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RESEARCH PAPER

# Tanshinones from Chinese Medicinal Herb Danshen (*Salvia miltiorrhiza* Bunge) Suppress Prostate Cancer Growth and Androgen Receptor Signaling

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## ABSTRACT

***Purpose*** To test whether tanshinones inhibit prostate cancer (PCa) growth at least in part through inhibiting androgen receptor (AR) signaling.

***Methods*** We evaluated cell growth, survival and AR signaling parameters of PCa cells after exposure to tanshinones in *in vitro* models. We also tested the *in vivo* inhibitory efficacy of tanshinone IIA (TIIA) against LNCaP xenograft model in athymic nude mice.

***Results*** For androgen-dependent LNCaP cells, a colony growth assay showed strong inhibitory potency following the order of TIIA≈cryptotanshinone>tanshinone I, being 10–30 folds higher than Casodex (racemic). TIIA inhibited growth of LNCaP cells more than several androgen-independent PCa cell lines. All 3 tested tanshinones were devoid of AR agonist activity under castrate condition. Mechanistically, tanshinones inhibited AR nuclear translocation within 2 h, decreased protein and mRNA abundance of AR and its target prostate-specific antigen within 12 h, and stimulated proteosomal degradation of AR. Oral administration of TIIA (25 mg/kg, once daily) retarded LNCaP xenograft growth and down-regulated tumor AR abundance in athymic nude mice.

***Conclusion*** AR targeting action of tanshinones was distinct from Casodex and contributed to prostate cancer growth suppression *in vitro* and *in vivo*.

**KEY WORDS** androgen receptor · nuclear translocation · prostate cancer · prostate specific antigen · proteosomal degradation · tanshinone

## ABBREVIATIONS

| | |
|---|---|
| AR | androgen receptor |
| ATCC | American type culture collection |
| Cas | Casodex |
| cPARP | cleaved poly (ADP)-ribose polymerase |
| CT | cryptotanshinone |
| DMSO | dimethylsulfoxide |
| Mib | mibolerone |
| PCa | prostate cancer |
| PSA | prostate-specific antigen |
| TI | tanshinone I |
| TIIA | tanshinone IIA |

## INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer death in American men and responsible for an estimated 30,000 deaths per year (1). Increasing laboratory and

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clinical evidence indicates that androgen receptor (AR) signaling plays a critical role in the survival and growth of PCa cells (2-7). Recent clinical trials of abiraterone acetate (a CYP17 blocker to inhibit the synthesis of androgenic steroids) and MDV-3100 (an antagonist to inhibit ligand activation of AR) have shown drug-induced decline of AR transcriptional target prostate specific antigen (PSA) and tumor regression in PCa patients including those with castration-resistant PCa (8,9). These observations strengthen the notion that AR signaling remains an important target for the prevention and therapy of PCa. The current AR antagonist drugs such as Casodex (also known as bicalutamide) and flutamide exhibit partial AR agonist activity toward some AR mutants and induce drug resistance through mutations of AR or increasing expression of anti-apoptotic molecules (10-12), calling for novel agents for improving PCa treatment and prevention.

Oriental medicinal herbals are a rich source of various bioactive compounds including anti-cancer agents. The roots of Chinese herb Danshen (*Salvia miltiorrhiza* Bunge) have been traditionally used for cardiovascular diseases in China and other Asian countries for centuries (13). Tanshinones including tanshinone I (TI), tanshinone IIA (TIIA) and cryptotanshinone (CT) (Fig. 1a) are the major hydrophobic bioactive compounds isolated from Danshen (13). Recent studies suggest that tanshinones exert broad-range growth inhibitory and cytocidal activities against various cancer cell lines such as prostate, lung, breast, leukemia, glioma, liver through inducing cell cycle arrest and apoptosis *in vitro* (14-23). In addition, TI has been shown to inhibit human androgen-independent PCa xenograft growth *in vivo* (24), as has TIIA to inhibit human breast cancer xenografts (25,26), providing preliminary evidence of efficacy and bioavailability.

Danshen has also been used clinically to treat acne, a dermal lesion in association with aberrant sebum secretion stimulated by increased androgen during adolescence (27). Anti-androgen action is considered as one of the major mechanisms of tanshinone against acne (28). Furthermore, a previous report demonstrated that *in vivo* administration of tanshinones decreased the androgen-dependent organ (prostate and seminal vesicle) weights in experimental animals, albeit the molecular mechanisms were not well delineated (29). Based on these androgen-related indications, we hypothesize that tanshinones may inhibit PCa growth, at least in part, through targeting AR signaling. In the present study we report that tanshinones inhibit AR-dependent signaling in androgen-dependent LNCaP model in addition to growth suppression of androgen-independent PCa cells and that TIIA suppresses LNCaP xenograft growth in athymic nude mice, with distinct actions from those of Casodex.

## MATERIALS AND METHODS

### Tanshinone Compounds and Other Chemicals

TI, TIIA and CT (purity 98.6%, 98.0% and 94.6%, respectively, Fig. 1a) were purchased from LKT Laboratories Inc. (St Paul, MN). The purity of each compound was confirmed by HPLC-diode array detection in our laboratory. Additional TIIA for *in vivo* study was isolated from Danshen at KyungHee University (Seoul, Korea) as previously reported (14) and showed similar inhibitory potency on AR signaling. Racemic bicalutamide (referred to as Casodex throughout this text) was purchased from LKT Labs (Cat# B3209). The antiandrogenic activity of Casodex is almost exclusively exhibited by the R-enantiomer while the S-enantiomer is essentially inactive (30).

### Cell Culture

Human LNCaP, DU145 and 22Rv1 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). C4-2 cells, a castration-resistant variant of LNCaP cells, were a generous gift from Dr. Donald Tindall (Mayo Clinic, Rochester, MN). All the cells were maintained and treated in the ATCC-recommended growth media supplemented with 10% fetal bovine serum (complete growth medium) without antibiotics under the standard 37°C and 5% $CO_2$ humidified environment, except when indicated otherwise.

### Cell Growth Assays

In addition to short-term exposure effect (3 day treatment), colony growth assay was used to evaluate the long-term growth response of PCa cells exposed to tanshinones as previously described (31,32). In brief, PCa cells were seeded on 6-well plates (~$10^4$ cells per well) and exposed to indicated concentrations of TI, TIIA and CT (LKT Laboratories Inc., St. Paul, MN) in complete growth medium or androgen-deprived growth medium (phenol-red free growth medium supplemented with 5% charcoal-stripped serum) without medium change for 9~14 days depending on the doubling time of each cell line. After treatment, the cells remaining attached were fixed, and then the cell number was estimated by staining the cellular protein with 0.02% crystal violet (31,32).

### Cell Cycle Distribution

LNCaP cells were exposed to indicated concentrations of tanshinones for 24 h, and then were incubated with 1 μM of BrdU for 30 min. The cells were collected for BrdU

