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Research Article

Androgen-Induced Coactivator ANCCA Mediates Specific Androgen Receptor Signaling in Prostate Cancer

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Abstract

Androgen receptor (AR) plays a pivotal role in prostate cancer, primarily by regulating different gene expression programs elicited by androgen, which is important for cancer cell proliferation, survival, and differentiation. It is believed that the transcriptional function of AR is mediated largely by distinct nuclear coregulators. We report here the identification of ANCCA (also known as ATAD2), a new member of the AAA+ ATPase family proteins, as a novel AR coactivator. ANCCA interacts directly with AR and enhances its transcriptional activity, and is required for androgen-stimulated expression of a specific subgroup of genes including *IGF1R, IRS-2, SGK1*, and *survivin*. Upon androgen stimulation, ANCCA together with AR is recruited to the specific AR target genes. Suppression of ANCCA expression strongly inhibited the proliferation of androgen-responsive or androgen-independent, AR-positive prostate cancer cells and caused a significant increase of cellular apoptosis. Strikingly, the *ANCCA* gene itself, located at chromosome 8q24, is highly induced by androgen in androgen-dependent prostate cancer cells and xenograft tumors. Although ANCCA is hardly detected in normal human prostate tissue, high levels of ANCCA are found in hormone-independent prostate cancer cell lines, xenograft tumor, and a subset of prostate cancers with high Gleason scores. Together, these findings suggest that ANCCA plays an important role in prostate cancer by mediating specific AR functions in cancer cell survival and proliferation. The possession of ATPase and bromodomain by ANCCA makes it an attractive target for the development of therapeutics for the disease. [Cancer Res 2009;69(8):3339–46]

Introduction

Prostate cancer is one of the most frequently occurring malignancies in aging men worldwide. It requires androgen and/or androgen receptor (AR)–mediated signaling for its development and progression as androgen ablation therapy and/or AR antagonist are effective in the initial treatment of the disease. In prostate cancer, the aberrant function of AR is likely the primary driving force of the initial androgen-dependent tumor growth and the later progression to androgen deprivation–independent or hormone refractory status. However, how AR function evolves from largely prodifferentiation in the normal, adult prostate to protumorigenesis in the malignant tissue is still poorly understood (1, 2). Intensive studies aimed at understanding prostate cancer progression from androgen-dependent to hormone refractory status has led to the proposition of multiple mechanisms to explain how AR is facilitated to override the need of physiologic levels of androgen for its activation. They include increased expression of AR protein, mutations in AR that broaden its hormone response spectrum, abnormal signaling by growth factors and/or their receptors that activates AR, and the aberrant function of AR-interacting proteins, particularly the coactivators (3, 4). Despite such progress, the pressing issue that remains is how the function of AR evolves to aberrantly promote the expression of genes that are critical for prostate cancer development and progression.

The primary functional mode of AR, a member of the nuclear hormone receptor superfamily, is believed to regulate gene transcription (5). Like other transcription factors, AR-mediated transcriptional activation or repression requires many coregulators (6), which were identified by virtue of their interaction with AR and coactivating AR on the prostate-specific antigen (*PSA*) gene promoter. Among them, a few have been strongly implicated in prostate cancer with evidence of being important for AR-mediated control of prostate cancer cell proliferation and/or survival, tumor growth, and aberrant expression in tumors (7–15). For example, members of the SRC/p160 nuclear receptor coactivator family were found overexpressed in subgroups of prostate cancer tumors, and their elevated levels correlate with disease recurrence and prostate cancer cell proliferation and survival (7, 12, 16). Genetic inactivation of SRC-3 expression in a mouse prostate cancer model strongly prevented the progression of prostate tumorigenesis to more aggressive forms (17). Further studies suggest that overexpressed SRCs such as SRC3 may promote androgen deprivation–independent prostate cancer cell proliferation by stimulating the expression of genes critical for cell proliferation and survival (18, 19). Interestingly, overexpressed p300, histone acetylases which can directly or indirectly interact with AR, may regulate nuclear matrix protein expression and promote prostate cancer aggressiveness (20). Recently, histone demethylases such as LSD1 and JMJD2C were found to coactivate AR and were aberrantly expressed in prostate cancer, further underscoring the role of AR-interacting proteins, particularly the chromatin coregulators, in prostate cancer progression (11, 21).

Here, we report our identification of ANCCA, a member of the AAA+ ATPase family of proteins (22), as a novel AR coactivator. ANCCA is overexpressed in prostate tumors and is critical for prostate cancer cell proliferation and survival. Strikingly, ANCCA is

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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highly induced by androgen and controls the expression of specific AR target genes such as insulin growth factor-1 receptor (*IGF1R*), insulin receptor substrate-2 (*IRS-2*), and serum and glucocorticoid–induced protein kinase-1 (*SGK1*), but not *PSA*. Together, our data suggest that androgen-dependent, aberrant AR signaling in prostate cancer involves *de novo* synthesis of specific coregulators such as ANCCA to mediate the tumorigenic gene expression program of AR, and that androgen-independent, elevated expression and/or function of these coregulators, as observed for ANCCA, may represent an important mechanism of prostate cancer progression to androgen deprivation–independent or hormone refractory status.

Materials and Methods

Cell culture, transfection, infection, gene expression, cell proliferation, and apoptosis assays. A detailed description of the cell culture and assays can be found in the Supplementary Data. Briefly, cells were transfected or infected with synthetic small interfering RNA (siRNA) targeting ANCCA (5′-GCTACTGTTTACTATCAGGCT-3′ in human ANCCA/ATAD2 cDNA) or control sequences, later treated with androgen, and then harvested at the indicated times for RNA and protein analysis or apoptosis and cell proliferation assay.

