Suppression of growth and invasive behavior of human prostate cancer cells by ProstaCaid™: Mechanism of activity

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Abstract. Since the use of dietary supplements as alternative treatments or adjuvant therapies in cancer treatment is growing, a scientific verification of their biological activity and the detailed mechanisms of their action are necessary for the acceptance of dietary supplements in conventional cancer treatments. In the present study we have evaluated the anti-cancer effects of dietary supplement ProstaCaid™ (PC) which contains mycelium from medicinal mushrooms (*Ganoderma lucidum, Coriolus versicolor, Phellinus linteus*), saw palmetto berry, pomegranate, pumpkin seed, green tea [40% epigallocatechin-3-gallate (EGCG)], Japanese knotweed (50% resveratrol), extracts of turmeric root (BCM-95®), grape skin, pygeum bark, sarsaparilla root, Scutellaria barbata, eleuthero root, Job's tears, astragalus root, skullcap, dandelion, coptis root, broccoli, and stinging nettle, with purified vitamin C, vitamin D3, selenium, quercetin, citrus bioflavonoid complex, β sitosterolzinc, lycopene, α lipoic acid, boron, berberine and 3.3'-diinodolymethane (DIM). We show that PC treatment resulted in the inhibition of cell proliferation of the highly invasive human hormone refractory (independent) PC-3 prostate cancer cells in a dose- and time-dependent manner with IC_{50} 56.0, 45.6 and 39.0 μg/ml for 24, 48 and 72 h, respectively. DNA-microarray analysis demonstrated that PC inhibits proliferation through the modulation of expression of *CCND1*, *CDK4*, *CDKN1A*, *E2F1*, *MAPK6* and *PCNA* genes. In addition, PC also suppresses metastatic behavior of PC-3 by the inhibition of cell adhesion, cell migration and cell invasion, which was associated with the down-regulation of expression of *CAV1*, *IGF2*, *NR2F1*, and *PLAU* genes and suppressed secretion of the urokinase plasminogen activator (uPA) from PC-3 cells. In conclusion, the dietary supplement PC is a promising natural complex with the potency to inhibit invasive human prostate cancer.

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Introduction

Prostate cancer is one of the leading causes of cancer-related death in American men due to its unpredictable hormonal independence and highly metastatic nature (1). Prostate cancers usually progress from androgen-dependent to androgen-independent phenotype with highly metastatic properties (2-4). Thus, the metastasis of prostate cancer remains the primary issue in improving prostate cancer patient survival. Moreover, hormone ablation therapy and chemotherapy for advanced stage prostate cancer seem not to offer more benefit in improving patient survival rate (5,6). Therefore, there is an urgent need for the identification of new therapies with anti-cancer effects in highly metastatic prostate cancers. Recent epidemiologic and experimental studies show that natural agents have potential chemopreventive and chemotherapeutic action for prostate cancer. Natural herbal and phytochemical agents are being recognized as an alternative therapy of prostate cancer patients (7,8).

ProstaCaid (PC) is a dietary supplement consisting of a 33-ingredient comprehensive polyherbal and nutrient preparation which inhibits aberrant cell proliferation and induces apoptosis in androgen dependent and independent human and mouse prostate cancer cell lines (9). PC contains mycelium from medicinal mushrooms (*Ganoderma lucidum*, *Coriolus versicolor* and *Phellinus linteus*), which separately demonstrated anti-cancer properties (7,10-12). *Ganoderma lucidum* (*G. lucidum*) (Ling Zhi, Reishi) is a popular medicinal mushroom used as a traditional medicine in China, Korea and Japan for more than 2,000 years to prevent or treat different diseases, including cancer (13,14). The anti-cancer properties of *G. lucidum* have been attributed to the polysaccharides, which are responsible for the modulation of the immune system, or triterpenes, which demonstrate cytotoxic activity against a variety of cancer cells including breast, prostate, lung, colon, sarcoma, hepatoma and leukemia cells (13-15). *G. lucidum* has been shown to inhibit proliferation by cell cycle arrest at the G2/M phase and induced apoptosis in human prostate cancer cells by down-regulation of transcription factors NF-κB (16), resulting in modulating the expression of NF-κB-regulated Bcl-2 and Bcl-xl. *G. lucidum*

