Supporting information

Materials and Methods

Cell culture and reporter gene assays. LNCaP, CWR22Rv1, PC-3, and PC346C (1) cells were cultured in RPMI 1640 (Invitrogen) with 10% fetal bovine serum (FBS; Omega). Culture of LNCaP-C4-2B cells was described previously (2). Cells were maintained at 37^{o}C in a humidified atmosphere of 5% CO2. Reporter gene assays were performed by transfecting PC-3 cells with firefly luciferase reporter plasmid 5XARE-TK-pGL3, gene expression constructs pcDNA3-hAR, pcDHCMV-ANCCA-HA and pCMV-β-gal for normalization. For transfection with constructs omitted for specific gene expression, corresponding empty vectors were used to ensure equal amount of total DNA used in different transfection. Cells seeded in 24-well plates in hormone-deprived medium supplemented with 10% cds-FBS (Hyclone) were transfected with Fugene 6 (Roche), then treated with 1 nm DHT at 24 hrs after transfection, and incubated for another 24 hrs before harvested for β-gal and luciferase assays. All reporter gene assays were performed in triplicate, with the entire experiment repeated at least twice.

siRNA transfection, adenovirus infection, cell proliferation and apoptosis assays. Cells were seeded at a density of 2-3 x 10^5 cells per well in 6-well plates with phenol-red free RPMI containing 10% cds-FBS for 48 hrs (for LNCaP), or 5% cds-FBS for 24 hrs (for LNCaP-C4-2B and CWR22Rv1). Cells were then transfected with synthetic siRNA targeting ANCCA (5'GCTACTGTTTACTATCAGGCT3' in human ANCCA/ATAD2 cDNA) or control sequences using Oligofectamine (Invitrogen) following the manufacturer's protocols. LNCaP cells were treated with 1 nM DHT or 0.1 nM R1881 24 hrs later. Cells were then harvested at indicated times for RNA and protein analysis or apoptosis assay. For LNCaP cell proliferation assay, 5000 LNCaP cells were seeded 24 hrs before infection in each well of a 96-well plate in the hormone-depleted medium, then infected with equal titers of adeno-RNAi-ANCCA or adeno-RNAi-GFP as control, and stimulated with 1 nm DHT 24 hrs after infection. Cell proliferation was measured every 2 days by colorimetric MTS assays (Promega). For LNCaP-C4-2B and CWR22Rv1, respectively 2.5x10^5 or 2x10^5 cells were seeded in each well of 6-well plates in phenol-red free RPMI containing 5% cds-FBS for 24 hrs prior to the infection. After the infection, cell proliferation was measured every two days by counting the cell number. Adenovirus vectors were prepared and used as previously described (2, 3). Early apoptotic cell death was measured by Annexin V staining by following the manufacture's protocol

(BD). FITC conjugated Annexin V together with vital dyes such as propidium iodine was used to identify early apoptotic cells. For quantification of apoptotic cells, transfected cells were detached, collected, stained and analyzed by flow cytometry.

ANCCA antibody, Western blotting and real-time reverse transcription-PCR (RT-PCR). Anti-ANCCA antiserum was generated at Covance by immunizing rabbits with recombinant ANCCA N-terminus protein (aa1-264) expressed and purified from E. coli. The antibody was then affinity-purified before use. Cell lysates for Western were prepared and analyzed as described previously (2) using antibodies from Cell Signaling against SGK1, IRS2, PARP and from Santa Cruz against AR, E2F1, cyclin D3, IGF1Rβ, and ANCCA antibody as described above. Real-time RT-PCR analysis of gene expression was described previously (2, 3).

1. Marques RB, van Weerden WM, Erkens-Schulze S*, et al.* The human PC346 xenograft and cell line panel: a model system for prostate cancer progression. Eur Urol 2006;49:245-57.
2. Zou JX, Zhong Z, Shi XB*, et al.* ACTR/AIB1/SRC-3 and androgen receptor control prostate cancer cell proliferation and tumor growth through direct control of cell cycle genes. Prostate 2006;66:1474-86.
3. Louie MC, Zou JX, Rabinovich A, Chen HW. ACTR/AIB1 functions as an E2F1 coactivator to promote breast cancer cell proliferation and antiestrogen resistance. Mol Cell Biol 2004;24:5157-71.