated with various concentrations of psoralidin and apoptotic cells were scored after 24 h by TUNEL assay.
inhibited by psoralidin in both the AIPC cell lines. A timedependent decrease of IKKα and a constant level of IKKβ expression were seen in PC-3 cells, whereas the opposite was observed in DU-145 cells, indicating the preferential binding of IKKs to the NF-κB dimers in AIPC cells. Also, phosphorylated IKKα/β (Thr23) was found to be decreasing in a time-dependent manner in PC-3 cells while it maintained its level of expression in DU-145 cells (Fig. 5A).

We then examined the total and phosphorylated levels of IκB-α following treatment with psoralidin, which revealed a decrease in the levels of phosphorylated IκB-α in both PC-3 and DU-145 cells. In contrast, nonphosphorylated level of IκB-α either remained constant or up-regulated in both the cell lines. Collectively, these results suggest that psoralidin has an inhibitory effect on the phosphorylation and degradation of IκB-α, which is preceded by the inhibition of the IKKs and NIK, resulting in the complete inhibition of NF-κB activation in both the AIPC cell lines (Fig. 5A). The net inhibitory effects of psoralidin have been found to be a complete inactivation of NF-κB in AIPC cells. Following observation of constant or increased IκB-α levels by Western blotting, we determined IκB-α activity, which revealed that in both the AIPC cell lines, nonphosphorylated IκB-α levels either remained constant or increased following treatment with psoralidin, which was in accordance with the Western blot results (Fig. 5B). As IκB degradation is responsible for the nuclear translocation of NF-κB, we infected the AIPC cells with the Ad5-IκB super-repressor. Twenty-four hours after infection, AIPC cells were treated with either vehicle control (DMSO) or psoralidin, and the results of Western blotting and DNA binding assays suggest that psoralidin sensitized the AIPC cells infected with Ad5-IκB super-repressor more significantly when compared with the controls (data not shown).

Bcl-2 and inhibitor of apoptosis (IAP) family members, many of which are regulated by the PI3K/Akt/NF-κB pathway, are the major regulators of apoptosis in both normal and transformed cells. Therefore, we explored the effect of psoralidin on the Bcl-2 and IAP family of proteins and found...
that psoralidin completely down-regulated prosurvival proteins, such as Bcl-2, Bcl-xL, and survivin, while maintaining constant levels of proapoptotic protein Bax in both PC-3 and DU-145 cells (Fig. 5C).

Overexpression of Bcl-2 is reported in a variety of human cancers, including prostate cancer, and is associated with tumor aggressiveness. Because psoralidin inhibits Bcl-2 levels in AIPC cells, we overexpressed Bcl-2 in PC-3 cells and then explored whether psoralidin effectively suppresses the growth of PC-3/Bcl2 cells. As expected, Bcl-2 overexpressed PC-3 cells imparts resistance to psoralidin treatment when compared with PC-3/Neo control cells harboring empty vector; however, a higher concentration of psoralidin sensitizes PC3/ Bcl-2 overexpressed cells by down-regulating ectopically overexpressed as well as basal levels of Bcl-2 in PC-3 cells (Fig. 5D), resulting in the induction of apoptosis (Fig. 5E).

Psoralidin inhibits cell viability and induces apoptosis in AIPC cells

To explore the potential antitumor properties of psoralidin, androgen-negative (PC-3 and DU-145), androgen-positive (22Rv1 and LNCAP), and normal immortalized prostate epithelial PzHPV-7 cell lines were selected to perform cell viability assays (trypan blue dye exclusion). At 24 hours post-treatment, psoralidin (25-100 μmol/L) significantly inhibited (P < 0.001) viability of AIPC and androgen-dependent prostate cancer cells when compared with normal prostate epithelial cells (Fig. 6A). These findings suggest that psoralidin selectively inhibit the survival of prostate cancer cells. To address the question of whether psoralidin induces apoptosis, we performed two different apoptotic assays (Annexin V-FITC and TUNEL) using PC-3, DU-145, 22Rv1, and LNCAP cells that showed that psoralidin induces significant (P < 0.0001) apoptosis in both androgen-negative and androgen-positive cell lines (Fig. 6B).