has also demonstrated anti-invasive and anti-angiogenic properties which were mediated by the suppression of secretion of plasminogen activator (uPA), vascular endothelial growth factor (VEGF), and transforming growth factor-β1 (TGF-β1) from prostate cancer cells, respectively (17,18). *Coriolus versicolor* (*C. versicolor*) (Yunzhi) is a medicinal mushroom traditionally used in Asia to treat cancers as well as improve immunomodulatory activities (19). *C. versicolor* contains biologically active structurally different protein-bound polysaccharide-K (PSK, Krestin) and polysaccharopeptide (PSP), which have been shown to inhibit the proliferation of various cancer cells including prostate cancer cells (12,20,21). In addition to the anti-cancer properties, extracts of *C. versicolor* demonstrated strong immunomodulatory effects such as elevated IL-2, natural killer cell activity and T-cell proliferation (11,22). *In vivo* studies showed that oral administration of *C. versicolor* extract or PSP to nude mice significantly suppressed the growth of inoculated prostate cancer cells (12,20,23,24). Although the detailed mechanisms of action of *C. versicolor* on the growth of cancer cells remains to be addressed, recent studies indicate that PSK and PSP of *C. versicolor* caused cell cycle arrest at the G0/G1 phase, induced apoptosis, and inhibited metastasis of prostate cancer cells (11,12,23,24). *Phellinus linteus* (*P. linteus*) was mainly used in Asian countries for the treatment of various human malignancies including prostate cancer (7,25). Although the major biologically active components in *P. linteus* are polysaccharides (25), *P. linteus* also contains a polysaccharide-protein complex (PPC) which stimulated the tumoricidal activities of macrophages and natural killer (NK) cells, and induced the proliferation of B cells *in vitro* (26). In addition, *P. linteus* inhibits growth and induces apoptosis of invasive prostate cancer cells *in vitro* (7,10,27,28), and sensitizes advanced prostate cancer cells to apoptosis in a xenograft model of prostate cancer (10).

In addition, some of the natural compounds in PC demonstrated a direct effect on prostate cancer cells. For example, resveratrol is a natural polyphenol present in various plants which have demonstrated anti-inflammatory, anti-oxidant, anti-invasive, and cardioprotective properties (29-32). Previous studies showed that resveratrol inhibited growth and increased apoptosis in prostate cancer cells (33,34) and a dimethyl ester derivative of resveratrol (Pterostibene) also inhibited MMP-9 and α methylacyl-CoA recemase of prostate cancer cells, two metastatic markers for the invasion and metastasis of prostate cancer cells (35). Vitamin D3 possesses anti-proliferative, anti-invasive, anti-migration, anti-metastasis, and anti-angiogenesis effect on prostate cancer cells (36,37), which are mediated through the arrest of cell cycle and the down-regulation of expression of caveolin and inhibition of MMP-9 activity (38,39). Epigallocatechin-3-gallate (EGCG), a major polyphenolic component in the green tea, induced cell cycle arrest and apoptosis in androgen-dependent and -independent human prostate cancer cell lines (40-43). Moreover, EGCG inhibited MMP-2 and MMP-9 via suppression of activation of mitogen-activated protein kinase (MAPK) and also inhibited inflammation-triggered MMP-2 activation and invasion in a murine TRAMP model of prostate cancer (44,45). The molecular mechanisms responsible for the anti-invasive activity of EGCG were associated with down-regulation of activation of c-Jun and NF-κB signaling (43,45).

In the present study, we evaluated anti-proliferative and anti-invasive properties of a dietary supplement PC on highly invasive human hormone refractory (independent) prostate cancer cells PC-3. Here, we show that PC inhibits PC-3 proliferation and modulates expression of prostate cancer-related biomarker genes. In addition, PC also suppresses invasive behavior of PC-3 cells by the inhibition of cell adhesion, migration and invasion. Our results demonstrate a novel mechanism of action of PC in the inhibition of growth and invasive behavior of prostate cancer cells.

