Diallyl Trisulfide Inhibits Activation of Signal Transducer and Activator of Transcription 3 in Prostate Cancer Cells in Culture and In vivo

Kumar Chandra-Kuntal and Shivendra V. Singh

Abstract

Signal transducer and activator of transcription 3 (STAT3) is an oncogenic transcription factor implicated in prostate carcinogenesis. The present study shows that diallyl trisulfide (DATS), a promising cancer-chemopreventive constituent of processed garlic, inhibits phosphorylation of STAT3 in prostate cancer cells in culture and in vivo. Exposure of DU145 and LNCaP human prostate cancer cells to growth-suppressive and pharmacologically relevant concentrations of DATS (20 and 40 μmol/L) resulted in suppression of constitutive (DU145) as well as interleukin-6 (IL-6)-induced (LNCaP) phosphorylation of STAT3 (Tyr705), which correlated with inhibition of Janus-activated kinase 2 phosphorylation. Constitutive and/or IL-6-induced nuclear translocation of pSTAT3 and STAT3 dimerization was also markedly inhibited on treatment with DATS in both cell lines. Inhibition of prostate cancer development in transgenic adenocarcinoma of mouse prostate mice by gavage of DATS correlated with a visible decrease in the levels of pSTAT3. Interestingly, the IL-6-mediated activation of STAT3 largely failed to confer protection against proapoptotic response to DATS in both cells. Likewise, DATS-mediated inhibition of cell migration was either not affected or minimally reversed by IL-6 treatment or ectopic expression of constitutively active STAT3. In conclusion, the present study indicates that DATS treatment suppresses STAT3 phosphorylation in prostate cancer cells in culture and in vivo, but activation of this oncogenic transcription factor is largely dispensable for cellular responses to DATS. Ability of DATS to overcome STAT3 activation is a therapeutic advantage for this chemopreventive agent. Cancer Prev Res; 3(11); OF1–11. ©2010 AACR.

Introduction

Signal transducer and activator of transcription 3 (STAT3) is an oncogenic transcription factor belonging to the seven-member STAT gene family (1). STAT3 is activated by phosphorylation of Tyr705 at the COOH terminus (1, 2). STAT3 is constitutively active in a large fraction of human prostate cancer cells and clinical prostate cancers (3–6), and its hyperactivity is associated with advanced stage of the disease (6, 7). Furthermore, STAT3 activation promotes survival and inhibits apoptosis in prostate cancer cells (8, 9). STAT3 regulates expression of genes common to wound healing and cancer (10). Agents that are safe but can either suppress activation of STAT3 or overcome STAT3 activation for their anticancer effects are attractive for prevention and treatment of prostate cancer.

Natural products have attracted tremendous attention for the discovery of novel agents potentially useful for prevention and treatment of human cancers (11–13). Epidemiologic studies continue to support the premise that dietary intake of Allium vegetables, such as garlic, may be protective against risk of various malignancies, including cancer of the prostate (14–16), which is a leading cause of cancer-related deaths among American men (17). In a population-based case-control study, the risk of prostate cancer was found to be significantly lower in men consuming >10 g/d of total Allium vegetables than in men with total Allium vegetable intake of <2.2 g/d (16). The anticancer effect of Allium vegetables, which have been used for medicinal purposes throughout the recorded history (18), is attributed to organosulfur compounds that are generated on cutting or chewing of these vegetables (19). Garlic-derived organosulfur compounds, including diallyl trisulfide (DATS), have been shown to afford significant protection against cancer in animal models induced by a variety of chemical carcinogens (20–25). The organosulfur compound–mediated prevention of chemically induced cancer in experimental rodents correlates with induction of phase 2 carcinogen-detoxifying enzymes as well as inhibition of phase 1 carcinogen-activating enzymes (26–28). Studies from our laboratory have shown that gavage of DATS not only retards growth of PC-3 human prostate...
cancer cells s.c. implanted in male athymic mice but also affords significant protection against cancer development in transgenic adenocarcinoma of mouse prostate (TRAMP) mice without causing any toxicity (29, 30).