Effect of psoralidin on xenograft models

To explore the in vivo effects of psoralidin on AIPC, we tested psoralidin on PC-3 xenografts in male nude mice. The animals were observed daily for 1 to 2 weeks until the tumors reached a volume of 50 mm³. At this time, a total of 27 mice were randomized into three groups (n = 9 per group). Psoralidin (15 and 25 mg/kg body weight) or vehicle (corn oil) was administered intratumorally, 5 days a week for 4 weeks and tumor measurement studies were done over a 4-week period. The data were analyzed using longitudinal analysis and computations were carried out using the SAS program PROC MIXED, which yielded a statistically significant difference in tumor volumes between the control and treatment groups at 2, 3, and 4 weeks, with respective P values of 0.006, 0.0001, and <0.0001 (Fig. 6C). Upon observing the tumor growth inhibition effected by psoralidin, we investigated whether the tumor regression by psoralidin was due to induction of apoptosis and not necrosis by using TUNEL staining in the tumor sections; a more significant number of TUNEL-positive cells were seen in psoralidin-treated tumors compared with corn oil–treated controls (Fig. 6C, see inset). The strong induction of TUNEL-positive cells in psoralidin-treated tumors indicates that psoralidin is a potent compound that inhibits the growth of AIPC. We sacrificed three animals for gross morphologic changes, such as color or texture of organs (liver, lung, kidney, heart, prostate, and bladder), and tissues were weighed and compared with healthy, nontumor-bearing animals; we noted no significant difference between the groups, indicating that psoralidin is a nontoxic agent in animal models.

Discussion

PI3K/Akt signaling is a major component of the cell signaling network, as it is a focal point for a number of prosurvival pathways that modulate numerous transcriptional factors and genes involved in the regulation of cell proliferation, cell survival, angiogenesis, and tissue invasion (27). PTEN is a negative regulator of Akt, which when gets mutated or deleted in AIPC, resulting in Akt-mediated survival signaling, which confers chemotherapeutic resistance in AIPC (28). To effectively target PTEN-negative prostate tumors, more selective therapeutic approaches are needed; however, the low degree of specificity of many agents that are currently under investigation has been an impediment in realizing this goal (29). In this study, we have identified a chloroform-extractable natural compound, psoralidin, from Rasagenthi Lehyam, an herbal preparation, and have found it to be effective for the treatment of AIPC (30). Psoralidin is one of the active ingredients in the Psoralea corylifolia plant, which is extensively used in traditional medicines against many diseases, including cancer. We found that psoralidin targets PI3K-mediated Akt signaling, resulting in the inhibition of cell survival and induction of apoptosis in AIPC cells.

Our findings suggest that psoralidin inhibits pAkt (Ser473) in PC-3 and DU-145 cells without altering total levels of Akt. Our results also indicate that psoralidin inhibits pAkt after shorter exposure (3 hours), compared with commercially available Akt inhibitors. The complete down-regulation of pAkt by psoralidin suggests that it could be an excellent candidate to inhibit Akt-mediated signaling in prostate cancer. Psoralidin also inhibits Akt kinase activation, and as a result its direct substrate GSK-3 (31). Akt has been shown to phosphorylate GSK-3 (Ser^β9), which via the regulation of genes involved in cell cycle progression and survival plays a role in cell proliferation. Psoralidin inhibits the kinetics of pGSK-3α/β in a dose-dependent manner, thereby revealing that GSK-3 is directly regulated by Akt in AIPC cells. Further, GSK-3 is involved in the phosphorylation and degradation of the cell cycle regulatory protein, cyclin D1 (32), and our results showed that psoralidin down-regulates cyclin D1 in both the prostate cancer cell lines, suggesting that psoralidin is capable of inhibiting the complete Akt signaling pathway in prostate cancer cells, including its downstream targets. Further, overexpression of myr-Akt induces Akt phosphorylation and enhances cell growth in PC-3 and DU-145 (results not shown). Psoralidin, however, overcomes Akt-mediated resistance and induces apoptosis in Akt-overexpressed AIPC cells. Recently, it was found that phosphorylation and activation of Akt correlates with prostate tumor invasiveness (33) and high Gleason grade prostate cancers (34). As we know that Akt is involved in tumor aggressiveness and metastasis, psoralidin-mediated inhibition of Akt signaling might possess a therapeutic potential in sensitizing prostate cancer to apoptosis.

PI3K is one of the key activators of Akt signaling, and accumulating evidence implicates the involvement of the
PI3K/Akt signaling as having a critical role in the development of several human malignancies, including prostate cancer (35, 36). Our results show that psoralidin inhibits the constitutive levels of PI3K p110 and p85 in a dose- and time-dependent manner. Our results also reveal that psoralidin blocks PI3K activation in both PC-3 and DU-145 cells. Several studies suggested that inhibition of the PI3K signaling results in the induction of apoptosis in AIPC (37, 38) and the PI3K pathway is currently a major therapeutic target for the treatment of cancer (39, 40); we believe that psoralidin is also one such compound that targets the PI3K/Akt pathway in AIPC cells.

We have previously shown that activation of NF-κB confers resistance to current treatments in AIPC (25), as activated NF-κB promotes tumor growth and curtails induction of apoptosis in AIPC (41, 42). Interestingly, psoralidin-mediated inhibition of NF-κB activation occurred in both PC-3 and DU-145 cells, suggesting that psoralidin not only selectively inhibits Akt but also targets other prosurvival signaling in prostate cancer. Although Akt-driven NF-κB activation is well established in many cancer cell types, epidermal growth factor receptor- and Her-2-mediated NF-κB activation via casein kinase II (CK-2) has also been reported in AIPC (43, 44). Inhibition of NF-κB activation by psoralidin suggests that not only Akt-mediated activation of NF-κB but also epidermal growth factor receptor- or Her-2-mediated NF-κB might be suppressed in prostate cancer.