Materials and methods

Cell culture and reagents. The human prostate cancer cell line PC-3 was obtained from ATCC (Manassas, VA, USA). PC-3 cells were maintained in DMEM/F-12 medium containing penicillin (50 U/ml), streptomycin (50 U/ml), and 10% fetal bovine serum (FBS). Medium and supplements came from Invitrogen (Grand Island, NY, USA). FBS was obtained from Hyclone (Logan, UT, USA). ProstaCaid (PC) a 33-ingredient comprehensive poly-herbal and nutrition preparation containing the following active weight components: *Curcuma longa* root extract complex with enhanced bioavailability (BCM-95®) 20%, quercetin 15%, *Coriolus versicolor*, *Ganoderma lucidum*, *Phellinus linteus* mushroom mycelium blend 10% [*Astragalus membranaceus* root extract (5:1), *Coix lacryma-jobi* seed extract (5:1), *Coptis japonica* rhizome extract (10:1), *Eleutherococcus senticosus* root extract (5:1), *Scutellaria baicalensis* root extract (5:1), *Scutellaria barbata* root extract (10:1), *Similax glabrae* extract (5:1), *Taraxacum officinale* herb (5:1)] herbal blend 9%, *Urtica dioica* herb extract (5:1) 6%, β sitostererol 6%, *Serenoa repens* berry 5%, *Brassica oleracea* var. italic herb extract (22:1) 4%, *Punica granatum* fruit (40% Ellagic acid) 4%, *Vitis vinifera* fruit skin extract (10:1) 4%, Vitamin C 4%, α lipoic acid 3%, 3.3'-diinodlylmethane (DIM) 3%, *Cucurbita pepo* seed 2%, *Prunus africana* bark extract (4:1) 2%, *Camellia sinensis* herb extract (40% EGCG; 95% phenols; 70% catechens) 1.5%, lycopene 0.6%, Zinc 0.4%, Vitamin D3 0.2%, resveratrol 0.2%, berberine 0.1%, boron 0.06%, selenium 0.004%, was supplied by the EcoNugenics, Inc. (Santa Rosa, CA, USA). PC stock solution was prepared by dissolving PC in dimethyl-sulphoxide (DMSO) at a concentration of 25 mg/ml and stored at 4˚C.

Cell proliferation. Cell proliferation was determined by the tetrazolium salt method (MTT method), according to the manufacturer's instructions (Promega, Madison, WI, USA). Briefly, PC-3 cells were cultured in a 96-well plate and treated with PC (0-80 μg/ml) for 24, 48 and 72 h. At the end of the incubation period, the cells were harvested and absorption was determined with an ELISA plate reader at 570 nm, as previously described (46). Data points represent mean ± SD in the representative experiment of triplicate determinations. Similar results were obtained in two independent experiments.

DNA microarrays. PC-3 cells were treated with PC (0-80 μg/ml) for 24 h and total RNA isolated with RNAeasy (Qiagen, Valencia, CA). This RNA was used for the evaluation of prostate cancer genes with Oligo GEArray® Human Prostate Cancer Biomarkers Microarray according to the manufacturer's protocol

(SABiosciences, Frederick, MD, USA), as previously described (47). The fold change of gene expression was determined by GEArray expression® analysis suite (SABiosciences).

Cell adhesion, migration and invasion assays. Cell adhesion was performed with Cytomatrix Adhesion Strips coated with human fibronectin (Chemicon International, Temecula, CA, USA). Briefly, PC-3 cells were treated with PC (0-80 μg/ml) for 24 h, harvested, and counted. Cell adhesion was determined after 1.5 h of incubation at 37˚C (46). Cell migration of PC-3 cells treated with PC (0-80 μg/ml) was assessed in Transwell chambers in the Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM/F12) medium containing 10% fetal bovine serum (FBS) (46). Invasion of PC-3 cells treated with PC (0-80 μg/ml) was assessed in Transwell chambers coated with 100 μl of Matrigel™ (BD Biosciences, Bedford, MA, USA) diluted 1:3 with DMEM/F12, after 24 h of incubation (46).

uPA secretion. DMEM/F12 media from PC-3 cells treated with PC (0-80 μg/ml) for 24 h were collected and concentrated, and the secretion of uPA was detected by Western blot analysis with anti-uPA antibody (Oncogene Research Products, Cambridge, MA, USA), as described (46). Quantification of uPA secretion was performed by measuring optical densities of autoradiograms with HP-Scanjet 550c and analyzed by UN-SCAN-IT software (Silk Scientific, Orem, UT, USA).