In human prostate cancer cells, DATS treatment has been shown to cause cell cycle arrest, apoptosis induction, and transcriptional repression of androgen receptor (31–37). DATS treatment also inhibits angiogenic features in human umbilical vein endothelial cells (38). The mechanisms underlying growth arrest and apoptosis induction by DATS have been thoroughly investigated in human prostate cancer cells (31–37). We previously showed that DATS-induced apoptosis in prostate cancer cells correlates with downregulation and phosphorylation of Bcl-2 (31). Because Bcl-2 is one of the targets of STAT3 (39), it was of interest to determine the role of this transcription factor in DATS-induced apoptosis. The present study shows that DATS inhibits activation of STAT3 in prostate cancer cells in culture as well as in vivo, but this transcription factor is largely dispensable for cellular anticancer responses to DATS.

Materials and Methods

Reagents

DATS (purity >98%) was purchased from LKT Laboratories. Cell culture reagents were purchased from Life Technologies, whereas interleukin-6 (IL-6), eosin, and anti-actin antibody were obtained from Sigma-Aldrich. Antibodies against phospho-STAT3 (pSTAT3; Tyr705), phospho-Janus-activated kinase 2 (pJAK2; Tyr1007/1008), total STAT3, and total JAK2 were obtained from Cell Signaling. The pSTAT3 (Tyr705) antibody used for immunofluorescence microscopy was procured from Santa Cruz Biotechnology. Cytoplasmic histone-associated apoptotic DNA fragmentation was measured using a kit from Roche.

Cell lines

Prostate cancer cell lines LNCaP and DU145 were purchased from the American Type Culture Collection (ATCC) in 2004 to 2005 and maintained as previously described (31, 36). Cell line authentication was done to test for interspecies contamination and alleles for nine different short tandem repeats (done by Research Animal Diagnostic Laboratory, University of Missouri, Columbia, MO) identifiable in the ATCC database. The cells were last tested in July 2010. Each cell line was found to be of human origin, and no mammalian interspecies contamination was detected. Moreover, the genetic profiles for LNCaP and DU145 were found to be consistent with the genetic profiles in the ATCC database. Stock solution of DATS was prepared in dimethylsulfoxide (DMSO), and an equal volume of DMSO (final concentration <0.2%) was added to the controls.

Immunoblotting

Cells were treated with DATS and/or IL-6 for specified time periods and lysed as described by us previously (40). Cell lysates were cleared by centrifugation at 14,000 rpm for 20 minutes. Lysate proteins were resolved by SDS-PAGE and transferred onto polyvinylidene fluoride membranes. After blocking with 5% (w/v) nonfat dry milk solution in TBS containing 0.05% Tween 20, the membrane was incubated with the desired primary antibody for 2 hours at room temperature or overnight at 4°C. The membrane was treated with an appropriate secondary antibody for 1 hour at room temperature. The immunoreactive bands were visualized by the enhanced chemiluminescence method. The blots were stripped and reprobed with anti-actin antibody to normalize for differences in protein loading. The intensity of the immunoreactive band was determined by densitometric scanning to quantify changes in protein levels. Quantitation of the immunoreactive bands was done using Image-QuaNT (version 4.2a) software (Molecular Dynamics).

Immunocytochemical analysis for nuclear localization of pSTAT3

Nuclear translocation of pSTAT3 was determined by immunocytochemistry. Briefly, DU145 or LNCaP cells (10^4 cells/mL) were cultured on coverslips and allowed to attach by overnight incubation. The cells were treated with 20 or 40 μmol/L DATS in the absence or presence of 25 ng/mL IL-6 for 3 or 24 hours at 37°C. After washing with PBS, the cells were fixed with 2% paraformaldehyde for 1 hour at room temperature and permeabilized using 0.05% Triton X-100 for 5 minutes. The cells were then incubated with PBS supplemented with 0.5% bovine serum albumin and 0.15% (w/v) glycine for 1 hour followed by overnight incubation with anti-pSTAT3 antibody (1:500 dilution) at 4°C. The cells were then treated with 2 μg/mL Alexa Fluor 568–conjugated secondary antibody (Molecular Probes) for 1 hour at room temperature. After washing with PBS, the cells were counterstained with 0.5 μmol/L SytoxGreen for 3 minutes at room temperature. The cells were washed with PBS, mounted, and observed under a Leica DCM00F microscope at ×100 objective magnification.

