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Independent Prostate Cancer

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)
Androgen-ablation therapy is the primary treatment for prostate cancer that has escaped local control through surgical excision or radiation (hormone sensitive, HS). While generally effective, the treatment is short-lived and hormone refractory (HR) cancer eventually develops. To identify the responsible mechanisms, we set out a microarray experiment using seven pairs of HS and HR xenografts and identified androgen receptor (AR) overexpression is the only consistent change in the progression of prostate cancer. In the last grand period, I confirmed by western blot analysis that androgen receptor protein is higher in HR than HS tumors. Through lentivirus and retrovirus systems, I was able to overexpress AR in both LNCaP and LAPC4 cells. In vitro and in vivo experiments demonstrated that overexpression of AR is sufficient for HS-to-HR transition. We are testing if AR is necessary for the growth of androgen independent prostate cancer

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INTRODUCTION

I proposed in the grant to examine if overexpression of androgen receptor (AR) is sufficient and necessary for prostate cancer progression from androgen -dependent (AD) to -independence (AI). The terms of AD and AI are changed to hormone sensitive (HS) and hormone refractory (HR), respectively, because androgen is still required after progression. Up to date, I have been able to establish the sufficiency by in vitro cell assay and in vivo mouse xenograft models. We have established a knockdown system to examine the necessity.

BOBY

Conformation of AR overexpression

By microarray studies, I identified overexpression of AR mRNA as the only consistent change between HS and HR xenografts. To confirm this results in protein level, xenograft tumors were lysed in 2xSDS buffer and subject to western blot analysis. Indeed, AR protein level is higher in each HR tumor than its counterpart (Figure 1).

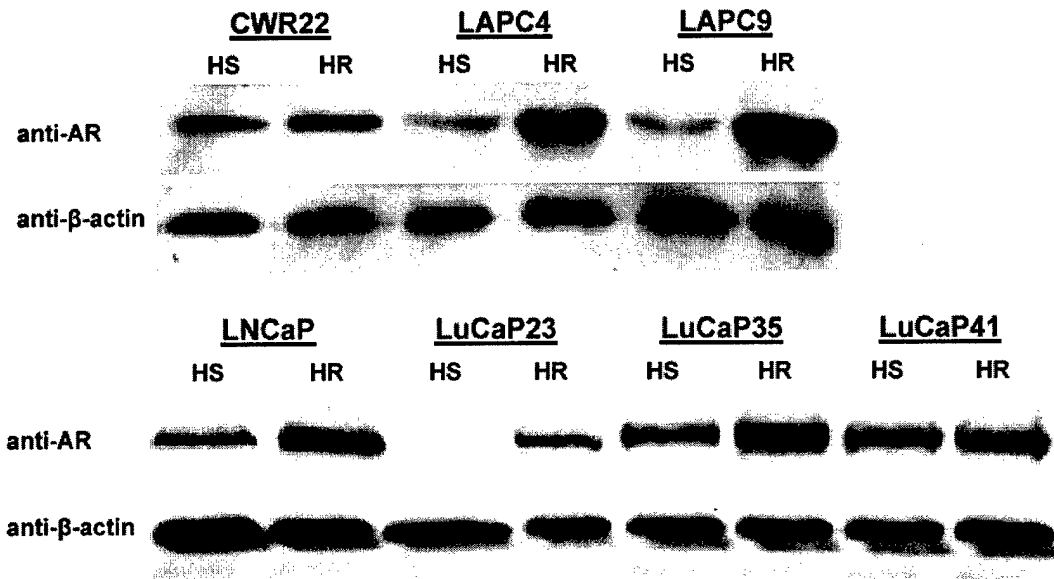


Figure 1. Increased AR protein levels in HR xenograft tumors. Western blot analysis of total cellular proteins from matched HS and HR xenografts for protein levels of AR (top panel) and β -actin (bottom panel). AR protein was detected in HS tumor lysate of LuCaP23 after a longer exposure (data not shown).

To establish AR overexpressing prostate cancer cells

Flag-tagged AR cDNA was subcloned into both lentivirus (pCSCG) and retrovirus vectors (pSRa), and viruses were generated to infect two prostate cancer cell lines: LNCaP and LAPC4. Overexpression of AR in infected cells was confirmed by western

blot analysis (Figure 2A and 3A). AR protein levels are about three-fold higher in overexpressing than in control cells, which are comparable to those in xenograft tumors.

