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Multitargeted prevention and therapy of cancer by diallyl trisulfide and related *Allium* vegetable-derived organosulfur compounds

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Abstract

Allium vegetables, such as garlic, have been used for medicinal purposes throughout the recorded history. The known health benefits of *Allium* vegetable constituents include cardiovascular effects, improvement of the immune function, lowering of blood glucose level, radioprotection, protection against microbial infections, and anticancer effects. Initial evidence for the anticancer effect of *Allium* vegetables was provided by population-based case-control studies. Subsequent laboratory studies showed that the *Allium* vegetable constituents, such as diallyl disulfide, S-allylcysteine, and ajoene can not only offer protection against chemically-induced cancer in animal models by altering carcinogen metabolism, but also suppress growth of cancer cells in culture and *in vivo* by causing cell cycle arrest and apoptosis induction. Suppression of angiogenesis and experimental metastasis by *Allium* constituents has also been reported. Defining the mechanism by which sulfur compounds derived from *Allium* vegetables inhibit cancer cell growth has been the topic of intense research in the last two decades. Some *Allium* vegetable constituents have also entered clinical trials to assess their safety and anticancer efficacy. This article summarizes preclinical and limited clinical data to warrant further clinical evaluation of *Allium* vegetable constituents for prevention and therapy of human cancers.

Keywords

Garlic; organosulfides; cell cycle; apoptosis; chemoprevention

1. Introduction

Allium vegetables have been used in the traditional medicine for centuries [1]. Recent scientific investigations have shown that *Allium* vegetables and their constituents reduce the risk of cardiovascular disease and diabetes, stimulate immune system, protect against infections, and have anti-aging as well as anti-cancer effects [1-4]. The anticancer effects of *Allium* vegetables are supported by epidemiological data from population-based case-control studies. For example, You et al [5] examined the association between *Allium* vegetable intake and the risk of gastric cancer in a population-based case-control study involving 564 patients and 1131 healthy controls. Subjects in the highest quartile of *Allium* vegetable intake had significantly lower risk of developing gastric cancer compared to those in the lowest quartile [5]. Similarly, another population-based case-control study conducted in Shanghai, China evaluated the effects of *Allium* vegetable intake on prostate cancer risk [6]. The results of this study indicated that intake of *Allium* vegetables was inversely associated with the risk of prostate cancer [6]. While these examples serve to illustrate protective effect of *Allium* vegetables against cancer

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risk [5;6], similar conclusions have been reached for certain other types of cancers in epidemiological studies extensively reviewed by Shukla and Kalra [7]. These epidemiological studies triggered intense research in the past two decades aimed not only at identification of the putative phytochemicals responsible for the anticancer effects of *Allium* vegetables but also elucidation of the mechanism of their action.

2. Anticancer phytochemicals in *Allium* vegetables

Research over the years has revealed that the anticancer effects of *Allium* vegetables are attributable to organosulfur compounds (OSCs), which are released from the vegetables upon their processing (mincing, chewing etc.) [8]. The γ -glutamyl-S-alk(en)yl-L-cysteines are the primary sulfur compounds in intact *Allium* vegetables, which are hydrolyzed and oxidized to yield S-alkyl(en)yl-L-cysteine sulfoxide (alliin) [8]. Alliin is the odorless precursor of the OSCs and naturally accumulates during storage of the *Allium* vegetables [8]. The OSCs are generated upon conversion of alliin to allicin and other alkyl alkane-thiosulfinates through mediation of alliinase, which is released from vacuoles upon cutting, crushing or chewing of the *Allium* vegetables. Allicin and related thiosulfinates are highly unstable and instantly decompose to yield various sulfur compounds including diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins, and ajoene [8]. The metabolic pathway and chemical structures of the widely studied OSCs are depicted in Figure 1.

3. Molecular mechanisms (targets) for anticancer effect of OSCs

3.1. Modulation of carcinogen metabolism

Carcinogenic chemicals often require metabolic activation mediated by cytochrome P450-dependent monooxygenases (phase 1 enzymes) for their neoplastic activity. Inactivation of activated carcinogenic intermediates is accomplished by phase 2 enzymes including glutathione transferases. Studies have revealed that OSCs can not only inhibit phase 1 enzymes but also increase the expression of phase 2 enzymes (reviewed extensively in [7;9]). For example, DAS and its metabolites diallyl sulfoxide and diallyl sulfone competitively inhibited activity of cytochrome P-450 2E1 in a time- and NADPH-dependent manner with a pseudo-first-order kinetics [10]. Wattenberg and colleagues showed that prevention of benzo[a]pyrene-induced forestomach and lung cancer in mice by garlic OSCs was accompanied by elevation of hepatic and target organ glutathione transferase activity [11]. Thus it is reasonable to conclude that OSCs function in prevention of chemically-induced cancers not only by inhibiting carcinogen activation but also by enhancing detoxification of the activated carcinogenic intermediates through induction of phase 2 enzymes [7;9-11].

