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ERG oncogene modulates prostaglandin signaling in prostate cancer cells

Ahmed A. Mohamed,[1] Shyh-Han Tan,[1] Chen Sun,[1] Syed Shaheduzzaman,[1] Ying Hu,[1] Gyorgy Petrovics,[1] Yongmei Chen,[1] Isabell A. Sesterhenn,[2,3] Hua Li,[1] Taduru Sreenath,[1] David G. McLeod,[1,3,4] Albert Dobi[1,3,*] and Shiv Srivastava[1,3,*]

[1]Center for Prostate Disease Research; Department of Surgery; Uniformed Services University of the Health Sciences; Rockville, MD USA; [2]Department of Genitourinary Pathology; Armed Forces Institute of Pathology; [3]US Military Cancer Institute; [4]Center for Prostate Disease Research; Urology Service; Walter Reed Army Medical Center; Washington, DC USA

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Abbreviations: CaP, prostate cancer; *ERG, ETS*-related gene; *HPGD,* 15-hydroxy-prostaglandin dehydrogenase; AR, androgen receptor; PGE_2, prostaglandin E_2; EP4, E prostanoid receptor type 4; uPA, urokinase-type plasminogen activator; COX-2, cyclooxygenase 2; IL-1β, interleukin 1beta; ChIP, chromatin immunoprecipitation; LCM, laser capture micro-dissection; BrdU, bromodeoxyuridine; DAB, 3'3'-diaminobenzidine

Androgen dependent induction of the ETS related gene (*ERG*) expression in more than half of all prostate cancers results from gene fusions involving regulatory sequence of androgen regulated genes (i.e., *TMPRSS2, SLC45A3 and NDRG1*) and protein coding sequence of the *ERG*. Emerging studies in experimental models underscore the functions of ERG in prostate tumorigenesis. However, biological and biochemical functions of *ERG* in prostate cancer (CaP) remain to be elucidated. This study suggests that *ERG* activation plays a role in prostaglandin signaling because knockdown of ERG expression in *TMPRSS2-ERG* fusion containing CaP cells leads to altered levels of the 15-hydroxy-prostaglandin dehydrogenase (HPGD), a tumor suppressor and prostaglandin catabolizing enzyme and prostaglandin E_2 (PGE_2). We demonstrate that *HPGD* expression is regulated by the binding of the ERG protein to the core promoter of this gene. Moreover, prostaglandin E_2 dependent cell growth and urokinase-type plasminogen activator (uPA) expression are also affected by ERG knockdown. Together, these data imply that the ERG oncoprotein in CaP cells positively influence prostaglandin mediated signaling, which may contribute to tumor progression.

Introduction

Prevalent gene fusions involving regulatory sequences of androgen receptor (AR) regulated prostate associated genes (predominantly *TMPRSS2*) and protein coding sequences of the nuclear transcription factors in the ETS transcription family (predominantly *ERG*) result in overexpression of *ERG* in two-thirds of prostate cancer (CaP) patients.[1-13] Emerging studies in experimental models suggest oncogenic functions of *ERG* and *ETV1* in CaP.[6,14-17] Our earlier report suggested a regulatory role of the ERG oncoprotein in prostate epithelial differentiation program and activation of *C-MYC* in CaP cells.[5] Recent studies in mouse models show cooperative effects of *ERG* overexpression and the PI3-Kinase pathway in CaP progression.[14,16,18] Thus, a better understanding of *ERG* functions in CaP biology may lead to rational therapeutic strategies for *ERG* positive tumors.

During our recent evaluation of *ERG* downstream transcriptional targets, we noted consistent induction of the 15-hydroxy-prostaglandin dehydrogenase gene (*HPGD*) in response to ERG knockdown.[5] HPGD is an important enzyme in prostaglandin metabolism that catalyzes the oxidation of prostaglandins into inactive keto-metabolites. HPGD physiologically antagonizes COX-2, a prostaglandin-synthesizing enzyme, thus playing a critical role in diverse physiological aspects ranging from inflammation to cancer.[19,20] Recent studies have indicated that *HPGD* is downregulated in a majority of lung, colon, breast and bladder cancers. Tumor suppressor functions of *HPGD* have been demonstrated in cell culture and mouse models.[21-26] Moreover, accumulating evidence suggests the involvement of HPGD in chemopreventive effects of non-steroid anti-inflammatory drugs (NSAIDs). Several NSAIDs, including Celecoxib, Indomethacin and Flurbiprofen, exert their anti-inflammatory effects by inducing *HPGD* or inhibiting COX-2.[27-29] However, other studies have shown *HPGD* involvement with cell differentiation and immune regulation.[30,31] Due to these diverse functions of *HPGD* and the suggested roles of inflammation in prostate cancer,[32] we have focused on the regulation of *HPGD* and related signaling events in the context of *TMPRSS2-ERG* fusion in prostate cancer cells.