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation (ChIP) assay was performed essentially as described previously (23, 24). The primers used for amplification of *PSA* gene fragments were described by Louie and colleagues (23), and the primers used for amplification of *SGK-1* gene enhancer were described by Shanmugam and colleagues (25). For *IRS-2* gene amplification, the primers used were 5′-AGCTCTGTGACTGCCTCCTGCTGT-3′ and 5′-GATGCTCCTCC-GATGCTCCAACGG-3′, which were designed based on the AR-binding region identified (26).

Coimmunoprecipitation and GST pull-down assays. LNCaP cells were stimulated with 1 nmol/L of DHT for 24 h and then harvested for preparation of nuclear extracts as described previously (24). The nuclear extracts were diluted to contain 150 mmol/L of NaCl, 20 mmol/L of Hepes (pH 7.9), 0.2 mmol/L of EDTA, 0.5% NP40, and 1 mmol/L of phenylmethylsulfonyl fluoride and protease inhibitor cocktails (Sigma). Equal amounts of nuclear extracts were incubated with the antibodies with or without 1 nmol/L of R1881 for 2 h at 4°C, followed by incubation with protein A beads (Zymed) for 1 h. After extensive washing, the immunoprecipitates were analyzed by Western analysis with anti-AR antibody (NeoMarker). *In vitro*–translated ANCCA proteins were incubated with anti-flag M2 antibody bead-bound with purified, flag-tagged AR protein (~100 ng) in the presence of 1 nmol/L of R1881 at 4°C for 1 h in the binding buffer described previously (24). The beads were then washed four times with the binding buffer containing 300 mmol/L of KCl and subjected to SDS-PAGE. ANCCA proteins retained on the beads were visualized by autoradiography.

Immunohistochemistry. Tissue sections from formalin-fixed, paraffin-embedded tissue blocks or arrays were dewaxed, rehydrated, and blocked for endogenous peroxidase activity. Antigen retrieving was performed with a microwave oven in 0.01 mol/L of sodium citrate buffer (pH 6.0). Nonspecific antibody binding was blocked by incubating in 10% normal horse serum for 30 min. Affinity-purified, anti-ANCCA antibody was added at 1:300 (for CWR22 xenograft tumors) or 1:100 (for normal or tumor human prostate tissues) dilutions, and incubated for 30 min. The sections

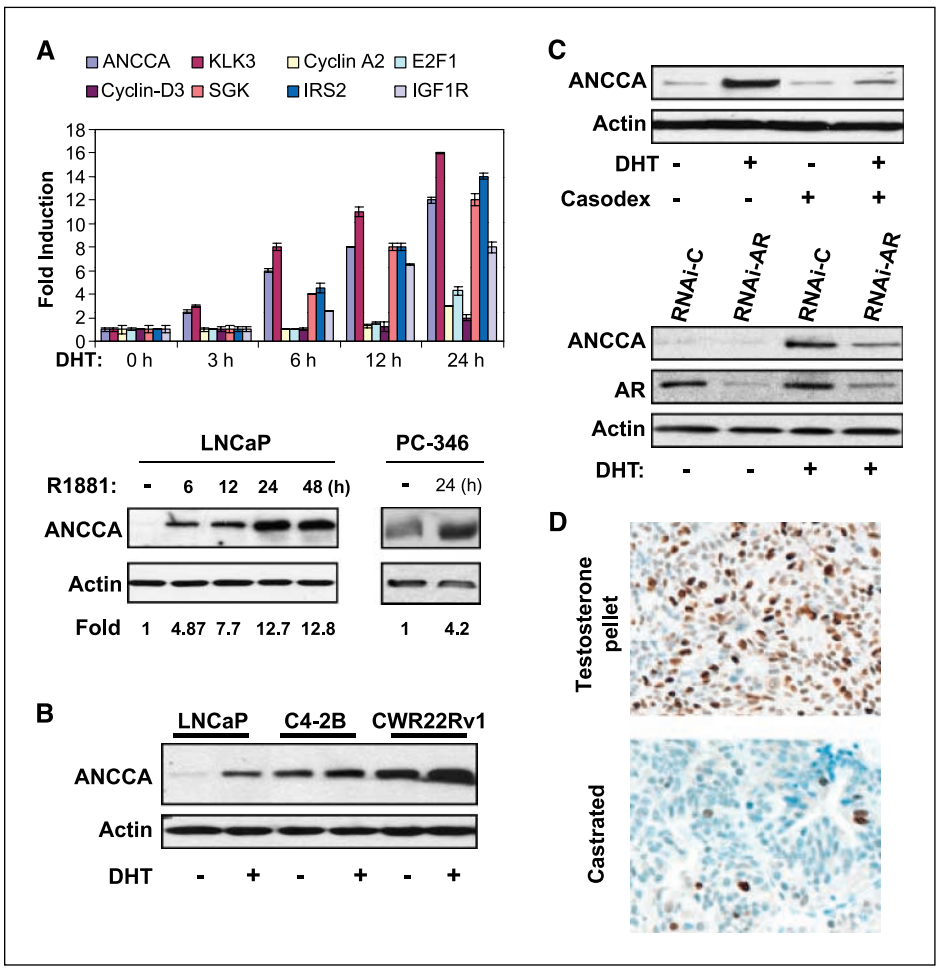

Figure 1. High levels of ANCCA are induced by androgen via AR or expressed independent of hormone in hormone-refractory cells. *A,* cells were hormone-deprived before being stimulated with the indicated androgens for RNA (*top*) or protein analysis (*bottom*). Fold induction was obtained by comparing the real-time reverse transcription-PCR data from cells treated with DHT for different time periods and data from untreated cells. *B,* cells were treated with 1 nmol/L of DHT for 24 h and harvested for Western analysis. *C,* LNCaP cells were treated with 1 nmol/L of DHT or 10 μmol/L of Casodex alone or with both for 24 h (*top*) or were infected with adeno-RNAi-AR or control and then treated with or without DHT (*bottom*) before Western analysis. *D,* sections of CWR22 xenograft tumors isolated from an intact mouse implanted with a testosterone pellet or from a mouse castrated for 2 weeks were examined by immunohistochemistry for ANCCA.