3.2. Inhibition of cell cycle progression

More recent studies have revealed that OSCs can halt cell cycle progression in neoplastic cells. Knowles and Milner [12] were the first to report G2/M phase cell cycle arrest in human colon cancer cells upon treatment with DADS, which was associated with a decrease in complex formation between cyclin-dependent kinase 1 (Cdk1) and cyclin B1 leading to suppression of the kinase activity of Cdk1/cyclin B1 complex. The DADS-induced G2/M phase cell cycle arrest has also been reported in other cell lines including PC-3 prostate cancer cell line [13], MGC80 human gastric cancer cell line [14], and A549 lung cancer cell line [15]. Our laboratory has more extensively studied the mechanism of DATS-induced cell cycle arrest using human prostate cancer cells (PC-3 and DU145) as a model [16-19]. We demonstrated that the DATS-mediated G2/M phase cell cycle arrest in prostate cancer cells was associated with reactive oxygen species (ROS)-dependent hyperphosphorylation and destruction of the cell division cycle 25C (Cdc25C) phosphatase [19]. The DATS-mediated G2/M phase cell cycle arrest appeared to be selective for cancer cells since a normal prostate epithelial cell line was resistant

to cell cycle arrest by DATS [19]. Subsequently we demonstrated that the ROS generation by DATS treatment in prostate cancer cells was caused by an increase in the level of labile iron due to c-Jun N-terminal kinase (JNK)-mediated degradation of the iron storage protein ferritin [16]. Further investigations revealed that the DATS-treated cells were also arrested in prometaphase, which was partly dependent on checkpoint kinase 1 (Chk1)-mediated inactivation of the anaphase promoting complex/cyclosome [17;18].

The cell cycle arrest in cancer cells has also been reported for other OSCs, including the water soluble sulfur compounds. For example, S-allylmercaptocysteine (SAMC) treatment resulted in G2 and/or mitotic arrest in SW-480 and HT-29 human colon cancer cells, and NIH3T3 fibroblasts [20;21]. Moreover, SAMC treatment caused microtubule depolymerization, disruption of the cytoskeleton, and fragmentation of the centrosome in interphase cells [21]. Similarly, ajoene was shown to cause G2/M phase cell cycle arrest in HL60 cells in association with disruption of the cytoskeleton [22]. It is interesting to note that allicin treatment arrested human mammary cancer cells in both G0/G1 and G2/M phases of the cell cycle [23]. Collectively, these studies indicate that the cell cycle arrest is a common cellular response to structurally diverse OSCs. The mechanisms of DATS-induced G2/M phase cell cycle arrest in prostate cancer cells are summarized in Figure 2.

