Caffeic Acid Phenethyl Ester Suppresses the Proliferation of Human Prostate Cancer Cells through Inhibition of p70S6K and Akt Signaling Networks

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Abstract

Caffeic acid phenethyl ester (CAPE) is a bioactive component derived from honeybee hive propolis. CAPE has been shown to have antimitogenic, anticarcinogenic, and other beneficial medicinal properties. Many of its effects have been shown to be mediated through its inhibition of NF-κB signaling pathways. We took a systematic approach to uncover the effects of CAPE from hours to days on the signaling networks in human prostate cancer cells. We observed that CAPE dosage dependently suppressed the proliferation of LNCaP, DU-145, and PC-3 human prostate cancer cells. Administration of CAPE by gavage significantly inhibited the tumor growth of LNCaP xenografts in nude mice. Using LNCaP cells as a model system, we examined the effect of CAPE on gene expression, protein signaling, and transcriptional regulatory networks using micro-Western arrays and PCR arrays. We built a model of the impact of CAPE on cell signaling which suggested that it acted through inhibition of Akt-related protein signaling networks. Overexpression of Akt1 or c-Myc, a downstream target of Akt signaling, significantly blocked the antiproliferative effects of CAPE. In summary, our results suggest that CAPE administration may be useful as an adjuvant therapy for prostate and potentially other types of cancers that are driven by the p70S6K and Akt signaling networks. Cancer Prev Res; 5(5): 788–97. ©2012 AACR.

Introduction

Prostate cancer is the most common noncutaneous carcinoma of men in the United States. Androgen ablation therapy is the primary and standard treatment for metastatic prostate cancer. However, most patients with prostate cancer receiving androgen ablation therapy ultimately develop recurrent castration-resistant tumors within 12 to 33 months after treatment with a mean survival of 2 to 3 years. Adjuvant therapies are therefore critically needed for improving the outcome of patients with prostate cancer. PTEN is frequently deleted in prostate cancer, resulting in activation of PI3K/Akt signaling (1). PI3K/Akt signaling plays an important role in survival and progression of prostate cancer cells (1). Akt is a serine/threonine protein kinase regulating a variety of cellular responses, including inhibition of apoptosis and stimulation of cell proliferation. Upregulation of tumor PI3K/Akt activity is associated with a poor clinical outcome of patients with prostate cancer (2). Therefore, small-molecule inhibitors that can suppress PI3K/Akt signaling with minimal side effects are potential candidates for prostate cancer treatment. Caffeic acid phenethyl ester (CAPE) is a bioactive component extracted from honeybee hive propolis. CAPE is a known inhibitor of NF-κB (3) that has strong antioxidant (4) properties. CAPE is marketed over-the-counter as a health food supplement that does not typically have substantial side effects. CAPE has been shown to suppress Akt signaling and cause growth inhibition in human CD4+ T cells (5) and human coronary smooth muscle cells (6). CAPE treatment has been shown to induce apoptosis through activation of p53-regulated Bax (7, 8), c-jun-NH2-kinase (JNK; ref. 7), and p38 mitogen-activated protein kinase (p38 MAPK; ref. 7), as well as through suppression of NF-κB activity (7, 9), and reduction of Bcl-2, cAP-1, cAP-2, and X-linked inhibitor of apoptotic protein (XIAP; refs. 9, 10) expression in several human cancer cell lines. In addition, CAPE has been shown to induce cell-cycle arrest in cancer cells through suppression of cyclin D1 (10, 11), cyclin E (10), and c-Myc expression (12), as well as via induction of the cyclin-dependent kinase inhibitors p21WAF1/CIP1 (10), p27Kip1 (10), and p16INK4A (10). We reasoned that CAPE might be a potentially useful therapeutic for prostate cancer.

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treatment. We thus applied several systems-level approaches to examine the molecular mechanisms by which CAPE inhibited the growth of prostate cancer cells.

Material and Methods

Chemicals

Chemicals were purchased from Sigma.

Cell culture

LNCaP 104-S, DU-145, and PC-3 cells were passaged and maintained as previously described (13–20).

Cell proliferation assay

Relative cell number was analyzed by measuring DNA content of cell lysates with the fluorescent dye Hoechst 33258 (Sigma) as described previously (13, 14, 17–20).

