Estrogens Promote Invasion of Prostate Cancer Cells in a Paracrine Manner through Up-Regulation of Matrix Metalloproteinase 2 in Prostatic Stromal Cells

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Accumulating evidence suggests an enhancing effect of estrogens on prostate cancer (PCa) progression. Matrix metalloproteinase 2 (MMP2), which plays an important role in prostate cancer invasion, is mainly expressed in prostatic stromal cells (PrSC). Here we show that estradiol (E2) treatment up-regulates MMP2 production in PrSC, which promotes PCa cell invasion in a paracrine manner. Conditioned medium (CM) was collected from E2-treated prostatic stromal cell line WPMY-1 and primary PrSC. The CM of E2-treated WPMY-1 and PrSC promoted invasion of PCa cells, as measured by Matrigel transwell assays. Treatment with E2 and 1,3,5-Tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole, an estrogen receptor-alpha (ERα) specific agonist, significantly up-regulated MMP2 expression in WPMY-1 and PrSC cells at both mRNA and protein levels. The CM treated with an anti-MMP2 antibody lost the stimulatory effect on invasion of PCa cells. The ER inhibitor ICI 182,780, as well as a TGFβ1 neutralizing antibody and ERα-specific small interfering RNA effectively suppressed E2-induced MMP2 expression in WPMY-1 cells. Mechanistic studies showed that E2 up-regulated MMP2 in an indirect manner: E2 induced TGFβ1 expression via ERα; TGFβ1 stimulated MMP2 expression in PrSC; the invasion of PCa cells were stimulated by elevated MMP2 expression induced by E2 in a paracrine manner. Our data show that E2 induces MMP2 expression in WPMY-1 and PrSC cells, which was mediated by TGFβ1. The effect of E2 on invasion of PCa cells is mediated by up-regulation of MMP2 in a paracrine mechanism. (Endocrinology 152: 773–781, 2011)

Invasion and metastasis are the most important causes of poor prognosis of prostate cancer (PCa) (1–2). The processes of invasion and metastasis are controlled by a large number of factors, such as hormones, growth factors, chemokines, and extracellular matrix (ECM) (3–5). Particularly, recent results showed that estrogens participate in the process of invasion and metastasis of PCa (6). Estrogens stimulate human PCa progression and metastasis in a recombinant tissue transplantation model using rat or mouse urogenital mesenchyme and prostatic epithelium transplanted into nude mice treated with estradiol (E2) and testosterone at a 1:10 ratio (7–8), and estrogen receptor α (ERα) plays a key role in this process (9). In addition, estrogens and selective estrogen receptor modulators (SERMs) can up-regulate expression of key proteins such as osteopontin, related to PCa bone metastasis (10).

Stroma–epithelium interaction in the prostate has been documented in recent years (11). Prostate stromal compartment plays a vital role in PCa invasion and metastasis. Human prostate fibroblasts induce growth and confer metastatic potential in lymph node metastatic lesion of prostate carcinoma (LNCaP) cells in an in vivo transplantation experiment (12–13). Stromal cells isolated from malignant prostate tissue stimulate PCa cell growth and invasion stronger than nor-
nal prostate stromal cells (14–15). Reactive stroma in human PCa is also involved in ECM remodeling (16).

Matrix metalloproteinase 2 (MMP2) is one of the key enzymes involved in ECM degradation. MMP2 activity significantly correlates with malignancy and metastasis of PCa (17–18). In the prostate, MMP2 is mainly expressed and secreted by stromal cells (19–21). Recent studies of mice deficient for MMP2 showed reduced prostate tumor burden, prolonged survival, and decreased lung metastasis (22). The expression of MMPs is regulated by many growth factors, such as TGFβ1. TGFβ1 up-regulates MMPs expression and induces ECM degradation in a variety of tissues and organs (23–25), including the prostate (26).

In this study, we hypothesize that E2 promotes PCa invasion in a paracrine manner through stimulating MMP2 expression in prostatic stromal cells (PrSC). We investigated the effect of PrSC on PCa cell invasion. We show that E2 induces MMP2 production in PrSC in an indirect manner, which involves induction of TGFβ1 expression and an autocrine effect of TGFβ1 on PrSC. We describe a novel mechanism of stroma–epithelium interaction demonstrating that the conditioned medium (CM) from E2-treated PrSC enhances PCa cell invasion through MMP2 secretion.