In unstimulated conditions, NF-κB is sequestered in the cytoplasm as a heterodimer by the inhibitory protein IκBα (45). In response to external stimuli, IκBα is rapidly phosphorylated, allowing the active dimers to translocate to the nucleus, thereby activating the target genes. In our results, we observed that psoralidin degrades the p65, the cytosolic and nuclear fractions of both PC-3 and DU-145 cells. Recently, it was reported that PDLIM2 causes ubiquitination and degradation of p65 in several cell types (46) and, hence, it might be possible that psoralidin stabilizes PDLIM2, thereby degrading p65 in AIPC cells. Interestingly, decreased expression of p50 was observed only in cytoplasm but not in nuclear extract of both the cell lines. This may be due to the fact that p50 is constitutively bound to the NF-κB binding sites in the promoter regions of the target genes, thereby inhibiting NF-κB activation in these cell lines (47). Additionally, NF-κB binding as well as promoter activation studies clearly suggested that psoralidin regulates NF-κB at the promoter level.

NF-κB regulates many prosurvival genes such as members of the Bcl-2 and IAP families, which suppress apoptosis (48). Therefore, we speculated that inhibition of NFκB by psoralidin might result in the down-regulation of antiapoptotic Bcl-2 and Bcl-xl proteins; our results confirmed that there is indeed a complete inhibition of prosurvival signaling in both PC-3 and DU-145 cells. Down-regulation of endogenous as well as ectopic expression of Bcl2 protein might lead to an alteration in the Bcl2 to Bax ratio, which could trigger apoptosis through the mitochondrial pathway in these cells. Survivin, also a member of the inhibitor of apoptosis family, is overexpressed in the AIPC (49), and treatment with psoralidin resulted in a decrease in survivin expression over time (undetectable levels in both PC3 and DU-145 cells after 12 hours). Survivin has been shown to inhibit apoptosis by binding to active caspase-3 and caspase-7 (50). Thus, we observed that treatment of both the prostate cancer cell lines with psoralidin resulted in increased expression of active caspase-3 and caspase-7, which resulted in the induction of apoptosis.

In summary, this study shows that direct modulation of PI3K/Akt/NF-κB signaling activity by psoralidin causes apoptosis induction in AIPC cells, which could provide the molecular basis for therapeutic targeting of advanced prostate cancer with this compound. Considering the pivotal role of PI3K/Akt signaling in the pathogenesis of human prostate cancer, these findings may have significant clinical relevance, in the context that psoralidin could be developed as an agent for the management of prostate cancer, as a novel chemoprevention strategy, and/or as an effective therapeutic approach. Ongoing studies focus on fully dissecting the mechanism of action of psoralidin in a physiologic setting in the AIPC models and functionally linking the anti-tumor action of this (relatively safe and well-tolerated) phytochemical with the prevention of prostate cancer during prostate tumor progression to metastatic disease using in vivo model systems. The observations made in our in vivo studies may also enable us to conduct clinical trials with psoralidin to determine its chemotherapeutic and chemopreventive effects in human subjects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Naoya Fujita (Japanese Foundation for Cancer Research, Tokyo, Japan) for kindly providing Akt plasmids.
Psoralidin Targets Akt Signaling


Downloaded from cancerpreventionresearch.aacrjournals.org on January 9, 2021. © 2009 American Association for Cancer Research.
Retraction: Psoralidin, an Herbal Molecule, Inhibits Phosphatidylinositol 3-Kinase–Mediated Akt Signaling in Androgen-Independent Prostate Cancer Cells

The Publisher retracts this article (Can Prev Res 2009;2:234–243), which was published in the March 2009 issue of Cancer Prevention Research (1), based on the recommendation of the Investigation Committee at the University of Kentucky after an institutional investigation made a finding of misconduct related to Figure 3A.

The authors were given an opportunity to retract the article, but declined.

Reference


Published OnlineFirst October 18, 2011.  
doi: 10.1158/1940-6207.CAPR-11-0467  
©2011 American Association for Cancer Research.
Psoralidin, an Herbal Molecule, Inhibits Phosphatidylinositol 3-Kinase–Mediated Akt Signaling in Androgen-Independent Prostate Cancer Cells

Raj Kumar, Sowmyalakshmi Srinivasan, Srinivas Koduru, et al.


Updated version  Access the most recent version of this article at: doi:10.1158/1940-6207.CAPR-08-0129

Cited articles  This article cites 48 articles, 15 of which you can access for free at: http://cancerpreventionresearch.aacrjournals.org/content/2/3/234.full#ref-list-1

Citing articles  This article has been cited by 2 HighWire-hosted articles. Access the articles at: http://cancerpreventionresearch.aacrjournals.org/content/2/3/234.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://cancerpreventionresearch.aacrjournals.org/content/2/3/234. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.