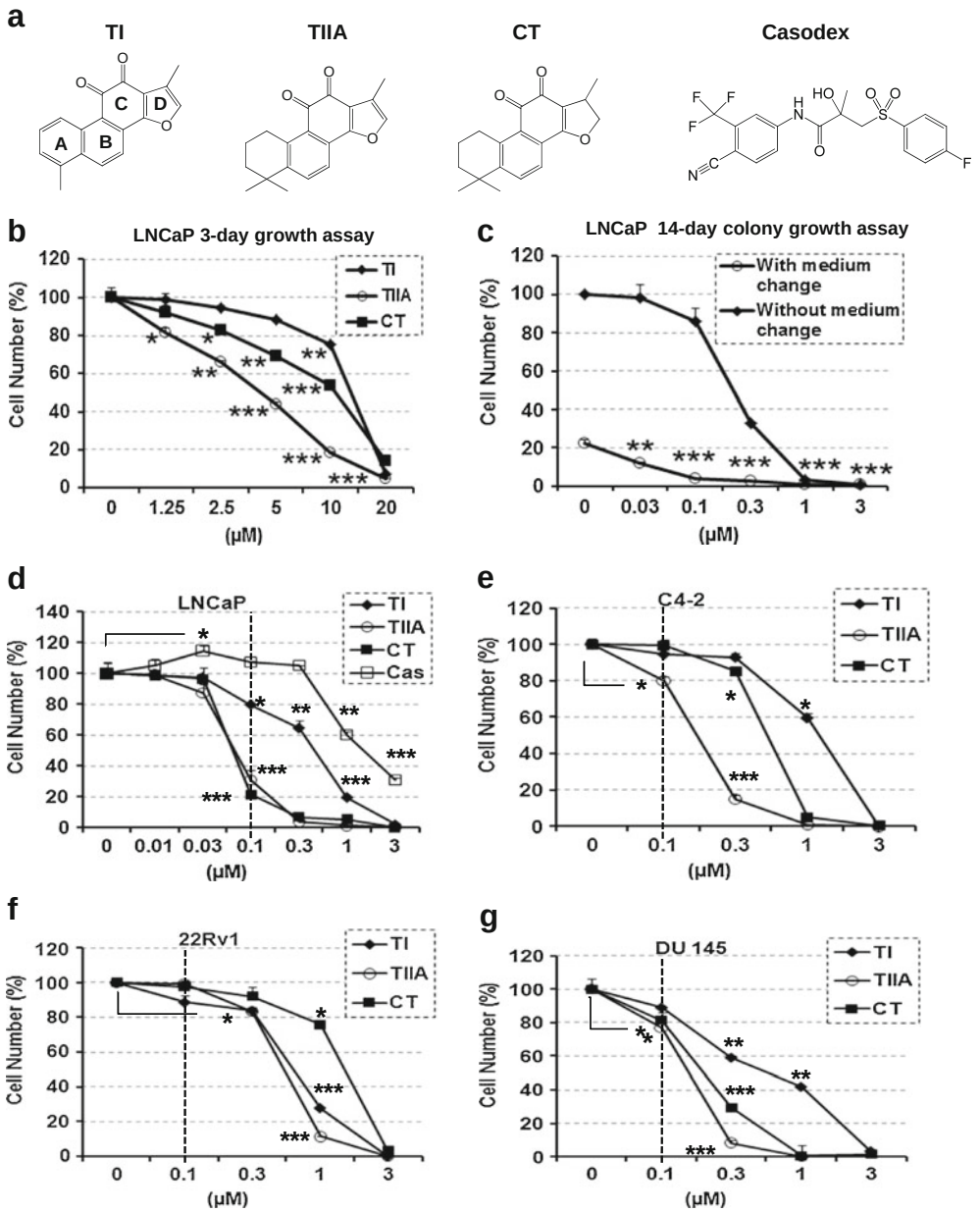



**Fig. 1** (**a**) Structures of tanshinone I (TI), tanshinone IIA (TIIA), cryptotanshinone (CT) and AR antagonist drug Casodex. (**b**) Effects of tanshinones on the growth of LNCaP cells in complete medium after 3 days. (**c**) Effects of chronic exposure to TIIA on the colony growth of LNCaP cells, comparing a one-time treatment protocol without changing medium with one involving changing medium with fresh TIIA every 3 days. Overall colony growth was estimated after 14 days. (**d**) Comparison of chronic effects of different tanshinones on the colony growth of LNCaP cells with Casodex (Cas) using the one-time treatment protocol (14 days). (**e**–**g**) Chronic effects of different tanshinones on the colony growth of androgen-independent (*E*) LNCaP C4-2 (14 days); (*F*) 22Rv1 (14 days) and (*G*) DU 145 cells (9 days) using the one-time treatment protocol. The attached cells were quantified using crystal violet staining of cellular protein. Each point represents Mean±SEM, $n=3$. Statistical significance from control: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

immunostaining and bivariate cell cycle analysis as previously described (33).

## Immunoblotting

The whole cell lysate or subcellular fractions were prepared for immunoblotting. Anti-PSA was obtained from DAKO (Glostrup, Denmark). Anti-AR and anti-α-tubulin were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-PARP and anti-cleaved PARP were obtained from Cell Signaling Technology (Beverly, MA).

## ELISA Assays of Secreted PSA

After exposure to indicated treatments, the growth medium of LNCaP cells was collected and the floating cells were

removed by brief centrifugation. The secreted PSA was quantified using a MAGIWEL PSA ELISA system (United Biotech, Mountain View, CA) as previously described (34,35). The PSA values were normalized against cell number as estimated by cellular protein content.

### RNA Isolation and Real-Time PCR

After exposure to indicated treatment, the total RNA was isolated from PCa cells by using RNeasy Mini Kit (Qiagen, Valencia, CA) according to the user manual. Total RNA (2 μg) from each sample was reverse transcribed by using SuperScript™ II RT Kit (Invitrogen, Carlsbad, CA). Real-time PCR was performed on an Applied Biosystems 7,500 machine using FastStart Universal SYBR Green Master (Rox) system (Roche Diagnostics GmbH, Mannheim, Germany) as previous described (36). AR primers: Forward 5′-CAG GAG GAA GGA GAG GCT TC-3′, Reverse 5′-AGC AAG GCT GCA AAG GAG TC-3′. PSA primers: Forward 5′-CCC ACT GCA TCA GGA ACA AA-3′, Reverse 5′-GAG CGG GTG TGG GAA GCT-3′. β-Actin was used for template normalization. The PCR condition was started as 1 cycle of 2 min at 50°C and 10 min at 95°C, then 40 cycles of 15 s at 95°C and 1 min at 60°C, and then followed by 1 cycle of 15 s at 95°C, 1 min at 60°C, 15 s at 95°C and 15 s at 60°C.

### AR Nuclear Translocation

Evaluation of androgen-stimulated AR nuclear translocation was carried out as previously described (31,37). Briefly, LNCaP cells were grown in androgen-deprived condition for 48 h, then were pre-treated with indicated tanshinones for 1 h, and then were stimulated with mibolerone (a stable dihydrotestosterone analog, kindly provided by Dr. Charles Young, Mayo Clinic, Rochester, MN) for another 2 h. The nuclear and cytosolic fractions of cell lysate were separated using NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit (Pierce, Rockford, IL) for immunoblotting detection of AR.

### Measurement of AR Degradation

LNCaP cells were treated with cycloheximide (CHX, Sigma-Aldrich, St Louis, MO) to block new protein synthesis in the absence or presence of tanshinones for 3, 6 and 12 h, and then the cellular AR abundance was measured as previously described (37,38). In addition, MG 132 (Sigma-Aldrich, St Louis, MO) was also included to investigate the involvement of 26S proteasomal-dependent degradation in the AR degradation induced by tanshinones as previously described (38). The band intensities were quantified with Quantity One software (Bio-Rad Laboratories, Hercules, CA).

### Fluorescence Polarization AR Competitor Binding Assay

The potential interaction of tanshinones with the hormone-binding pocket of AR-LBD was evaluated using the PolarScreen™ Androgen Receptor Competitor Assay Green Kit according to the technical manual (Invitrogen, Carlsbad, CA). Both wild type AR-LBD and mutant T877A (found in LNCaP cells) AR-LBD (Invitrogen, Carlsbad, CA) were tested. The fluorescence polarization was read three times by a TECAN microplate reader (TECAN, Switzerland). Mibolerone and Casodex were included in the assays as positive controls.

### *In Vivo* Xenograft Study

The *in vivo* study was approved by Kyunghee University Animal Care Committee and conducted in that institution. Male athymic Balb/C nude mice at 8–10 weeks of age (NARA Biotech, Seoul, Korea) were subcutaneously inoculated with $3\times10^6$ LNCaP cells into the right flanks. One group of mice (designated as Normal) was not inoculated with cancer cells. After 2 weeks of inoculation, fifteen tumor-bearing mice were randomly separated into three groups and once daily received vehicle only (corn oil), TIIA (25 mg/kg) or racemic Casodex (25 mg/kg) through oral gavage, respectively. The mouse body weight and tumor size were measured every 3 days. The tumor volume was calculated based on the formula $V = L \times W^2 \times 0.52$. After 28 days of treatment, the mice were euthanized to collect blood and xenograft tumors. The serum was used for ELISA assay of circulating PSA. Each xenograft tumor was separated into two parts: one part was lysed for ELISA assay of intra-tumor PSA, and the other part was processed for histopathological analysis. TUNEL assay of apoptotic signal was done using the Apo-Direct™ Kit (Calbiochem, San Diego, CA) as previously described (39). Immunohistochemistry (IHC) staining of AR and CD34 (Millipore, Billerica, MA) were done as previously described (39,40). The percentage of positive-stained cells was counted based on ten high power microscopic fields from each sample.

### Statistical Analysis

ANOVA were applied over data sets with multiple dose/time variables to detect overall differences. Two-tailed student *t*-test was used to determine the statistical significance between the specific groups.

