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Independent Prostate Cancer

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13. ABSTRACT (Maximum 200 Words) Hormone therapy is an effective treatment for advanced hormone sensitive (HS) prostate cancer. However, the treatment is short-lived and hormone refractory (HR) cancer eventually develops. Through gene profiling using seven pairs of HS and HR xenografts, we identified overexpression of androgen receptor (AR) is the only consistent change in the progression of prostate cancer. In the first grand period (01/02-10/03), we confirmed that AR protein is elevated in HR tumors. Using lentivirus and retrovirus systems, we were able to overexpress AR in HS prostate cancer cells. In vitro and in vivo experiments demonstrated that overexpression of AR is sufficient for HS-to-HR transition. We also developed a system (shRNA) to knockdown AR in HR LNCaP cells and shown that AR-knockdown abolished HR phenotype in vitro. In the last grand period (01/03-01/04), we demonstrated that overexpression of AR is required for hormone refractory prostate cancer in vivo using both LAPC4 and LNCaP cells. Although, we were unable to obtain conclusive results to determine the mechanisms for AR overexpression, we demonstrated that gene amplification is not the sole cause of the overexpression.				
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INTRODUCTION

I proposed in the grant to examine if overexpression of androgen receptor is necessary and sufficient for prostate cancer progression from androgen dependent to independence (Here the terms are changed to hormone sensitive to hormone refractory). In the first grant period, I demonstrated that overexpression of androgen receptor is sufficient for the progression in vitro and in vivo. In this grant period, I demonstrated that overexpression of androgen receptor is necessary for the progression.

BODY

Necessity test

To knockdown AR in HR LNCaP cells by RNA interference

In the first grant period, we demonstrated that AR is required for growth of HR LNCaP cells using shRNA. To determine that the phenomenon is not specific to HR LNCaP cells, we also knocked down AR in LAPC4, another HR cell line, using the same technology. When cells were implanted into the flanks of castrated male mice, both knockdown cells (LNCaP and LAPC4) grew slower than vector-infected controls (Figure 1). Moreover, those tumors that did grow did not express GFP when compared to vector-infected controls (insets) and still expressed AR protein (data not shown), indicating selection for cells that escaped AR knockdown. These data indicate that increased AR levels are necessary for hormone refractory prostate cancer.

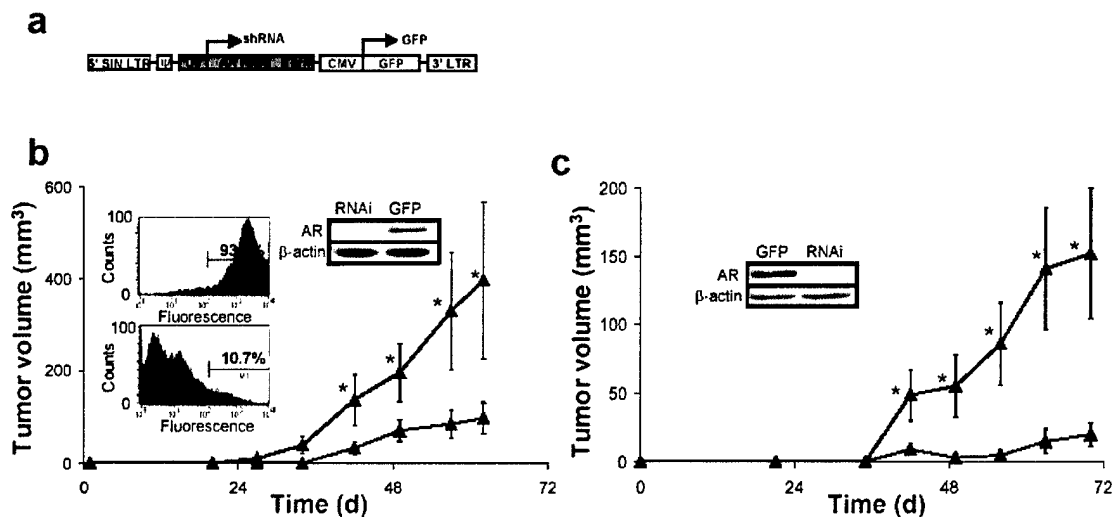


Figure 1. Androgen receptor expression is necessary for hormone sensitive-to-hormone refractory progression (a) Schematic of shRNA lentivirus directed against androgen receptor (b) Tumor volume (\pm SEM) of hormone refractory LAPC4 cells infected with either the shRNA-expressing (blue, $n = 8$) or control virus (grey, $n = 8$) (* signifies $P < 0.05$, t -test). Right inset, western blot of shRNA-expressing and control (empty vector) cells. Left inset, flow cytometry for GFP-positive cells of representative tumors

(control virus, top; androgen receptor shRNA virus, bottom). (c) Tumor volume (+/- SEM) in a similar experiment using hormone refractory LNCaP cells.

Mechanisms of AR overexpression. In the original proposal, we proposed to study the mechanisms responsible for the overexpression of AR in AI tumors. Up to now we are unable to generate data for the original proposed work, because we could not get enough number of single cells for each pair of xenografts. We have already tried this experiments three times. We are optimizing the digestion protocol to improve the yield.

It was demonstrated that hormone refractory prostate cancer samples have AR gene amplification (Visakorpi et al., 1995). To examine if the gene amplification can explain our xenograft finding, we initiated collaboration with the Visakorpi group in Finland. We performed the comparative genomic hybridization (CGH) on hormone sensitive and hormone refractory pairs of LAPC4, 9, and LNCaP. Using this method, we did not observe any AR gene amplification in hormone refractory xenografts compared to their respective hormone sensitive counterparts, suggesting that gene amplification cannot be the sole cause, if at all, for the overexpression.

Table 1. Comparative genomic hybridization (CGH) on hormone sensitive (HS) and hormone refractory (HR) LAPC4 and 9, and hormone sensitive LNCaP.

Sample	Gains	Losses
9HS	7p14-pter,	1p13-p21, 3p21-pter, 6cen-q22, 8p21-pter, 17q24-qter
9HR	7p14-pter,	1p13-p21, 3p21-pter, 6cen-q22, 8p21-pter, 17q24-qter
4HS	1p32-pter, 8q24, 9q34, 16p, 17q24-qter, 19, 22, Xq26-q28	1p22-31, 2q22-q34, 4p14-qter, 5p14-qter, 6cen-q24, 8q21-q23, 13q21-qter
4HR	1p32-pter, 8q24, 9q34, 16p, 17q24-qter, 19, 22, Xq26-q28	1p22-31, 2q22-q34, 4p14-qter, 5p14-qter, 6cen-q24, 8q21-q23, 13q21-qter
LNCaP HS	19p	2p13-p23, 4q26-q32, 6cen-q21, 13q21-q25
LNCaP HR	not done	not done

KEY RESEARCH ACCOMPLISHMENT

1. Demonstrated that AR overexpression is necessary for hormone refractory prostate cancer by RNA knockdown technology
2. Demonstrated that AR gene amplification is not the sole cause of the overexpression

REPORTABLE OUTCOMES

None

CONCLUSION

In the last grant period, I was able to demonstrate that AR overexpression is necessary for hormone refractory prostate cancer progression in vivo. We also demonstrated that gene amplification is not the sole cause of the AR overexpression.

REFERENCES

Visakorpi, T., Hyytinen, E., Koivisto, P., Tanner, M., Keinanen, R., Palmberg, C., Palotie, A., Tammela, T., Isola, J., and Kallioniemi, O. P. (1995). In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet* 9, 401-406.