Soft agar colony formation assay

Eight thousand cells were suspended in 0.3% low melting agarose (Lonza) with 10% FBS in Dulbecco’s Modified Eagle’s Media (DMEM) and then layered on top of 3 mL of 0.5% low melting agarose plus 10% FBS in DMEM in 6-cm dishes. Cells were allowed to grow at 37°C with 5% CO₂ for 14 days. The plates were stained with 0.005% crystal violet in 30% ethanol for 6 hours.

Flow cytometric analysis

After 96 hours of culture in the presence of different concentrations of CAPE, cells were processed, cell-cycle profiles were determined by flow cytometric analysis using a BD FACScan flow cytometer (BD Biosciences), and data were analyzed using ModFit LT software (Verity Software House) as described (17–20).

Western blotting analysis

Cells were lysed in SDS lysis buffer (240 mmol/L Tris-acetate, 1% SDS, 1% glycerol, 5 mmol/L EDTA, pH 8.0) with dithiothreitol (DTT), protease inhibitors, and a cocktail of phosphatase inhibitors. Expression of proteins including Akt1/2/3 (with an antibody that did not discriminate between the proteins), Akt1, Akt2, Akt3, Skp2, α-tubulin, cyclin A, phospho-Cdk2 (T160), phospho-GSK3α (S21), phospho-GSK3β (S9), GAPDH, phospho-Rb (S807/811), phosphoP38 MAPK (T180/Y182), phospho-Akt (S473), phospho-Akt (T308), and phospho-P90RSK (S380) were detected by antibodies against each protein. Antibodies were from Cell Signaling Technology. Anti-rabbit and anti-mouse IgG secondary antibodies were from Invitrogen and LI-COR BioSciences. p21WAF1/CIP1 antibody was from Santa Cruz Biotechnology and p27Kip1 antibody was from BD Biosciences. Blots were scanned and quantified using a LI-COR Odyssey near-infrared imaging system. α-Tubulin, β-actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as loading controls.

Micro-Western arrays

104-S cells were treated with vehicle (ethanol) or 10 μmol/L CAPE by replacing medium with new medium [DMEM + 8% FBS + 1 nmol/L dihydrotestosterone (DHT)] and the treatment lasted for 0, 30, 60, 120, 240, and 480 minutes. Cells were lysed at the indicated time points, and micro-Western arrays (MWA) were conducted to measure protein expression and modification as previously described (21, 22).

Protein overexpression

Ectopic expression of Akt1 and c-Myc was achieved by infecting LNCaP 104-S cells with pSRα or pBABE retroviruses carrying the cDNA of the indicated proteins, respectively. Antibiotic-resistant (G418 and puromycin) colonies were expanded and screened for increased target protein expression by Western blot analysis. Cells infected with retroviruses carrying empty vectors were used as controls.

PCR arrays

LNCaP 104-S cells were treated with 0 or 10 μmol/L CAPE for 48 hours. Total RNA was isolated with the TRIzol reagent (Invitrogen), and contaminating DNA was removed using DNase I (DNA-free, Ambion). cDNA was synthesized from total RNA using SMARTScribe Reverse Transcriptase (Clontech). Expression of mRNA of selected genes was assayed using precoated 96-well SYBR Green real-time PCR arrays (SABiosciences), including the human Cancer Pathway Finder PCR Array (PAHS-033) and the human PI3K-AKT Signaling Pathway PCR Array (PAHS-058), with RT² qPCR Master Mixes (SABiosciences) and StepOnePlus Real-Time PCR System (Applied Biosystems) according to the manufacturer’s protocol. Experiments were carried out with 4 biologic replicates and 1 to 2 technical replicates per each sample. Five housekeeping genes (B2M, HPRT1m, RPL13A, GAPDH, and ACTB) were included in each array as RNA content controls. Gene expression was determined using the 2ΔΔCt method and fold changes were calculated as the difference in gene expression between the CAPE-treated and control groups. Data were analyzed using the RT² Profiler PCR Array Data Analysis website (http://www.sabiosciences.com/pcr/arrayanalysis.php). Functional annotation enrichment for both up- and downregulated genes after CAPE treatment was conducted using Database for Annotation, Visualization and Integrated Discovery (DAVID) with the entire set of genes on both quantitative real-time PCR (RT-PCR) arrays as background. P values represent a minus log transformation of the geometric mean of all of the enrichment P values (EASE scores) for each annotation term associated with the gene members in the group.