*Correspondence to: Albert Dobi and Shiv Srivastava; Email: adobi@cpdr.org and ssrivastava@cpdr.org
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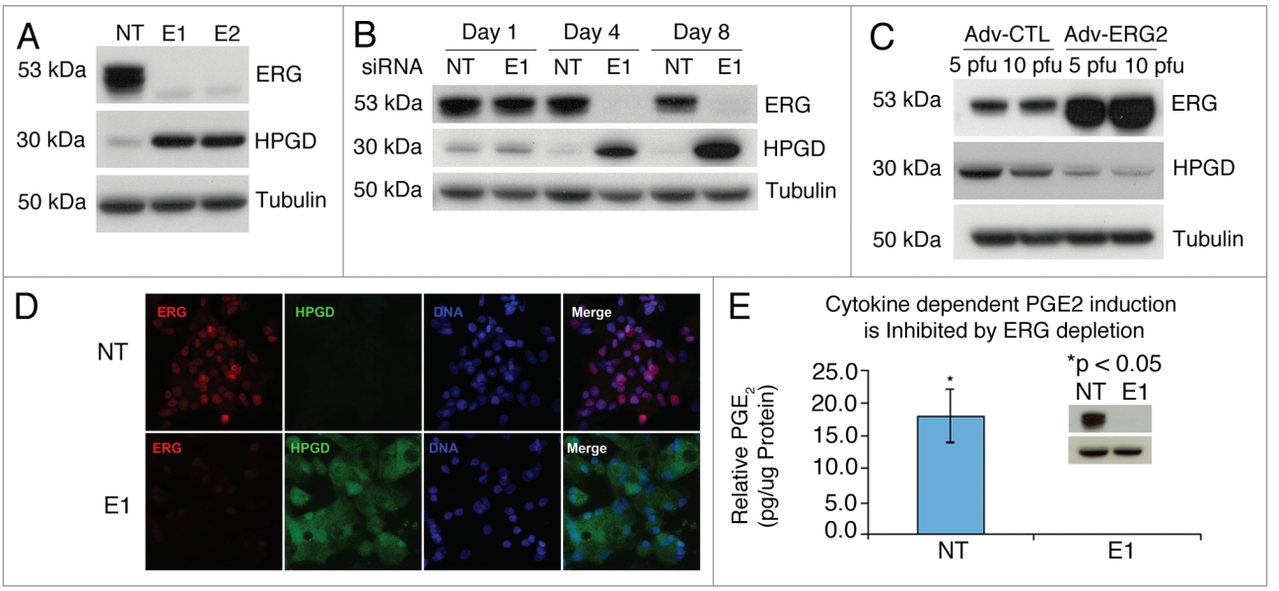

Figure 1. ERG regulates HPGD expression and PGE_2 in VCaP cells. (A) VCaP cells transfected with ERG siRNA (E1, E2) or with non-targeting siRNA (NT) from triplicate experiments were harvested on day 4 post transfection and processed for immunoblot analysis for detecting ERG and HPGD. Tubulin expression was used as the input control. (B) Induction of HPGD expression in response to ERG knockdown in a time dependent fashion. Transfected VCaP cells, as described in the Materials and Methods, were harvested on days 1, 4 and 8 post-transfection and processed for immunoblot assay for detecting ERG and HPGD proteins. (C) Ectopic expression of ERG decreased HPGD protein levels. VCaP cells were infected with either wild type ERG2 adenovirus expression vector (Adv-ERG2) or control adenovirus expression vector (Adv-CTL). (D) Sub-cellular localization of ERG and HPGD in response to ERG siRNA (E1) or non-targeting siRNA (NT) was assessed by immunofluorescence assay in VCaP cells. (E) PGE_2 levels were measured in the conditioned medium of VCaP cells transfected with either non-targeting siRNA (NT) or ERG siRNA (E1) in the presence of IL-1β. Cells were harvested after 24 h and total lysate were prepared for PGE_2 normalization (insert).

Results

HPGD expression is upregulated in response to ERG inhibition. Evaluation of ERG siRNA (E1, E2) treatment in the *TMPRSS2-ERG* expressing human prostate cancer cell line (VCaP cells) revealed robust upregulation of HPGD (**Fig. 1A and B**). Consistent with this observation, VCaP cells infected with an adenovirus vector expressing wild type ERG-2 (Adv-E2) inhibited HPGD protein expression (**Fig. 1C**). Further, immunofluorescence staining showed that cells expressing siRNA to ERG showed a robust reduction of ERG transcription factor in the nuclei of VCaP cells as well as an overexpression of cytoplasmic HPGD (**Fig. 1D**).

Cytokine mediated PGE_2 induction is inhibited by ERG knockdown. To assess the effect of ERG inhibition on prostaglandin E_2 (PGE_2), VCaP cells transfected with ERG siRNA (E1) or non-targeting siRNA (NT) were analyzed for Interleukin-1beta (IL-1β) induced PGE_2. PGE_2 was significantly inhibited in ERG siRNA transfected VCaP cells in comparison to the control NT siRNA transfected VCaP cells (**Fig. 1E**).

ERG is recruited to the core promoter of *HPGD*. An ETS binding site was identified in the core promoter at -200 bp upstream of the *HPGD* transcription initiation site by using MatInspector software (Genomatix GmbH, Munich, Germany), consistent with earlier reports showing the presence of ETS transcription factor binding sites within the *HPGD* promoter upstream sequences.[33,34] Chromatin immunoprecipitation assays (ChIP) confirmed the specific recruitment of the ERG oncoprotein to the predicted ETS site of the *HPGD* core promoter, which was significantly reduced in ERG siRNA treated VCaP cells (**Fig. 2**). The observations of ERG-induced alterations in *HPGD* gene expression, along with the recruitment of ERG to the *HPGD* promoter suggested that ERG directly regulates *HPGD* expression in prostate tumor cells.

