Research Article

Curcumin-Targeting Pericellular Serine Protease Matriptase Role in Suppression of Prostate Cancer Cell Invasion, Tumor Growth, and Metastasis

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Abstract
Curcumin has been shown to possess potent chemopreventive and antitumor effects on prostate cancer. However, the molecular mechanism involved in curcumin’s ability to suppress prostate cancer cell invasion, tumor growth, and metastasis is not yet well understood. In this study, we have shown that curcumin can suppress epidermal growth factor (EGF)-stimulated and heregulin-stimulated PC-3 cell invasion, as well as androgen-induced LNCaP cell invasion. Curcumin treatment significantly resulted in reduced matrix metalloproteinase 9 activity and downregulation of cellular matriptase, a membrane-anchored serine protease with oncogenic roles in tumor formation and invasion. Our data further show that curcumin is able to inhibit the induction effects of androgens and EGF on matriptase activation, as well as to reduce the activated levels of matriptase after its overexpression, thus suggesting that curcumin may interrupt diverse signal pathways to block the protease. Furthermore, the reduction of activated matriptase in cells by curcumin was also partly due to curcumin’s effect on promoting the shedding of matriptase into an extracellular environment, but not via altering matriptase gene expression. In addition, curcumin significantly suppressed the invasive ability of prostate cancer cells induced by matriptase overexpression. In xenograft model, curcumin not only inhibits prostate cancer tumor growth and metastasis but also downregulates matriptase activity in vivo. Overall, the data indicate that curcumin exhibits a suppressive effect on prostate cancer cell invasion, tumor growth, and metastasis, at least in part via downregulating matriptase function. Cancer Prev Res; 1–11. ©2013 AACR.

Introduction
Prostate cancer is currently the second leading cause of cancer-related death in men in the western world (1). Localized prostate cancer is highly curable by surgery or chemotherapy to remove or destroy the cancerous lesions. Yet, there currently exist no efficacious therapies for advanced prostate cancer with hormone refractory or metastatic phenotypes (2). Therefore, it is a critical issue to discover a chemical or compound valuable for developing new therapies that inhibit the progression or invasive abilities of prostate cancer.

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It has been shown that the expression levels of matriptase, a membrane-anchored serine protease, are increased in prostate cancer and correlated with tumor grades and poor prognosis (3). Several agents including androgens, epidermal growth factor (EGF), sphingosine-1-phosphate, and suramin exhibit stimulatory effects on the matriptase activation in mammalian epithelial, prostate, and breast cancer cells (4–6). In matriptase transgenic mice, matriptase overexpression promoted tumorigenicity and carcinogen-induced tumor formation (7). Matriptase is also involved in ErbB-2-induced prostate cancer cell invasion (6). This suggests that dysregulation of matriptase exhibits oncogenic effects and can also promote the progression of human cancer including prostate cancer. Inhibition of matriptase may be an opportunity to reduce prostate tumor growth and progression (8). Thus, matriptase may present a new potential target for prostate cancer therapies.

Curcumin has emerged as a compound with multiple biologic properties for health maintenance and cancer prevention. The antiinflammatory and antioxidant activities of curcumin have been proposed via inhibiting NF-kB, COX-2, iNOS, and cytokine production (9, 10). In rodents, curcumin can prevent carcinogenesis induced by various carcinogens (11). A more recent phase II clinical study has
pointed out that allotting low concentrations of curcumin in patients have a similar biologic impact on NF-kB, COX-2, and phospho-STAT-3 in peripheral blood mononuclear cells, as those observed in the in vitro studies with 5 to 50 μmol/L of curcumin treatments (12, 13). In hepatocellular cancer cells, curcumin can inhibit cancer cell invasion and matrix metalloproteinase 9 (MMP-9) secretion (14). In prostate cancer cells, curcumin can downregulate androgen receptors (10, 15), decrease cell proliferation (16), and inhibit DU145 cell invasion via decreasing MMP-2/-9 activity (17). However, the more detailed molecular mechanisms how curcumin inhibits prostate cancer cell invasion, tumor growth, and metastasis have not been yet clearly elucidated. In this study, we show that curcumin can significantly suppress prostate cancer cell invasion, tumor growth, and metastasis. Curcumin not only inhibits MMP-9 activity, but also decreases the cellular levels of matriptase. Furthermore, the invasive ability of prostate cancer cells induced by matriptase overexpression was also significantly suppressed by curcumin. In summary, the data indicate that curcumin exhibits a suppressive effect on prostate cancer cell invasion, tumor growth, and metastasis, at least in part by downregulating matriptase function. Thus, the data suggest that curcumin may exhibit a therapeutic potential for invasive prostate cancer.