3.3. Induction of apoptosis

Numerous publications indicate that the suppression of cancer cell growth by OSCs correlates with apoptosis induction. The first report on OSC-mediated apoptosis came from Milner and colleagues who observed DNA fragmentation and other morphological changes indicative of apoptosis in DADS-treated human colon cancer cells [24]. Elucidation of the mechanism(s) of apoptosis induction by OSCs has been the topic of intense research in the last few years. Most studies implicate involvement of Bcl-2 family proteins in regulation of OSC-mediated apoptosis. For example, the DAS and DADS-mediated apoptosis in SH-SY5Y neuroblastoma cell line and lung cancer cells (H460 and H1299) correlated with an increase in the ratio of Bax/Bcl-2 [25;26]. Upregulation of Bax protein level with a concomitant decrease in the level of Bcl-xL protein was observed in DADS-treated MDA-MB-231 breast cancer cell line [27]. The ajoene-induced apoptosis in HL-60 cells correlated with caspase-mediated cleavage of Bcl-2 [28]. We have shown that PC-3 and DU145 prostate cancer cells are more sensitive to apoptosis induction by DATS compared with DAS or DADS [29]. The DATS-induced apoptosis in PC-3 and DU145 cells correlated with a decrease in Bcl-2 protein level as well as its hyperphosphorylation leading to reduced Bcl-2:Bax interaction and activation of the mitochondria-mediated intrinsic caspase cascade [29]. The DATS-induced hyperphosphorylation of Bcl-2 in PC-3 and DU145 cells was mediated by JNK, and to a smaller extent by extracellular signal-regulated kinase 1/2 (ERK1/2) [29]. The DATS treatment decreased Bcl-2 and Bcl-xL protein levels and increased Bak protein expression in LNCaP human prostate cancer cell line, which correlated with loss of the mitochondrial membrane potential [30]. Knockdown of the Bax and Bak proteins conferred significant protection against DATS-induced apoptosis in LNCaP cells [30]. However, ectopic expression of Bcl-2 protein protected against DATS-induced apoptosis in PC-3 cells but not in the LNCaP cell line [29; 30]. The PC-3 cell line is androgen-independent and lacks functional p53, whereas the LNCaP cell line is androgen-responsive with wild type p53 expression. It is possible that the differential effect of Bcl-2 overexpression on DATS-induced apoptosis in PC-3 *versus* LNCaP cells is related to differences in their androgen responsiveness or p53 status. Further studies are needed to explore these possibilities.

It is intriguing to note that DATS treatment causes only a modest increase in protein levels of Bax and Bak yet knockdown of these proteins confers statistically significant protection against DATS-induced apoptosis [30]. Even though the mechanism(s) by which Bax and Bak regulate

DATS-induced cell death are not fully elucidated, it is possible that DATS induces a conformational change and oligomerization of Bax/Bak resulting in their translocation to the mitochondria. This speculation is partially substantiated: (a) certain apoptotic stimuli induce Bax activation in an ROS-dependent manner and DATS causes ROS generation [16;30]; (b) microtubule damaging agents induce Bax activation, and DATS treatment was shown to disrupt tubulin network [18;31]. For example, DATS, but not DADS or DAS, has been shown to disrupt microtubule network in human colon cancer cells *via* oxidative modification of the β tubulin at cysteine residues in positions 12 and 354 [31].

Recent studies have implicated ROS generation as well as an increase in intracellular calcium level in apoptosis induction by OSCs. For instance, the DADS-induced apoptosis in HL-60 cells correlated with ROS generation [32]. The DADS-induced ROS formation in SH-SY5Y neuroblastoma cells was evident as early as 15 min after treatment and accompanied by oxidation of cellular lipids and proteins [33]. Overexpression of Cu, Zn- superoxide dismutase or pretreatment with a spin trapping molecule (5,5'-dimethyl-1-pyrroline *N*-oxide) offered protection against DADS-induced ROS generation, oxidative damage of cellular macromolecules and apoptosis in SH-SY5Y cells [33]. Our studies have implicated ROS production in apoptotic response to DATS in the LNCaP cell line [30]. In another study using human glioblastoma cells, Das et al [34] demonstrated OSC-mediated ROS generation and an increase in the free intracellular calcium level. The DADS treatment in N18 retina ganglion cells resulted in an increase in free intracellular calcium [35]. The ajoene-induced apoptosis in human promyeloleukemic cells was accompanied by generation of ROS and activation of nuclear factor-kappaB [36].

It is important to point out that even though OSCs induce apoptosis in cancer cells they have little or no effect on normal cells. For instance, ajoene did not cause apoptosis in peripheral mononuclear blood cells from healthy donors but caused apoptosis in human leukemia cells [36]. Similarly, DAS or DADS treatments caused apoptosis in SH-SY5Y neuroblastoma cells but did not have any appreciable effect on viability of the primary neurons [26]. The PrEC normal prostate epithelial cells were significantly more resistant to apoptosis induction by DATS compared with prostate cancer cells [30]. However, the mechanism for selectivity of OSCs for apoptosis induction in cancer cells remains elusive. The mechanisms involved in DATS-induced apoptosis are summarized in Figure 2.