## RESULTS

### Tanshinones Inhibited PCa Cell Growth *In Vitro* with a Preference for Androgen-Dependent Stage

To define the concentration ranges of TI, TIIA and CT in terms of growth inhibitory responses on LNCaP cells (super-functional T877A-mutant AR), we seeded these cells into 6-well plates and exposed them to increasing concentrations of each compound in complete growth medium (when the cells were ~30–40% confluence) for 3 days and estimated the number of adherent cells by crystal violet staining of the cellular protein (Fig. 1b). The relative inhibitory potency order was established as TIIA>CT>TI. To more sensitively compare the growth inhibitory effects of these tanshinones, we next used a colony growth assay in which cells were seeded very sparsely and allowed to grow for multiple cell division cycles, first with TIIA in LNCaP cells (Fig. 1c). We chose lower exposure concentrations of TIIA than the acute exposure levels previously reported by us and others (14,24) due to the cumulative growth inhibitory effects of the chronic growth assay. Because LNCaP cells did not attach tightly to plastic surface of the culture plates, we further compared a one-time treatment protocol without changing medium with one involving changing medium with fresh TIIA every 3 days and evaluated overall colony cell number after 14 days (Fig. 1c). As is apparent, changing medium caused a loss of cells in DMSO-treated controls (20% *vs.* one-time protocol). Regardless of the treatment protocols, TIIA effectively inhibited colony cell growth with $IC_{50}$ in the sub-micro-molar range.

Using the one-time treatment protocol (without medium change), we compared TI, TIIA and CT with Casodex in androgen-dependent LNCaP cells (Fig. 1d). Casodex caused a minor stimulatory action, peaking at ~30 nM (+16%) and an inhibitory effect at 1 μM and higher concentrations ($IC_{50}$~2 μM, Fig. 1d). Tanshinone IIA and CT inhibited LNCaP cell growth with similar potency ($IC_{50}$~0.06 μM) and were more potent than TI ($IC_{50}$~0.5 μM) (Fig. 1d). All three tanshinones were more potent than Casodex, and lacked the minor stimulatory effect of Casodex on LNCaP cell growth.

Following the same protocol, we evaluated colony growth responses of castration-resistant LNCaP derivative line C4-2 (T877A mutant AR+) (Fig. 1e), castration-resistant 22Rv1 cells (mutant AR+) (Fig. 1f) and androgen-independent DU 145 cells (AR-) (Fig. 1g) in complete media for 9~14 days (depending on the doubling time of each cell line). Prostate cancer cells that were AR(+) but castration-resistant such as C4-2 and 22Rv1 cells also responded to tanshinones, but with less sensitivity than LNCaP cells ($IC_{50}$ for TIIA, CT and TI in C4-2 cells~0.2, 0.5 and 1.5 μM, respectively; in 22Rv1 cells ~0.5, 1.5 and 0.6 μM, respectively, Fig. 1e–f). The AR(-) androgen-independent DU 145 cells showed $IC_{50}$ for TIIA, CT and TI ~0.15, 0.2 and 0.6 μM, respectively (Fig. 1g). The difference in the inhibitory potency among the cell lines was more evident when the exposure concentration of 0.1 μM was compared (Fig. 1d–g, marked by a dash vertical line). Taken together, the data indicated that tanshinones, especially TIIA and CT, inhibited the growth of PCa cells, with androgen-dependent LNCaP cells being more sensitive.

### Tanshinones Lacked Agonist Action of Casodex on Basal AR Signaling

To further contrast tanshinones from Casodex under androgen-deprived condition, we maintained LNCaP cells in phenol-red free growth medium plus 5% charcoal-stripped serum with the indicated treatments for 9 days. As expected, the stable dihydrotestosterone analog mibolerone caused a concentration-dependent stimulation of PSA secretion as a read-out for AR signaling and cell growth (Fig. 2a) from 10 to 30 pM, plateauing through 50 pM. Such data are in agreement with previous reports that physiological level of androgen plays a stimulatory role but supra-physiological level of androgen could be cytotoxic to LNCaP cells (41). Casodex in the range of 5–20 μM exerted a modest but clear stimulation of PSA expression in a concentration-dependent manner, consistent with our previous reports and others (31,38), in spite of a trend for declining LNCaP cell number. However, none of the three tanshinones stimulated PSA expression or cell growth (Fig. 2a) within the tested concentration ranges. Acute exposure (24 h) experiments showed that TIIA was devoid of the stimulating activity of Casodex on cellular and secreted PSA expression under androgen-deprived condition (Fig. 2b), whereas both TIIA and Casodex suppressed PSA expression under the androgen-stimulated condition. Taken together, these data suggested that tanshinones might lack the agonist effect on basal AR signaling in the LNCaP model in contrast to the Casodex used here.

### Tanshinones Induced Cell Cycle Arrest and Apoptosis in LNCaP Cells

We next examined the cellular consequences of acute tanshinone exposure (24 h) on cell cycle distribution in LNCaP cells. The acute treatment regimens necessitated the use of higher exposure concentrations than the colony growth assay to elicit measurable cell cycle and biochemical changes within the shorter time frame of exposure. As shown in Fig. 2c, TI in the range of 2.5–10 μM, TIIA and CT at 2.5 and 5 μM, respectively, induced $G_1$ arrest as indicated by the accumulation of $G_0/G_1$ phase cells (77.3%, 81.5% and 77.6% for TI, 86.4% and 83% for TIIA and 84.0% and 81% for CT *vs.* 70.4% for control, respectively) and

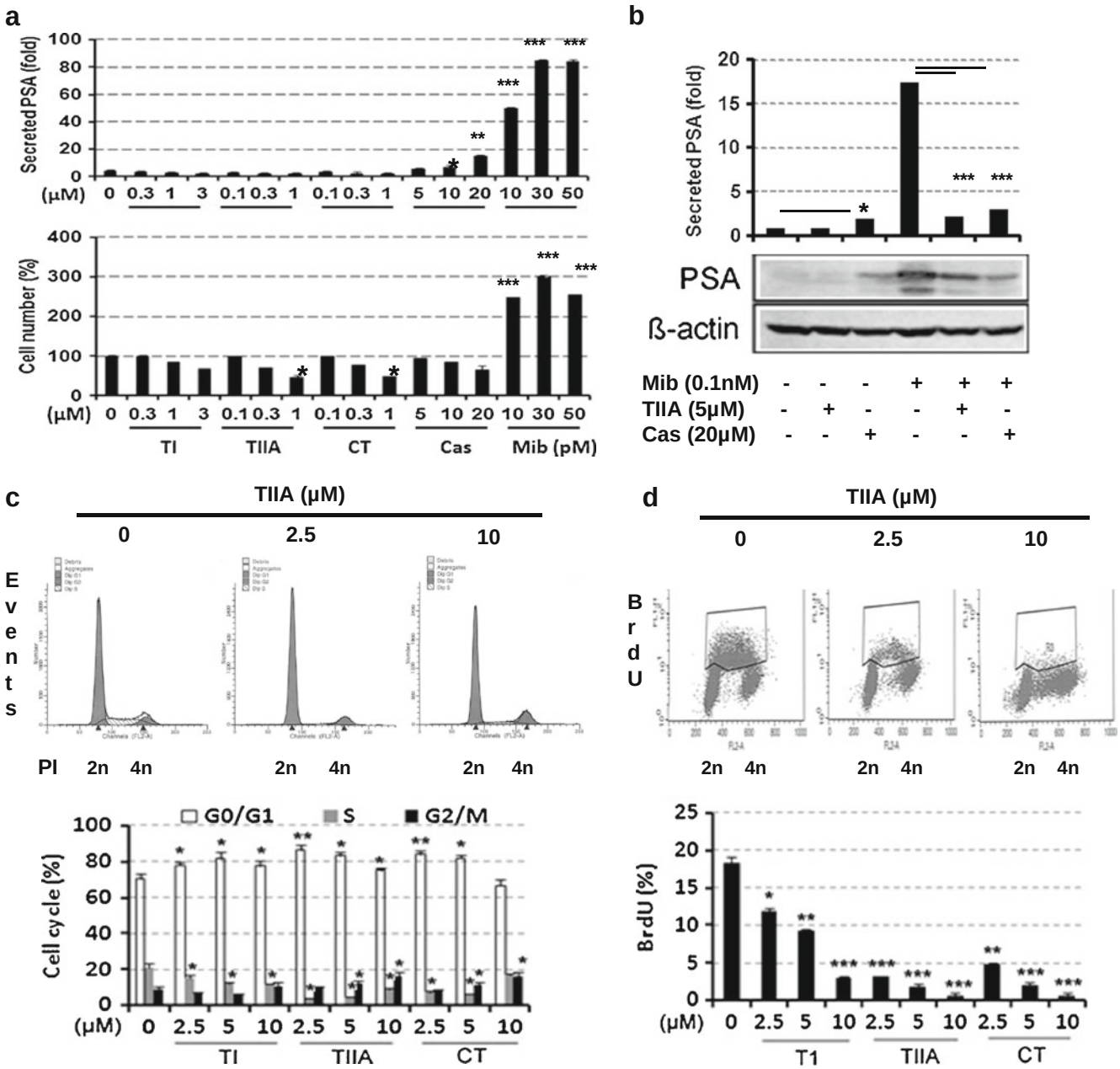



**Fig. 2** (**a**) Comparison of effects of tanshinones with Casodex on PSA secretion and colony growth in LNCaP cells under androgen-deprived condition. Mibolerone (Mib) was included as an agonist control. LNCaP cells were seeded in phenol-red free growth medium supplemented with 5% charcoal-stripped serum (CSS) and the indicated treatments for 9 days without medium change, and then the medium was collected for ELISA assay of secreted PSA and cells were stained with crystal violet to estimate the number. (**b**) Comparison of acute (24 h) effects of tanshinone IIA with Casodex on PSA secretion and cellular expression in LNCaP cells under androgen-deprived condition. LNCaP cells were seeded in complete medium and then depleted of androgen in phenol-red free growth medium supplemented with 5% CSS for 48 h. Treatments as outlined in the label were carried out for 24 h. (**c**–**d**) Cell cycle distribution (**c**) and BrdU incorporation rate (**d**) in LNCaP cells after exposure to tanshinones in complete medium for 24 h. Representative flow cytometric results were shown for TIIA. Data as Mean±SEM, $n=3$. Statistical significance from vehicle control, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