Reverse transcription-polymerase chain reaction (RT-PCR). PC-3 cells were treated with different concentrations of PC (0-80 μg/ml) for 24 h. The total RNA from PC-3 cells was isolated by RNeasy® mini kit (Qiagen, Valencia, CA, USA) according to instruction of manufacture. RT-PCR was performed as previously described (48). Briefly, PCR for CDK4, CNKN1A and E2F1 was run for 30 cycles at 95˚C for denaturation for 45 sec, 60˚C for annealing for 45 sec and 72˚C for extension for 1 min. PCR for CAV1 was run for 38 cycles at 95˚C for denaturation for 45 sec, 60˚C for annealing for 1 min and 72˚C for extension for 1 min. The primer sequences for CDK4 were 5'-TGGTGAGGGTGGGGTGAGG-3' (sense) and 5'-TGGCCACTGTGGGGATCACG-3' (antisense); the primer sequences for CDKN1A were 5'-CCTGCCCTCATG GCCCCTCT-3' (sense) and 5'-TGGGACCCTCACCCCCA CAG-3' (antisense); the primer sequences for E2F1 were 5'-GGC CGTCCTCCCAGCCTGTT-3' (sense) and 5'-CCCACGCGC ACACATGGACT-3' (antisense); the primer sequences for CAV1 were 5'-CGCCCTCTGCTGCCAGAACC-3' (sense) and 5'-GGCCCGTGGCTGGATGAAAA-3' (antisense); and the primer sequences for β-actin were 5'-ACGAGTCCGGCCC CTCCATC-3' (sense) and 5'-GGGGGCACGAAGGCTCA TCA-3' (antisense). The final RT-PCR products (10 μl) were run on a 1.5% agarose gel containing ethidium bromide and quantified using imager Fluor Chem HD2 (Cell Biosciences, Santa Clara, CA, USA). The results are presented as the ratio of a specific target gene to β-actin.

Statistical analysis. Data are presented as the means ± SD. Statistical comparison between the control group (0 μg/ml of PC) and groups with different PC doses were carried out using one-way analysis of variance (ANOVA). P<0.05 was considered to be significant.

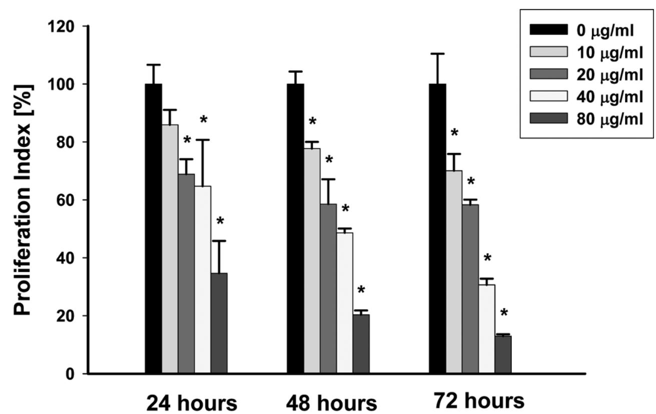

Figure 1. Effect of PC on the growth of prostate cancer cells. PC-3 cells were treated with PC (0-80 μg/ml) for 24, 48 and 72 h. (A) Cell proliferation was determined by MTT assay as described in Materials and methods. Data are the means ± SD of triplicate determinations. Similar results were obtained in at least two additional experiments. *P<0.05.