Materials and Methods

Cell culture

Two types of stromal cells were used for this study. Human PrSC were isolated from fresh surgical prostate specimens of seven individual patients with benign prostatic hyperplasia and cultured as previously described (27). Informed consent was obtained from each patient, and this study was approved by the Institutional Review Board of the First Central Hospital, Tianjin, China. The expression patterns of ER, TGFβ1, and MMP2 of the PrSC from different benign prostatic hyperplasia patients are summarized in Supplemental Fig. 1 (published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org/). The human prostate stromal cell line WPMY-1, which was derived from bovine serum (FBS, Invitrogen) at 37°C under 5% CO2. The cells were routinely maintained in RPMI 1640 phenol red-free medium (Sigma-Aldrich, St. Louis, MO) supplemented with 100 U/ml penicillin and 100 g/ml streptomycin (P/S, Invitrogen, Carlsbad, CA) and 5% fetal bovine serum (FBS, Invitrogen) and cultured as previously described (27). Informed consent was obtained from each patient, and this study was approved by the Institutional Review Board of the First Central Hospital, Tianjin, China. The expression patterns of ER, TGFβ1, and MMP2 of the PrSC from different benign prostatic hyperplasia patients are summarized in Supplemental Fig. 1 (published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org/). The human prostate stromal cell line WPMY-1, which was derived from normal stromal cells of the peripheral zone in the adult prostate (28), was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were routinely maintained in DMEM phenol red-free medium (Sigma-Aldrich, St. Louis, MO) supplemented with 100 U/ml penicillin and 100 g/ml streptomycin (P/S, Invitrogen, Carlsbad, CA) and 5% fetal bovine serum (FBS, Invitrogen) at 37°C under 5% CO2. The human PCa cell line DU145 and LNCaP were obtained from ATCC, the human PCa cell line PC3 was from the Deutsche Sammlung fuer Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). All PCa cell lines were cultured in RPMI 1640 phenol red-free medium (Sigma) supplemented with P/S and 10% FBS (Invitrogen).

Collection of CM

WPMY-1 cells and PrSC were seeded in 75 cm2 flasks in DMEM with 2.5% charcoal-dextran treated FBS (CDS, Invitrogen) and grown for 48 h. Then the medium was changed to serum-free DMEM/F12 with 10 μg/ml transferrin (Transf), 5ng/ml sodium selenite, 40 μg/ml L-proline, 1% nonessential amino acids, and 100 U/ml penicillin and 100 g/ml streptomycin. The cells were cultured in this medium with only ethanol (used as vehicle), 10 nm E2, or 10 nm TGFβ1-neutralizing antibody (BD Biosciences, San Jose, CA) for 2 h. Then the cells were stimulated or not with 10 nm E2. The conditioned media were collected, centrifuged, and named as CM-E2, CM-TGFβ1, and CM-PT, respectively. Similarly, after 1 h pretreatment with 100 nm ICI 182,780 (Sigma), WPMY-1 and PrSC were stimulated or not with 10 nm E2. The conditioned media were collected, centrifuged, and named as CM-E2-ICI and CM-ICI. After collecting the CM, the two types of stromal cells were trypsinized and counted. All CM volumes used in subsequent experiments were normalized to the same cell number.

MMP2 and TGFβ1 neutralization in the CM

CMs were depleted of MMP2 by immunoprecipitation of the MMP2 by using MMP2 antibody from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Protein G Plus/Protein A-Agarose from Calbiochem (Merck, Hamburg, Germany). Immunoprecipitation was performed according to the manufacturer’s instructions with 10 μg antibody for 1 ml CM. The CMs, after immunoprecipitation with nonspecific IgG or anti-MMP2 antibody, were named as CM-con-IgG, CM-con-MMP2 Ab, CM-E2-IgG, CM-E2-MMP2 Ab, CM-PPT-IgG, or CM-PPT-MMP2 Ab. In other experiments, WPMY-1 and PrSC were pretreated with 50 μg/ml TGFβ1-neutralizing antibody (BD Biosciences, San Jose, CA) for 2 h. Then the cells were stimulated or not with 10 nm E2 in the presence of the antibody for another 48 h. The CMs were collected and named as CM-con-TGFβ1Ab, CM-E2-TGFβ1 Ab, and CM-PPT-TGFβ1 Ab (29).