reduction of S phase cells (15.0%, 12.0% and 11.8% for TI, 3.9% and 4% for TIIA and 7.6% and 5.5% for CT *vs.* 20.6% for control, respectively). Higher concentration (10 μM) of TIIA and CT led to $G_2$/M arrest indicated by more cells accumulating in the $G_2$/M phases (15.9% for TIIA and 16.3% for CT *vs.* 9.0% for control, respectively). Measurement of the BrdU incorporation rate after 24 h of exposure indicated the enrichment of a subpopulation of S-phase arrested cells with DNA replication shut off at the higher exposure concentrations (Fig. 2d).

We examined cleaved poly (ADP)-ribose polymerase (cPARP), a well-known biomarker of caspase-mediated

apoptosis, by immunoblotting for evidence of death execution. Exposure concentration of 10–20 μM TIIA and CT dramatically induced caspase-mediated apoptosis as indicated by increased cPARP after 48 h of exposure (Fig. 3a). Taken together, the data suggested that tanshinones induced potent $G_1$ arrest at lower micromolar concentrations, with additional S and $G_2$/M arrests and/or apoptosis at higher concentrations.

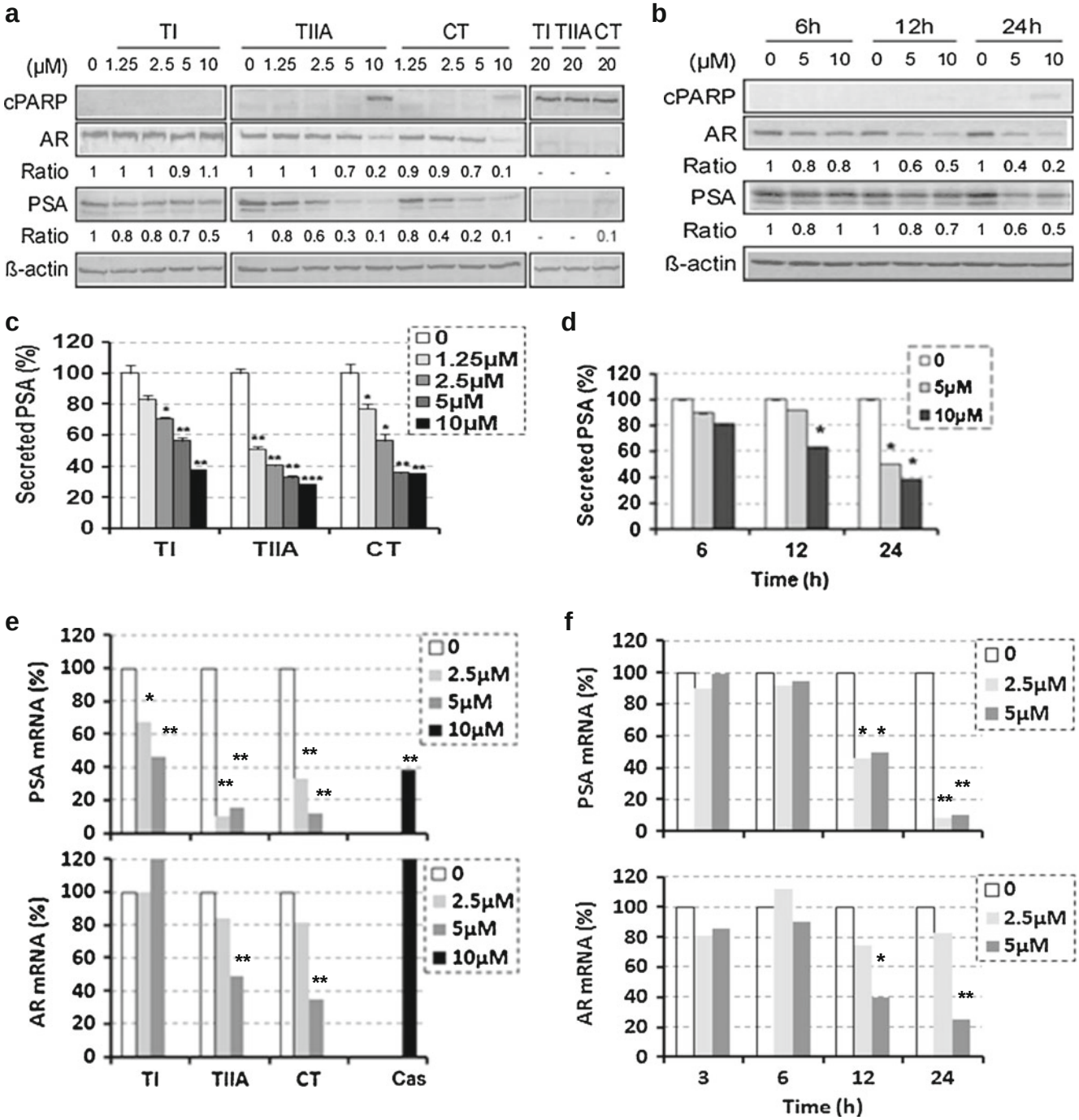



**Fig. 3** (**a**, **c**) Concentration-dependent effect of tanshinones on cellular cPARP, AR and PSA (**a**) and secreted PSA (**c**) in LNCaP cells after 48 h of exposure. (**b**, **d**) Temporal effect of TIIA on cellular cPARP, AR and PSA (**b**) and secreted PSA (**d**) in LNCaP cells. The secreted PSA was normalized to the cell number estimated by cellular protein yield, and then converted to the% of control. (**e**, **f**) Effects of tanshinones on AR and PSA mRNA steady state level in LNCaP cells. (**e**) Real-time PCR detection of concentration-dependent effect of tanshinones on steady state levels of PSA (*top graph*) and AR (*bottom graph*) mRNA in LNCaP cells after 24 h of exposure. Casodex at 10 $\mu$M was included as a comparison. (**f**) Real-time PCR detection of time course change of PSA (*top graph*) and AR (bottom graph) mRNA levels in LNCaP cells exposed to TIIA for indicated time. Each column represents Mean±SEM, $n$=2–4 replicates from two experiments. *$P<0.05$, ** $P<0.01$.

### Tanshinones Decreased AR and PSA in LNCaP Cells

To interrogate the relationship between the inhibitory action of tanshinones against cell growth and AR signaling, we exposed LNCaP cells to acute exposure protocols in the next sets of experiments. Exposure to TIIA and CT for 48 h resulted in a decline of cellular PSA detected by immunoblotting in a concentration-dependent manner, as did the AR protein level (Fig. 3a). Notably, the concentrations of tanshinones to induce apoptosis as indicated by cPARP were clearly higher than those to suppress PSA expression in LNCaP cells (Fig. 3a). For example, for TIIA and CT, we detected increased cPARP at 10 μM, whereas we detected decreased cellular PSA at 1.25–2.5 μM. Furthermore, in time course experiments, decreased cellular AR and PSA were detected by 12 h, whereas a weak cPARP signal was detected by 24 h (Fig. 3b). The secreted PSA, normalized against cell number, in the growth medium was decreased in a concentration-dependent manner by each tanshinone (Fig. 3c), in particular TIIA. Time course of TIIA-induced reduction of secreted PSA (Fig. 3d) followed the cellular PSA changes. These temporal changes were consistent with AR signaling suppression ahead of apoptosis.