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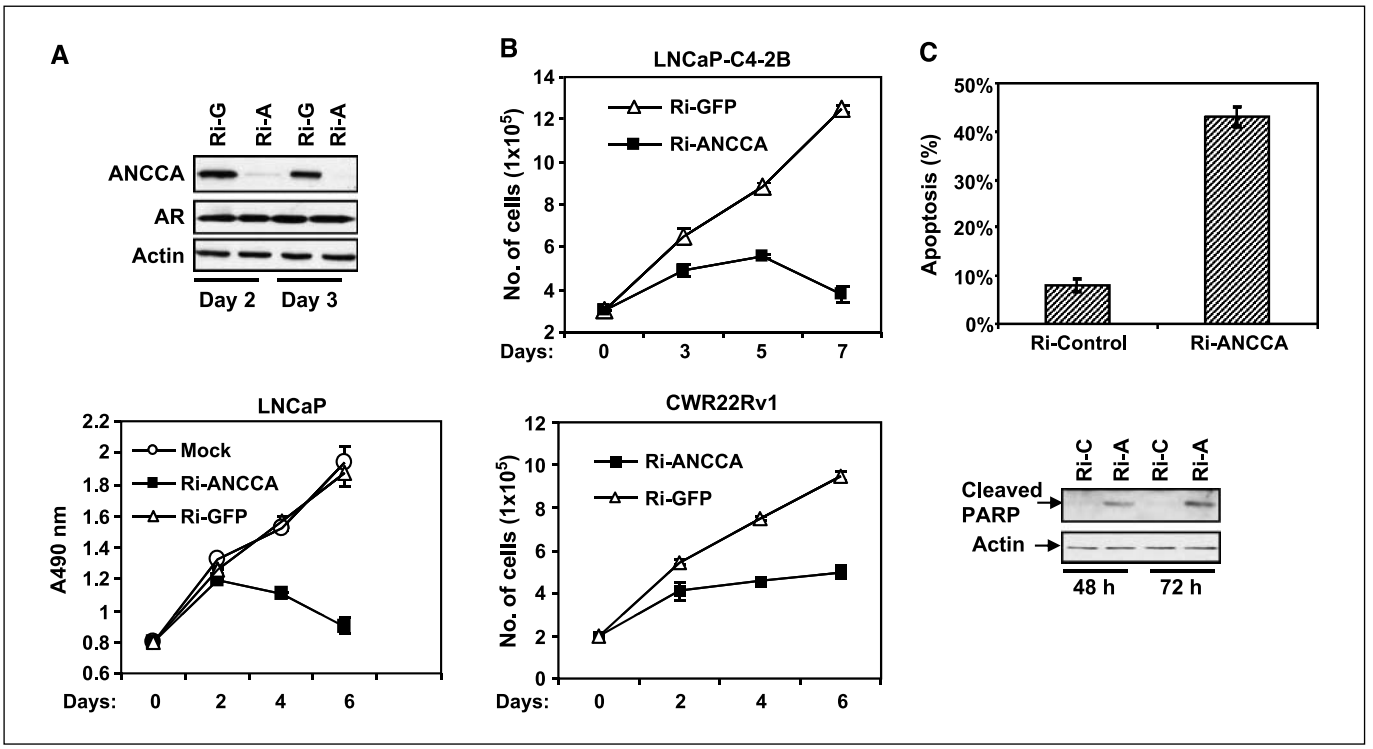

Figure 2. Suppression of ANCCA expression inhibits the proliferation and survival of androgen-dependent and hormone-refractory prostate cancer cells. Cells were plated in hormone-deprived medium for 24 h before being infected with adeno-RNAi-ANCCA (*Ri-A*) or adeno-RNAi-GFP (*Ri-G*) control, or mock-infected (for LNCaP and CWR22Rv1 in *A* and *B*), or transfected with synthetic RNAi (for C4-2B in *B* and LNCaP in *C*). Twenty-four hours after the treatments, cells were stimulated with 0.1 nmol/L of R1881 (*LNCaP*) or changed to fresh hormone-deprived medium. Cells were harvested on different days after infection for Western or proliferation (*A* and *B*) or for detection of apoptosis (*C*). For apoptosis, RNAi-treated LNCaP cells were incubated with Annexin V-FITC and propidium iodide and quantified for apoptotic cells by flow cytometry. Cells were also harvested 72 h after transfection for detection of cleaved PARP (85 kDa) by Western analysis.

were then washed and incubated with biotin-conjugated secondary antibodies for 30 min, followed by incubation with avidin DH-biotinylated horseradish peroxidase complex for 30 min (Vectastain ABC Elite Kit, Vector Laboratories). The sections were developed with the diaminobenzidine substrate kit (Vector Laboratories) and counterstained with hematoxylin (Sigma). The specificity of the ANCCA antibody was determined using LNCaP cell cultures and CWR22 tumors harvested with and without androgen stimulation. CWR22 xenografts were generated as described previously (19). Human tumor blocks were collected from the Department of Pathology of Zhujiang Hospital in accordance with the hospital's Institutional Review Board on Biomedical Specimen standards. Tissue microarray slides were obtained from U.S. Biomax, Inc. Tumors were evaluated and graded by pathologists according to guidelines (27). The tissue section immunoreactivity to ANCCA antibody was scored negative if <1% of prostate epithelial cells displayed any staining, and scored positive if >5% of the cells displayed staining with moderate to high intensity. Differences in the immunostaining among groups were analyzed with the χ^2 test.