Small interfering RNA (siRNA) transfection

All siRNAs used were ordered from GenePharma (Shanghai, China). For siRNA transfection, WPMY-1 cells were seeded at 200,000 cells per six wells and allowed to attach overnight. Twenty-four hours before transfection, the medium was changed into phenol red-free DMEM with 2.5% CD5. Then the cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions.

RNA extraction and real-time RT-PCR

Total RNA was prepared from WPMY-1 and PrSC using Trizol reagent (Invitrogen) as described previously (30). RT was performed at 37°C for 2 h. The 20-μl reaction mix included 1 μg RNA, 0.5 mM dNTPs, 5 μM random hexamers, 10 mM DTT, 200 μM Moloney murine leukemia virus reverse transcriptase (M-MLV-RT), and 20 U ribonuclease inhibitor (all from Promega). SYBR Green I-based real-time quantitative PCR was carried out on a MJ Research DNA Engine Opticon Continuous Fluorescence Detection System (Opticon Monitor II, Bio-Rad, Hercules, CA) as described previously (31). The relative expression of each gene was determined using the comparative Crt method. The expression units of MMP2 and TGFβ1 were normalized to the housekeeping gene hypoxanthine-guanine phosphoribosyl transferase. The following primers were synthesized by Sangon (Shanghai, China): MMP2: (F) 5'-AGGGCGCTCTGTCTCCTGGG-3', (R)
Western blot

After 48-h culturing of WPMY-1 cells and PrSC in DMEM phenol red–free media with 2.5% CDS, the medium was changed to DMEM/F12 serum-free medium containing 10 μg/ml transferrin. Then 10 nM E2, 10 nM PPT (Sigma), and 100 nM ICI were added to the culture and cells were maintained for another 48 h. The CMs of the stromal cells were collected, lyophilized, and redissolved in RIPA buffer. Protein was quantified using the bicinchoninic acid method. Equal amounts of protein (30 μg) were loaded to each well of a polyacrylamide gel. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane. The membrane was stained with Ponceau S and scanned. As the serum-free medium contained 10 μg/ml transferrin, this protein could be stained clearly and used as loading control of CM. Then the membrane was blocked with 4% skim milk for 1 h and incubated with primary antibody of MMP2 (1:400, Santa Cruz) at 4 C overnight. After washing three times with Tris-buffered saline with 0.1% Tween-20, the membrane was incubated with the secondary antibody (goat-antirabbit horseradish peroxidase-conjugated, 1:5000, Bio-Rad) at roomtemperature for 1 h. After the treatment with enhanced chemiluminescence reagent (PIERCE, Rockford, IL), the membrane was exposed to an x-ray film (Kodak, Rochester, NY) and analyzed by densi-
Gelatin zymography
The CM of WPMY-1 and PrSC was collected, lyophilized, and redissolved in RIPA buffer. Thirty micrograms of total protein was loaded on a 10% polyacrylamide gel containing 0.1% gelatin. After electrophoresis, the gel was washed for 1 h in 2.5% (vol/vol) Triton X-100 to remove SDS and then incubated overnight at 37 °C in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5 mM ZnCl₂, and 10 mM CaCl₂ to allow proteolysis of the gelatin substrate. Bands corresponding to activity were visualized by negative staining using 0.5% Coomassie brilliant blue R-250 (Bio-Rad).

Transwell assay
For invasion assays, the transwell inserts with 8-μm pore size (BD Biosciences) for 24-well plates were coated with 50 μl Matrigel (BD Biosciences). PCa cells were seeded in the upper chamber at 500,000 cells per well in DMEM/F12 serum-free medium with 5 ng/ml Mitomycin C to inhibit cell growth. The CM was added in the bottom chamber. Twenty-four hours later the cells on the upper surface of the filter were removed using a cotton swab. Cells that had invaded through the ECM and the filter to the lower surface were fixed with 4% paraformaldehyde and stained with 10% crystal violet. Five fields of each chamber were randomly selected and the cell numbers were counted under the microscope.