### Tanshinones Decreased AR mRNA and PSA mRNA in LNCaP Cells

To determine whether tanshinones affected AR signaling at the transcriptional level, we measured the steady state mRNA level of AR and PSA in LNCaP cells exposed to tanshinones for 24 h (Fig. 3e). Casodex at 10 μM decreased PSA mRNA by ~60% but did not affect AR mRNA level, as expected. TIIA and CT were more potent than Casodex to decrease PSA mRNA, with the added reduction of AR mRNA. Time course experiments (Fig. 3f) showed the reduction of PSA and AR mRNA in a timeframe congruent with their protein changes (Fig. 3b). Taken together, these data suggested that tanshinones decreased AR mRNA abundance, distinct from the lack of such action by Casodex.

### Tanshinones Inhibited AR Nuclear Translocation and Caused AR Proteosomal Degradation

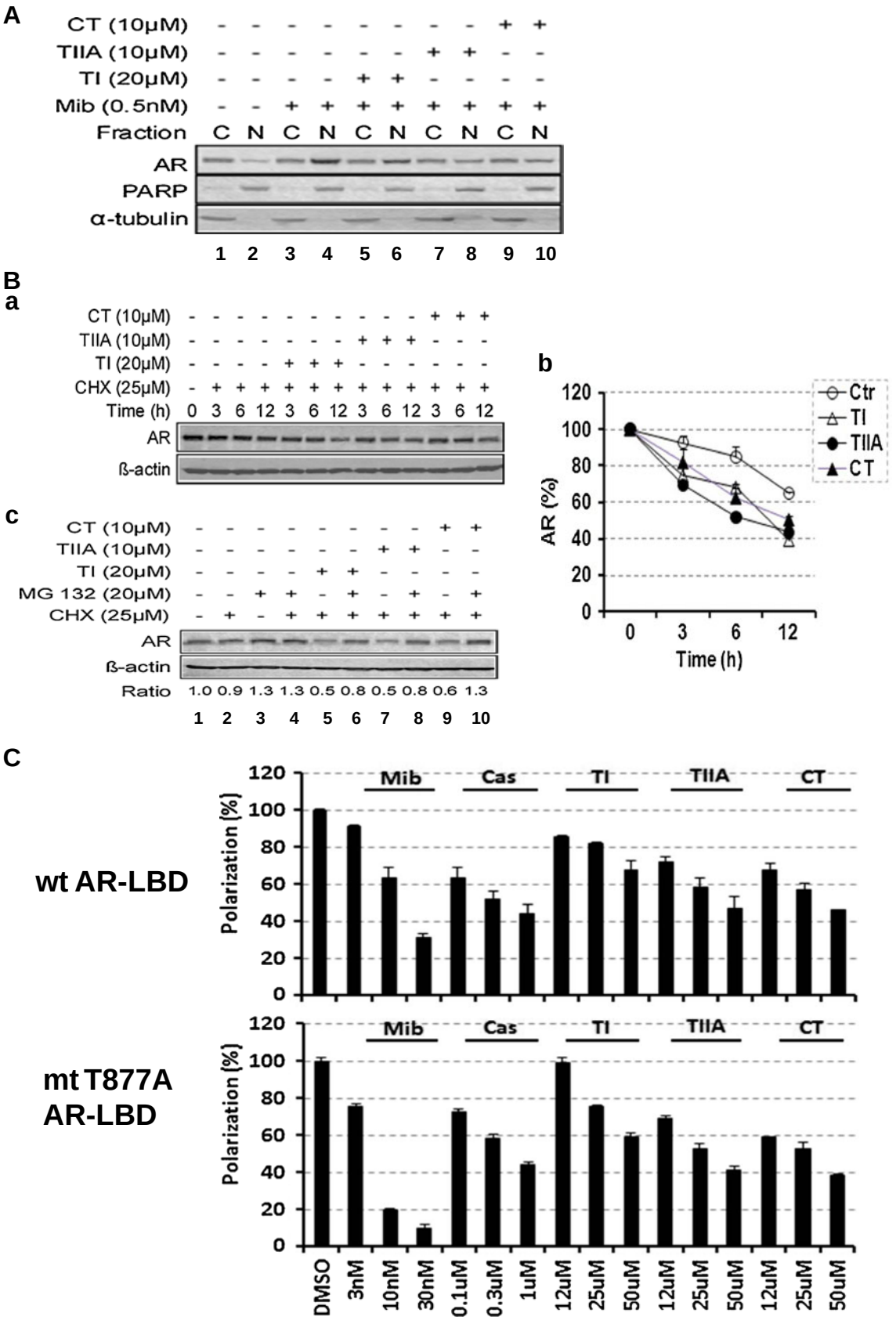
As a nuclear transcriptional factor, the activity of AR closely relates to its translocation from the cytoplasm to the nucleus after ligand binding activation. To examine whether tanshinones interfered with this translocation process, we maintained LNCaP cells in phenol-red free medium supplemented with 5% charcoal-stripped serum for 48 h to deplete nuclear AR. After 1 h of pretreatment with tanshinones *vs.* with solvent vehicle DMSO, we stimulated the cells with mibolerone for another 2 h, and then examined the AR protein abundance in the cytoplasm *vs.* nuclear fractions. Figure 4a showed that most of AR resided in the cytoplasm in androgen-deprived cells (lane 1 *vs.* 2). Mibolerone rapidly stimulated AR translocation from cytoplasm to nucleus (lane 4 *vs.* 2). TIIA (lane 7 and 8) and CT (lane 9 and 10) significantly retarded the mibolerone-induced AR nuclear translocation, whereas TI (lane 5 and 6) was less potent even at twice the exposure concentration.

Cytosolic retention of un-liganded AR is known to promote AR protein degradation. We tested this by blocking new protein synthesis with cycloheximide and followed the AR level in TI-, TIIA- and CT-treated LNCaP cells by immunoblotting. The data suggested an accelerated degradation of AR after exposure to tanshinones (Fig. 4b). To test the involvement of the well-known 26S proteasomal pathway in tanshinone-induced AR protein degradation, we co-treated LNCaP cells with cycloheximide and MG132 (an inhibitor of 26S proteasomal pathway). MG132 attenuated AR protein degradation induced by TI (lane 5 and 6), TIIA (lane 7 and 8) and CT (lane 9 and 10), supporting the promotion of AR protein degradation via 26S proteasomal pathway as another potential mechanism for tanshinones to decrease AR availability and signaling.

### Tanshinones Bind AR Ligand-Binding Domain (AR-LBD) with Low Affinity

We carried out binding study in the test tube for evidence of a potential direct interaction of tanshinones with both wild type AR-LBD and mutant T877A AR-LBD using PolarScreen Androgen Receptor Competitor Assay Kit (Invitrogen, Carlsbad, CA). Mibolerone and Casodex were used as positive controls. If the tested compound competitively interacts with the hormone-binding pocket of AR-LBD, the fluorescent androgen ligand (tracer) will be released into the solution. The free tracer causes a lower fluorescence polarization value than that of the protein-bound tracer. Non-competing compounds will not release the tracer ligand from the AR-LBD, so

**Fig. 4** (**A**) Effect of tanshinones (pre-treatment for 1 h) on AR nuclear translocation stimulated by mibolerone (Mib) for 2 h in androgen-deprived LNCaP cells. PARP and α-tubulin were used as markers of the nuclear and cytosolic fractions, respectively. C: cytosolic fraction, N: nuclear fraction. (**B**) Effect of tanshinones on AR degradation. (**a**) Immunoblotting detection of AR protein in LNCaP cells treated with tanshinones after new protein synthesis was blocked by cycloheximide (CHX). (**b**) The intensity of AR band was normalized to that of β-actin, and then plotted relative to the initial control. The data were from two experiments, each run in duplicate. Each point represents Mean ± SEM. (**c**) Attenuation of tanshinone-induced AR degradation by the co-treatment with a 26S proteasomal inhibitor MG-132 for 6 h in the presence of CHX. (**C**) Fluorescence polarization AR competitor binding assay of tanshinones interacting with the wild type (wt) AR-LBD and mutant (mt) T877A AR-LBD. Mibolerone (Mib) and Casodex were included as positive controls. Each column represents Mean ± SEM, $n=3$.