3.4. Histone modification

Modification of histone acetylation by OSCs has also been documented [37-41]. For example, DADS induced differentiation of DS19 mouse erythroleukemia and K562 human leukemia cells which was associated with increased acetylation of histones H4 and H3 [39]. Additional experiments revealed that the DADS and/or its metabolite, allyl mercaptan, inhibited histone deacetylases in rat hepatoma and human breast cancer cells [39]. Increased histone acetylation and correlated with it growth inhibition was observed in cell culture models in response to a variety of OSCs including allicin, SAMC and S-allyl cysteine (SAC) on DS19 cells and SAMC on Caco-2 human colon and T47D human breast cancer cells [40]. Druesne et al [37;38] observed that DADS treatment resulted in accumulation of Caco-2 and HT-29 colon tumor cells in G2/M phase of the cell cycle, which correlated with inhibition of histone deacetylase resulting in the observed hyperacetylation of H3 and H4 histones, as well as upregulation of p21 mRNA and protein level [37;38].

3.5. Inhibition of angiogenesis

Mousa et al [42] demonstrated inhibition of fibroblast growth factor-2 and vascular endothelial growth factor (VEGF)-induced tube formation in human endothelial cells and inhibition of *ex vivo* neovascularization in chick chorioallantoic membrane assay by alliin. The anti-angiogenic

effects of alliin were mediated, at least in part, by increase in cellular nitric oxide and p53 protein expression [42]. The DATS treatment inhibited capillary-like tube formation and migration of human umbilical vein endothelial cells (HUVECs) [43]. The anti-angiogenic effect of DATS correlated with suppression of VEGF secretion, down-regulation of VEGF receptor-2 protein level and inactivation of Akt [43]. The OSCs were demonstrated to affect remodeling of the extracellular matrix. For example, DADS not only inhibited HUVEC cell proliferation but also attenuated activation of matrix metalloproteinase-2 (MMP-2) and MMP-9 [44]. Intraperitoneal administration of ajoene to C57BL/6 mice injected with B16/BL6 melanoma cells strongly inhibited lung metastasis [45].

4. *In vivo* evidence for cancer preventive/therapeutic effects of OSCs

Belman [46] was the first to show inhibition of chemically-induced skin carcinogenesis in mice by garlic oil. Inhibition of skin carcinogenesis by ajoene has also been reported [47], Wargovich et al [48] showed prevention of 1,2-dimethylhydrazine-induced colon cancer by oral gavage of DAS. The DAS administration dramatically inhibited formation of esophageal tumors induced by *N*-nitrosomethylbenzylamine in rats [49]. Interestingly, DAS was effective if administered during the initiation phase but not after the carcinogen challenge or during the promotion stage [49]. Dietary administration of 100 and 200 ppm DADS inhibited the incidence and multiplicity of the invasive colon adenocarcinomas induced by azoxymethane in rats [50]. Chemopreventive effects for AGE and other garlic derived OSCs have also been reported. For example, AGE treated F344 rats had significant reduction in glutathione S-transferase-P positive hepatocellular foci [51]. Garlic powder with varying alliin content inhibited carcinogen-induced DNA damage in rat liver and colon [52]. Prevention of the chemically-induced cancers of the lung, stomach, breast, cervix, breast and oral cavity in experimental animals by garlic or OSCs has been reported (reviewed in [7]).

In addition to prevention of chemically-induced cancers, garlic constituents have been shown to inhibit cancer cell growth *in vivo* in xenograft models. Milner and colleagues were the first to report inhibitory effect of DADS on the growth of HTC-15 human colon cancer cells implanted in nude mice [53]. Studies from our laboratory have revealed that oral administration of DADS, three times per week beginning the day of tumor cell injection, suppressed growth of H-ras oncogene transformed tumor xenografts in nude mice without causing weight loss or any other side effects [54]. The appearance of measurable tumors was also delayed in DADS-treated mice relative to controls [54]. The DADS-mediated suppression of H-ras oncogene transformed tumor growth correlated with a decrease in hepatic and tumoral HMG-Co A reductase activity leading to inhibition of membrane association of p21 [54]. These studies were the first published reports to document activity of DADS against H-ras oncogene transformed tumors. The DADS treatment also attenuated growth of breast cancer cells implanted in nude mice [27]. The DADS-mediated inhibition of breast cancer cell growth correlated with reduced cell proliferation as judged by immunohistochemical staining for proliferating cell nuclear antigen (PCNA) [27]. Oral gavage of DATS retarded growth of PC-3 cells implanted in male athymic mice, which correlated with increased apoptosis in the tumor tissue [55]. SAC administration inhibited CWR22R human androgen independent prostate cancer xenograft in nude mice without any side effects [56]. Recent advances in nanotechnology have also been utilized to explore the potential of OSCs in targeted therapy. Zhang et al [57] used polybutylcyanoacrylate nanoparticles filled with DATS (DATS-PBCA-NP, administered i.v.) in therapy targeted against orthotopically transplanted HepG2 cells in BALB/c nude mice. Treatment with DATS-PBCA-NP retarded the growth of the implanted tumors in association with increased apoptotic index and decreased cell proliferation [57].