Results

ANCCA is strongly induced upon androgen stimulation or highly elevated independent of androgen. In our attempt to identify genes that may play critical roles in androgen induction of prostate cancer cell proliferation, we compared three sets of microarray data generated from LNCaP cells, one treated by DHT, one ectopically expressing the nuclear hormone receptor coactivator/proto-oncogene ACTR, and one ectopically expressing the kinase-dead mutant of MAK, a serine/threonine kinase that can act as a coactivator of AR (28). We found that an ORF named PRO2000 (also known as ATAD2), with no functions reported at the time of the analysis, is one of a small group of genes (data not shown) with expression induced by DHT, up-regulated by ACTR, and down-regulated by the kinase-dead mutant MAK, which suppresses LNCaP proliferation (28). Given its predicted domain structures (AAA+ ATPase and bromodomain) and its functional association with AR and cancer (described below),[4] we thus named it ANCCA, for AAA+ nuclear coactivator cancer–associated.

To determine the expression kinetics of ANCCA induced by androgen in prostate cancer cells, we treated LNCaP cells with DHT or synthetic androgen R1881 for different time periods and harvested cells for ANCCA mRNA and protein analysis. As shown in Fig. 1*A*, ANCCA protein (~170 kDa) was barely detected in the absence of androgen. Remarkably, 24 hours of androgen treatment resulted in a >10-fold induction in ANCCA mRNA and protein, and its androgen induction can be observed as early as 3 hours after androgen treatment, similar to that of PSA (Fig. 1*A*). Interestingly, androgen induction of ANCCA can be detected earlier than many androgen-induced genes that play critical roles in cancer cell proliferation and survival including *SGK1, IRS2, IGF1R*, and some of the cell cycle/E2F target genes examined. We next extended our analysis to PC346C, an androgen-sensitive prostate cancer cell line with wild-type AR (29), and found that ANCCA is also highly induced by androgen (Fig. 1*A, bottom*). Next, we examined ANCCA expression in androgen deprivation–independent prostate

[4] Manuscript in preparation.

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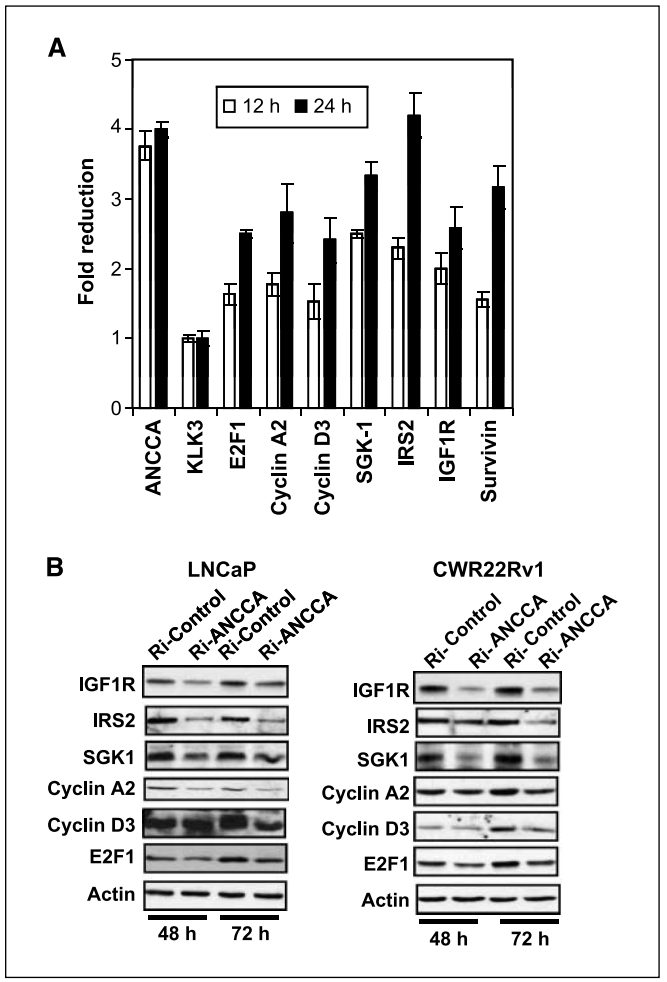

Figure 3. ANCCA is required for the expression of androgen-induced genes controlling cancer cell proliferation and survival. LNCaP or CWR22Rv1 cells were cultured in hormone-deprived medium for 48 h before being transfected with siRNA targeting ANCCA or control sequence. Twenty-four hours after the transfection, LNCaP cells were stimulated with 0.1 nmol/L of R1881 and harvested at the indicated times for real-time reverse transcription-PCR (*A*) and Western analysis (*B*). CWR22Rv1 cells were analyzed without hormone treatment.

cancer cells and tumor xenografts. Compared with androgen-induced ANCCA in LNCaP, high levels of ANCCA protein were detected in LNCaP-derived C4-2B and CWR22 xenograft–derived CWR22Rv1 cells both in the absence and presence of DHT (Fig. 1*B*), indicating that ANCCA expression in these androgen deprivation–independent cells is largely independent of androgen. Similar results were obtained from other hormone-refractory prostate cancer cells such as LNCaP-cds, LNCaP-p53-R273H (30, 31), and AR-negative PC-3 cells (data not shown).