A
CT (10μM)
TIIA (10μM)
TI (20μM)
Mib (0.5nM)
Fraction C N C N C N C N C N
AR
PARP
α-tubulin
1 2 3 4 5 6 7 8 9 10
B
a
CT (10μM)
TIIA (10μM)
TI (20μM)
CHX (25μM)
Time (h) 0 3 6 12 3 6 12 3 6 12 3 6 12
AR
ß-actin
b
Ctr
TI
TIIA
CT
AR (%)
Time (h)
c
CT (10μM)
TIIA (10μM)
TI (20μM)
MG 132 (20μM)
CHX (25μM)
AR
ß-actin
Ratio 1.0 0.9 1.3 1.3 0.5 0.8 0.5 0.8 0.6 1.3
1 2 3 4 5 6 7 8 9 10
C
wt AR-LBD
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Mib
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DMSO
3nM
10nM
30nM
0.1uM
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1uM
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50uM
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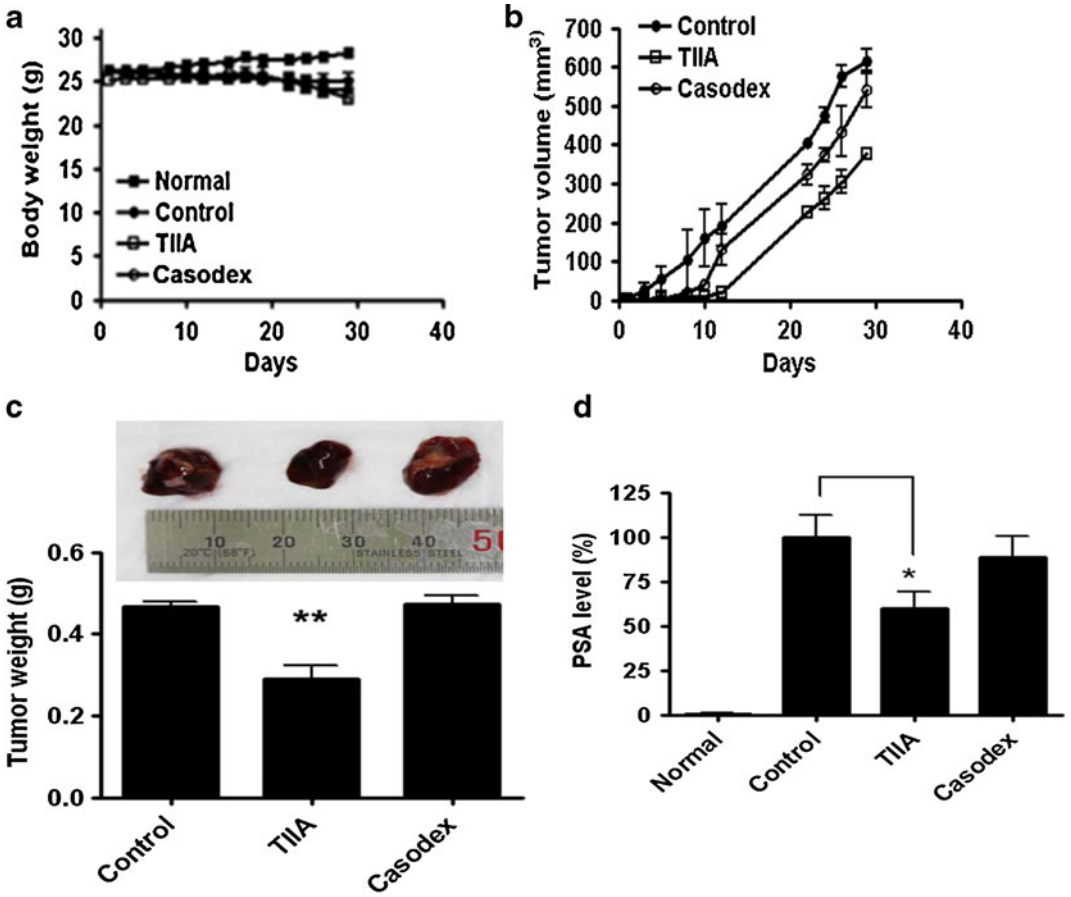



**Fig. 5** Suppression of LNCaP xenograft growth by oral TIIA in athymic nude mice. TIIA or Casodex was dissolved in corn oil and was orally administrated (once daily) to each mouse (25 mg/kg) using a feeding needle starting 2 weeks after tumor inoculation until termination. One group of mice which was not inoculated with cancer cells served as "Normal". (**a**) Mouse body weight. (**b**) LNCaP xenograft volume. (**c**) Final tumor weight and a representative tumor from each group at necropsy. (**d**) Serum PSA in mice of different groups. Mean±SEM, $n=5$. Statistical significance from vehicle control, * $P<0.05$, ** $P<0.01$.

the fluorescence polarization value remains high. As expected, mibolerone competed with the tracer binding to the wild type AR-LBD with high potency ($IC_{50}$~15 nM) (Fig. 4c) and its binding to the mutant T877A AR-LBD was even tighter ($IC_{50}$~5 nM) (Fig. 4c). Casodex showed less interfering potency ($IC_{50}$ ~300–400 nM) than that of mibolerone, regardless of the mutational status of AR.

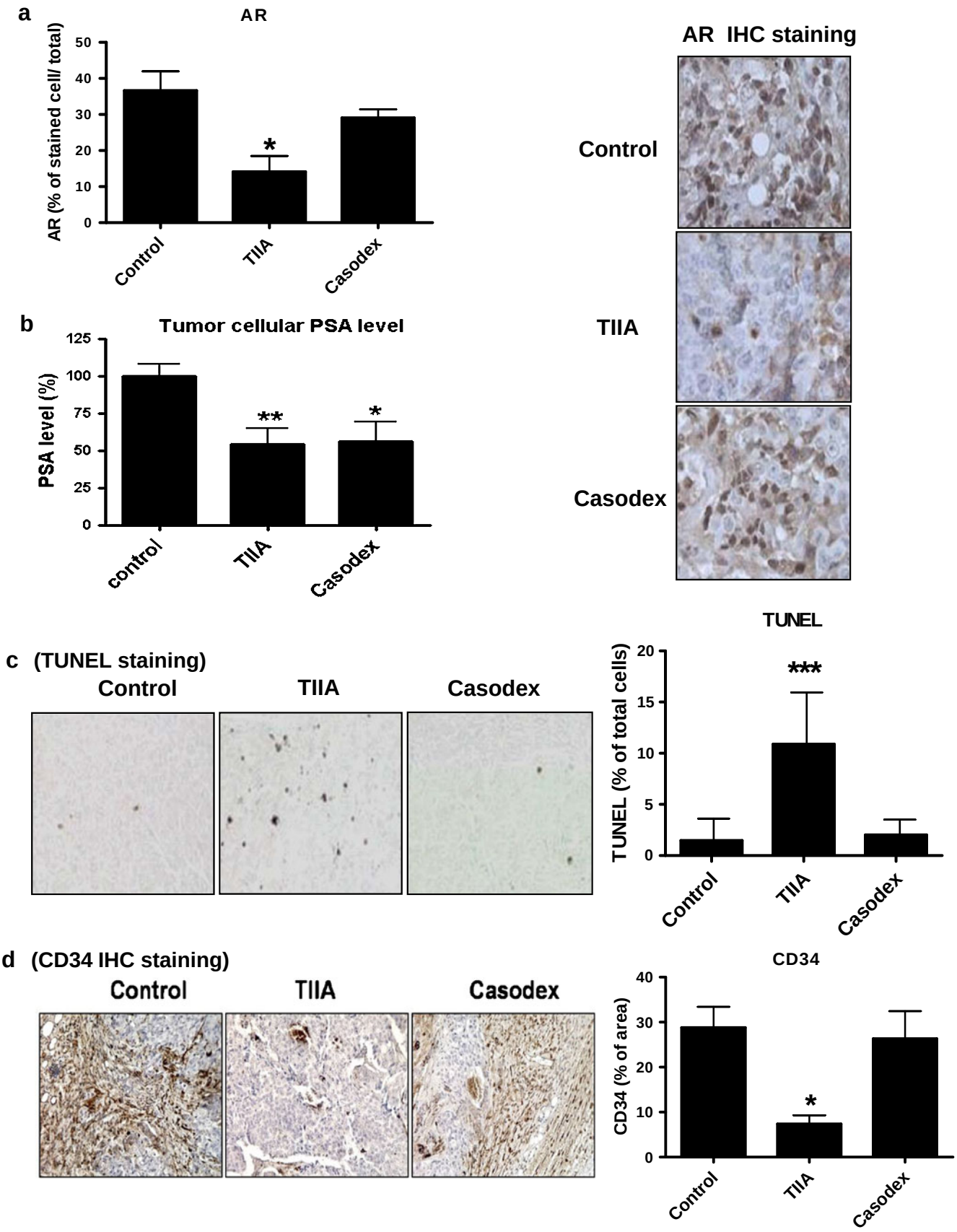
Tanshinones decreased the fluorescence polarization values, each in a concentration-dependent manner, for wild type AR-LBD and mutant T877A AR-LBD, with the tracer-ligand displacing potency in the order of CT≈TIIA>TI, in general agreement with their growth and PSA/AR suppression effects in LNCaP cells. However, their ligand displacing potency was much weaker than those of mibolerone and Casodex, inconsistent with tanshinones as direct AR-LBD binding antagonists.