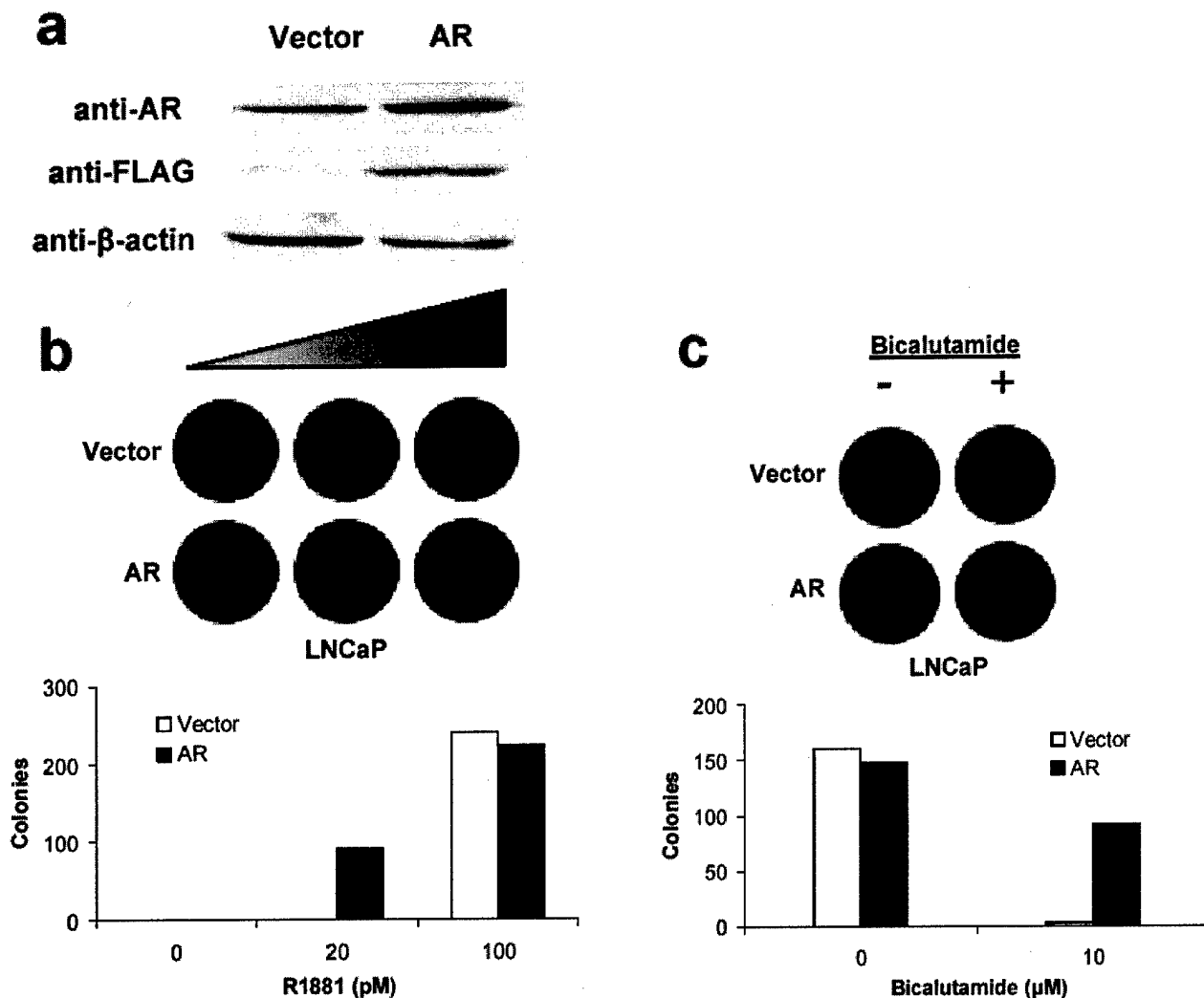


Figure 2. Overexpression of AR caused HR growth in vitro. LNCaP cells were infected with retrovirus expressing empty vector or flag-tagged, wild-type human AR, and stable lines were derived by G418 selection. A, Western blot analysis of AR-overexpressing LNCaP cells. Lysates from control and AR-overexpressing LNCaP cells were resolved and probed with antibodies against AR (top), FLAG (middle), or β-actin (bottom). B, Effect of AR overexpression on growth response of LNCaP cells to R1881. Control and AR overexpressing LNCaP cells were grown in 10% charcoal-stripped FBS in addition of various concentrations of R1881. Colonies were stained and the number was counted at 14 days. Quantification is shown in the bottom. C, Effect of AR overexpression on growth inhibition of LNCaP cells to anti-androgen, Casodex. Control and AR overexpressing LNCaP cells were grown in 10% charcoal-stripped FBS supplemented with 1nM of R1881 in the presence or absence of 10 μM of Casodex. Colonies were stained and the number was counted at 14 days. Quantification is shown in the bottom.

Sufficiency test

In vitro. Control and AR-overexpressing LNCaP cells were plated in media containing 10% charcoal-stripped serum (this serum has low concentration of androgen) in addition of different concentrations of a synthetic androgen, R1881. Cell growth was examined by colony formation. AR-overexpressing cells grew in media