Results and Discussion

Effects of PC on the growth of the highly invasive prostate cancer cells. Chemopreventive and therapeutic studies in Asia have demonstrated the beneficial effects of herbal supplements upon a variety of diseases including cancer (43). Although chemotherapy and hormone therapy demonstrated initial efficacy for metastatic prostate cancer patients, after the long-term anti-androgen treatment, prostate cancer patients lose their responsiveness to treatment and prostate cancers progress to androgen-independent phenotype with highly metastatic properties (2-4). Moreover, some of these chemotherapeutic drugs have undesirable toxic side effects (43,49). Therefore, there is a significant clinical application in the identification of natural complexes demonstrating anti-proliferative and anti-metastatic properties. As recently demonstrated PC suppresses proliferation of a variety of prostate cancer cells and this effect is associated with cycle arrest at G2/M phase and induction of apoptosis (9). However, the effect of PC on invasive behavior of prostate cancer cells was not previously addressed. First, we evaluated if PC inhibits growth of highly invasive androgen independent PC-3 prostate cancer cells. As seen in Fig. 1, the increased concentration of PC (0-80 μg/ml) markedly suppressed proliferation of PC-3 cells in a dose- and time-dependent manner. The IC_{50} of PC for 24, 48 and 72 h treatment was 56.0, 45.6 and 39.0 μg/ml, respectively. Thus, our results are consistent with the recent report by Yan and Katz (9). Although previous studies with mushroom extracts or isolated components of PC demonstrated anti-proliferative and pro-apoptotic effects in prostate cancer cells, the advantage in the use of complex PC is in the low dose of these isolated components which can be explained by their synergistic or additive effects. For example, resveratrol inhibited proliferation of prostate cancer cells PC-3 at 50 μM corresponding to 11.4 μg/ml (50), vitamin D3 at 100 nM corresponding to 38.5 ng/ml (51), and EGCG at 80 μM corresponding to 36.7 μg/ml (52), whereas the concentration of resveratrol, vitamin D3 and EGCG in PC corresponds to 1.6 μg/mg PC, 2 μg/mg PC, 5.8 μg/mg PC, respectively. Therefore, the final concentration of resveratrol

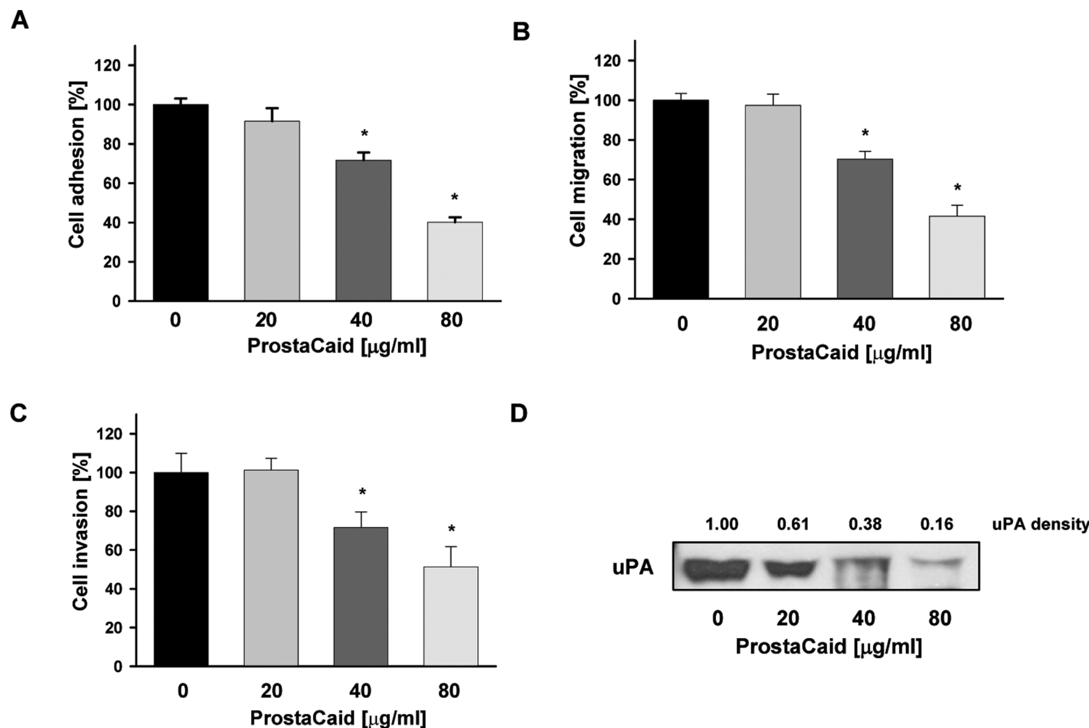

Figure 2. Effect of PC on invasive behavior of prostate cancer cells. (A) Cell adhesion. PC-3 cells were treated with PC (0-80 μg/ml) for 24 h and cell adhesion to fibronectin determined as described in Materials and methods. Each bar represents the mean ± SD of three experiments. *P<0.05. (B) Cell migration. Cell migration was determined after 24 h of incubation in the presence of PC (0-80 μg/ml) in Boyden Chambers as described in Materials and methods. Each bar represents the mean ± SD of three experiments. *P<0.05. (C) Cell invasion. Cell invasion was determined after 24 h of incubation in the presence of PC (0-80 μg/ml) in Boyden Chambers coated with Matrigel as described in Materials and methods. Each bar represents the mean ± SD of three experiments. *P<0.05. (D) uPA secretion. PC-3 cells were treated with PC (0-80 μg/ml) for 24 h and the expression of uPA detected in conditioned media with anti-uPA antibody by Western blot analysis as described in Materials and methods. The results are representative of three separate experiments.