Overall, the data indicated that tanshinones inhibited AR nuclear translocation (within 2 h) and down-regulated cellular AR abundance, by inducing proteosomal degradation and down-regulating its mRNA level. These biological attributes are distinct from those of Casodex which has been documented to bind AR and stimulate AR nuclear translocation and stabilize AR in the LNCaP model (42).

## TIIA Suppressed LNCaP Xenograft Growth in Athymic Nude Mice

To validate the *in vivo* anti-PCa and anti-AR activities of tanshinones, we selected TIIA, owing to its greater *in vitro* potency than the other two tested forms, for efficacy evaluation in LNCaP xenografts alongside of Casodex. Figure 5a showed that oral TIIA did not significantly affect mouse body weight, which was similar to that of the Casodex group. TIIA inhibited LNCaP xenograft growth but the

**Fig. 6** Biomarker changes in TIIA-treated LNCaP xenografts. (**a**) IHC staining of AR in LNCaP xenografts. A representative image from each group was shown and the summary data were plotted in graph. (**b**) ELISA detection of intra-tumor PSA in LNCaP xenografts. (**c**, **d**) IHC staining of TUNEL (**c**) and CD34 (**d**) in LNCaP xenografts. A representative image from each group was shown (*left image*) and the summary data were plotted (*right graph*). Each column represents Mean±SEM, $n=5$. Statistical significance from vehicle control, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.


a
AR
AR (% of stained cell/ total)
50
40
30
20
10
0
*
Control
TIIA
Casodex
AR IHC staining
Control
TIIA
Casodex
b
Tumor cellular PSA level
PSA level (%)
125
100
75
50
25
0
**
*
control
TIIA
Casodex
c (TUNEL staining)
Control
TIIA
Casodex
TUNEL
TUNEL (% of total cells)
20
15
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Control
TIIA
Casodex
d (CD34 IHC staining)
Control
TIIA
Casodex
CD34
CD34 (% of area)
40
30
20
10
0
*
Control
TIIA
Casodex

same dose of racemic Casodex did not (Fig. 5b–c). The serum PSA level reflected the impact of TIIA or Casodex on tumor growth (Fig. 5d).

TIIA significantly decreased intra-tumor protein abundance of AR (Fig. 6a) and PSA (Fig. 6b) in LNCaP xenografts, whereas Casodex merely decreased PSA (Fig. 6b) without affecting AR abundance (Fig. 6a). In contrast to Casodex, TIIA increased TUNEL apoptosis signal (Fig. 6c) and decreased CD34 positive vessel count (Fig. 6d), indicating an induction of apoptosis and potential inhibition of neo-angiogenesis. Taken together, the data suggested that oral administration of TIIA exerted anti-AR signaling as well as potential pro-apoptosis and anti-angiogenesis properties in LNCaP xenografts.

## DISCUSSION

Development of resistance to current androgen deprivation therapies and evolution of anti-androgen withdrawal syndrome in PCa patients call for the discovery of novel agents that inhibit AR signaling pathway with distinct actions from the existing antagonist drugs such as Casodex and flutamide. Our data presented above on selected tanshinones suggest these compounds have novel anti-AR signaling activities that contribute to their overall potent anti-proliferative effects on androgen-dependent PCa cells, in addition to their multi-targeting actions against AR-independent PCa cells and a broad range of other cancer cell types through non-AR related pathways.

With respect to AR signaling, we detected a minor stimulatory activity of racemic Casodex on LNCaP cell growth in the complete growth medium (Fig. 1d) and on PSA expression in the androgen-deprived growth medium (Fig. 2a–b), consistent with previous reports of ours and others (11,31,38). In contrast, tanshinones lacked detectable agonist activity under the same experimental conditions (Figs. 1c–d and 2a–b). Tanshinones were more potent (10–30 folds) at inhibiting cellular growth than racemic Casodex for LNCaP cells (lower $IC_{50}$ values) (Fig. 1d). Tanshinones exerted a rapid (within 2 h) interference with the androgen-stimulated AR nuclear translocation (Fig. 4a) and led to increased AR proteosomal degradation (Fig. 4b) as well as decreased AR mRNA level (Fig. 3e). These actions were distinct from those of Casodex which has been known to increase AR stability and promote AR dimerization and nuclear translocation, though eventually leading to an inhibitory action through the formation of inactive transcriptional complexes on the androgen response element sites of the targeting promoters in the LNCaP model (42). The suppression of LNCaP xenograft growth (Fig. 5) and the observed reduction of AR and PSA expression in the TIIA-treated LNCaP xenografts (Fig. 6a–b) indicated delivery of this tanshinone and/or its metabolites to the target tissue *in vivo*.

While this manuscript was under revision, a report was e-published describing the selective AR-targeting action of a new tanshinone PTS33 (43), which is a more water-soluble derivative of CT. The data showed that PTS33 selectively inhibited AR activities, but did not repress the activities of other nuclear receptors such as ERα, GR, and PR. At a low concentration, 2 μM of PTS33 effectively suppressed the growth of AR(+) PCa cells, and had little effect on AR(-) PCa cells. PTS33 modulated AR transactivation activity and suppressed AR target genes PSA, TMPRSS2 and TMEPA1 expression in AR(+) PCa cells. The study suggested the inhibition of AR function might be in association with down-regulation of AR protein expression, interference of N-C terminal interaction and co-regulators recruitment. This independent report extends mechanistic insights into the AR targeting action of tanshinones that we reported in the current paper.

Aside from the AR signaling inhibitory effects identified in the present study, other molecular targets may also account for the anti-PCa activities of tanshinones. For example, the growth of AR(-) DU145 cells was inhibited by these tanshinones, albeit requiring higher levels of exposure than for LNCaP cells (Fig. 1g), supporting AR-independent mechanisms for higher concentrations of tanshinones. Consistent with this point, several recent studies have shown inactivation of aurora kinase A by TI, TIIA and CT (24), STAT3 by CT (44), and activation of JNK and p38 MAPK by CT (45). Our finding of altered angiogenesis indicators in LNCaP xenografts (Fig. 6d) suggested a potential involvement of endothelial cellular targets as well.

In summary, our data point to tanshinones as potential novel anti-AR signaling agents without agonist activity. They are more potent than Casodex (racemic) both *in vitro* and *in vivo*, and with distinct mechanisms of action, particularly through inhibiting AR nuclear translocation, reducing AR protein abundance and mRNA and promoting AR proteosomal degradation. The AR targeting actions reported here and by Xu, *et al.* (43) and the additional cellular and molecular anti-cancer effects reported in multiple cancer cell lines support the merit of developing tanshinones and their derivatives as new drug leads for prostate cancer chemoprevention and therapy.