Enzyme-linked immunosorbent assay
WPMY-1 cells were seeded in six-well plates and grown in 2.5% CDS for 48 h. Then the medium was changed to DMEM/F12 serum-free medium. E₂ and specific inhibitors were added to the cells for another 48 h. The conditioned media were collected and cells were trypsinized and counted. TGFβ1 and MMP2 concentrations in the CM were determined by ELISA kit (BOSTER, Wuhan, China) according to the manufacturer’s instructions.

Statistical analysis
The data were analyzed with the statistical software SPSS 16.0 (Chicago, IL). Differences among groups were assessed using ANOVA, and the least significant difference test was used to compare the differences between every combination of two groups. Differences between two groups were assessed using Student’s t test. A P value less than 0.05 was considered statistically significant.

Results
E₂ up-regulates MMP2 expression in both WPMY-1 and PrSC and stimulates PCa cells invasion by a paracrine mechanism
To examine the effect of CM from E₂-treated WPMY-1 and PrSC cells on the invasion of different PCa cells, we treated the PCa cells with CM-con, CM-E₂, CM-ICI, and CM-E₂-ICI from the two stromal cell types in Matrigel transwell assays. We used a series of prostate epithelial cell lines including the highly invasive malignant PCa cell lines PC3, DU145, and the less invasive malignant PCa cell line LNCaP as model, the basal invasive capacities of the PCa cells are shown in the Supplemental Fig. 2. The results showed that after culturing for 24 h with the CM-E₂ invasion of all the prostate epithelial cells was significantly promoted compared with CM-con, while ICI pretreatment inhibited the effect of CM-E₂ (P < 0.01; Fig. 1, A–C).

To characterize the component in CM from stromal cells that promoted the invasion of PCa cells, the CM was boiled. The boiled CM-E₂ lost the ability to pro-
mote invasion of PC3 and LNCaP (Fig. 1, D and E). These results suggested that heat-sensitive components in the CM-E2, most likely proteins, stimulated the invasive ability of PCa cells. We hypothesized that the heat-sensitive factor is MMP2, because WPMY-1 cells express high level of MMP2, which plays an important role in PCa metastasis. We also examined the effect of E2 on MMP2 expression in WPMY-1 and PrSC. We found that E2 up-regulated MMP2 expression at both mRNA and protein level in WPMY-1 and PrSC (Fig. 2, A–C). Next, we used immunoprecipitation with an anti-MMP2 polyclonal antibody to deplete MMP2 from the CM of the two cell types (Fig. 2D). We found that the MMP2-deficient CM lost the stimulatory effect on the invasion of PC3 cells (Fig. 2E).

**Mechanism of E2-induced MMP2 expression in WPMY-1 cells**

**E2-induced MMP2 expression is mediated by ERα**

Next we aimed to investigate the mechanism by which E2 induces MMP2 expression in PrSC. The results showed that

![Graph](https://example.com/graph1.png)

**FIG. 3.** E2 and ERα agonist PPT up-regulate MMP2 expression in WPMY-1 cells. A, Real-time RT-PCR analysis of MMP2 mRNA expression in WPMY-1 cells treated with E2 and ICI. Results are presented as mean ± SD, n = 3. **, P < 0.01 vs. controls. B, Western blot analysis of MMP2 concentrations in CM collected from WPMY-1 cells treated with E2 and ICI. Thirty micrograms of protein were loaded per lane. Transferrin present in the medium served as loading control. C, Gelatin zymography analysis of MMP2 activity in CM collected from WPMY-1 cells treated with E2 and ICI. Twenty-five micrograms of protein were loaded per lane. D, Real-time RT-PCR analysis of MMP2 mRNA expression in WPMY-1 cells treated with E2 or PPT. Results are presented as mean ± SD, n = 3. **, P < 0.01 vs. control. E, Western blot analysis of MMP2 concentrations in CM collected from WPMY-1 cells treated with E2 or PPT. Thirty micrograms of protein were loaded per lane. F, Gelatin zymography analysis of MMP2 activities in CM collected from WPMY-1 cells treated with E2 or PPT. Twenty-five micrograms of protein were loaded per lane.