Xenografts in athymic mice

Experiments involving mice were approved by the University of Chicago (Chicago, IL) Institutional Animal Care and Use Committee. Six- to 8-week-old male Balb/c nu/nu mice (NIC Frederick, Bethesda, MD) were injected subcutaneously in both flanks with 1 × 10⁶ LNCaP 104-S cells suspended in 50 μL DMEM mixed with 50 μL of Matrigel (BD Bioscience). CAPE (10 mg/kg/d in 0.2 mL sesame oil) or vehicle (sesame oil) was administered to mice by gavage starting 1 week after cancer cell injection. Tumors were...
measured weekly using the formula: volume = length × width × height × 0.52 (14–16, 20). The CAPE treatment group comprised 10 mice with 17 tumors whereas the vehicle control group comprised 9 mice with 16 tumors. CAPE and vehicle treatment stopped at the sixth week and tumors were allowed to grow for another 2 weeks.

**NF-κB reporter assays**

For measuring NF-κB activity, 3 × 10⁴ cells were seeded per well in 48-well plate for 24 hours, then transfected with phRL-CMV-Renilla luciferase plasmid (1 ng per well), 4 × NF-κB (50 ng per well), and Bluescript SKII + (750 ng per well) using the calcium phosphate co-precipitation method (13). Twenty-four hours after transfection, cells were treated with increasing concentrations of CAPE for 24 hours and were lysed in 50 μL passive lysis buffer (Promega). Luciferase activity was measured using a Dual-Luciferase kit (Promega) in a Monolight luminometer (BD Biosciences).

**Results and Discussion**

**CAPE treatment suppressed the proliferation of human prostate cancer cells**

The proliferation of LNCaP 104-S, DU-145, and PC-3 human prostate cancer cells was dosage dependently suppressed by CAPE treatment (Fig. 1A) with an IC₅₀ values of 0.68, 9.54, and 18.65 μmol/L, respectively. We thus chose...
LNCaP 104-S cells for further investigation. The growth-inhibitory effect of CAPE was evident within 24 hours of treatment but the suppressive effect accumulated over time (Fig. 1B). A colony formation assay revealed that treatment of 1 μmol/L CAPE reduced colony formation by 50% whereas treatment with 10 μmol/L CAPE completely blocked the formation of LNCaP 104-S colonies (Fig. 1C), confirming the anti-cancer effect of CAPE in prostate cancer cells.

CAPE caused retardation of xenograft growth and G1 cell-cycle arrest in LNCaP cells

Administration of CAPE by gavage (10 mg/kg body weight per day) for 6 weeks resulted in a 50% reduction of tumor volume ($P = 0.0008$; Fig. 1D). CAPE treatment did not affect the body weight of the mice (data not shown), suggesting that the dosage used was not overtly toxic. Termination of CAPE treatment at the sixth week resulted in the return of 104-S tumors growth to a rate comparable with the control group, indicating that CAPE inhibited tumor growth but did not completely eliminate the xenografted tumor cells. Following 96 hours of treatment with at least 3 μmol/L CAPE, flow cytometric analysis revealed a significant reduction of cells in the S-phase and an increase of cells in either the G0 or G1 population phases (Fig. 1E). We did not observe any sub-G1 population. Annexin V staining (data not shown) confirmed that apoptosis was not induced in 104-S cells by CAPE, indicating that CAPE treatment inhibited LNCaP cell proliferation through induction of either a G1 cell-cycle arrest or a return of cells to a quiescent G0 state.

CAPE treatment affected expression of genes related to Akt signaling and cell-cycle regulation