To examine whether androgen induction of ANCCA is mediated by AR, LNCaP cells were treated with DHT in the absence or presence of antiandrogen bicalutamide (Casodex). Results in Fig. 1*C* clearly show that androgen-induced ANCCA expression in LNCaP can be effectively blocked by the antiandrogen (*top*). To directly show the involvement of AR, we also knocked down AR expression in LNCaP cells and found that suppression of AR significantly impeded androgen-induction of ANCCA (Fig. 1*C, bottom*). The growth of CWR22 xenograft tumor in nude mice is androgen-dependent (32). Immunohistochemical analysis with anti-ANCCA antibody clearly showed that ANCCA was highly expressed as a nuclear protein in tumor cells growing in the intact animal with a testosterone pellet implanted (Fig. 1*D*). Strikingly, when the animal was castrated and the

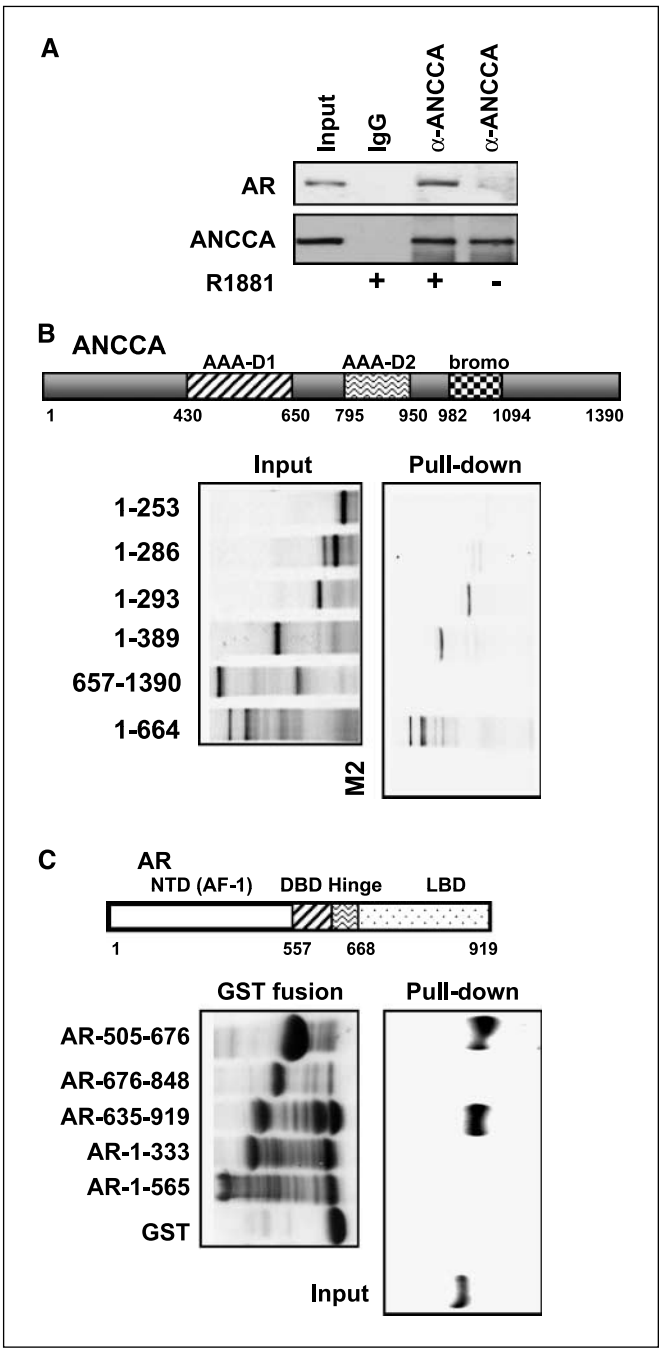

Figure 4. ANCCA interacts directly with AR. *A,* LNCaP cells were stimulated with 1 nmol/L of DHT for 24 h before being harvested for nuclear extract preparation. Equal amounts of extracts were incubated with anti-ANCCA or normal rabbit antibody in the presence or absence of androgen. *Input,* 10% of the extract used in coimmunoprecipitation. *B,* various *in vitro*–translated ANCCA fragments were incubated with M2 agarose beads bound with flag-AR in the presence of 0.1 nmol/L of R1881. The M2 agarose beads without AR were incubated with an NH_2-terminal fragment (*1–664*) and used as a control. After extensive washing, ANCCA proteins retained on the beads were separated by SDS-PAGE and visualized by autoradiography. *C,* GST-AR fragments or GST alone were incubated with purified, flag-tagged ANCCA. Retained ANCCA was detected by Western analysis with anti-ANCCA antibody.

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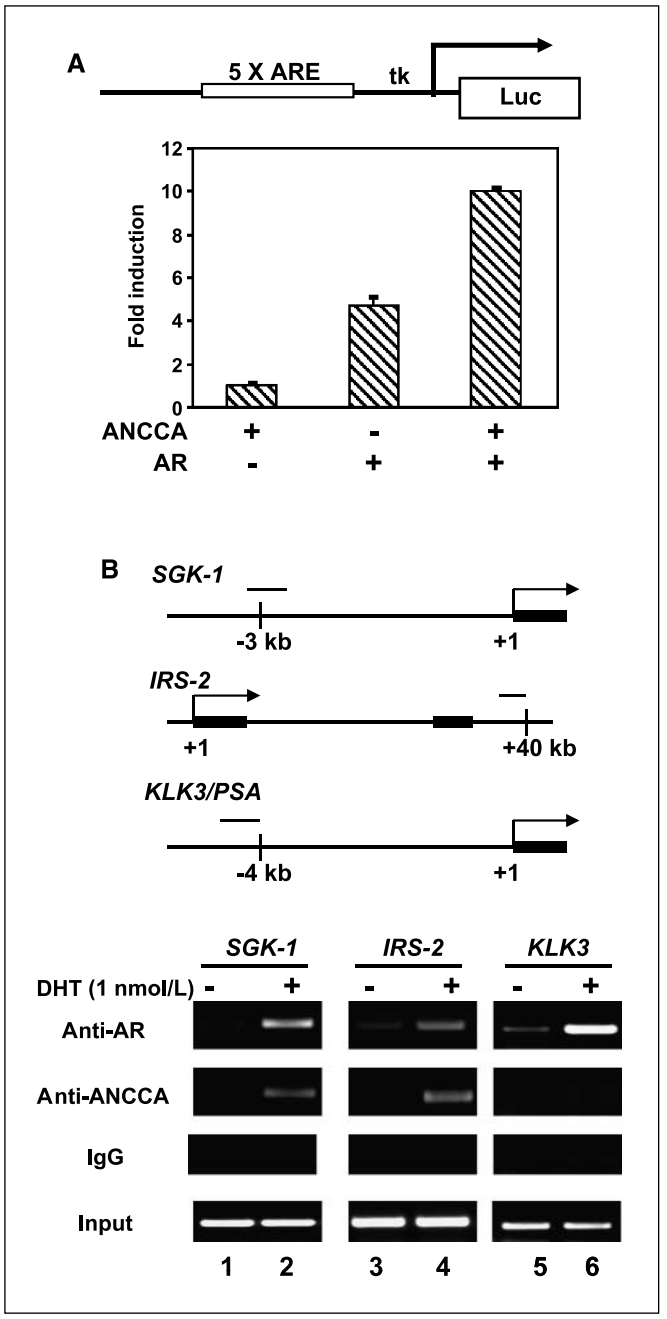