Analysis of STAT3 dimerization

Cells were treated for 24 hours with 20 or 40 μmol/L DATS in the absence (DU145) or presence of 10 ng/mL IL-6 (LNCaP). Subsequently, protein extracts were prepared as described by Shin et al. (41). Proteins were resolved by 6% nondenaturing gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membrane was probed with anti-STAT3 antibody as described above.

Immunohistochemical analysis of pSTST3 in TRAMP tissues

We used prostate tissues from control and DATS-treated TRAMP mice to determine the effect of DATS administration on pSTST3 expression in vivo (30). Prostate tissues were sectioned at 5-μm thickness, quenched with 3% hydrogen peroxide, and blocked with normal serum. The sections were then incubated with the pSTAT3 primary
antibody and washed with TBS followed by incubation with an appropriate secondary antibody. The characteristic brown color was developed by incubation with 3,3’-diaminobenzidine. The sections were counterstained with Mayer’s hematoxylin (Sigma) and examined under a Leica microscope.

**Cell viability and apoptosis assays**

The viability of DU145 and LNCaP cells following 24 hours of treatment with DATS (20 or 40 μmol/L) and/or IL-6 (25 ng/mL) was determined by trypan blue dye exclusion assay as described by us previously (31). Apoptosis in DATS and/or IL-6-treated cells (24-hour exposure) was assessed by analysis of histone-associated DNA fragment release into the cytosol (31).

**Reverse transcription-PCR**

Total RNA from DU145 or LNCaP cells treated for 24 hours with DATS (20 or 40 μmol/L) and/or IL-6 (25 ng/mL) was extracted using RNeasy kit (Invitrogen). cDNA was synthesized from 2 μg of total RNA with reverse transcriptase and oligo(dT)20. Reverse transcription-PCR was carried out using High Fidelity Taq Polymerase (Invitrogen), gene-specific primers, and cDNA. The primers were as follows: (a) Bcl-2, forward 5′-TGCACTGACGCCTT- CAC-3′ and reverse 5′-TACTGTACGCTTGGCT- GA-3′ (product size 560 bp), and (b) Bcl-xL, forward 5′-CCGAGAAAGGATACAGCTGG-3′ and reverse 5′-GGATCCGACTCACCAATAC-3′ (product size 448 bp). Amplification conditions were as follows: Bcl-2: 94°C/ 2 minutes, 25 cycles of 94°C/15 seconds, 69°C/1 minute, 72°C/2 minutes, and 72°C/8 minutes; Bcl-xL: 94°C/2 minutes, 25 cycles of 94°C/45 seconds, 56°C/30 seconds, 72°C/60 seconds, and 72°C/8 minutes. The housekeeping gene β-actin was used as an internal control and amplified using the following primers: forward 5′-CAAGAGCCCTGCTACC- CAAC-3′ and reverse 5′-CATACTCTGCTTGGCT- GATCC-3′ (product size 277 bp) and amplification conditions of 95°C/3 minutes, 18 cycles 95°C/60 seconds, 56°C/60 seconds, 68°C/60 seconds, and 68°C/10 minutes. The PCR products were resolved by 2% agarose gel prestained with ethidium bromide.

**Migration assay**

Migration of DU145 or LNCaP cells was determined using Transwell Boyden chamber (Corning) containing a polycarbonate filter with a pore size of 8 μm as previously described by us (38). Briefly, 0.2 mL cell suspension containing 4 × 10⁴ cells was mixed with 40 μmol/L DATS or DMSO (control), and the suspension was added to the upper compartment of the chamber. The lower compartment of the chamber contained 0.6 mL of cell culture medium (chemoattractant) containing the same concentrations of DATS or DMSO. After 24 hours of incubation at 37°C, nonmigrant cells from the upper surface of the membrane were removed using a cotton swab. The membrane was washed with PBS and the migrated cells on the bottom face of the membrane were fixed with 90% ethanol and stained with eosin for 3 minutes. Four randomly selected fields were examined under a microscope at ×10 objective magnification.

**Stable overexpression of constitutively active STAT3**

LNCaP cells were stably transfected with pIRES empty vector or vector encoding for constitutively active STAT3 (S3c; kindly provided by Dr. Beverly F. Barton, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ; ref. 9) using FuGene6 (Roche Applied Science). Several clones were selected and screened for overexpression of STAT3.