As PI3K/Akt signaling plays an important role in survival and progression of prostate cancer cells (1, 23), we used PCR arrays to quantitatively monitor the expression of many genes with known involvement in cancer- and Akt-related signaling pathways (Supplementary Table S1). Of the 162 total genes examined, 20 (12.3%) were significantly downregulated and 23 (14.2%) significantly upregulated after CAPE treatment ($P < 0.05$; Fig. 2A). In the Akt signaling array, 95% (19 of 20) of the genes affected by CAPE treatment were significantly downregulated, indicating that CAPE suppressed Akt-related transcriptional activity. Many genes important for cell proliferation were downregulated following CAPE treatment, including BRCA1, Akt1, Akt2, Myc, E2F1, TERT, HRAS, RPS6KB1, and RHOA (Fig. 2A). Simultaneously, the cell-cycle-inhibitory gene p21 (CDKN1A) was upregulated. SERPINB5 (also known as maspin) expression was upregulated by 5-fold. Maspin is a tumor suppressor protein and a matrix metalloproteinase (MMP) inhibitor–like protein whose expression has been linked to repression of prostate cancer metastasis (24). Recently, expression of maspin by stable transfection was shown to decrease the survival of lung cancer tumors in nude mice through reduction of Akt phosphorylation (25). We tested the $-2,000$ bp to $+200$ bp regions of all
43 differentially expressed genes for enrichment of any known transcription factor–binding sites via position weight matrices from Jasper and Transfac 6.0 using TFM-Explorer (26) relative to all mRNAs on the RT-PCR arrays ($P < 0.05$). Enriched binding site motifs included OCT1, MEF2, TCF11, MEIS1A/HOXA9, BRN2, and VMAF. Many previous studies have reported that CAPE suppresses NF-κB activity in the time frame of minutes to hours (3). However, we observed that treatment of LNCaP cells with 10 μmol/L CAPE for 48 hours caused upregulation of genes that were significantly enriched for known NF-κB targets according to a database of known NF-κB targets ($P = 9 \times 10^{-6}$, permutation analysis), http://bioinfo.lifl.fr/NF-KB/ (27), and Transcriptional Regulatory Element Database (28).

Our analysis showed that 16 of 23 (69.6%) upregulated genes were known NF-κB target genes versus 46 of 162 (28.4%) NF-κB target genes examined in the analysis. Upregulated NF-κB target genes included the proinflammatory cytokines (IFNB1, TNF, and IL8), the matrix metalloproteases (MMP1, MMP2, and MMP9), regulator of morphogenesis and metastasis (TWIST; ref. 29), and the cell-cycle inhibitor p21Cip (Fig. 2A). To further address whether CAPE treatment caused activation of NF-κB, we examined the transcriptional output of an NF-κB–driven luciferase reporter construct transfected into LNCaP 104-S cells (Fig. 2B). Treatment with 1 to 10 μmol/L CAPE did not cause a significant change in luciferase activity. Therefore, CAPE appeared to cause the upregulation of many known NF-κB target genes without activation of NF-κB activity.

**CAPE squelched early phosphorylation events following replenishment of cell medium in LNCaP 104-S cells**

To determine the global short-term effects of CAPE on the changes in cellular signaling proteins related to Akt signaling, cell stress, and cell proliferation in LNCaP 104-S cells, we conducted MWA analysis (21, 22, 30, 31) of 28 protein abundances and modifications in LNCaP 104-S cells at 6 time points from 0 to 480 minutes following replenishment of serum- and androgen-containing medium in the absence or presence of 10 μmol/L CAPE (Fig. 3; Supplementary Fig. S1). In the absence of CAPE, replenishment of growth medium resulted in the phosphorylation of cAMP-responsive element-binding protein CREB (S133) and GSK3β (S9) within 30 minutes (Fig. 3). The phosphorylation of CREB returned to baseline levels at 4 hours consistent with the known burst-attenuation kinetics of cAMP (32). In the time between 1 and 4 hours, the phosphorylation of serum and glucocorticoid-inducible kinase SGK (S78), FoxO3a (T32), GSK3α (S21), Akt (S473), and Akt (T308) were also subtly increased, consistent with a role for NF-κB in driving a proliferative response following replenishment of growth medium (Fig. 4). Following CAPE treatment, almost all of the previous changes in protein abundance and modification were lost or substantially delayed (Fig. 3). Not only did we observe an absence of upregulation of p-CREB, p-GSK3β, p65/RelA, and other proteins, we observed subtle decreases in the levels of Stat1, p-Stat1, p65/RelA, and p-SGK following CAPE treatment. S-phase kinase–associated protein 2 (Skp2), a member of the F-box protein family which is responsible for ubiquitination and downregulation of p27$^{kip1}$ cell-cycle inhibitors, was subtly downregulated. These data helped to provide mechanistic understanding for...
the observed cell-cycle arrest. Our results suggested that CAPE treatment altered cell signaling networks in a manner that resulted in a lack of cell proliferation in response to the availability of nutrition. Notably, the effect of CAPE on cell signaling was observed at 30 minutes, the earliest measured time point. The identity of the targets effected at 30 minutes included p-CREB (S133) and p-GSK3β (S9), suggesting that CAPE acted at early time points through inhibition of mTOR and p70S6K followed by a downstream effect on Akt. Akt (S473) is a known substrate of mTORC2. The kinetics of Akt (S473) and p70S6K (T421/S424) phosphorylation were similarly delayed by CAPE treatment (Fig. 3). We constructed a model of protein influences based on the literature and based on the timing of changes that we observed (Fig. 4).