Table I. Effect of ProstaCaid on the expression of prostate cancer related genes.

Gene	Description	Fold change
CDKN1A	Cyclin-dependent kinase inhibitor (p21)	3.49
CAV1	Caveolin 1	0.57
CCND1	Cyclin D1	0.77
CDK4	Cyclin-dependent kinase 4	0.83
E2F1	E2F transcription factor 1	0.54
ELAC2	ElaC homolog 2 (*E. coli)*	0.66
IGF2	Insulin-like growth factor 2	0.62
MAPK6	Mitogen-activated protein kinase 6	0.54
NR2F2	Nuclear receptor subfamily2, group F, member 2	0.52
PCNA	Proliferating cell nuclear antigen	0.47
PLAU	Plasminogen activator, urokinase	0.35

DNA-microarray analysis was performed with PC-3 cells treated with PC (0-80 μg/ml) for 24 h as described in Materials and methods. The data are representative of two independent experiments.

corresponds to 128 ng/ml, vitamin D3 to 160 ng/ml, and EGCG to 464 ng/ml at the highest used dose of 80 μg/ml of PC in our experiments.

Effect of PC on the invasive behavior of prostate cancer cells. Tumor invasion and metastasis are multifaceted processes including cell adhesion, proteolytic degradation of tissue barriers, cell migration, invasion, and angiogenesis (43,53). Invasive behavior of prostate cancer cells is associated with their ability to migrate and invade the surround tissues and is mediated through uPA/uPAR complex (43,53,54). To investigate if PC has an inhibitory effect on invasive behavior of

