Supporting Information

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SI Materials and Methods

Immunohistochemistry. Sections were subjected to boiling in 10 mM citrate buffer (pH 6.0) in a microwave oven for 30 min (1). The cooled sections were incubated in 1% H2O2 for 30 min to quench endogenous peroxidase and then incubated with 1% Triton X-100 in PBS for 10 min. To block nonspecific antibody binding, sections were incubated in normal goat serum for 1 h at 4 °C. Sections were then incubated with anti-Wnt-5a (Imgenex) or anti-AR (androgen receptor) (Santa Cruz Biotechnology) in 3% BSA overnight at 4 °C. Negative controls were incubated in 3% BSA without primary antibody. The sections were then incubated with secondary antibodies for 2 h at room temperature, washed with PBS, and incubated with an avidin–biotin complex for 1 h (Vector Laboratories). Signals were visualized with 3,3′-diaminobenzidine tetrahydrochloride substrate (Sigma), and then slightly counterstained with eosin, dehydrated through an ethanol series and xylene, and mounted.

For immunofluorescence double staining (2, 3), the sections were treated as above and first stained with anti-Wnt-5a followed by signal amplification with TSA Plus Fluorescein Systems (PerkinElmer). After biotin blocking, the sections were then stained with anti-Wnt-5a, and signal was amplified with TSA Plus Cy3 Fluorescence Systems (PerkinElmer). Confocal microscopy analysis was carried out on a Zeiss LSM 510 confocal laser scanning system.

siRNA. Knockdown was induced using siRNA duplex targeting human Wnt-5a. The transfection was performed using Lipofectamine 2000 (Invitrogen) with an siRNA concentration of 300 μM for ~8 × 105 cells per 10 cm-dish. Forty-eight hours after the transfection, these cells were subjected to assays. For a cell proliferation assay, 24 h after the transfection, cells (~2 × 105 cells/mL) were plated on 12-well plates (Corning).

ON-TARGETplus SMARTpool Human WNT5A (siWnt5a) was purchased from Thermo Scientific Dharmacon (L-003939-00), and Silencer Select Negative Control #1 (siControl) was purchased from Ambion (4390844).

RNA Preparation and Real-Time RT-PCR. Ventral prostate lobes were homogenized in ISOGEN (Nippon Gene) and total RNA was extracted according to the manufacturer’s instructions. Oligo-dT-primed cDNA was synthesized from 1 μg of prostate RNA by using SuperScript reverse transcriptase (Gibco-BRL) in a 20-μL reaction volume, 1 μL of which was then diluted serially. Primer sets for PCR were as follows: Wnt-5a forward, 5′-GGCCCATATTTTTCTCCTTCG-3′; Wnt-5a reverse, 5′-CAGAGGCTGTCGTGCCTCTATAA-3′; GAPDH forward, 5′-ACCACAGTCCATGGCCAATC-3′; GAPDH reverse, 5′-TCCACCACTCTGTTGCTGTA-3′. Real-time quantitative RT-PCR was performed using SYBR Premix Ex TaqII (Takara) with a Thermal Dice Cycler (Takara) according to the manufacturer’s instructions. Experimental samples were matched to a standard curve generated by amplifying serially diluted products using the same PCR protocol. To correct for variations in both RNA recovery and the efficiency of reverse transcription, GAPDH cDNA was amplified and quantified in each cDNA preparation (4, 5).

Cell Proliferation Assay. LNCaP cells transfected with siRNA in RPMI 1640 with 10% charcoal-stripped FBS and antibiotics were plated on 12-well culture plates (Corning) at ~2 × 105 cell/mL (day 0) and cultured with 5α-dihydrotestosterone (DHT), hydroxyflutamide (HF), or bicalutamide (BIC) for 6 d. In the absence of the ligands, 100% EtOH was added as a control. Cell numbers were counted using a trypan blue exclusion test at each point as shown in Fig. 4D. All values are means ± SD from at least three independent experiments.

Plasmids. Expression vectors for full-length human AR were inserted into a pcDNA3 vector (Invitrogen). The ARE-tk-Luc reporter vectors were constructed as described (6).

Reporter Assays. LNCaP cells (all 70–80% confluent) were transfected with plasmids using Superfect Reagent (QIAGEN) on 12-well plates. DHT (10−6 M), BIC (10−7 M), and HF (10−7 M) were added 12 h after transfection and then cells were incubated for 12 h. Luciferase activities were determined using the Dual Luciferase Assay System (Promega). As a reference plasmid to normalize transfection efficiency, 2 ng pRL-CMV plasmid (Promega) was cotransfected in all experiments. For RNAi, a two-step transfection was performed with Lipofectamine 2000 (Invitrogen) and Superfect Reagent (QIAGEN) following the manufacturer’s recommendation.