PGE_2 dependent cell growth is abrogated by depletion of ERG. Because ERG knockdown resulted in the overexpression of HPGD, which is known to metabolize PGE_2 into inactive keto-metabolites, we hypothesized that ERG knockdown would lead to inhibition of PGE_2 associated biological and biochemical functions. PGE_2 has been shown to induce growth in prostate cancer cell culture models.[35] VCaP cells were transfected with either of ERG siRNA or NT siRNA in the presence or absence of PGE_2. As expected, PGE_2 treatment increased the incorporation of Bromodeoxyuridine (BrdU) into the nucleus of control NT siRNA transfected VCaP cells. In contrast, significantly less BrdU incorporation was observed in ERG siRNA treated cells (**Fig. 3A and B**). These findings indicate that PGE_2 mediated cell growth is inhibited when ERG is depleted from prostate cancer cells.

ERG knockdown reduced PGE_2 receptor 4 (E prostanoid receptor type 4, EP4) expression. To further investigate ERG involvement in the prostaglandin signaling, we evaluated the relationship between ERG and the PGE_2 receptor, EP4. ERG depletion decreased EP4 protein expression in VCaP cells, indicating

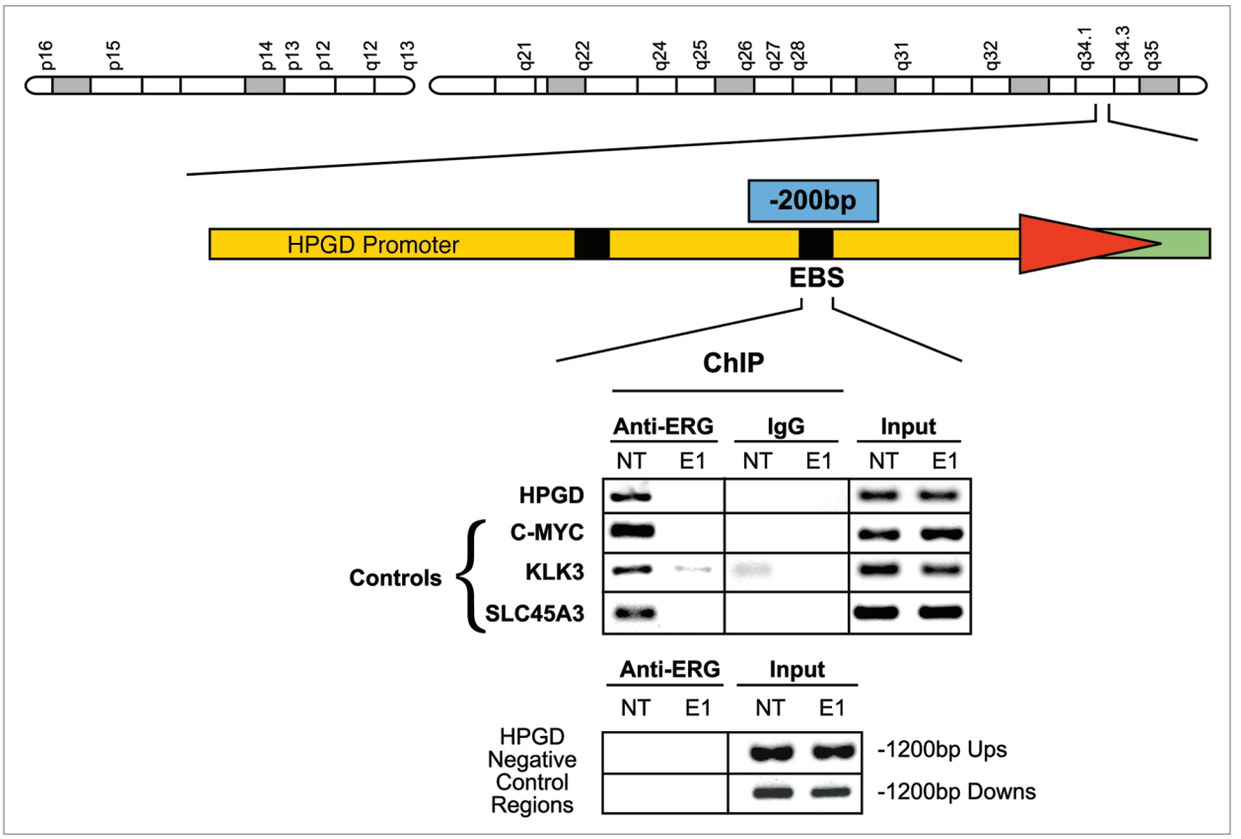

Figure 2. ERG is recruited to the HPGD core promoter ETS binding site in VCaP cells. ERG recruitment is specific to the core ETS binding site of HPGD and is eliminated by ERG siRNA treatment. Upstream and downstream sequences with no ETS binding element were used as negative controls. In the ChIP assay recruitment of ERG to the KLK3/PSA, C-MYC and SLC45A3 gene promoter upstream regions were also tested as positive controls of ERG binding as similar data have been reported before (controls).[5] Input indicates control genomic DNA amplicons.

that ERG does affect EP4, a key regulator of the prostaglandin pathway (**Fig. 4A**).

PGE_2 induced urokinase-type plasminogen activator (uPA) expression in prostate cancer cells is inhibited by ERG knockdown. Urokinase-type plasminogen activator (uPA) mediated signaling has been implicated in tumor cell invasion, survival and metastasis in a variety of cancers.[36,37] PGE_2 has been reported to increase cell growth, induce uPA expression and enhance tumor metastasis and angiogenesis in prostate cancer.[38] Studies evaluating *ERG* functions have also shown that *ERG* induces cell invasion associated genes including uPA.[15,17] To determine the effect of ERG on PGE_2 induced uPA expression, *TMPRSS2-ERG* harboring VCaP cells were treated with PGE_2. Expression of uPA protein in response to PGE_2 treatment was inhibited by ERG knockdown (**Fig. 4B**).