5. Clinical trials

Positive results of the *in vitro* and *in vivo* studies were followed up in a few intervention trials which examined the chemopreventive effects of *Allium* vegetable constituents in human population. First double-blind intervention trial examined the effects of high dose of DATS (also known as allitridum; 200 mg/day) and micro-doses of selenium (100 µg every other day) [58]. Both supplements were taken by the intervention group (2,526 subjects) for a period of one month, while control group (2,507 subjects) was given placebo [58]. All participants were followed for five years and incidence of various cancers was recorded. Results of this trial revealed that the intervention group had 22% lower incidence of all cancers and 47.3% lower incidence of gastric cancer [58]. After adjusting for age, gender, family history, smoking, alcohol consumption, and medical history of stomach illness, the relative risk (RR) for all cancers was 0.67 (95% confidence limit CL=0.43-1.03) and RR = 0.48 for gastric cancer (95% CL=0.21-1.06) [58]. Results of this trial suggested that large doses of DATS can be used without any noticeable side effects. Despite the promising results of the above mentioned trial, beneficial effects of AGE was not observed against stomach cancer in a later study by You et al [59]. In this study, 3365 eligible subjects were randomly assigned in a factorial design to three intervention groups or placebo control, including amoxicillin and omeprazole for 2 weeks (*H. pylori* treatment), vitamin C, vitamin E, and selenium for 7.3 years (vitamin supplement), and a blend of AGE and steam-distilled garlic oil (which may contain DADS and DATS) for 7.3 years (garlic supplement) [59]. The *H. pylori* treatment resulted in statistically significant decreases in the combined prevalence of severe chronic atrophic gastritis, intestinal metaplasia, dysplasia, or gastric cancer (OR= 0.77; 95% CI = 0.62 to 0.95). On the other hand, no statistically significant favorable effects were evident either for garlic or vitamin supplements [59]. However, a clinical study performed in China supports beneficial effect of AGE against colorectal cancer [60]. Tanaka et al [60] examined the effects of high (2.4 mL/day) or low (0.16 mL/day) doses of AGE given to patients with colorectal polyps over a 12-month period. Administration of high dose AGE significantly reduced the number and size of colon adenomas after 12 months of treatment [60]. Therapeutic role of ajoene was also demonstrated in skin cancer [61]. Tili et al [61] topically applied ajoene onto tumors of 21 patients with nodular or superficial basal carcinoma for six months. They recorded a significant reduction in tumor size in 17 cases [61]. Immunohistochemical analyses of the tumors revealed reduction in expression of anti-apoptotic protein Bcl-2 [61]. Clearly, carefully designed clinical trials are needed to further evaluate chemopreventive and therapeutic potential of *Allium* vegetable constituents in humans.

6. Bioavailability, pharmacokinetics, and metabolism of the OSCs

One of the key factors affecting the clinical application of OSCs is their bioavailability and plasma concentrations. Analyses of the compounds reveal that one gram of freshly blended garlic can provide up to 2.5 mg of allicin and about 60 µg of SAC [62]. Similarly, it has been estimated that one gram of fresh garlic contains about 900-1100 µg of DATS and 530-610 µg of DADS [7]. Thus it is possible that the concentrations of the OSCs needed to bring about cellular responses (e.g., cell cycle arrest and apoptosis) in cancer cells may be generated through dietary intake of garlic. However, further studies are needed to determine whether biologically effective concentrations of OSCs can be achieved in plasma through dietary intake of *Allium* vegetables or through pharmacological intervention with pure compounds. In prostate cancer cells, exposure for 24 h is sufficient to cause DATS-mediated cell cycle arrest and apoptosis induction [16-19;29]. Carefully designed cellular studies are also needed to determine the minimum exposure time required for initiation of cellular effects of DATS and related compounds in cancer cells.