Materials and Methods

Materials

DMEM, FBS, and RPMI1640 media were obtained from Hyclone. Protein Assay kits were from Bio-Rad. Anti-V5 Ab and Lipofectamine™ TM 2000 reagent were purchased from Invitrogen. Curcumin (Cat.# C7727, purity 94% curcuminoid, 80% curcumin) and all other reagents were purchased from Sigma-Aldrich, unless otherwise noted. M24, M69, and M19 antibodies were gifts from Dr. Chen-Yong Lin at the Georgetown University, DC.

Cell culture

PC-3, CWR22Rv1, and WPMY-1 cells were obtained from American Type Culture Collection (ATCC). PNT-2 cells were purchased from Sigma-Aldrich. PC-3 and WPMY-1 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and 2 mmol/L glutamine in a 5% CO2 and incubated at 37°C. DU145, C-33, and C-81 LNCaP cells were gifts from Dr. Ming-Fong Lin at the University of Nebraska Medical Center, NE. CWR22Rv1, C-33, C-81 LNCaP, DU145, and PNT-2 cells were maintained in 5% FBS, 2 mmol/L glutamine RPMI1640 medium. No authentication was done by the authors for all cell lines used.

Cell growth and cytotoxicity assays

Cells were seeded at a density of 1.5 × 10⁴ or 3 × 10⁵ cells/cm² for cell growth and cytotoxicity assays, respectively. For growth assay, 1 day after seeding, cell amounts were daily analyzed by MTT assays according to the manufacturer’s protocol, and other cells were accordingly refreshed with media. For cell cytotoxicity assay, 1 day after seeding, the cells were treated with indicated concentrations of curcumin for 16 hours and the viable cell numbers were counted by a hemacytometer with a trypan blue exclusion method.

Cell invasion and migration assays

Transwell assays were conducted according to the previously described procedures (18). In brief, transwells were coated with or without 20 μg of matrigel (BD biosciences) for cell invasion or migration assay. Serum-starved cells were then seeded at a density of 3 × 10⁵ cells/cm² in the upper chambers of transwells with serum-free medium. The lower chambers were filled with the medium containing 10% FBS or growth factors as chemoattractants. After 16-hour incubation, cells were fixed and stained with 1% GIEMSA dye. The penetrating cells were photographed (100 ×) and counted using a light microscope. All experiments were conducted in triplicate.

RNA extraction and quantitative real-time PCR

Both assays were conducted according to the previously described procedures (19). Briefly, 16-hour, curcumin-treated cells were used for total RNA extraction using Trizol reagent (Invitrogen), and RNA was reversely transcribed using SuperScript One-step RT-PCR System (Invitrogen) according to the manufacturer’s instructions. Quantitative real-time PCR (Q-PCR) was conducted using the StepOne Real-time PCR system (Applied Biosystems). The primers for the experiments were listed as follows: Matriptase: forward, 5′-CACCTCAGTGTCGTTCC-3′ and reverse, 5′-GCGTGCAGGCCAAAGCT-3′; GAPDH: forward, 5′-AAAGATCCACTGGCG-3′ and reverse, 5′-GAATTCGTCATGGATGACCTTGGCCAG-3′. HAI-1 primers were obtained from ABI (Cat.#Hs00173678). All Q-PCR reactions were carried out 3 times.

Gelatin zymography

For gelatin zymography, cells were treated with different concentrations of curcumin for 16 hours. The supernatants of the conditioned media were collected and concentrated using Amicon Ultra-4 centrifuge filter devices (Millipore) at 3,000 r.p.m. at 4°C for 30 minutes. Without heating or reducing, samples underwent 0.1% (w/v) gelatin SDS-PAGE. After electrophoresis, the gel was incubated in a renaturation buffer (50 mmol/L Tris-HCl, pH7.5, 10 mmol/L NaCl, 2.5% v/v Triton X-100) at 37°C for 1.5 hours, and following with a developing buffer (50 mmol/L Tris-HCl, pH7.5, 5 mmol/L CaCl₂) at 37°C for 18 hours with gentle agitation. Gels were then stained and clear bands were shown on a blue background. The bands were detected by a luminescent image analyzer (LAS-4000; Fujifilm).