HPGD expression is attenuated in TMPRSS2-ERG fusion positive prostate cancer specimens. HPGD expression is down-regulated in many solid tumors.[22,23] On the basis of our data, we hypothesized that *HPGD* expression will be decreased in CaP cells harboring the *TMPRSS2-ERG* fusion. We compared *HPGD* expression levels between *TMPRSS2-ERG* positive and negative prostate tumor specimens in 28 patients. For this analysis, we selected well-to-moderately differentiated tumor specimens in order to minimize potentially confounding genetic alterations associating with more advanced tumors with poorly differentiated phenotypes. Laser capture micro-dissection (LCM) derived RNA samples were evaluated for *HPGD* expression levels. The results, although not reaching statistical significance, revealed a trend towards decreased *HPGD* RNA expression in *TMPRSS2-ERG* positive tumors, which is consistent with the reciprocal relationship of *HPGD* expression observed in response to ERG knockdown in VCaP cells (**Fig. 5**). A more uniform suppression of the *HPGD* gene was found in the fusion positive tumor cells while the fusion negative tumor cells showed heterogeneity of expression with most of the samples showing higher *HPGD* expression.

Discussion

It is now established that *ERG* expression in CaP is activated by gene fusions involving androgen regulated promoter sequences, such as, *TMPRSS2, SLC45A3, NDRG1* and the ERG protein coding sequence, which represent one of the most common oncogenic alterations in CaP.[1,2,8,39,40] However, the biochemical mechanisms by which elevated ERG contributes to the development and/or progression of CaP needs further clarification. Our findings showing that *ERG* alterations may influence the components of the prostaglandin signaling pathway in CaP cells; *HPGD* in particular, suggest a new biological function of *ERG* in prostate cancer cells.