Figure 4. Putative model of CAPE describing the cause–effect relationship of the activity of 18 measured and 27 inferred proteins in LNCaP 104-S cells following addition of fresh serum-containing growth medium in the absence (A) or presence (B) of 10 μmol/L CAPE in the time between 0 minutes and 4 hours. Protein nodes measured in the MWA experiment were arranged in the figure along with unmeasured protein nodes based on relationships suggested from the literature. Nodes with measured or inferred upregulation in activity are colored pink, those with a measured or inferred downregulation in activity are colored green, those with no measured or inferred change are colored white, and those proteins downstream of opposing influences are colored half green and half pink. Measured nodes have a deep black outline whereas inferred (unmeasured) nodes have no black outline (in the case of colored nodes) or a thin black outline (in the case of noncolored nodes). Protein nodes are depicted in small ovals whereas cellular behaviors are depicted in large boxes.

CAPE caused a reduction in abundance and activity of cell-cycle–promoting proteins and Akt signaling pathway proteins

We used traditional Western blotting to further examine the effects of CAPE on cells following longer term treatment. Following 96-hour treatment, changes in protein expression and modification induced by CAPE were similar to those observed following short-term treatment but effects on the Akt pathway were more evident. GSK3 phosphorylation was reduced and the abundance and modification of components of the cell cycle changed in a manner consistent with a reduction in cell proliferation. About 10 μmol/L CAPE treatment caused a modest reduction in the phosphorylation of GSK3α (S21) and a major (70%) reduction in the phosphorylation of GSK3β (S9; Fig. 5). The reduction in phosphorylation of GSK3β would be expected to result in an increase in its activity (34), which would result in phosphorylation and targeted destruction of cell-cycle stimulatory proteins such as β-catenin, cyclin D1, cyclin E, and c-Myc (35–37). The abundance of c-Myc and Skp2 decreased by several fold (Fig. 5; Supplementary Fig. S2), whereas the abundance of cell-cycle–inhibitory proteins p27Kip1 and p21Cip1 (38) increased by 2-fold. Furthermore, CAPE treatment
resulted in a dramatic reduction in the abundance of cyclin A and phospho-Cdk2 (T160). Cyclin A binds to Cdk2 and is required for cells to progress through the S-phase. The cyclin A/Cdk2 complex is inhibited by cell-cycle inhibitor p21Cip. Phosphorylation of Cdk2 on T160 is necessary for its activation (39) and is required for traversing the G1–S checkpoint through phosphorylation of pRb. Consistent with inactivation of Cdk2, phospho-pRb (S807/S811) was decreased by 70%. CAPE treatment led to increased levels of phospho-c-Raf (S259; Fig. 5). Phosphorylation of c-Raf on S259 and S621 creates 14–3-3 binding sites which are thought to maintain it in an autoinhibited state (40). Downregulation of cyclin A, c-Myc, Skp2, phosphorylated Rb, and Cdk2 coupled with increased phospho-c-Raf (S259), p21Cip, and p27kip abundance likely contributed to the sustained induction of G1 cell-cycle arrest following 96-hour CAPE treatment.

However, the long-term signaling data highlighted changes in Akt and p38 MAPK signaling that were not apparent following short-term treatment. While CAPE did not affect total Akt levels, it caused a 20% reduction in phospho-Akt (S473) and an 80% reduction in phospho-Akt (T308) levels (Fig. 5). While S473 is a known target of mTOR, T308 is a known target of PDK1. CAPE treatment led to increased Akt2 levels and caused a complete loss of Akt3 protein expression. CAPE treatment caused an increase in phospho-p38 MAPK (T180/Y182) and phospho-p90 ribosomal S6 kinase (p90RSK; S380). p38 MAPK activity can negatively regulate cell-cycle progression both at the G1–S and the G2–M transitions, in part, through phosphorylation and stabilization of cell-cycle inhibitor p21Cip1 (41–43). Increased phosphorylation of p90RSK (S380) could indicate either increased extracellular signal-regulated kinase or JNK activity. However, given the role of JNK in responding to cell stress, we speculate that increased JNK activity was likely responsible for this phosphorylation.