containing 0.02 nM of R1881, which is 5-fold less than what is required for the control cells (Figure 2B). Also, in contrast to the control cells, AR overexpressing cells grew in the presence of 10 μ M casodex, a clinical anti-androgen drug (Figure 2C).

In vivo. Control and AR-overexpressing LAPC4 cells were injected into intact and castrated SCID mice. Tumor formation (Figure 3B), defined as a tumor reaching 40 mm³, was monitored each week. Although AR overexpressing and the control cells have a similar growth rate in intact mice, AR overexpression conferred faster growth in castrated mice.

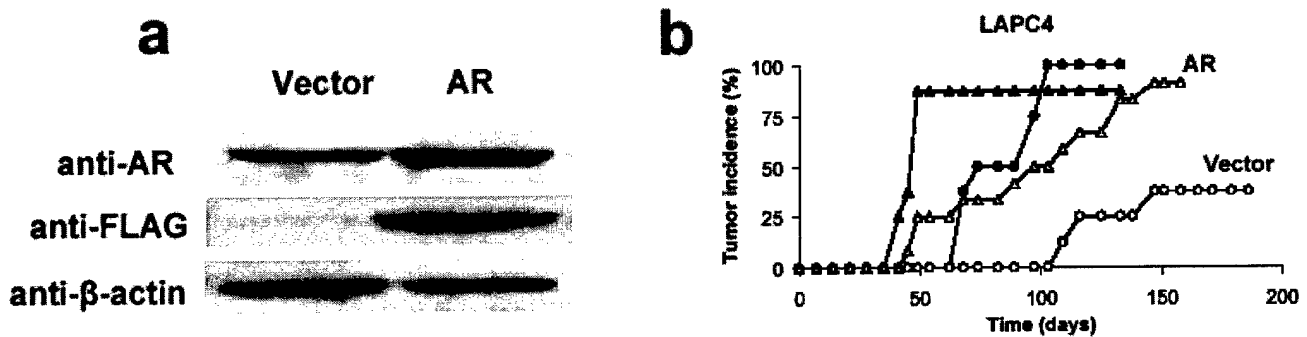


Figure 3. AR overexpression was sufficient to cause HS to HR progression in vivo. LAPC4 cells were infected with lentivirus expressing empty vector or flag-tagged, wild-type human AR. (A) Western blot analysis of AR-overexpressing LAPC4 cells. Lysates from control and AR-overexpressing LAPC4 cells were resolved and probed with antibodies against AR (top), FLAG (middle), or β -actin (bottom). (B) One million vector-infected (circles) or AR-infected (triangles) cells were grown subcutaneously in the flank of SCID mice which were either intact (closed symbols, n=8) or castrated (open symbols, n=12). Tumor formation, defined as a tumor reaching 40 mm³, was monitored each week.

Taken together, in vitro and in vivo data demonstrate that overexpression of AR is sufficient for the growth of HR prostate cancer.