Figure 5. ANCCA coactivates AR-mediated transcription. *A,* PC-3 cells were transfected with the indicated constructs, stimulated with 1 nmol/L DHT or vehicle for 24 h, and then harvested for the β-gal and luciferase assays. *B,* LNCaP cells stimulated with 1 nmol/L of DHT or vehicle for 12 h were harvested for ChIP assays with indicated antibodies. The eluted DNA fragments were amplified with primers encompassing the ARE sequences in the promoter or enhancer region of *SGK-1, IRS2*, or *KLK3/PSA* genes. The locations of the regions amplified relative to the gene loci are indicated by a short line on the gene locus diagrams. The filled boxes indicate exons.

pellet removed, ANCCA expression was dramatically reduced. Together, these results indicate that ANCCA is a novel nuclear protein that is strongly induced by androgen through AR in AR-positive prostate cancer cell lines and tumors, and is highly elevated when prostate cancer cells become androgen-independent or hormone-refractory.

ANCCA is required for proliferation and survival of androgen-dependent and hormone-refractory prostate cancer cells. Given its remarkable induction by androgen, we began to examine the potential role of ANCCA in the control of prostate cancer cell proliferation and survival. LNCaP cells were infected with recombinant adenoviruses that express short hairpin RNA specifically targeting ANCCA, and then treated these cells with androgen. As shown in Fig. 2*A*, ANCCA-specific RNAi inhibits the expression of ANCCA but not AR. However, suppression of ANCCA induction dramatically inhibited androgen-stimulated LNCaP cell proliferation. Similar inhibitory effects of ANCCA knockdown were observed in LNCaP cells grown in the absence of androgen

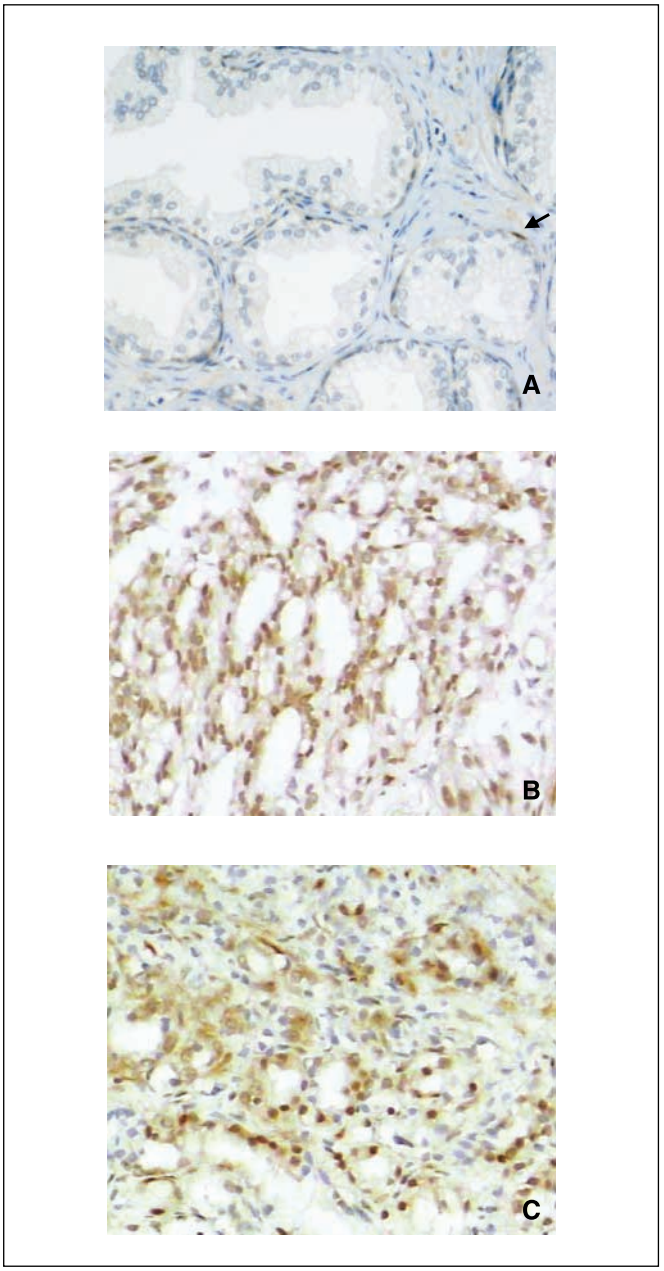

Figure 6. ANCCA is overexpressed in prostate cancer. Tissue sections from formalin-fixed, paraffin-embedded tissue blocks or arrays were processed for immunohistochemical analysis with an affinity-purified ANCCA antibody. Examples of immunohistochemistry in normal prostate (*A*) and prostate cancer tissues (*B* and *C*, with Gleason scores of 7 and 9, respectively) are shown.