**Statistical analysis**

Statistical significance of the difference in measured variables between control and treated groups was determined by one-way ANOVA followed by Bonferroni’s test. The difference was considered significant at P < 0.05.

**Results**

**DATS inhibited constitutive and IL-6–induced activation of STAT3 in human prostate cancer cells**

As shown in Fig. 1A, treatment of DU145 cells with DATS resulted in ~40% to 70% decrease in constitutive STAT3 phosphorylation at the 6- and 24-hour time points. The pSTAT3 level was increased by 1.5- to 8.2-fold in the presence of 10 ng/mL IL-6 in DU145 cells. IL-6–induced phosphorylation of STAT3 was decreased only slightly in the presence of DATS at the 24-hour time point (Fig. 1A). DATS-mediated inhibition of constitutive STAT3 phosphorylation in DU145 cells did not correlate with decrease in the level of total STAT3 protein. Constitutive phosphorylation of JAK2, an upstream kinase implicated in activation of STAT3 (42, 43), was decreased by DATS treatment (Fig. 1A). Constitutive pSTAT3 was not detectable in the LNCaP cell line. Exposure of LNCaP cells to IL-6 for 6 or 24 hours resulted in a robust increase in phosphorylation of STAT3 at both time points. The IL-6–inducible activation of STAT3 was decreased in a concentration-dependent manner upon cotreatment with DATS (Fig. 1B), which was not due to downregulation of total STAT3 protein (Fig. 1B). DATS treatment inhibited IL-6–induced phosphorylation of JAK2 in the LNCaP cell line. DATS-mediated inhibition of IL-6–inducible phosphorylation of JAK2 was comparatively more pronounced after 24 hours of treatment relative to the 6-hour time point (Fig. 1B). Nevertheless, these results showed that constitutive and/or IL-6–inducible activation of pSTAT3 in human prostate cancer cells was inhibited in the presence of DATS.

**DATS treatment inhibited nuclear translocation of pSTAT3**

Tyr705 phosphorylation of STAT3 results in its homodimerization or heterodimerization with other STATs, enabling nuclear translocation of STAT3 for binding to specific sequences of target genes (1, 2, 43). Because DATS
treatment decreased the level of pSTAT3 in both cells, we designed experiments to determine the effect of DATS on nuclear translocation of pSTAT3. Immunostaining for pSTAT3 (red fluorescence) and SytoxGreen-associated nuclear staining (green fluorescence) in DU145 cells following 3 or 24 hours of treatment with DMSO (control) or the indicated concentrations of DATS are shown in Fig. 2A. In DMSO-treated control DU145 cells, pSTAT3 was detectable in both cytoplasm and nucleus at the 3-hour time point, but its enrichment in the nucleus was clearly evident after 24 hours. The nuclear level of constitutively active pSTAT3 in DU145 cells was visibly reduced in the presence of DATS at both 20 and 40 μmol/L concentrations (Fig. 2A). Consistent with the immunoblotting results shown in Fig. 1B, immunostaining for pSTAT3 was very weak in DMSO-treated LNCaP cells at both the 3-hour (Fig. 2B) and 24-hour time points (Fig. 2C). The nuclear level of pSTAT3 was increased on stimulation with IL-6 as evidenced by the merging of pSTAT3-associated red fluorescence and SytoxGreen-associated green fluorescence. The IL-6 stimulated nuclear translocation of pSTAT3 in LNCaP
Fig. 2. A, immunocytochemical analysis for pSTAT3 in DU145 cells following 3- or 24-h treatment with DMSO (control) or DATS (20 or 40 μmol/L). B, immunocytochemical analysis for pSTAT3 in LNCaP cells following 3-h treatment with DMSO (control) or DATS (20 or 40 μmol/L) in the absence or presence of 25 ng/mL IL-6. C, immunocytochemical analysis for pSTAT3 in LNCaP cells following 24-h treatment with DMSO (control) or DATS (20 or 40 μmol/L) in the absence or presence of 25 ng/mL IL-6. Staining for pSTAT3 and nuclear DNA is indicated by red and green fluorescence, respectively (x100 objective magnification). Each experiment was repeated twice, and representative data from one such experiment are shown.
cells was inhibited in a concentration-dependent manner in the presence of DATS at both the 3-hour (Fig. 2B) and 24-hour time points (Fig. 2C). In addition, DATS treatment (24 hours) resulted in suppression of constitutive (DU145) as well as IL-6–stimulated (LNCaP) dimerization of STAT3 (Fig. 3A). Collectively, these results showed DATS-mediated inhibition of constitutive and IL-6–induced nuclear translocation of pSTAT3 as well as suppression of STAT3 dimer formation.