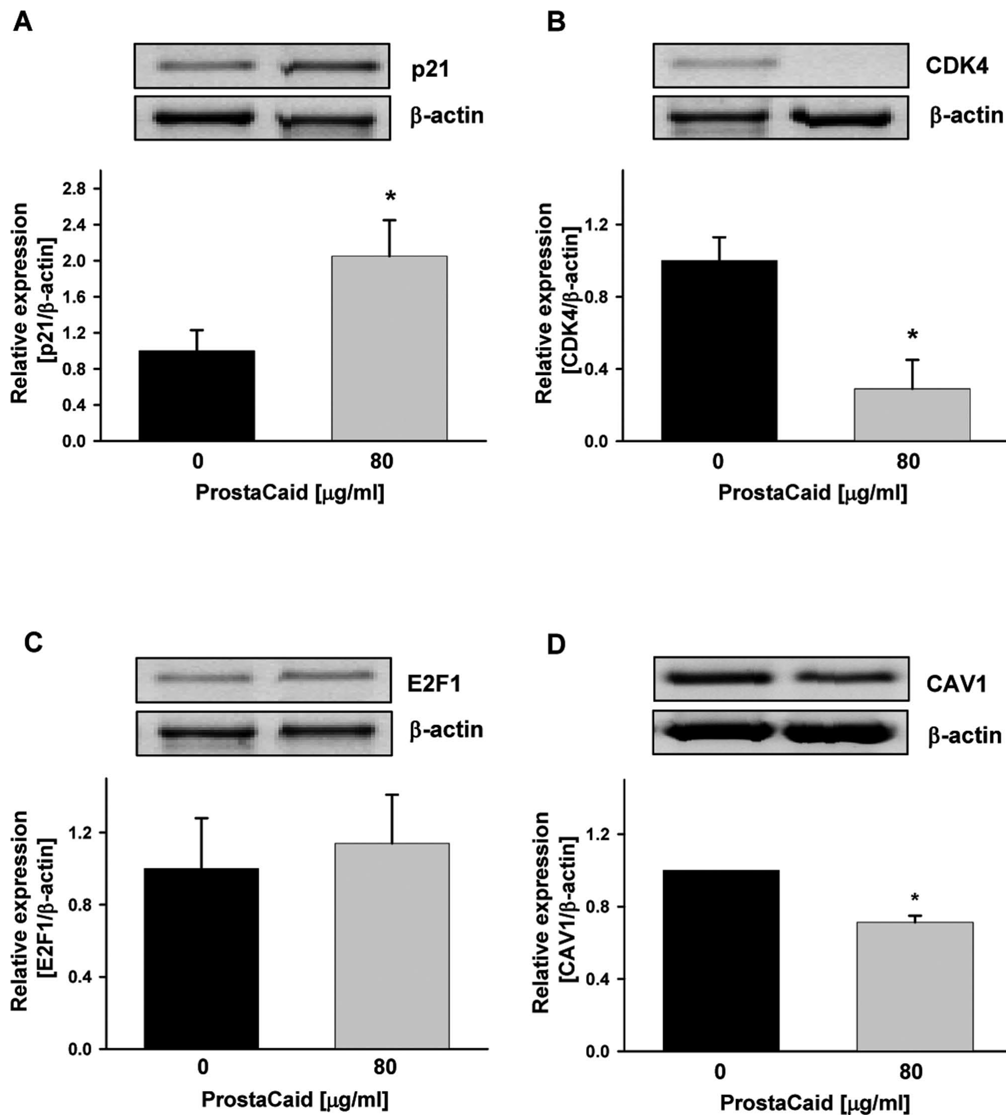

Figure 3. Effect of PC on the mRNA expression on prostate cancer biomarkers in prostate cancer cells. The mRNA expression of (A) p21, (B) CDK4, (C) E2F1 and (D) CAV1 in PC-3 cells was determined by RT-PCR analysis. Total RNA was isolated from PC-3 cells treated with vehicle or PC (80 μg/ml) and RT-PCR was performed with specific primers as described in Materials and methods. RT-PCR for β-actin was used as an internal loading control. The results are expressed as the relative expression ratios of specific mRNA to β-actin. Each bar represents the mean ± SD of three experiments. *P<0.05.

highly invasive prostate cancer cells, PC-3 cells were pretreated with PC (0-80 μg/ml) for 24 h and their adhesion to fibronectin was determined on strips coated with human fibronectin as described in Materials and methods. As seen in Fig. 2A, adhesion of PC-3 cells to fibronectin was markedly suppressed by the PC treatment by 28.3 and 59.9% at 40 and 80 μg/ml, respectively. The effect of PC on migratory potential of prostate cancer cells was evaluated in PC-3 cells pretreated with PC (0-80 μg/ml) for 1 h and cell migration was determined after additional 24 h of incubation. As expected, PC significantly decreased the migration rate of PC-3 cells by 29.7 and 58.5% at 40 and 80 μg/ml, respectively (Fig. 2B). Cell invasion is another key factor involved in cancer progression and metastasis (3,43,46). To examine the effect of PC on the invasive ability of PC-3 cells, cell invasion assays were performed in Transwell chambers coated with Matrigel as described in Materials and methods. As seen in Fig. 2C, PC markedly inhibited invasion of PC-3 cells in a dose-response manner by 28.3 and 48.7% at 40 and 80 μg/ml, respectively. In order to evaluate the molecular mechanism of action of PC on the invasion of prostate cancer cells, conditioned media from PC-3 cells treated with PC (0-80 μg/ml) were collected and secretion of uPA was determined by Western blot analysis. As expected, PC markedly decreased secretion of uPA from PC-3 cells (Fig. 2D). This observation is consistent with our previous report demonstrating the anti-invasive effect of *G. lucidum* in human prostate cancer cells through the mechanisms including uPA/uPAR signaling (18,46). As in the inhibition of proliferation by PC described above, the concentration of *G. lucidum* in PC was markedly lower (19.5 μg/mg PC which corresponds to the final concentration of *G. lucidum* at 1.56 μg/ml at the highest used dose of 80 μg/ml of PC), than in the original experiments with individual *G. lucidum* extracts (0.5-2.5 mg/ml) (18).

