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PRINCIPAL INVESTIGATOR: Steven P. Balk

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center
Boston, MA 02215

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14. ABSTRACT We have examined a series of prostate cancer xenografts to identify models that progress to castration resistant prostate cancer (CRPC) with reactivation of androgen receptor (AR) activity and increased expression of aldo-keto reductase family 1, member C3 (AKR1C3). Significantly, we found that tumor progression after castration in the VCaP xenograft is associated with reactivation of AR (including expression of the TMPRSS2-ERG fusion gene), and with an increase of ~5-fold in AKR1C3 gene expression. We are currently assessing whether inhibition of AKR1C3 will impair growth in this model. To assess androgen synthesis in patients progressing to CRPC, we have measured serum levels of androgen and metabolites and found that a subset have increased levels of the androgen metabolite 3alpha-diolG. We are currently assessing androgen levels in CRPC patients treated with agents to further suppress androgen synthesis to determine whether androgen levels correlate with responses.					
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INTRODUCTION

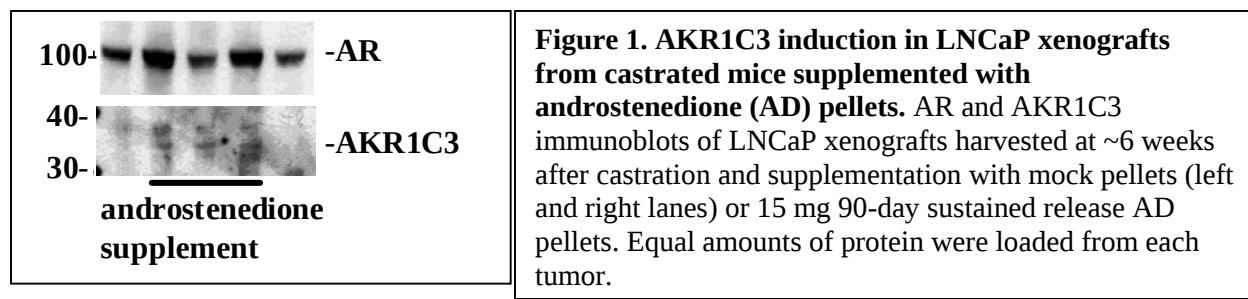
The majority of prostate cancers (PCa) are androgen dependent, and androgen deprivation therapies remain as the standard treatment for non-organ confined disease. Unfortunately, patients treated with androgen deprivation therapies invariably relapse with rapidly progressive systemic PCa, which has been termed hormone refractory, androgen independent, or castration resistant prostate cancer (CRPC). Significantly, the androgen receptor (AR) is highly expressed in most cases of CRPC and appears to be transcriptionally active, but the molecular events mediating the progression to CRPC and apparent reactivation of AR transcriptional activity remain to be defined. Our data indicate that increased intratumoral production of testosterone from precursors (adrenal androgens and possibly endogenous sterols) may contribute to the reactivation of AR transcriptional activity in CRPC. We propose that tumors adapt to androgen deprivation therapy by increasing their synthesis of potent androgens from available weak adrenal androgens (and possibly from endogenous precursors), and that AKR1C3 is a key enzyme in this process. Our objective is to test this hypotheses using cell line and xenograft models (Aim 1) and by measuring androgen and androgen metabolite levels in patients who progress to CRPC. If the intracellular production of potent androgens from adrenal or endogenous precursors is indeed a mechanism for progression to CRPC, then this pathway would become a new therapeutic target.

BODY

Aim 1. Test the hypothesis that increased expression of AKR1C3 enhances conversion of androstenedione to testosterone and stimulates tumor growth after androgen deprivation therapy in cell line and xenograft models (months 1-36)

1a. Determine whether increased AKR1C3 stimulates testosterone synthesis and expression of downstream androgen catabolic enzymes and enhances tumor growth under castrate conditions when supplemented with androstenedione (months 1-18)

Research findings: One important difference between humans and rodents is that the production of weak androgens (DHEA and androstenedione) is unique to human adrenal glands, so that levels of these hormones are much lower in castrated mice. Therefore, one approach to model progression to CRPC was to supplement mice with androstenedione (AD) pellets. In initial studies we found that 15 mg AD 90-day sustained release pellets (Innovative Research of America) would increase serum AD levels in castrate mice to levels that were at least equal to those in castrated men (data not shown). Significantly, we find that AKR1C3 protein expression in LNCaP xenografts in castrated mice is induced under these conditions (Fig. 1).