DATS administration decreased the level of pSTAT3 in the prostate of TRAMP mice

We have previously shown that gavage of 1 and 2 mg DATS per day (three times per week for 13 weeks) significantly inhibits incidence as well as burden (affected area) of poorly differentiated cancer in male TRAMP mice without causing weight loss or any other side effects (30). Specifically, the incidence of poorly differentiated cancer was decreased by ~34% to 41% in DATS-treated TRAMP mice compared with vehicle-treated controls (30). In the present study, we used the prostate tissues from the mice of control and 2 mg DATS groups of the same experiment to determine the in vivo relevance of the cellular results showing DATS-mediated inhibition of pSTAT3 (Fig. 1). Representative images for pSTAT3 expression in the dorsolateral prostate of two mice of both groups are shown in Fig. 3B. Immunostaining for pSTAT3 was not uniform but detectable in epithelial cells as well as in the stroma of dorsolateral prostate from control mice. The level of pSTAT3 was markedly lower in the prostate of DATS-treated mice compared with control (Fig. 3B). These results provided evidence for DATS-mediated suppression of STAT3 phosphorylation in vivo.

IL-6–stimulated STAT3 activation failed to confer resistance against DATS-induced apoptosis

Expression of many antiapoptotic genes, including Bcl-2, Bcl-xl, and survivin, is transcriptionally regulated by STAT3 (39, 43). Consistent with antiapoptotic role for STAT3, its direct inhibition has been shown to induce apoptosis in prostate cancer cell lines (9). We have also shown previously that DATS treatment decreases the protein level of Bcl-2 in PC-3 human prostate cancer cell lines and ectopic expression of Bcl-2 confers partial but statistically significant protection against DATS-induced apoptosis in PC-3 cells (31). We therefore questioned if DATS-induced
apoptosis was blunted by STAT3 activation. We tested this possibility by determining the growth-suppressive and proapoptotic effects of DATS in the absence or presence of IL-6 (25 ng/mL). As shown in Fig. 4A, 24-hour treatment with DATS inhibited the viability of both DU145 and LNCaP cells. Inhibition of cell viability resulting from DATS exposure was not reversed in the presence of IL-6 in either cell line (Fig. 4A). Consistent with these results, IL-6 failed to confer resistance against DATS-induced cytoplasmic histone-associated DNA fragmentation enrichment over DMSO-treated control in both cell lines, except for a marginal protection observed in the LNCaP cells at 20 μmol/L DATS concentration (Fig. 4B). To our surprise, IL-6 did not increase expression of either Bcl-2 or Bcl-xL mRNA in either cell line (Fig. 4C). We conclude that STAT3 activation resulting from cotreatment with IL-6 is largely dispensable for proapoptotic response to DATS in DU145 and LNCaP cells.

DATS treatment inhibited migration of DU145 and LNCaP cells

Cell migration and invasion are characteristics of metastatic disease. STAT3 signaling has been linked to cancer cell invasion and migration (39, 43). We therefore determined the effect of DATS treatment on migration of DU145 and LNCaP cells with or without IL-6. Migration by DU145 cells was significantly inhibited in the presence of 40 μmol/L DATS (Fig. 5A). IL-6 increased migration of DU145 cells by ~1.7-fold compared with DMSO-treated controls. DATS-mediated inhibition of DU145 cell migration was maintained in the presence of IL-6 (Fig. 5A). The DU145 cell line was relatively more migratory compared with LNCaP cells, which may, at least in part, be attributed to status of constitutively active STAT3. The LNCaP cells were also sensitive to IL-6-induced migration similar to the DU145 cell line. Similar to DU145 cells (Fig. 5A), DATS was able to inhibit migration of LNCaP cells.