Western blot analysis

For Western blotting by M24, M69, and M19 mAbs, equal amounts of cell lysates were mixed with protein loading dye in a nonreducing and nonboiling condition (20). For the other samples, equal amounts of cell lysates were mixed with regular protein loading dye and boiled for 10 minutes.
Samples were separated by SDS-PAGE and transferred to nitrocellular membranes (Whatman). The membranes were blocked with 5% skim milk in Tris-buffered saline and overnight incubated with primary antibodies at 4°C overnight, followed by secondary antibody incubation. The protein images were visualized using an Enhanced Luminol Reagent Plus (Perkin Elmer) and detected by a luminescent image analyzer (LAS-4000; Fujiﬁlm).

Tumor xenografts and bioluminescence analysis

All procedures for animal experimental protocols were approved by Institutional Animal Care and Use Committee (IACUC) of Academia Sinica and NTU. For xenograft study, 6-week-old male nude mice were inoculated subcutaneously into the dorsal flank with 2 x 10⁶ luciferase-expressed PC3 (PC3-Luc) cells. After 2 weeks, mice were randomly assigned into 2 groups (6 mice/group): 1 group receiving 100 mg/kg of curcumin and the other receiving vehicle (corn oil) by daily intraperitoneal injection. The tumor volume and body weight of each mouse was monitored weekly. After 3-week treatment, mice were sacriﬁced, and both brachial lymph nodes were extracted and snap frozen in liquid nitrogen for Western blot analysis. For ex vivo bioluminescence images of lymph nodes, the images were acquired with Xenogen IVIS50 Imaging System, and measurements of bioluminescent signals were conducted with Living Image 2.50 software. D-Luciferin of 150 mg/kg was injected into the mice 10 minutes before imaging. Mice were humanely sacriﬁced, and both brachial lymph nodes were taken and imaged for 10 seconds.

Statistical analysis

A mean ± SE was calculated from 3 repeated groups in all experiments. A statistical signiﬁcance between groups was determined by Student t test. A P-value less than 0.05 was considered as a signiﬁcant difference between the 2 groups.

Results

Curcumin inhibition of prostate cancer cell migration and invasion

To examine the effects of curcumin on the growth of PC-3 cells, and ensure that the cell growth kept in the log phase after 5-day culture, cells were seeded at a density of 1.5 x 10⁴ cells/cm² and treated with 5, 25, and 50 μmol/L of curcumin. With MTT assays, Fig. 1A shows that curcumin ably inhibits PC-3 cell growth in a time- and dose-dependent manner. We then tested the cytotoxicity of curcumin on PC-3 cells in a cell density of 3 x 10⁵ cells/cm², because this cell density would be used in following transwell assays. Using trypan blue exclusion assays, the results displayed that there was no signiﬁcant cytotoxicity on PC-3 cells after the curcumin treatment (Fig. 1B). That 25 or 50 μmol/L of curcumin dramatically decreased PC3 cell growth without any significant effect on cell cytotoxicity may be partly due to the difference of cell-seeding density and/or the inhibitory effect of curcumin on dehydrogenases (21). To further investigate the effect of curcumin on prostate cancer cell migration and invasion, we conducted transwell assays and found that the migration and invasion of PC-3 cells were signiﬁcantly decreased by approximately 50% after 5 μmol/L-curcumin treatment and suppressed up to 80% upon 25 and 50 μmol/L curcumin treatments (Fig. 1C and D). Taken together, these data indicate that curcumin can signiﬁcantly suppress PC-3 cell migration and invasion.