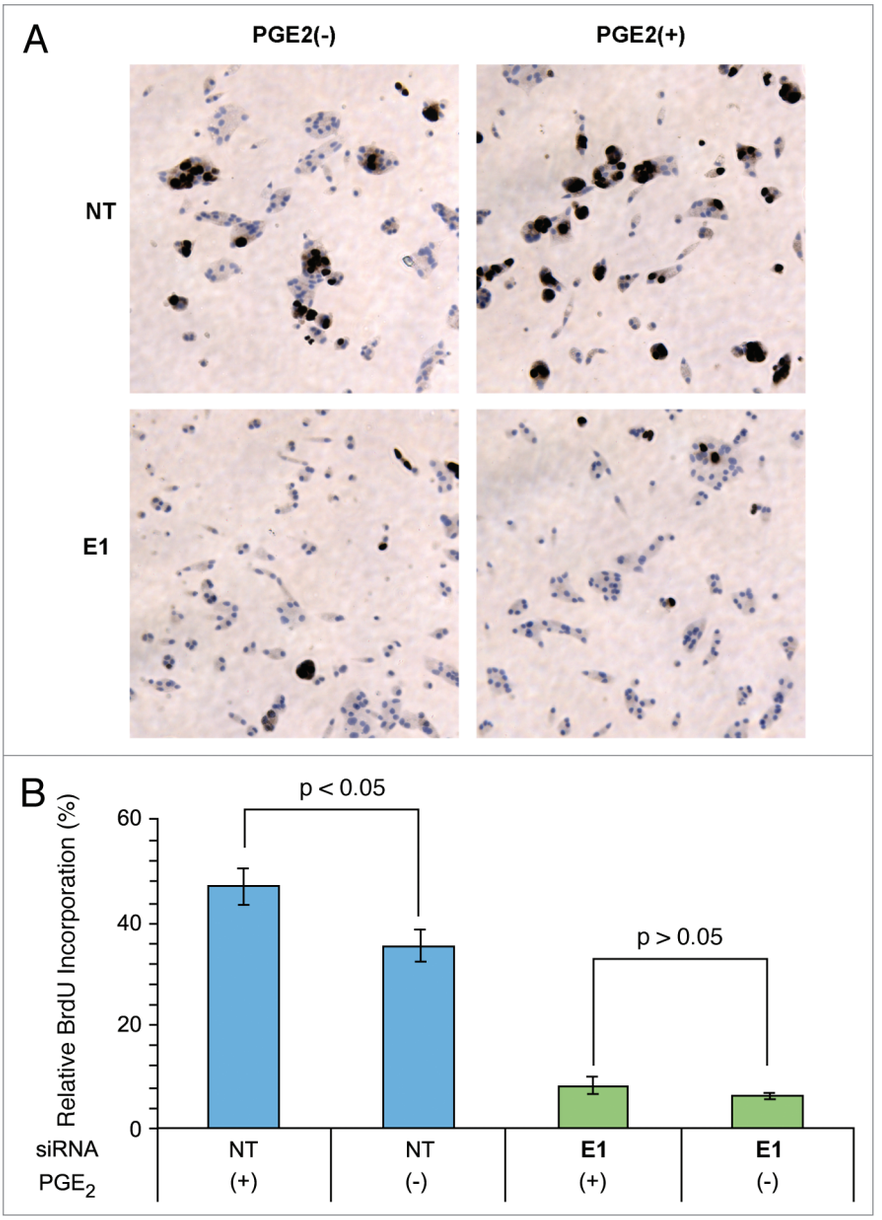

Figure 3. PGE_2 induced CaP cell growth is inhibited by ERG knockdown. VCaP cells were evaluated for cell growth in the presence (+) or absence (-) of PGE_2 in response to non-targeting siRNA (NT) or ERG siRNA (E1). (A) BrdU nuclear staining of proliferating cells. In the presence of PGE_2, VCaP cells with control NT siRNA showed dramatically increased BrdU nuclear staining in response to PGE_2 treatment. In contrast, ERG siRNA transfected VCaP cells show significantly decreased BrdU nuclear staining both in the presence or absence of PGE_2. (B) Relative percent of BrdU positive cells. In control NT siRNA transfected cells, BrdU incorporation is higher in PGE_2 treated cells than in the untreated group. In contrast, BrdU incorporation is low in both ERG siRNA treated groups irrespective to PGE_2 treatment.

On the basis of data presented in this study we propose that elevated expression of *ERG* leads to decreased *HPGD*, increased prostaglandin E_2 and its EP4 receptor; the cumulative effects of these changes may contribute to increased tumor cell growth and invasion (**Fig. 6**). Signal transduction pathways such as C-MYC, the PI3K/PTEN/AKT axis or AR-mediated signaling may also influence *ERG* functions in CaP progression as defined by recent studies.[5,14,16,18,41,42] Our study shows that *ERG* regulates the expression of *HPGD*, suggesting that *ERG* plays a role in prostate tumorigenesis in part through modulation of prostaglandin signaling pathway. We have previously reported that *ERG* activates *C-MYC* and abrogates differentiation genes (*KLK3*/PSA, *MSMB, SLC45A3*).[5] Although the role of *HPGD* in prostate cells differentiation is unclear, it is intriguing that other reports have shown a role of *HPGD* regulating cell differentiation.[30]

Several biological processes that are important in tumorigenesis such as angiogenesis, cell proliferation and motility, inhibition of the immune responses and apoptosis are known to be regulated by prostaglandins, specifically PGE_2.[43] Steady state cellular levels of PGE_2 depend on the relative rates of COX-2/PGE synthase dependent biosynthesis and HPGD dependent degradation.[25,43] Inflammation, as well as alterations in enzymes involved in prostaglandin synthesis or degradation have been suggested to play roles in CaP.[22,23,32,35,44,45] Our study suggests the biological potential for *ERG* activation to interfere with prostaglandin signaling and the associated physiological context such as inflammation, suspected of fueling prostate tumorigenesis.[32,44] It is also important to point out that in contrast to HPGD, ERG does not affect COX-2 expression in ERG siRNA treated VCaP cells (data not shown).

In summary, this study establishes that ERG knockdown results in overexpression of *HPGD*, downregulation of EP4, inhibition of PGE_2 induction of cell growth and uPA expression, suggesting a role for the ERG oncoprotein in inflammation and prostate tumorigenesis. Given that *TMPRSS2-ERG* fusion occurs relatively early and is a common oncogenic activation in prostate tumorigenesis, ERG or ERG network components such as HPGD may be further evaluated as a target for prevention, as well as early therapeutic intervention in CaP.

Methods and Materials

Cell culture and treatment. Human prostate tumor cell line, VCaP, was purchased from American Type Culture Collection (ATCC). Cells were maintained in DMEM (cat.# 30-2002, ATCC), supplemented with 10% of fetal bovine serum (cat.#21640-079, Invitrogen) in a humidified CO_2 (5%) incubator at 37°C. Synthetic androgen analog, R1881 (cat.# NLP005005MG, Perkin Elmer) at 0.1 nM concentrations was used for androgen treatment. For prostaglandin E_2 treatment,