![Fig. 4.](image_url)
regardless of IL-6 treatment (Fig. 5B). These results indicated that the ability of DATS to inhibit cell migration was not affected by IL-6–mediated activation of STAT3. We confirmed these results by using LNCaP cells stably transfected with constitutively active STAT3 (S3c). As shown in Fig. 6A, the level of total STAT3 and pSTAT3 was higher by ∼1.4- and 3.5-fold, respectively, in S3c cells compared with empty vector–transfected control LNCaP cells. Ectopic expression of pSTAT3 in S3c cells resulted in potentiation of cell migration as expected (Fig. 6B). However, the S3c cell line exhibited only marginal resistance toward DATS-mediated inhibition of cell migration compared with empty vector–transfected cells (Fig. 6C).

Discussion

The primary objective of the present study is to test whether anticancer responses to DATS, which is a highly promising cancer-chemopreventive constituent of processed garlic, are related to inhibition of STAT3. STAT3 is a valid chemopreventive/therapeutic target for prostate cancer because (a) elevated STAT3 activation has been observed in a large fraction of human prostate tumors compared with matched adjacent normal prostate tissues (3, 6); (b) STAT3 regulates gene expression of many prosurvival molecules (39, 43); (c) inhibition of STAT3 activation triggers apoptotic cell death in human prostate cancer cells (7, 9); and (d) stable expression of constitutively active STAT3 alone has been shown to alter the phenotype of benign prostate epithelial cells to resemble malignant cells (4). Moreover, STAT3 is implicated in transition of hormone-sensitive prostate cancer to androgen-independent state (also referred to as “castration-resistant” or “hormone-refractory”; refs. 8, 44). The present study reveals that DATS treatment inhibits constitutive as well as IL-6–inducible activation of STAT3, which is not a cell line–specific effect. However, the underlying mechanism for IL-6–induced STAT3 phosphorylation is likely different between DU145 and LNCaP cells. IL-6 treatment clearly increases phosphorylation of JAK2 in LNCaP cells but not in the DU145 cell line. Activation of STAT3 can also be mediated by other kinases (e.g., Src and Rac1; refs. 1, 2, 42, 45), and the possibility that such kinases are involved in the activation of STAT3 in DU145 cells cannot be excluded. Noticeably, DATS-mediated inhibition of STAT3 activation is evident at pharmacologically relevant concentrations based on a rat pharmacokinetic study (46). We also show that DATS-mediated decrease in STAT3 phosphorylation is not restricted to cultured cells because dorsolateral prostates from DATS-treated mice exhibit reduced Tyr705 phosphorylation of STAT3 (Fig. 3B).

Tyr705 phosphorylation of STAT3 causes its dimerization (43). The STAT3 dimer translocates to the nucleus for binding to specific DNA sequences in the promoter of target genes (43). We found that DATS-mediated inhibition of STAT3 phosphorylation is accompanied by not only...
suppression of nuclear translocation of pSTAT3 but also its dimerization. Inhibition of the nuclear level of pSTAT3 by DATS treatment is evident at 3 hours and occurs even in IL-6–stimulated cells. Likewise, DATS-mediated suppression of STAT3 dimerization is observed in cells without (DU145) or with IL-6 treatment (LNCaP; Fig. 3A).

Another critical goal of the present study is to determine whether activation of STAT3 confers protection against cellular responses to DATS. This was a strong possibility considering the expression of many antiapoptotic genes (e.g., Bcl-2, Bcl-xL, and survivin) and those involved in angiogenesis and metastasis (e.g., MMP2, MMP9, and VEGF) are regulated by STAT3 (39, 43). Consistent with antiapoptotic role of STAT3 activation, inhibition of STAT3 triggers apoptosis in human prostate cancer cells (9). Peptide-mediated inhibition of STAT3 has been shown to sensitize ErbB2-overexpressing breast cancer cells to growth inhibition by Taxol (47). Likewise, blockade of STAT3 activation by treatment with inhibitors of epidermal growth factor receptor and JAK2 or by transfection with a dominant-negative STAT3 renders 435B cells more sensitive to chemotherapeutic-induced apoptosis (48).