Overexpression of Akt1 or c-Myc blocked the suppressive effect of CAPE on cell proliferation

To confirm that CAPE suppressed cell proliferation through suppression of Akt, we overexpressed Akt1 and c-Myc in LNCaP 104-S. Akt overexpression would be predicted to repress GSK3β activity resulting in increased β-catenin and NFAT activity whereas overexpression of c-Myc would be expected to have less effect as many parallel pathways would still be downregulated in response to increased GSK3β activity (44). As expected, the antiproliferative effect of CAPE was significantly reduced in 104-S cells transfected with Akt1. c-Myc overexpression was not as effective as Akt1 in rescuing the antiproliferative effect.
of CAPE (Fig. 6A). Somewhat surprisingly, overexpression of c-Myc caused a complete loss in Akt3 expression (Fig. 6B; Supplementary Fig. S3). Similarly, Akt1 overexpression caused a dramatic reduction in c-Myc expression (Fig. 6B; Supplementary Fig. S3), indicating that the expression of Akt isoforms and c-Myc proteins were coordinately regulated and that each Akt protein isoform was differentially regulated in LNCaP cells.

Finally, we sought to determine the effect of CAPE on the proliferation of normal prostate cells. We found that RWPE-1 cells, a model of normal prostate cells, were more resistant than LNCaP 104-S cells to the antiproliferative effects of CAPE. The IC50 value of RWPE-1 cells (4.83 μmol/L) was 8-fold higher than that of 104-S cells (Fig. 6C). CAPE treatment of RWPE-1 cells for 96 hours led to substantial reduction in Akt (S473) levels but only a modest reduction in Akt (T308) and GSK3β (S9; Fig. 6D). While RWPE-1 is a model for normal prostate epithelial cells, it has been transfected with a single copy of the human papilloma virus 18 (HPV-18; ATCC CRL-11609). It is not clear whether the HPV-18 virus renders the cells more or less vulnerable to CAPE treatment. However, as normal prostate epithelial cells usually do not proliferate, we believe that normal prostate epithelial cells in patients will be more resistant to CAPE treatment, especially given that the growth of xenograft-derived prostate tumors was inhibited by CAPE without an apparent drop in body weight or appetite of the mice harboring the tumors (data not shown).

CAPE has been shown to inhibit RANK ligand (RANKL)-induced osteoclastogenesis of precursor cells of the monocyte/macrophage lineage when added in the early stages of development (45). This effect was shown to occur concurrently with suppression of NF-κB and NFAT transcriptional activity consistent with previous findings of Marquez and colleagues (46). Very recent work has shown that Akt and GSK3β signaling are required for RANKL-induced activation of NFATc1 during osteoclast differentiation (47). Taken together with our own data, these results are consistent with a model whereby CAPE blocks the activity of p70S6K, and/or other upstream kinases such as mTOR, resulting in reduced GSK3β phosphorylation and increased GSK3β activity. Following short-term CAPE treatment, these changes may result in phosphorylation, nuclear export, and decreased transcriptional activity of NFATc1. However, following long-term treatment, our results showed dramatic downregulation of Akt concurrent with upregulation in p38 kinase phosphorylation and inferred activity. The p38 activity has been shown to be necessary for induction of NFAT in response to RANKL (48). Our data support a model whereby long-term CAPE...
treatment leads to suppressed Akt activity and increased p38 activity which may induce NFAT activity leading to the upregulation of many NF-kB target genes (Fig. 2). Both NF-kB and NFAT family proteins bind DNA through Rel homology domains and our data indicated that transcription from an NF-kB reporter was not upregulated. Our results provided systems-level insight into the molecular mechanism of the antiproliferative effect of CAPE in prostate cancer cells. As the achievable concentration of CAPE in human serum is around 5.0 μg/mL (17 μmol/L; ref. 49) and our study indicated that CAPE treatment at 10 μmol/L could effectively suppress different prostate cancer cell lines (Fig. 1A), we believe that CAPE is a promising adjuvant therapeutic candidate for prostate cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interests were disclosed.

Authors’ Contributions
Conception and design: C.-P. Chuu, M.F. Ciaccio, R.A. Hiipakka, S. Liao, R.B. Jones
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