Since recent studies have shown that oncogenic signaling of EGFR/ErbB-2 participates in metastatic prostate cancer (22), the effect of curcumin on ErbB ligands (EGF or heregulin)-induced prostate cancer cell invasion was further analyzed. As shown in Fig. 1E, EGF or heregulin was able to increase PC-3 cell invasion and this induced cancer cell invasion was ably antagonized by curcumin. Since androgen signaling has been another important factor for prostate cancer progression, to further explore the effect of curcumin on androgen-induced prostate cancer cell invasion, we treated androgen-sensitive LNCaP cells with DHT in the presence or absence of curcumin. The result (Fig. 1F) has shown that DHT signiﬁcantly enhanced the invasion of LNCaP cells and curcumin suppressed DHT-induced prostate cancer cell invasion. Thus, the data indicate that curcumin can block ErbB ligands (EGF and heregulin)- and DHT-induced prostate cancer cell invasion.

Identification of matriptase as a curcumin-targeting serine protease in prostate cancer cells

It has been shown that both MMPs and serine proteases play important roles in cancer cell invasion (23, 24). To further explore the involvement of MMPs or serine proteases in curcumin-inhibited prostate cancer cell invasion, we analyzed the effects of a broad MMP inhibitor GM6001, a serine protease inhibitor 4-(2-Aminoethyl) benzenesulfonyl ﬂuoride hydrochloride (AEBSF), or curcumin on PC-3 cell invasion. As shown in Fig. 2A, curcumin, GM6001, and AEBSF suppressed the invasion of PC-3 cells approximately by 76%, 38%, and 70%, respectively. With the combination treatment of GM6001 and curcumin, curcumin further reduced the invasion of PC-3 cells approximately by 35% in comparison with GM6001 alone. Moreover, curcumin only had a marginal effect to further reduce the invasion of AEBSF-treated cells, and the degree of curcumin to inhibit prostate cancer cell invasion was quite similar to the combined effect of GM6001 and AEBSF on this event. The data suggest that curcumin-inhibited prostate cancer cell invasion is mainly via suppressing both MMPs and serine proteases. Indeed, the activity of secreted MMP-9 in prostate cancer cells was signiﬁcantly suppressed by curcumin, with a less effect on MMP-2 (Fig. 2B). Since the expression of matriptase has been shown to be correlated with the prostate cancer progression (3) and also that matriptase is involved in ErbB-2-induced prostate cancer cell invasion (6), we then proposed that matriptase was a curcumin-targeting serine protease in prostate cancer cells, by examining the effect of curcumin on matriptase using Western blotting with anti-total matriptase (M24), anti-activated matriptase (M69) and anti-HAI-1 (M19) mAbs. As shown in Fig. 2C, curcumin profiably decreased
the total levels of matriptase including latent matriptase (70 kDa) and activated matriptase (a 120 kDa complex of activated matriptase and its cognate inhibitor HAI-1) in a dose-dependent manner. The decrease in matriptase/HAI-1 complexes by curcumin was further validated by the Western blotting with M69 Ab. In addition, the protein levels of HAI-1 or in a complex with activated matriptase were also reduced by curcumin. To further assess the effect of curcumin on the transcription levels of matriptase and HAI-1, we conducted Q-PCR and showed that curcumin had no significant effect on the expression of both genes (Fig. 2D). Thus, the data indicate that the reduction of matriptase and HAI-1 protein levels by curcumin is not via transcription regulation, but may utilize a posttranslational mechanism for decreasing cellular matriptase.

Since epithelial–mesenchymal transitions (EMT) have been shown to be implicated in the prostate cancer progression (25), we then investigated if curcumin functioned as an antagonist for EMT to reduce prostate cancer cell invasion, by using the immunoblotting analyses of several EMT markers including E-cadherin, β-catenin, vimentin, and snail. As shown in Fig. 2E, 25 μmol/L curcumin had no significant effect on those biomarkers in PC-3 cells.

Next, we examined the time-kinetic effect of curcumin on matriptase in PC-3 cells. The data showed that the levels of activated matriptase dramatically decreased at 30 minutes after curcumin treatment, and up to 16 hours, whereas the level of latent matriptase began to increase after 8-hour treatment, lasting to 24 hours (Fig. 2F). Moreover, to determine whether this decrease in matriptase by curcumin was a ubiquitous phenomenon in prostate cancer and prostate cells, we analyzed the effect of curcumin on matriptase in various human prostate cancer cells, prostatic stromal myofibroblast WPMY-1 cells, and immortalized prostatic epithelial PNT-2 cells. The result (Fig. 2G) has shown that curcumin also suppress the activated levels of matriptase in androgen-sensitive C-33 LNCaP cells and androgen-independent prostate cancer cells including C-81 LNCaP, DU145, and CWR22Rv1 cells. Moreover, matriptase protein in WPMY-1 cells and the activated level of matriptase in PNT-2 cells were negligibly detectable. Apparently, curcumin can reduce the level of latent
matriptase in PNT-2 cells (Fig. 2G). Thus, the data indicate that curcumin exhibits an inhibitory role in matriptase in prostate cancer cells, with a less effect on prostatic epithelial PNT-2 cells.