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## REFERENCES


1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277–300.
2. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004;10:33–9.
3. Kungand HJ, Evans CP. Oncogenic activation of androgen receptor. Urol Oncol. 2009;27:48–52.
4. Li TH, Zhao H, Peng Y, Beliakoff J, Brooks JD, Sun Z. A promoting role of androgen receptor in androgen-sensitive and -insensitive prostate cancer cells. Nucleic Acids Res. 2007;35:2767–76.
5. Petrylak DP. The current role of chemotherapy in metastatic hormone-refractory prostate cancer. Urology. 2005;65:3–7. discussion 7–8 (2005).
6. Scherand HI, Sawyers CL. Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. J Clin Oncol. 2005;23:8253–61.
7. Heinleinand CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev. 2004;25:276–308.
8. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, Wongvipat J, Smith-Jones PM, Yoo D, Kwon A, Wasielewska T, Welsbie D, Chen CD, Higano CS, Beer TM, Hung DT, Scher HI, Jung ME, Sawyers CL. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science (New York, NY). 2009;324:787–90.
9. Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkerd E, Messiou C, Molife LR, Maier G, Thompson E, Olmos D, Sinha R, Lee G, Dowsett M, Kaye SB, Dearnaley D, Kheoh T, Molina A, de Bono JS. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol. 2009;27:3742–8.
10. Kawata H, Ishikura N, Watanabe M, Nishimoto A, Tsunenari T, Aoki Y. Prolonged treatment with bicalutamide induces androgen receptor overexpression and androgen hypersensitivity. Prostate. 2010;70:745–54.
11. Culig Z, Hoffmann J, Erdel M, Eder IE, Hobisch A, Hittmair A, Bartsch G, Utermann G, Schneider MR, Parczyk K, Klocker H. Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. Br J Cancer. 1999;81:242–51.
12. Hara T, Miyazaki J, Araki H, Yamaoka M, Kanzaki N, Kusaka M, Miyamoto M. Novel mutations of androgen receptor: a possible mechanism of bicalutamide withdrawal syndrome. Cancer Res. 2003;63:149–53.
13. Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol. 2005;45:1345–59.
14. Won SH, Lee HJ, Jeong SJ, Lee EO, Jung DB, Shin JM, Kwon TR, Yun SM, Lee MH, Choi SH, Lu J, Kim SH. Tanshinone IIA induces mitochondria dependent apoptosis in prostate cancer cells in association with an inhibition of phosphoinositide 3-kinase/AKT pathway. Biol Pharm Bull. 2010;33:1828–34.
15. Lee CY, Sher HF, Chen HW, Liu CC, Chen CH, Lin CS, Yang PC, Tsay HS, Chen JJ. Anticancer effects of tanshinone I in human non-small cell lung cancer. Mol Cancer Ther. 2008;7:3527–38.
16. Sarker SD, Nahar L. Natural medicine: the genus Angelica. Curr Med Chem. 2004;11:1479–500.
17. Mosaddik MA. In vitro cytotoxicity of tanshinones isolated from Salvia miltiorrhiza Bunge against P388 lymphocytic leukemia cells. Phytomedicine. 2003;10:682–5.
18. Nizamutdinova IT, Lee GW, Son KH, Jeon SJ, Kang SS, Kim YS, Lee JH, Seo HG, Chang KC, Kim HJ. Tanshinone I effectively induces apoptosis in estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. Int J Oncol. 2008;33:485–91.
19. Su CC, Lin YH. Tanshinone IIA inhibits human breast cancer cells through increased Bax to Bcl-xL ratios. Int J Mol Med. 2008;22:357–61.
20. Wang J, Wang X, Jiang S, Yuan S, Lin P, Zhang J, Lu Y, Wang Q, Xiong Z, Wu Y, Ren J, Yang H. Growth inhibition and induction of apoptosis and differentiation of tanshinone IIA in human glioma cells. J Neurooncol. 2007;82:11–21.
21. Liu JJ, Zhang Y, Lin DJ, Xiao RZ. Tanshinone IIA inhibits leukemia THP-1 cell growth by induction of apoptosis. Oncol Rep. 2009;21:1075–81.
22. Sung HJ, Choi SM, Yoon Y, An KS. Tanshinone IIA, an ingredient of Salvia miltiorrhiza BUNGE, induces apoptosis in human leukemia cell lines through the activation of caspase-3. Exp Mol Med. 1999;31:174–8.
23. Lee WY, Chiu LC, Yeung JH. Cytotoxicity of major tanshinones isolated from Danshen (Salvia miltiorrhiza) on HepG2 cells in relation to glutathione perturbation. Food Chem Toxicol. 2008;46:328–38.
24. Gong Y, Li Y, Lu Y, Li L, Abdolmaleky H, Blackburn GL, Zhou JR. Bioactive tanshinones in Salvia miltiorrhiza inhibit the growth of prostate cancer cells in vitro and in mice. Int J Cancer (2010 Sep 16. [Epub ahead of print]).
25. Lu Q, Zhang P, Zhang X, Chen J. Experimental study of the anticancer mechanism of tanshinone IIA against human breast cancer. Int J Mol Med. 2009;24:773–80.
26. Wang X, Wei Y, Yuan S, Liu G, Lu Y, Zhang J, Wang W. Potential anticancer activity of tanshinone IIA against human breast cancer. Int J Cancer. 2005;116:799–807.
27. Wang DB, Liu AS. Tanshinone in the treatment of acne (a primary report of 20 cases (author's transl). Zhongguo yi xue ke xue yuan xue bao Acta Academiae Medicinae Sinicae. 1980;2:187–8.
28. Ju Q, Yin X, Shi J, Xin Y, Kang X, Chen Y, Cui P, Cao Y, Xia L. Effects of cryptotanshinone and tanshinone IIA on proliferation, lipid synthesis and expression of androgen receptor mRNA in human sebocytes *in vitro*. Chinese Journal of Dermatology. 2005;38(2).
29. Gao Y, Wang L, Tang J. Sex hormone-like activity of tanshinone. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 1980;2:189–91.
30. Cockshott ID. Bicalutamide: clinical pharmacokinetics and metabolism. Clin Pharmacokinet. 2004;43:855–78.
31. Zhang Y, Kim KH, Zhang W, Guo Y, Kim SH, Lu J. Galbanic acid decreases androgen receptor abundance and signaling and induces G(1) arrest in prostate cancer cells. Int J Cancer (2011 Feb 15. [Epub ahead of print]).
32. Zhang Y, Shaik AA, Xing C, Chai Y, Li L, Zhang J, Zhang W, Kim SH, Lu J, Jiang C. A synthetic decursin analog with increased in vivo stability suppresses androgen receptor signaling in vitro and in vivo. Investigational new drugs (2011).
33. Wang Z, Lee HJ, Chai Y, Hu H, Wang L, Zhang Y, Jiang C, Lu J. Persistent P21Cip1 induction mediates G(1) cell cycle arrest by methylseleninic acid in DU145 prostate cancer cells. Curr Cancer Drug Targets. 2010;10:307–18.
34. Wang F, Ezell SJ, Zhang Y, Wang W, Rayburn ER, Nadkarni DH, Murugesan S, Velu SE, Zhang R. FBA-TPQ, a novel marine-derived compound as experimental therapy for prostate cancer. Invest New Drugs. 2010;28:234–41.
35. Cho SD, Jiang C, Malewicz B, Dong Y, Young CY, Kang KS, Lee YS, Ip C, Lu J. Methyl selenium metabolites decrease prostate-specific antigen expression by inducing protein degradation and suppressing androgen-stimulated transcription. Mol Cancer Ther. 2004;3:605–11.
36. Zhang Y, Zhang J, Wang L, Quealy E, Gary BD, Reynolds RC, Piazza GA, Lu J. A novel sulindac derivative lacking cyclooxygenase-inhibitory

activities suppresses carcinogenesis in the transgenic adenocarcinoma of mouse prostate model. Cancer Prev Res (Phila). 2010;3:885–95.
37. Jiang C, Lee HJ, Li GX, Guo J, Malewicz B, Zhao Y, Lee EO, Lee JH, Kim MS, Kim SH, Lu J. Potent antiandrogen and androgen receptor activities of an Angelica gigas-containing herbal formulation: identification of decursin as a novel and active compound with implications for prevention and treatment of prostate cancer. Cancer Res. 2006;66:453–63.
38. Guo J, Jiang C, Wang Z, Lee HJ, Hu H, Malewicz B, Lee HJ, Lee JH, Baek NI, Jeong JH, Kim DK, Kang KS, Kim SH, Lu J. A novel class of pyranocoumarin anti-androgen receptor signaling compounds. Mol Cancer Ther. 2007;6:907–17.
39. Wang L, Bonorden MJ, Li GX, Lee HJ, Hu H, Zhang Y, Liao JD, Cleary MP, Lu J. Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit. Cancer Prev Res (Phila Pa). 2009;2:484–95.
40. Kim KH, Lee HJ, Jeong SJ, Lee EO, Kim HS, Zhang Y, Ryu SY, Lee MH, Lu J, Kim SH. Galbanic acid isolated from ferula assafoetida exerts in vivo anti-tumor activity in association with anti-angiogenesis and anti-proliferation. Pharm Res. 2011;28:597–609.
41. Joly-Pharaboz MO, Ruffion A, Roch A, Michel-Calemard L, Andre J, Chantepie J, Nicolas B, Panaye G. Inhibition of growth and induction of apoptosis by androgens of a variant of LNCaP cell line. J Steroid Biochem Mol Biol. 2000;73:237–49.
42. Masiello D, Cheng S, Bubley GJ, Lu ML, Balk SP. Bicalutamide functions as an androgen receptor antagonist by assembly of a transcriptionally inactive receptor. J Biol Chem. 2002;277:26321–6.
43. Xu D, Lin TH, Zhang C, Tsai YC, Li S, Zhang J, Yin M, Yeh S, Chang C. The selective inhibitory effect of a synthetic tanshinone derivative on prostate cancer cells. The Prostate (2011).
44. Shin DS, Kim HN, Shin KD, Yoon YJ, Kim SJ, Han DC, Kwon BM. Cryptotanshinone inhibits constitutive signal transducer and activator of transcription 3 function through blocking the dimerization in DU145 prostate cancer cells. Cancer Res. 2009;69:193–202.
45. Park IJ, Kim MJ, Park OJ, Park MG, Choe W, Kang I, Kim SS, Ha J. Cryptotanshinone sensitizes DU145 prostate cancer cells to Fas(APO1/CD95)-mediated apoptosis through Bcl-2 and MAPK regulation. Cancer Lett. 2010;298:88–98.