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(Supplementary Fig. S1) in C4-2B and in CWR22Rv1 cells (Fig. 2*B*), or by transfection of siRNA sequence targeting a different region of ANCCA (data not shown). Interestingly, the growth curve of ANCCA-suppressed cells turned downward after a few days of ANCCA suppression, suggesting that ANCCA expression is required for cancer cell survival. To determine whether ANCCA depletion resulted in the activation of apoptosis, we stained the cells with FITC-conjugated Annexin V (*green*) together with propidium iodide (*red*) and quantified apoptotic cells (positive for Annexin V and negative for propidium iodide) by flow cytometry (Fig. 2*C*; Supplementary Fig. S2). Remarkably, >43% of cells were apoptotic 3 days after transfection with synthetic siRNA targeting ANCCA. To ascertain that components of the apoptosis pathway are indeed activated, using Western analysis, we examined the appearance of cleaved poly(ADP-ribose) polymerase (PARP), a marker for apoptosis. Indeed, the cleaved PARP was readily detected in cells transfected with ANCCA siRNA but not in control siRNA-treated cells (Fig. 2*C*). Interestingly, depletion of ANCCA in AR-negative PC-3 cells did not significantly affect their proliferation (data not shown). Together, these data suggest that high levels of ANCCA are required for the proliferation and survival of AR-positive prostate cancers.

ANCCA is specifically required for the expression of androgen-induced genes that control cancer cell proliferation and survival. Having found that ANCCA is critical for prostate cancer cell proliferation and survival, we began to address its functional mechanism. Given its role as a transcriptional coregulator, as revealed in our recent study (24), we examined the effect of silencing ANCCA on androgen-induced expression of genes with demonstrated function in the control of cancer cell proliferation and survival. Among them are *IGF1R, IRS-2, SGK1, survivin, E2F1, cyclin A2*, and *cyclin D3* (18, 19, 25, 33–39). As shown in Fig. 3*A*, LNCaP cells transfected with ANCCA siRNA and treated with androgen showed a 3-fold to 4-fold reduction in the transcript levels of these genes when compared with cells transfected with control siRNA. Similar inhibitory effects on their protein levels were obtained by Western analysis (Fig. 3*B, left*). Intriguingly, ANCCA suppression did not seem to have any detectable negative effect on androgen-induced KLK3/PSA (Fig. 4*A*) and KLK2 expression (data not shown). In AR-positive but androgen-independent CWR22Rv1 cells, a similar effect of ANCCA suppression on cell proliferation and the survival of genes was observed (Fig. 3*B, right*). Together, these data suggest that high levels of ANCCA are required for the expression of an androgen-dependent and potentially AR-regulated subset of genes critical for cancer cell proliferation and survival.

ANCCA via its NH_2 terminus directly associates with AR at the DBD and hinge region. We next examined whether the function of ANCCA in the control of androgen-AR target genes is due to its ability to directly interact with AR. Thus, we first performed a coimmunoprecipitation assay. LNCaP cells were treated with androgen to induce the expression of ANCCA. Nuclear extracts were then immunoprecipitated with ANCCA antibody in the presence or absence of androgen. Remarkably, in the presence of androgen, a significant proportion (>10% of input) of AR was coprecipitated by anti-ANCCA antibody, indicating that endogenous ANCCA and AR are strongly associated and that the association is largely androgen-dependent (Fig. 4*A*). To identify the potential domain of ANCCA which mediates the interaction, we performed pull-down experiments in the presence of androgen, using purified, flag-tagged, recombinant AR and *in vitro*–translated, S^{35}-methionine–labeled ANCCA-truncated forms. Results in Fig. 4*B* indicate that ANCCA interacts specifically with AR primarily through the NH_2 terminus of ANCCA, with amino acids 284 to 293 being one of the critical regions. To localize the interface of AR that mediates the interaction with ANCCA, we performed GST pull-down assays with GST-AR fusions containing different domains of the receptor and full-length ANCCA protein. Results in Fig. 4*C* show that ANCCA interacts primarily with the DBD-hinge region of AR as fragments containing NTD (1–565) and LBD (676–848) do not show any interaction, but fragments containing DBD and hinge (505–676 or 635–919) showed strong binding. Similar results were obtained when the *in vitro* pull-down experiments were performed in the absence of hormone (data not shown).

ANCCA coactivates AR-mediated transcription. To determine whether ANCCA acts as a transcriptional coactivator for AR, we performed a reporter gene assay using synthetic ARE-driven luciferase construct in PC-3 cells which lack endogenous AR expression. As expected, expression of AR activated the reporter ~4-fold in the presence of androgen (Fig. 5*A*). Expression of ANCCA alone, however, has no significant effect on the reporter. Remarkably, coexpression of ANCCA and AR resulted in >10-fold activation of the reporter, strongly suggesting that ANCCA coactivates AR-mediated transcription. To extend our analysis to the potential, natural targets of ANCCA, we performed ChIP assay with LNCaP cells stimulated by androgen. As reported previously (23, 25, 26, 40, 41), androgen-stimulated AR recruitment to the AR-binding regions of *IRS-2* (~9 kb downstream of *IRS-2* gene), *SGK1* (~3 kb upstream of its transcription start site), and *PSA* genes (Fig. 5*B*). Analysis of anti-ANCCA ChIP DNA revealed that androgen also strongly stimulated ANCCA recruitment to the same AR-binding regions of SGK1 and IRS2 but not PSA, consistent with our finding that ANCCA is required for androgen induction of SGK1 and IRS-2 but not PSA. Taken together with the data that ANCCA directly interacts with AR, these findings indicate that ANCCA is a novel AR coactivator and functions by selectively mediating the expression of a subset of AR target genes.