In addition, we examined the effects of 2 curcumin analogues, dimethoxycurcumin (DMC) and EF24, on matriptase in PC3 cells and found that both analogues can also reduce the activated levels of matriptase (Fig. 2H). Taken together, these data suggest that curcumin-decreased prostate cancer cell invasion is at least partly due to decreasing both MMP-9 activity and cellular matriptase, rather than altering the EMT process.

Curcumin-reduced matriptase function in PC-3 cells

Next, we examined the pretreatment effect of curcumin on the FBS-induced matriptase activation in prostate cancer cells. The data have shown that the pretreatment of curcumin dramatically depressed the stimulatory effects of FBS on matriptase activation (Fig 3A). To further evaluate the duration of curcumin on reducing cellular matriptase in prostate cancer cells, the effect of 1-hour acute curcumin treatment on matriptase was examined. The data showed that curcumin could decrease the activated matriptase up to 8 hours after the acute treatment (Fig. 3B). Four hours after the acute treatment, the decreased levels of latent matriptase began to rebound. We then analyzed the effect of the acute curcumin treatment on prostate cancer cell invasion and found that the acute treatments of 25 and 50 μmol/L curcumin suppressed PC3 cell invasion by approximately 50% and approximately 64% (Fig. 3C). This result indicates that an acute exposure of curcumin remains effective in reducing both cellular matriptase and prostate cancer cell invasion.
Inhibitory effects of curcumin on DHT- and EGF-induced matriptase activation

Since androgens can proficiently induce matriptase activation in prostate cancer cells (26), we then examined the effect of curcumin on androgen-induced matriptase activation in LNCaP cells. As shown in Fig. 3D, curcumin had an inhibitory effect on DHT-induced matriptase activation in a dose-responsive manner. Moreover, we examined if curcumin also exhibited an antagonized effect on EGF-induced matriptase activation in prostate cancer cells. As shown in Fig. 3E, EGF could dramatically induce matriptase activation in LNCaP cells, and curcumin ably reduced EGF-induced matriptase activation. Likewise, we further analyzed the acute effect of curcumin on EGF-induced matriptase activation. After the treatment with EGF for 2 hours and followed by curcumin treatment for 30 minutes, the results showed that curcumin could quickly decrease the stimulatory effect of EGF on matriptase in LNCaP cells (Fig. 3F). These results display that curcumin can potently inhibit the stimulatory effects of androgens and EGF on matriptase in prostate cancer cells.

Curcumin promotion of matriptase shedding and suppression of matriptase-induced prostate cancer cell invasion

The shedding of matriptase–HAI-1 complexes to extracellular environments is thought of as a mechanism to remove matriptase from cells (26). Since Fig. 2 shows that curcumin can reduce the cellular content of matriptase but has no effect on the gene expression, we accordingly examined whether curcumin could promote the shedding of matriptase, leading to decreasing cellular matriptase in prostate cancer cells. The effect of curcumin on matriptase shedding was examined by using Western blotting to analyze the shed matriptase in the conditioned media. The data revealed that curcumin promoted the shedding of the matriptase–HAI-1 complex in a dose-dependent manner, with less effect on the latent form of matriptase (Fig. 4A). We then further analyzed whether the shed matriptase in the conditioned media retains its function for cancer cell invasion. Following the curcumin treatment, the conditioned media were collected, washed out of curcumin and used to treat PC-3 cells. Through cell invasion assays, the data showed that the shed matriptase
had no significant effect on prostate cancer cell invasion (Fig. 4B).