To our surprise, growth-inhibitory or proapoptotic effects of DATS are not significantly impaired by IL-6–mediated activation of STAT3 (present study). The antimigratory effect of DATS is also either not altered or only modestly blunted by IL-6 stimulation or by forced expression of constitutively active STAT3. These effects are not cell line–specific because similar responses are evident in both DU145 and LNCaP cells. It is interesting to note that STAT5 has been shown to modify the effects of STAT3 from the level of gene expression to cellular phenotype (49). Coactivation of STAT5 and STAT3 in breast cancer cells results in decreased cellular proliferation and increased sensitivity to paclitaxel and vinorelbine (49). It is plausible that DATS treatment causes activation of STAT5 to counteract the antiapoptotic effect of STAT3 activation resulting from the IL-6 treatment. Further studies are needed to systematically explore this possibility.

The mechanism of the growth-suppressive effect of DATS has been extensively studied in prostate cancer cells (31–37). For example, we have previously shown that DATS treatment causes activation of checkpoint kinase 1 (Chk1) in human prostate cancer cells regardless of androgen responsiveness or p53 status, and activation of Chk1 is critical for both mitotic arrest and apoptotic cell death (32, 35, 50). Moreover, Chk1 dependence of DATS-induced cell cycle arrest and apoptosis induction is not unique to prostate cancer cells because similar effects are seen in both DU145 and LNCaP cells. It is interesting to note that STAT5 has been shown to modify the effects of STAT3 from the level of gene expression to cellular phenotype (49). Coactivation of STAT5 and STAT3 in breast cancer cells results in decreased cellular proliferation and increased sensitivity to paclitaxel and vinorelbine (49). It is plausible that DATS treatment causes activation of STAT5 to counteract the antiapoptotic effect of STAT3 activation resulting from the IL-6 treatment. Further studies are needed to systematically explore this possibility.

The mechanism of the growth-suppressive effect of DATS has been extensively studied in prostate cancer cells (31–37). For example, we have previously shown that DATS treatment causes activation of checkpoint kinase 1 (Chk1) in human prostate cancer cells regardless of androgen responsiveness or p53 status, and activation of Chk1 is critical for both mitotic arrest and apoptotic cell death (32, 35, 50). Moreover, Chk1 dependence of DATS-induced cell cycle arrest and apoptosis induction is not unique to prostate cancer cells because similar effects are seen in a human colon cancer cell line (50). Previous studies have also established that the initial signal for cellular effects of DATS (apoptosis and cell cycle arrest) is derived from the generation of reactive oxygen species (31, 33), which causes activation of c-Jun NH2-terminal kinase and extracellular signal-regulated kinase, leading to phosphorylation and hence inactivation of Bcl-2 (31). Suppression of Akt leading to mitochondrial translocation of proapoptotic protein BAD and activation of caspases have also been
documented in DATS-treated prostate cancer cells (34). Thus, it is safe to conclude that DATS is capable of targeting multiple signaling pathways to execute cell death.

In summary, the present study shows the inhibition of STAT3 activation by DATS in human prostate cancer cells in culture and mouse prostate tumors in vivo. Equally exciting is our finding that DATS can overcome the survival advantage imparted by STAT3 activation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

4. Huang HF, Murphy TF, Shu P, Barton AB, Barton BE. Stable expression of constitutively-activated STAT3 in benign prostatic epithelial cells changes their phenotype to that resembling malignant cells. Mol Cancer 2005;4:2.
12. Huang HF, Murphy TF, Shu P, Barton AB, Barton BE. Stable expression of constitutively-activated STAT3 in benign prostatic epithelial cells changes their phenotype to that resembling malignant cells. Mol Cancer 2005;4:2.

Acknowledgments

We thank Abhai Kumar for technical assistance.

Grant Support

USPHS grant 2 RO1 CA113363-06 awarded by the National Cancer Institute.

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Received 06/01/2010; revised 08/24/2010; accepted 08/25/2010; published OnlineFirst 10/19/2010.

Chandra-Kuntal and Singh


Cancer Prevention Research

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Cancer Prev Res  Published OnlineFirst October 19, 2010.

Updated version  Access the most recent version of this article at: doi:10.1158/1940-6207.CAPR-10-0123

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