E2 significantly increased MMP2 mRNA expression in WPMY-1 cells (P < 0.05; Fig. 3A). Pretreatment of the cells with the ER inhibitor ICI 182,780 blocked E2-induced MMP2 expression. E2 treatment also increased MMP2 protein expression and activity in the CM of WPMY-1 cells (P < 0.01; Fig. 3, B and C). These observations suggest that the effect of the E2 on MMP2 expression is mediated by ER. The ERα-selective agonist PPT had a similar effect as E2 on MMP2 expression (P < 0.01; Fig. 3, D–F). WPMY-1 cells express two types of estrogen receptors: ERα and a membrane-bound G protein–coupled Receptor 30 (GPR30) (Supplemental Fig. 3). To rule out the role of GPR30, the siRNA of each receptor was transfected into

![Graph](https://example.com/graph2.png)

**FIG. 4.** ERα siRNA inhibits E2 induced up-regulation of MMP2 expression in WPMY-1 cells. A, Western blot analysis of the effect of siRNA knockdown of ERα and GPR30 expression. B, Real-time RT-PCR analysis of MMP2 mRNA expression in WPMY-1 cells transfected with different siRNAs and treated with E2. Results are presented as mean ± SD, n = 3. **, P < 0.01 vs. normal cultured WPMY-1 cells. C, Western blot analysis of MMP2 levels in CM collected from WPMY-1 cells transfected with different siRNAs and treated or not with E2. Thirty micrograms of protein were loaded per lane. Transferrin present in the medium served as loading control. D, Gelatin zymography analysis of MMP2 activities in CM collected from WPMY-1 cells transfected with different siRNAs and treated or not with E2. Twenty-five micrograms of protein were loaded per lane.
WPMY-1 cells. Three different siRNAs for each gene were tested in the knockdown experiments, all having a similar inhibiting effect (Supplemental Fig. 4). Only ERα siRNA transfection blocked E2-induced MMP2 expression and activity (\(P < 0.01\); Fig. 4, A–D), thereby confirming the role of ERα.

**E2 up-regulates TGFβ1 expression in WPMY-1 cells via ERα**

A, Real-time RT-PCR analysis of TGFβ1 and MMP2 mRNA expression in WPMY-1 treated with E2 for different time periods. B, ELISA analysis of TGFβ1 and MMP2 concentration in CM collected from WPMY-1 treated with E2 for different time periods. C, Real-time RT-PCR analysis of TGFβ1 mRNA expression in WPMY-1 cells treated with E2 or PPT for 8 h. D, Real-time RT-PCR analysis of TGFβ1 mRNA expression in WPMY-1 cells treated with 10 nM E2 and 100 nM ICI for 8 h. E, ELISA analysis of TGFβ1 concentration in CM collected from WPMY-1 treated with 10 nM E2 or 10 nM PPT, and 100 nM ICI for 16 h. Results are presented as mean ± SD, \(n = 3\). **, \(P < 0.01\).

**FIG. 5.**

**E2 induces MMP2 expression via induction of TGFβ1**

TGFβ1 is implicated in regulation of phenotypic modulation, ECM accumulation, and invasion of PCa. We first did a time course experiment to examine both TGFβ1 and MMP2 mRNA and protein expression levels. We found that after E2 treatment, up-regulation of both TGFβ1

**FIG. 6.**

**E2 induces MMP2 expression via induction of TGFβ1**

A, Treatment of CM from E2 or PPT-stimulated WPMY-1 cells with a TGFβ1-neutralizing antibody reduces significantly the TGFβ1 levels of CM. B, Real-time RT-PCR analysis of MMP2 mRNA expression in WPMY-1 cells treated with 10 nM E2, 10 nM PPT, and 30 ng per 10^6 cell TGFβ1 with and without pretreatment of the cells with a TGFβ1 neutralizing antibody. Results in A and B are presented as mean ± SD, \(n = 3\). **, \(P < 0.01\). C, Western blot analysis of MMP2 concentrations in CM from WPMY-1 cells after different treatments. Thirty micrograms of protein were loaded per lane. Transferrin present in the medium served as loading control. D, Gelatin zymography analysis of MMP2 activity in CM from WPMY-1 cells after different treatments. Twenty-five micrograms of protein were loaded per lane.
mRNA and protein occurred much earlier compared with MMP2; this applies particularly for mRNA up-regulation (Fig. 5, A and B). We further observed that the TGFβ1 increase in WPMY-1 cells was mediated through ERα because treatment with E2 and PPT caused a similar induction which could be prevented by ICI 182,780 (Fig. 5, C–E). We therefore hypothesized that E2 may induce MMP2 expression via TGFβ1. This was confirmed by an experiment in which WPMY-1 cells were pretreated with a TGFβ1-neutralizing antibody before addition of E2, PPT, or TGFβ1, which showed that MMP2 can be induced by E2 and PPT as well as TGFβ1, the MMP2 levels in the antibody-treated CM being similar to those observed in control CM (Fig. 6, A–D). Treatment of CM from E2 and PPT-stimulated cells with a TGFβ1-neutralizing antibody reduced TGFβ1 levels to control levels (Fig. 6A) and MMP2 up-regulation by E2, PPT, and TGF β1 was also inhibited to control values by pretreatment with TGFβ1 neutralizing antibody (Fig. 6B). From these data it can be concluded that MMP2 induction by E2 was mediated by TGFβ1 and that the process is triggered by activation of ERα.