Moreover, to recapitulate the increased levels of matriptase in advanced prostate cancer, PC3 cells were transiently transfected with V5-tagged matriptase plasmids. We then analyzed whether curcumin could also affect the activated levels of matriptase caused by overexpression. As shown in Fig. 4C, the overexpression of matriptase increased the activated levels of matriptase, and curcumin effectively reduced the cellular levels of matriptase, at least in part by promoting the shedding of the matriptase–HAI-1 complex into the conditioned media. Thus, the data indicate that curcumin-induced matriptase shedding may play an important role in reducing the cellular level of activated matriptase.

Since matriptase overexpression is observed in a variety of human carcinomas including prostate cancer (3, 27), we further examined the role of matriptase in prostate cancer cell invasion and the effect of curcumin on matriptase-induced cancer malignancy by establishing stable pools of matriptase-overexpressing CWR22Rv1 cells. The data displayed that the total and activated levels of matriptase increased in matriptase-overexpressing CWR22Rv1 cells (Fig. 4D), which were concurrent with increased invasion capabilities (Fig. 4E). We then examined the effect of curcumin and AEBSF on the invasion of matriptase-overexpressing CWR22Rv1 cells. As shown in Fig. 4F, similar to AEBSF, curcumin significantly inhibited the matriptase-induced prostate cancer cell invasion, up to 80%, down to the levels of curcumin-treated control cells. Thus, the data denote that curcumin exhibits a strong inhibitory potential for prostate cancer cell invasion, especially that caused by matriptase overexpression or dysregulation.

**Curcumin-inhibited tumor growth and metastasis in a prostate cancer xenograft model**

To further assess if curcumin can inhibit prostate tumor growth and metastasis, we examined the effects of curcumin on the tumor growth of PC3-Luc cells using a xenograft model. As shown in Fig. 5A, there was a significant regression of tumor volume in the group of mice receiving the curcumin treatment. No animal body weight was significantly altered during the treatment, indicating that the curcumin treatment had no toxicity in mice (Fig. 5B). After sacrifice, the tumor mass was measured and significantly reduced in the group of curcumin-treated mice (Fig. 5C). Moreover, we checked if there was any metastatic nodule in the lymph nodes nearby the xenografted tumors, and found that curcumin treatment significantly reduced the metastatic lesions at brachial lymph nodes in the xenografted mice, indicating that curcumin exhibited an inhibition effect on prostate cancer metastasis (Fig. 5D). We then further analyzed the total and activated levels of matriptase in the xenografted tumors with or without curcumin treatment. As shown in Fig. 5E, the level of activated matriptase was remarkably deceased in the xenografted tumors treated with curcumin, compared with vehicle control. Taken together, the data indicate that curcumin can inhibit prostate cancer tumor growth and metastasis, at least in part due to curcumin’s effect on downregulating the activated matriptase in tumors.

**Discussion**

Recent findings have recommended curcumin as a beneficial chemotherapeutic and chemopreventive agent for...
prostate cancer (28). However, its functions in prostate cancer cell invasion and metastasis are still lacking. In this study, we found that curcumin exhibits an inhibitory effect on prostate cancer cell invasion, tumor growth, and metastasis, not only in terms of inhibiting MMP-9 activity, but also on the downregulation of cellular matriptase, achieved through inhibiting the stimulatory effects of androgens or EGFR ligands on the protease and promoting the protease shedding.

For cancer cell invasion or metastasis, the EMT and the degradation of ECM have been proposed as 2 important processes. ECM degradation has been mainly attributed to dysregulation of pericellular proteolysis. In curcumin-inhibited prostate cancer cell invasion, our results suggest that some proteases from the families of MMPs and serine proteases are critically inhibited by curcumin, whereas the EMT is apparently not affected by this treatment. Moreover, our data indicate that in prostate cancer cells, MMP-9 activity is significantly inhibited by curcumin with less effect on MMP-2. Unexpectedly, we further observed that the combination treatment of GM6001 and AEBSF could reach to the similar inhibitory effect of curcumin on prostate cancer cell invasion through reducing the cellular matriptase. In addition, curcumin also can inhibit prostate tumor growth and metastasis, at least partly via downregulating the level of activated matriptase in prostate cancer xenografted tumors (Fig. 5). Thus, curcumin exhibits a chemopreventive and therapeutic potential in matriptase-involved prostate cancer malignancy.