Effect of PC on the gene expression profiles of prostate cancer-related biomarkers in prostate cancer cells. In order to evaluate

whether anti-proliferative and anti-invasive effects of PC are associated with the expression of genes previously identified in prostate cancer, we used cDNA microarray analysis with human prostate cancer biomarker genes. PC-3 cells were treated with PC (0-80 μg/ml) for 24 h and cDNA microarray analysis performed as described in Materials and methods. As seen in Table I, PC up-regulated the expression of CDKN1A, and down-regulated expression of *CAV1*, *CCND1*, *CDK4*, *E2F1*, *ELAC2*, *IGF2*, *MAPK6*, *NR2F2* and *PLAU* genes in the PC-3 cells. Furthermore, we have confirmed the expression of some genes by RT-PCR. PC-3 cells were treated with PC (80 μg/ml) for 24 h. Total RNA was isolated and RT-PCR analysis was performed. Consistent with the DNA microarray data, PC significantly induced the expression of the CDKN1A mRNA and down-regulated the expression of CDK4 mRNA and CAV1 mRNA (Fig. 3). Interestingly, expression of E2F1 mRNA was not changed by the PC treatment. Therefore, PC regulates the cell cycle progression network through binding to cell cycle regulators such as cyclin D1, Rb, and the transcription factor E2F1 (49,55-58). The cell progression is regulated by cyclins, cyclin dependent kinases (Cdks) and Cdk inhibitors such as p15, p16, 21, and p27; cyclin D1 (CCND1) and CDK4 form a complex to accelerate cell cycle progression, while Cdk inhibitors slow cell cycle progression (48,55,56,59,60). Therefore, the up-regulation of CDKN1A (p21) and down-regulation of *CCND1* and *CDK4* genes will cause cell cycle arrest at G1/G0 phase. Up-regulation of p21 induced strong downstream inhibition of CDK4 and cyclin D1 and hypophosphorylation of Rb, further leading to the inhibition of transcription factor E2F1. Nevertheless, p21 can bind to proliferating cell nuclear antigen (product of *PCNA* gene) (61) and PCNA regulated the expression of ERK3/MAPK6 (product of *MAPK6* gene) which affect cell viability and regulate the cell cycle (62). Thus, the induction of p21 also resulted in the inhibition of PNCA expression, leading to the reduction of ERK3 protein. In addition to the cell cycle regulatory genes, PC treatment also modulated expression of other genes previously identified in prostate cancer *CAV-1*, *IGF2*, *ELAC2* and *PLAU* (Table I). For example, Caveolin-1 (product of *CAV-1* gene) is a major structural component of caveolae, specialized in plasma membrane invaginations involved in endocytosis, cell adhesion, and signal transduction (63). Further, Caveolin-1 is overexpressed in advanced prostate cancer where it promotes migration, invasion, and angiogenesis in prostate cancer cells (63,64). The precise role of the insulin-like growth factor 2 (IGF2) on progression of tumor remains unclear. However, polymorphism of the *IGF2* gene is associated with increased prostate cancer risk (65,66) and an activation of autocrine IGF2 loop is linked to the neoplastic progression (67). uPA (product of *PLAU* gene) and its receptor (uPAR) are important in cancer adhesion, migration, and invasion. uPA interacts with uPAR, which further form the multi-complex with integrin receptor $\alpha_3\beta_1$ or $\alpha_v\beta_3$ and regulate the invasive behavior (adhesion, migration and invasion) of cancer cells (18,43,68,69). Although suggested polymorphism of the elaC homolog-2/hereditary prostate cancer (*ELAC2/HPC2*) gene and prostate cancer risk demonstrated conflicting results in a variety of studies, a recent meta-analysis showed that ELAC2 is associated with prostate cancer risk (70,71).

In summary, our data clearly demonstrate that PC modulates expression of specific genes related to prostate cancer growth and invasiveness, and special ingredients in the PC may contribute to the inhibition of prostate cancer cells through distinct signaling pathways.

In conclusion, ProstaCaid is a novel dietary supplement that contains multiple ingredients which show an anti-proliferation effect on androgen-dependent and -independent prostate cancer cells. Our results show that PC inhibits proliferation and invasive behavior of prostate cancer cells by the modulation of the expression of genes associated with prostate cancer. Our data suggest that PC has multiple targets for its therapeutic effect and the biological activity of PC is mediated by the additive or synergistic effects of its individual ingredients. In summary, PC may have potential clinical application for an alternative prostate cancer therapy.

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