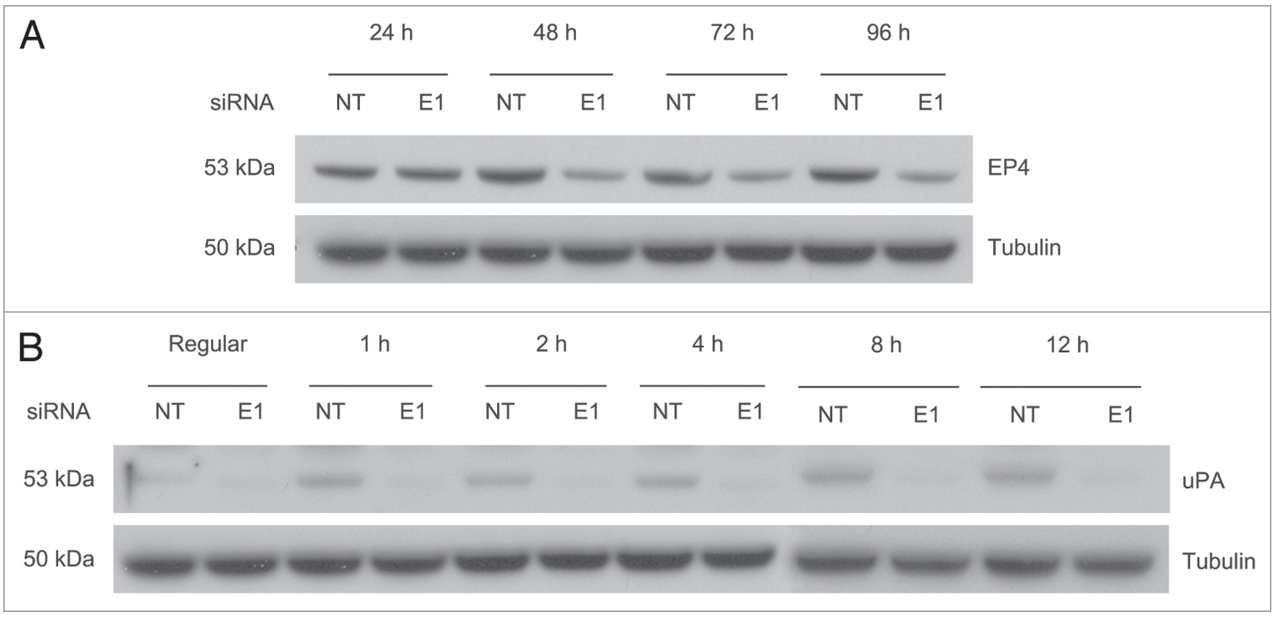

Figure 4. EP4 levels are reduced and uPA induction is inhibited in response to ERG knockdown. (A) EP4 protein levels were evaluated by immunoblot assays. Result shows that EP4 is downregulated in response to ERG knockdown. (B) Expression of uPA was assessed by immunoblotting at 1, 2, 4, 8 and 12 hours time points in VCaP cells treated with PGE_2 as described in the Methods and Materials. PGE_2 induces uPA in NT siRNA treated cells, whereas, ERG knockdown prevents uPA induction.

10 μM of prostaglandin E_2 (cat.# 14010, Cayman Chemical) was used. Five pico grams of IL-1β (cat.#19401-5UG, Sigma) was used for IL-1β treatment. Ten millimolar of BrdU was used for BrdU cell proliferation assay (cat.#550891, BD Biosciences).

Inhibition of endogenous ERG by siRNA. Small interfering RNA (siRNA) oligo duplex (5'-CGA CAU CCU UCU CUC ACA UAU-3' and 5'-UGA UGU UGA UAA AGC CUU A-3') against human ERG gene (E1, E2 respectively) (Gene ID: 2078, Accession: NM_004449) was purchased from Dharmacon. Two different siRNA each targeting *ERG* were used to assure that the effect we observed was specific and was not due to off target or none specific effect of siRNA (**Fig. 1A**). Since both siRNA showed identical results, one siRNA was selected and used in subsequent experiments. Non-targeting (NT) siRNA duplexes were used as control (D-001206-13-20; Dharmacon). Adenovirus expression vectors encoding the wild type protein products of *ERG* isoform 2 (Accessions: NM_004449.4) and adenovirus control vector were previously described in reference 5. VCaP cells were cultured in DMEM supplemented with 10% of Charcoal/Dextran stripped serum (cFBS), (cat.#100-119, Gemini Bio-Products) for 72 hours followed by transfection with 50 nM of NT siRNA or ERG siRNA using Lipofectamine 2000 (cat.#11668-109, Invitrogen). Twenty-four hours after transfection, cells were treated with 0.1 nM of R1881. Cells were harvested at days 1, 4 and 8 post treatments for western blot analysis. For ectopic expression of *ERG,* VCaP cells were infected with adenoviral *ERG* (Adv-ERG2) or control (Adv-CTL) expression vectors. Infected cells were incubated for 48 hours followed by cell harvesting and western blot analysis.

PGE_2 treatment. VCaP cells were transfected with either NT siRNA or ERG siRNA. Cells were treated with 10 μM of PGE_2 (cat.# 14010, Cayman Chemicals) at the time of R1881 treatment and cell growth medium were changed every two days with fresh DMEM containing 0.1 nM of R1881 and 10 μM of PGE_2. Cells were then harvested at days 1, 4 and 8 for cell growth assay. For assaying PGE_2 mediated signaling read-out, cells were

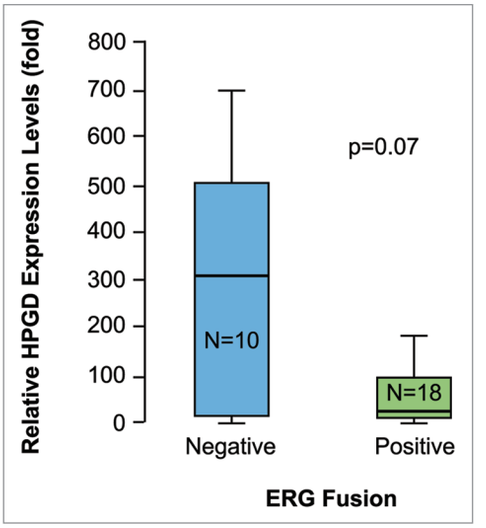

Figure 5. *HPGD* transcript levels are lower in *TMPRSS2-ERG* fusion positive prostate tumor epithelial cells than in fusion negative tumor cells. Total RNA isolated from LCM prostate epithelial tumor cells and VCaP cells were used for evaluation of *HPGD* and *GAPDH* expression. Tissue expression levels of *HPGD* normalized for *GAPDH* are shown in comparison to the normalized expression levels in VCaP cells. Evaluation of *HPGD* expression between *TMPRSS2-ERG* fusion positive (+) and negative (-) prostate tumors revealed a trend towards decreased *HPGD* RNA expression in fusion positive tumors.