As shown in Fig. 6, matriptase function is mainly regulated by 3 steps: activation, inhibition, and ectodomain shedding. Matriptase is synthesized as a single-chain zymogen, through 2 sequential endoproteolytic cleavages for activation: the first cleavage at G149 for matriptase maturation (30) and the second cleavage at Arg614 for activation (4). Matriptase overexpression, HAI-1 knockdown, S1P, EGF, Heregulin, or DHT can induce the protease activation (4–6, 26, 31). After activation, 2 mechanisms are proposed for inhibiting matriptase activity: (i) its inhibitor HAI-1-mediated inhibition by forming 120-kDa complexes and (ii) the ectodomain shedding of the activated matriptase–HAI-1 complex from cells into the extracellular environments with a molecular mass of 95 or 110 kDa (30, 32). Based on this model, we propose 2 possible mechanisms in which curcumin can downregulate cellular matriptase. First, perhaps curcumin promotes the shedding process to reduce the cellular levels of matriptase, leading to decreased prostate cancer cell invasion. This suggests that curcumin’s ability to decrease the cellular content of matriptase...
provides a way to reduce the protease’s oncogenic effect on prostate cancer. However, how curcumin enhances the matriptase shedding is still elusive and remains under further investigation. Second, curcumin can interrupt the stimulatory effects of EGF, heregulin, and androgens in the process of matriptase activation. This may be explained by curcumin’s multiple inhibitory effects on several signal molecules including androgen receptor, HER-2, EGFR, PI3K/Akt, etc. (9, 10, 33). Thus, curcumin may block the androgen- and ErbB ligand-induced signal pathways for matriptase activation, leading to reducing prostate cancer cell invasion. In conclusion, the data suggest that curcumin may be a potent phytocomponent able to efficiently inhibit matriptase-mediated prostate cancer malignancy.

The inhibitory effects on the activated matriptase in prostate cancer cells after an acute curcumin treatment only last up to 16 hours. This may be due to curcumin consumption by the cells or the instability of curcumin in the culture medium, since curcumin has been shown to be unstable at neutral and basic pH and decomposed approximately by 50% in the culture medium after 8-hour incubation (34). Therefore, 2 curcumin analogs (DMC and EF24) with an enhanced metabolic stability and a higher potency than curcumin (35, 36) were used in this study, and had shown that both analogs can also reduce the cellular levels of activated matriptase in prostate cancer cells. Thus, this study points out that enhancing the stability and potency of curcumin will improve the efficiency of curcumin in the chemoprevention or cancer therapy, especially in cancers with matriptase dysfunction.

Curcumin has been shown to downregulate urokinase-type plasminogen activator (uPA) in prostate cancer cells (37). Pro-uPA has been shown to be one of matriptase substrates (38, 39). Consequently, curcumin’s ability to inhibit the activities of uPA may be partly attributed to its inhibitory effects on matriptase.

In conclusion, our data reveal that matriptase can be activated by multiple factors, including its overexpression, growth factors or androgens, and ultimately lead to prostate cancer cell invasion. Curcumin can significantly decrease the cellular levels of matriptase in prostate cancer cells, by promoting protease shedding. Therefore, this study provides a novel mechanism for establishing how curcumin can inhibit prostate cancer cell invasion, tumor growth and metastasis by the downregulation of matriptase function.

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

Authors’ Contributions
Conception and design: T.S. Cheng, Y.Y. Lin, C.I. Liao, M.S. Lee
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Figure 6. Model of curcumin’s effects on matriptase in prostate cancer cells. Matriptase is synthesized as a single-chain polypeptide and its activation requires 2 sequential endoproteolytic cleavages: the first cleavage at G149 for matriptase maturation (30) and the second cleavage at R614 for activation (4). Active matriptase is quickly inhibited by HAI-1 with formation of 120 kDa complexes. Then, the matriptase–HAI-1 complex is shed with a molecular mass of 95 or 110 kDa into extracellular environments. The mechanism for curcumin to affect matriptase activity is proposed as follows: First, curcumin induces matriptase shedding, leading to decreasing cellular matriptase and prostate cancer cell invasion. Second, curcumin may inhibit matriptase activation by targeting multiple signaling pathways, such as androgens, EGF, and heregulin signaling, even by its overexpression.
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For Cancer Research.


Curcumin-Targeting Pericellular Serine Protease Matriptase Role in Suppression of Prostate Cancer Cell Invasion, Tumor Growth, and Metastasis

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