Early studies using aqueous garlic extracts indicated that OSCs are highly bioavailable. For example, Nagae et al [63] documented that SAC was rapidly absorbed in the gastrointestinal tract and distributed in plasma, liver and kidney of rats, mice and dogs. The bioavailability of the compound was 98.2% in rat, 103 % in mice and 87.2% in dogs [63]. The bioavailability and metabolism of oil-soluble OSCs were also investigated. When allicin was passed through rat liver, it was very efficiently metabolized into DADS and allyl mercaptan, with DADS being the most likely precursor for allyl mercaptan [64]. Germain et al [65] demonstrated that after single oral administration of 200 mg/kg of DADS to rats, the highest concentration was observed in the stomach within the first 24 h. A single intravenous administration of 10 mg DATS to rats resulted in peak blood concentration of about 31 μ M [66]. However, further studies are needed to determine pharmacokinetic and pharmacodynamic parameters for OSCs in humans. Likewise, it remains to be determined if biologically active concentrations of DATS and other garlic-derived sulfur compounds can be achieved through traditional dietary intakes or through pharmacological interventions in humans.

7. Concluding remarks and future directions

Research over the years has revealed that naturally occurring OSCs target multiple pathways to inhibit growth of cancer cells, which include impairment of carcinogen metabolism, cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis. Because OSCs exhibit other pharmacological effects, such as cardiovascular and anti-microbial effects, these compounds can be classified as being “promiscuous” rather than “selective”. However, promiscuity is not unique to OSCs since many known successful drugs as well as a number of promising natural anti-cancer agents are promiscuous. The best known example of a widely used drug that can be classified as “promiscuous” is aspirin. In addition to its pain relieving and anti-arthritis effects, aspirin exhibits many other pharmacological effects including blood thinning, reduction of platelet aggregation, prevention of preeclampsia (an hypertensive disorder during pregnancy), and anticancer effects. Likewise, recent studies have revealed that many promising dietary cancer chemopreventive agents (e.g., cruciferous vegetable constituent sulforaphane) target multiple signaling pathways in various cell types to inhibit cancer cell growth *in vitro* and *in vivo*. Etiology and pathogenesis of cancer is highly complex and involves abnormalities in multiple cellular checkpoints and signal transduction pathways therefore promiscuity may be an advantageous feature of potential anticancer agents. Promiscuous agent such as OSCs may be especially promising since recently developed highly specific anticancer agents have failed to live up to the expectations.

Future research on OSCs should focus on (a) preclinical assessment on cancer incidence and burden using transgenic animal models to further support chemopreventive potential of this highly promising class of natural products, (b) clinical assessment of these compounds for prevention/treatment of cancers in humans, and (c) pharmacokinetic and pharmacodynamics in humans. Because OSCs affect multiple signal transduction pathways, it is likely that they may find use in combination chemotherapy for treatment of human cancers. However, this idea needs to be explored experimentally.

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Figure 1.
Chemical structures of commonly astudies organosulfur compounds.

Figure 2.

Mechanisms of cellular responses to DATS in human prostate cancer cells. Based on the results of our previously published studies, it is reasonable to conclude that DATS causes degradation of ferritin thus increasing the levels of chelatable iron and leading to ROS generation in PC-3 and DU145 human prostate cancer cells. DATS-induced ROS generation results in down-regulation of Cdc25C which can be reversed in the presence of antioxidants such as N-acetylcysteine (NAC) and superoxide dismutase and catalase mimetic EUK134 (EUK). DATS also down-regulates Cdk1 expression in prostate cancer cells. The mechanism of DATS-mediated Cdk1 protein down-regulation is not yet clear but the net result of these effects is accumulation of inactive (Tyr15 phosphorylated) Cdk1/cyclin B1 kinase. DATS also causes ATR/Chk1-dependent prometaphase arrest in cancer cells. The mechanism behind the DATS-induced ATR activation remain elusive but may involve ROS-dependent DNA double strand breaks (DNA-DSB). The Chk1 activation correlates with inactivation of anaphase-promoting complex/cyclosome (APC/C). The DATS treatment of PC-3 and DU145 cells also causes c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinases (ERK1/2)-dependent phosphorylation of Bcl-2 leading to reduced interaction between Bax and Bcl-2. The net result of these effects is initiation of mitochondria-mediated caspase activation and apoptosis. DATS also inactivates Akt resulting in phosphorylation and mitochondrial translocation of pro-apoptotic protein Bad. Ectopic expression of constitutively active Akt (CA-Akt) and/or

combined knockdown of Bax and Bak proteins significantly inhibits DATS-induced apoptosis in prostate cancer cells.