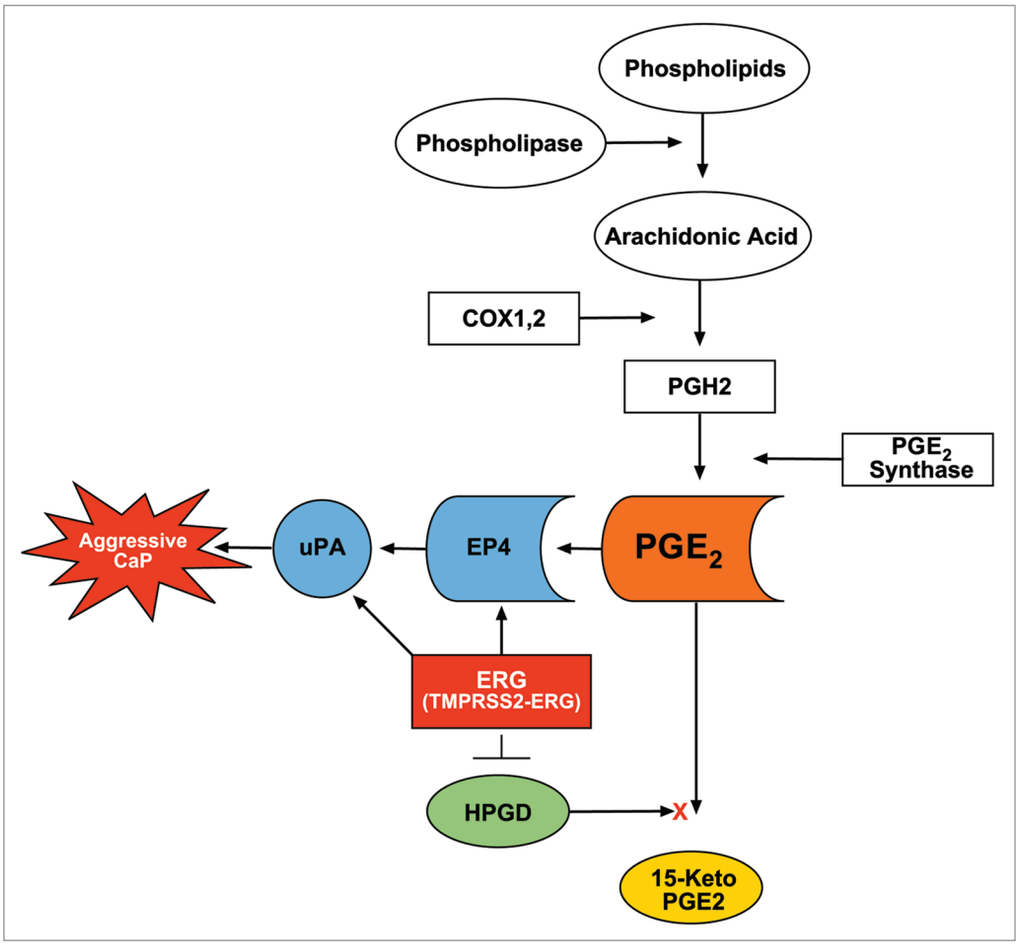

Figure 6. Proposed model for ERG functions in prostaglandin signaling pathway. Inhibition of HPGD as result of TMPRSS2-ERG overexpression prevents PGE_2 catabolism, thus accumulation of PGE_2 will result in uPA activation and cell growth, contributing to the progression of CaP. ERG directly binds to the promoter of uPA.

treated with 10 μM of PGE_2 and harvested after 1, 2, 4, 8 and 12 hours post treatment.

Western blot assays. Cells were lysed in Mammalian Protein Extraction Reagent (M-PER) (cat.# 78501, Pierce) containing protease inhibitor cocktail and phosphatase inhibitor cocktails I and II (cat.# P8340, P2850 and P5726, respectively, Sigma). Cell lysates equivalent to 50 μg of protein were separated on 4–12% Bis-Tris Gel (cat.#NP0335BOX, Invitrogen) and transferred to PVDF membrane (cat.# LC2005, Invitrogen). Membranes were incubated with primary antibodies: Anti-ERG monoclonal antibody (CPDR ERG-MAb, generated in our laboratory),[46,47] anti-HPGD (cat.# sc-48910, Santa Cruz Biotechnology), anti-α-Tubulin (cat.# sc-5286, Santa Cruz Biotechnology), at 4°C for 12 hours. Membranes were washed three times for five minutes each at room temperature followed by treatment with secondary antibodies: sheep anti-mouse IgG-HRP (cat.# NXA931, GE Health Care) or bovine-anti goat IgG-HRP (cat.# sc-2352, Santa Cruz Biotechnology) at 24°C for one hour. Finally membranes were washed three times and developed with ECL western blot detection reagent (cat.#RPN2209, GE Health Care).

BrdU cell proliferation assay. VCaP cells were grown in DMEM containing 10% hormone depleted cFBS for 72 hours followed by transfection with 50 nM of NT siRNA or ERG siRNA. Twelve hours post-transfection, cells were treated with 0.1 nM R1881 alone, or with 0.1 nM R1881 and 10 μM PGE_2. Bromodeoxyuridine (BrdU) incorporation technique was used to determine the effect of the ERG knockdown and PGE_2 treatment on cell proliferation. In brief, forty eight hours post transfection; cells were pulse-label with 10 mM BrdU (cat.#550891, BD Biosciences) for additional 24 h. Cells were then washed twice with 1x PBS, fixed with 4% paraformaldehyde for 20 minutes at room temperature and centrifuged onto silanized slides (cat.#89033-034, VWR, West Chester, PA) with a cytospin centrifuge. Slides were boiled at 90°C for 20 minutes, washed once with PBS after cooling down to 30°C, blocked in H_2O_2 for 10 minutes followed by ddH_2O wash. Biotinylated mouse monoclonal anti-BrdU antibody (Cat.#B8434, Sigma, St. Louis, MO) was added to the slides and were incubated for 30 min at room temperature followed by PBS wash. Visualization of incorporated BrdU was carried out with Peroxidase substrate kit DAB (cat.# SK-4100, Vector Laboratories) as recommended by the

supplier. The slides were analyzed under Leica DMLB upright microscope using x20 objective lens. BrdU incorporation was calculated from the number of stained BrdU-positive cells per 100 cells counted in each field. A total of five fields per experiment were evaluated.