Necessity test

To knockout AR in LAPC4 cells by somatic recombination

By fluorescence in situ hybridization, we determined that LAPC4 cells have one AR loci and LNCaP cells have two. Therefore, we set out to target the AR gene in LAPC4 cells using somatic recombination. However, we failed to obtain any recombinant after two rounds of transfection and selection. The failure may be interpreted as that LAPC4 cells do not have efficient recombination machinery and forced us to look for an alternative.

To knockdown AR in LNCaP cells by RNA interference

Using double strand small inhibitory RNA (siRNA), we were able to transiently and specifically knockdown AR expression by more than 90% (data not shown). A similar efficient and stable knockdown was achieved in LNCaP cells (Figure 4B) using a lentivirus system, which expresses the small inhibitory RNA of AR in a hairpin structure (Figure 4A). Preliminary results indicate that AR knockdown diminished the growth of HR cells in castrate level of androgen (Figure 4C). This result suggests that AR is required for HR prostate cancer. We are examining this issue in vivo.

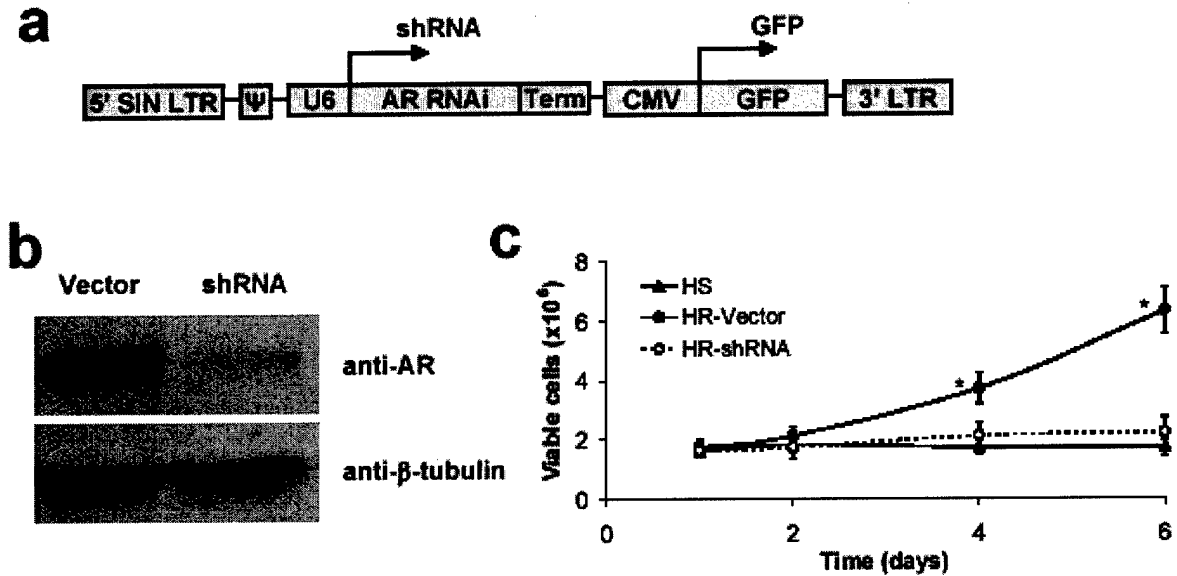


Figure 4. AR knockdown diminished HR growth of HR LNCaP cells. (A) Schematic of the lentivirus that expresses GFP and a shRNA directed against AR. (B) HR LNCaP cells were infected with the lentivirus with or without the U6-shRNA cassette. Cells were then sorted for the top 50th percentile of GFP-positive cells and analyzed by western blotting. (C) Sham-sorted HS LNCaP, or sorted HR LNCaP cells were androgen starved for 4 days and then grown in media containing 10% charcoal-stripped serum supplemented with 20 pM R1881. Cell counts were performed over 6 days.

KEY RESEARCH ACCOMPLISHMENT

1. Establish AR overexpressing prostate cancer cells
2. Demonstrate that AR overexpression is sufficient for HR progression of prostate cancer in vitro and in vivo
3. Knockdown AR in LNCaP cells by RNA interference

REPORTABLE OUTCOMES

None

CONCLUSION

In the last grant period, I was able to demonstrate that AR overexpression is sufficient for HR progression of prostate cancer in vitro and in vivo. I have also established a system to knockdown AR expression, which will be used to examine if AR overexpression is necessary for HR prostate cancer in vivo.