ANCCA is overexpressed in tumors of the prostate cancer and its overexpression correlates with disease progression. Given the critical role of ANCCA in prostate cancer cell proliferation and survival, we surmised that ANCCA protein might be aberrantly expressed in human prostate cancers. We thus examined ANCCA expression in surgical specimens of human prostate by immunohistochemistry. Eighty-five tumor tissues and nine normal tissues were analyzed. In all of the normal prostate tissue sections examined, virtually no staining was seen in the epithelial cells (Fig. 6; Supplementary Table S1). A relatively low ANCCA immunoreactivity was observed in the cytoplasm of some of the basal cells and occasionally in the nucleus (Fig. 6*A, arrow*). In contrast, among 85 tumors examined, positive ANCCA immunoreactivity was observed in 28 tumors (33%). When the results were grouped according to Gleason score, 51% of specimens with Gleason scores of 7 to 10 were positive for ANCCA, whereas 14% or 19% of tumors with Gleason scores of 1 to 3 or 4 to 6, respectively, were positive (Supplementary Table S1). These data suggest that ANCCA is overexpressed in a subset of human prostate cancers and that its overexpression seems to correlate significantly ($P = 0.0032$) with disease progression. The results also warrant future investigation with large cohorts of samples with clinical data for potential prognostic value of ANCCA overexpression.

Discussion

In our study on the key factors involved in AR signaling in prostate cancer cell proliferation, we identified ANCCA as a highly

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androgen-responsive gene and showed that its androgen induction plays a critical role in prostate cancer cell proliferation and survival. Suppression of ANCCA expression resulted in strong inhibition of androgen-stimulated as well as hormone-independent prostate cancer cell proliferation. We also found that ANCCA is overexpressed in a significant proportion of prostate cancer tumors and that its overexpression correlates with high tumor grades. Interestingly, high levels of ANCCA are not required for AR-negative prostate cancer cell proliferation (data not shown), suggesting that the function of ANCCA in prostate cancer cells is AR-dependent. Together, these results suggest that ANCCA plays an important role in prostate cancer progression.

AR controls the expression of genes with diverse functions ranging from cell differentiation, survival, proliferation, and metabolism. Whether specific coregulators are involved in mediating AR control of specific target genes has not been clearly addressed. In this study, we found that ANCCA does not seem to play a significant role in androgen induction of a group of genes such as *KLK2* and *KLK3/PSA* that are expressed in normal adult prostate but elevated in prostate tumors. Instead, we found that ANCCA is crucial for androgen-stimulated expression of *SGK1, IRS-2, IGF1R*, and *survivin*. Further ChIP analyses showed that ANCCA and AR are recruited to the AR-binding regions in an androgen-dependent manner, indicating that they are direct targets of ANCCA. Given the fact that ANCCA-AR target genes such as *IRS-2, survivin*, and *SGK1* are well-known for their function in promoting cancer cell proliferation and survival, it is attempting to speculate that coregulators such as ANCCA might be involved in the integration of a specific gene expression program among the functionally diverse gene targets of AR, suggesting a functional mode that is different from that of many other coactivators such as the p160/SRCs.

One salient feature of ANCCA is its robust induction of expression by androgen in hormone-sensitive prostate cancer cells, which suggests a feed-forward mechanism of ANCCA function in mediating androgen signaling. From the perspective of AR function, such a feature of ANCCA, combined with its selective involvement in specific AR target gene programs, argues for a notion that androgen-dependent, aberrant AR signaling in prostate cancer may entail the induction of specific coregulators to mediate the specific tumorigenic gene expression program of AR. Although the majority of the AR coregulators identified thus far may not be subject to androgen regulation in their expression, recently, a few coregulators including p300/CBP, Tip60, TIF2, and FHL2, as well as the serine/threonine kinase MAK which might regulate ANCCA expression (28), have been found to be regulated by androgen. FHL2 was shown to be induced by androgens through the activation of serum response factor (42). Interestingly, the other coregulators are negatively controlled by androgens as androgen withdrawal resulted in their elevated expression and androgen exposure led to their decreased expression (7, 9, 43). Therefore, due to such dynamic control of the coregulator expression in androgen-sensitive prostate cancer cells, it is quite possible that androgen stimulation and withdrawal triggers a reshuffle of AR coregulator complexes at specific AR target genes. Further study of the dynamics of coregulator expression, and their complex assembly and function at distinct AR target genes, will be of importance to our understanding of AR function in hormone-sensitive tumors as well as the mechanism of androgen-deprivation refractory state.

Several AR coregulators possess or recruit chromatin-modifying or chromatin-remodeling activities, underscoring changing chromatin structure and/or function as one of the crucial mechanisms of AR-mediated control of gene transcription. Interestingly, like Brg1 or Brm, the ATPase component of the human SWI/SNF chromatin remodeling complexes, ANCCA possesses an ATPase domain and a bromodomain (24), suggesting that ANCCA has the potential to remodel the chromatin structure. Alternatively, ANCCA may play a role in the dynamic assembly or disassembly of protein complexes at the regulatory region of AR targets. Such a function is fully in line with the well-established biochemical activities of AAA+ proteins, which are evolutionarily conserved proteins harnessing energy from ATP hydrolysis to alter the conformation of their substrate proteins. For instance, eukaryotic DNA replication involves a large number of ATPase proteins with many of them being AAA+ proteins including ORCs, Cdc6, MCMs, and the clamp loader RFCs (44). We recently showed that ANCCA possesses ATPase activity and that it is required for estrogen-induced recruitment of CBP to specific ER target genes (24), therefore supporting the idea that one of the mechanisms of ANCCA function is to facilitate the loading/assembly of coregulator complexes at the chromatin target. We also found that the bromodomain of ANCCA recognizes specific histone modification marks and is critical for ANCCA to serve as a coactivator.[5] Future studies will be needed to better understand the role of ANCCA in the dynamics of AR coregulator complexes and in mediating specific AR target gene programs that are critical for prostate tumorigenesis, and to examine the potential of ANCCA as a therapeutic target for prostate cancer intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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[5] Unpublished results.

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