Immuno-fluorescence assay. VCaP cells transfected with either ERG siRNA or non-targeting siRNA were fixed with 4% paraformaldehyde and centrifuged onto silanized slides (Sigma) with a cytospin centrifuge. Cells were immunostained with anti-HPGD antibody (cat.# 160615, Cayman Chemical), or CPDR ERG-MAb[46,47] followed by goat anti-rabbit Alexa-594 or anti-mouse Alexa-594 secondary antibodies (cat.s#A11037 and A11032, respectively, Invitrogen). Images were captured using a 40x/0.65 N-Plan objective on a Leica DMLB upright microscope with a QImaging Retiga-EX CCD camera (Burnaby) controlled by OpenLab software (Improvision). Images were converted into color and merged by using Adobe Photoshop.

PGE_2 measurement. PGE_2 was measured in the conditioned medium by using an enzyme linked immunoassay kit (cat.#930-001, Assay designs) according to the manufacturer's recommendation. VCaP Cells were grown in DMEM containing 10% of FBS for 48 hours and then transfected with 25 nM of NT siRNA or ERG siRNA. Twenty-four hours post-transfection, cell growth media was replaced with fresh pre-warmed DMEM containing 10% FBS. Seventy-two hours post transfection, the old medium was exchanged with fresh pre-warmed DMEM containing 10% FBS and 5 pg/ml of IL-1β for each groups of cells for 24 hours and the conditioned media were collected for PGE_2 analysis and cells were harvested for total protein extraction which is used for PGE_2 normalization.

Quantitative reverse transcription-polymerase chain reaction (QRT-PCR). Radical prostatectomy specimens were obtained under an Institutional Review Board (IRB) approved protocol (#20405). Total RNA from LCM derived tumor epithelial cells were used for QRT-PCR as previously described in reference 10 by using Sybr green PCR Master Mix Kit (cat.#4304437, Applied Biosystems). Relative expression of *HPGD* (normalized to *GAPDH*) was determined as described before in reference 48. Primers used for *HPGD* gene amplifications were as follows: forward primer: 5'-AAC CTC AGA AGA CTC TGT TCA TCC A-3', and reverse primer: 5'-CCA AAA TGT CCA GTC TTC CAA AGT-3'. *GAPDH* gene expression was detected using forward primer: 5'-GAG CCA CAT CGC CTC AGA CAC C-3'; and reverse primer: 5'-GTT CTC AGC TTG ACG GTG CC-3'. The *HPGD* expression level in VCaP prostate cancer cell line was used as the standard for generating relative expression value.

ChIP assay. VCaP cells were seeded in DMEM containing 10% cFBS and were grown for three days. Cells were transfected with 50 nM of ERG siRNA or with 50 nM of control NT siRNA and were further incubated in 10% FBS containing DMEM for 48 hours. Cells were processed for ChIP assay as described before in reference 5 and 49. Amplification reactions were carried out on T-Gradient Thermoblock (Biometra) by using 95°C, 15 s; 55°C 30 s; 72°C 60 s program setting. For detecting genomic input DNA and specific ChIP products 35 and 40 PCR amplification cycles were used, respectively. ETS binding sites within the target regions were identified by matrix match analysis using the MatInspector software (Genomatix GmbH). ERG protein was detected by an anti-ERG monoclonal antibody (CPDR ERG-MAb).[46,47] For amplifying the human *HPGD* gene (Gene ID: 3248, Accession: U63296 NM_000860) core promoter 5'-GCGAGTCCGGAAGGCAAAGAT-3' ETS binding site (V$ETS1/ELK10.2) the forward 5'-GGGCACTGAAGGAAACTCTTCTT-3' and reverse 5'-GTTCTGGAGCGCCAAGCTT-3' primer pair was used. Two sites that lack ETS binding sites of approximately 1,200 bp upstream and other 1,200 pb downstream of the *HPGD* transcription initiation site were used as negative internal controls. Primers used for the negative controls are upstream forward 5'-GCC AGG GAG GCA GTG TAT AA-3' and reverse 5'-AGA ACA CGG GGC AAA TTA AA-3'. Downstream negative control primers are: downstream forward 5'-GGG ACT AAG AGA CAT TGC TTG CTC-3' and reverse 5'-CTG GCA GCT TGG TAAG AATG CA-3'. For assaying the negative control regions, 40 cycles of PCR amplification cycles were used for both ChIP and input amplifications. Amplicons were detected only in input amplification reaction. As another negative control, we used ChIP with IgG controls.

Statistical analysis. Wilcoxon Rank-sum test was used to compare HPGD transcript expressions between *TMPRSS2-ERG* fusion positive and fusion negative prostate epithelial samples and data is presented with median ± standard deviation (SD). A Student's t-test was used for the comparison of the effect of prostaglandin E_2 induced cell growth, cytokine induced PGE_2. All data are presented as means ± standard deviation (SD), n = 3.

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