Fig. S1. (A) The growth curves of $AR^{flox/Y}$ and $AR^{pe-T877A/Y}$ mice were normal and comparable to that of wild-type ($AR^{WT/Y}$) mice. (B) Endocrine markers of mice at 20 wk of age. Serum testosterone levels were up-regulated in $AR^{flox/Y}$ and $AR^{pe-T877A/Y}$ mice. LH, luteinizing hormone. (C) Ventral prostate (VP), anterior prostate (AP), dorsal prostate (DP), and seminal vesicle (SV) of $AR^{flox/Y}$ mice at 10 wk of age were remarkably more hypoplastic than those of $AR^{WT/Y}$ mice. (D) Decreased wet weights in VP, AP, and DP of $AR^{flox/Y}$ mice.
Fig. S2. (A) LacZ staining of the ventral prostates of KLKB1-Cre-ER<sup>T2</sup>; CAG-CAT-Z mice. KLKB1-Cre-ER<sup>T2</sup> mice were crossed with CAG-CAT-Z mice. Tamoxifen (TAM) was administered to the offspring at 6 wk of age followed by resection of the ventral prostate. LacZ expression, resulting from Cre-mediated excision, induced by TAM, was seen exclusively in the prostate epithelial cells. (B) Sequence analysis of cDNA extracted from the ventral prostates of AR<sup>pe-T877A/Y</sup> mice. Sequences from exon 6 to exon 8 of the AR gene locus were analyzed, and the point mutation of T to A at codon 877 was confirmed. (C) No significant alterations were detected in AR mRNA levels in the prostates of AR<sup>flox/Y</sup> and AR<sup>pe-T877A/Y</sup> mice by semiquantitative RT-PCR. (D) Reduced expression level of AR protein in the prostates of AR<sup>flox/Y</sup> and AR<sup>pe-T877A/Y</sup> mice. The indicated proteins were visualized by Western blotting with α-AR or α-β-actin antibody. (E) Immunohistochemical staining demonstrating increased levels of cleaved caspase-3 and BrdU incorporation in prostatic cells. The number of BrdU-positive cells increased more in AR<sup>pe-T877A/Y</sup> mice than in AR<sup>flox/Y</sup> mice following DHT treatment for 3 d. The sections were counterstained with hematoxylin. (F) Hematoxylin and eosin staining of prostate lobes from 24-wk-old transgenic murine prostate cancer model (TRAMP)-AR<sup>flox/Y</sup> mice and 36-wk-old TRAMP-AR<sup>pe-T877A/Y</sup> mice revealed no histological difference.
Fig. S3. Alterations in gene expression of several major prostate cancer oncogenes (A) and tumor suppressor genes (B). Quantitative RT-PCR analysis of tumor-related genes was performed in the mice, and the results of representative five independent experiments are shown.

Fig. S4. Prostate tumors from TRAMP-AR<sup>pe-T877A/Y</sup> and TRAMP-AR<sup>fl/Y</sup> mice were transplanted into the ventral prostate lobes of nude mice at 8 wk of age. (A) Schematic representation of the experimental procedure for transplantation of prostate tumors. (B) Photographs of representative nude mice (Upper) and tumors (Lower). (C) Transplanted prostate weights are shown (**P < 0.01 with one-way ANOVA). (D) Hematoxylin and eosin staining of transplanted tumors.
Fig. S5. (A) The effect of castration in nude mice on the growth of transplanted tumors from TRAMP-AR<sup>pe-T877A/Y</sup> mice (*P < 0.05 with one-way ANOVA). Black columns indicate prostate tumors of TRAMP-AR<sup>pe-T877A/Y</sup> mice, and white columns indicate prostate tumors of TRAMP-AR<sup>flox/Y</sup> mice. (B) Photographs of representative nude mice (Upper) and tumors (Lower).

Fig. S6. Efficiencies of the indicated siRNA toward human Wnt-5a in LNCaP cells were analyzed by quantitative RT-PCR with the primer sets for human Wnt-5a.

Fig. S7. No significant alterations of cell proliferation were detected in BIC-treated LNCaP cells. LNCaP cells transfected with siRNAs were subjected to a cell proliferation assay. BIC was incubated with the cells for 6 d. Each point represents the mean ± SD of three separate experiments.
Dataset S1. Human prostate specimens

Pathological diagnosis was based on World Health Organization histological grading and Gleason grade.