E2 induces MMP2 expression in WPMY-1 and PrSC cells via induction of TGFβ1 and stimulates PCa cells invasion in paracrine manner

Finally, we investigated the effects of blocking TGFβ1 or MMP2 on the invasion of PCa cells. The CM from WPMY-1 and PrSC were treated with neutralizing antibodies to MMP2 or TGFβ1. The CM from E2 and PPT stimulated cells treated with neutralizing antibody lost the ability to promote invasion of PC3 and LNCaP cells (Fig. 7, A and B). This data suggests that prostate stromal cells treated with E2 produce TGFβ1, and TGFβ1 up-regulates MMP2 expression and secretion in PrSC, which enhances the invasion of PCa cells in a paracrine manner (Fig. 8).

Discussion

The prostate has a glandular texture and is mainly composed of epithelial and stromal components. Stroma–epithelium interaction in the prostate has been documented to constitute a determining process of prostate function in recent years (32–34). Prostate stromal compartment plays a critical role in stroma–epithelium interaction by secreting growth factors and ECM components. In PCa these components are involved in the invasion and metastasis.

Results of the present study demonstrated that the CM of prostate stromal cells treated with E2 significantly increased the invasion of PCa cells and that MMP2 participated in this process. MMP2 is a well-known MMP related to cancer cell invasion and metastasis; its overexpression can be induced by cytokines, growth factors, and oncogenes (35–36). In the Noble rat model, after long time androgen and estrogen treatment, MMPs are up-regulated in the fibromuscular layer surrounding the prostatic adenomas and adenocarcinomas, and the epithelial basement membranes are degraded (37). In the present study, we...
provide evidence that E₂ up-regulates MMP2 expression indirectly by stimulating TGFβ1 expression in prostate stromal cells, and the stimulation of PCa cell invasion by E₂ using PC3, DU145, and LNCaP cells as model is mediated by a complex paracrine mechanism.

TGFβ1 is a multifunctional cytokine that is implicated in cell proliferation, apoptosis, phenotypic modulation, invasion, and metastasis (38–39). TGFβ1 can be regulated by many hormones in human prostate. In this study, we demonstrated that E₂ up-regulated TGFβ1 expression in WPMY1, and E₂ induced MMP2 expression in both WPMY1 and PrSC via TGFβ1 in an autocrine manner. These results suggested a novel role of hormone and growth factor in regulating expression of a key MMP that is involved in modulating extracellular matrix accumulation and PCa cell invasion.

Myofibroblasts are important components of the prostate stroma. The mutual interaction through direct cell–cell contacts or paracrine signals between cancer cells and myofibroblasts is essential for invasive growth and is translated into a poor clinical prognosis (40). It has been shown that loss of ERα prevented hormonal carcinogenesis and invasion (9). However, it was unclear whether stromal or epithelial ERα is responsible for the hormonal carcinogenesis and invasion processes. In the present study, we demonstrated the importance of ERα of stromal cells in regulating PCa invasion.

In conclusion, we show in this study that estrogens up-regulate MMP2 through ERα in prostate stromal cells, and the induction of MMP2 enhances the invasion of PCa cells in a paracrine manner. These results provide novel evidence of estrogen effect on PCa invasion via stroma–epithelial interaction.

Acknowledgments

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