1b. Test the hypothesis in androgen dependent PCa xenograft models that adrenal androgens select for tumor cells with increased conversion of androstenedione to testosterone and DHT (months 12-36)

Research findings: We recently extended our studies to look at the VCaP prostate cancer xenograft, as these cells express the androgen regulated TMPRSS2-ERG fusion. As shown in figure 2, expression of ERG, TMPRSS2, and PSA mRNA fall rapidly when the androgen dependent xenografts (D) are androgen deprived by castration (C). However, expression of these transcripts is reactivated when the tumors start progressing after ~6 weeks (androgen independent, I).

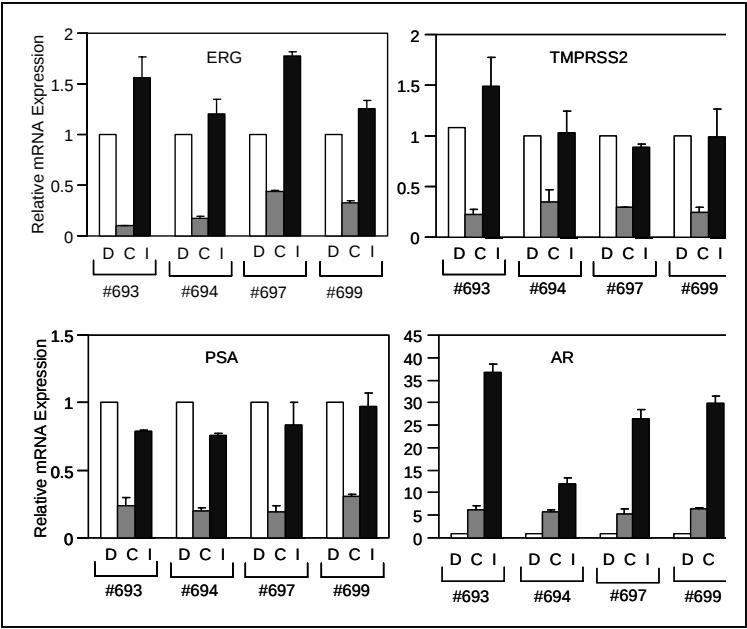


Figure 2. AR reactivation after castration in VCaP xenografts. VCaP xenografts in scid mice were biopsied prior to castration (D) , at 5 days after castration (C), or at ~6 weeks after castration when tumors were again growing (I). Expression of ERG, TMPRSS2, PSA, and AR were assessed by RT-PCR.

To determine whether there was selection for increased androgen production, we assessed a series of enzymes that mediate androgen synthesis and metabolism. An outline of androgen metabolism is shown in figure 3. As shown in figure 4, AKR1C3 and other enzymes mediating testosterone synthesis from adrenal androgens were increased after castration. Significantly, CYP17A1, which is deficient in rodent adrenal glands, was also increased in the VCaP xenografts after castration. These results indicate that prostate cancers may progress to CRPC both by increasing their ability to generate testosterone from adrenal androgens, and by

increasing their synthesis of androgen precursors from cholesterol. Further xenograft studies are now being focused on the VCaP model.

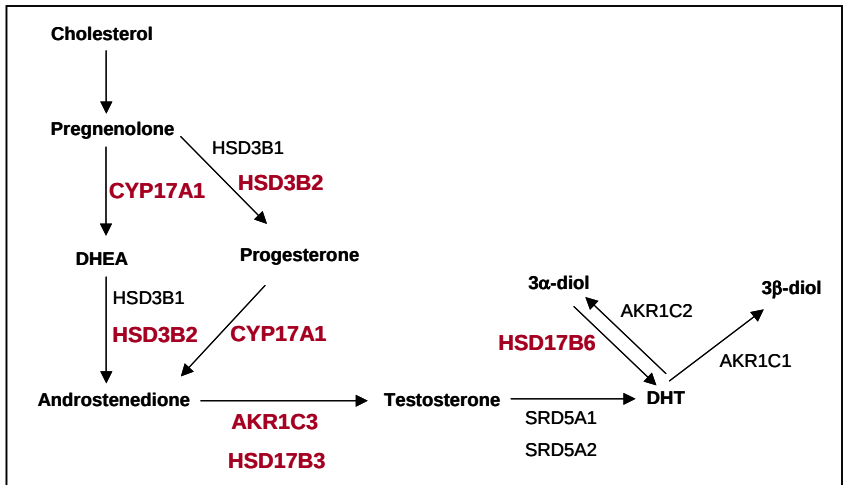


Figure 3. Outline of androgen synthesis. Testosterone synthesis from AD is mediated normally by AKR1C3 in prostate and by HSD17B3 in testes.

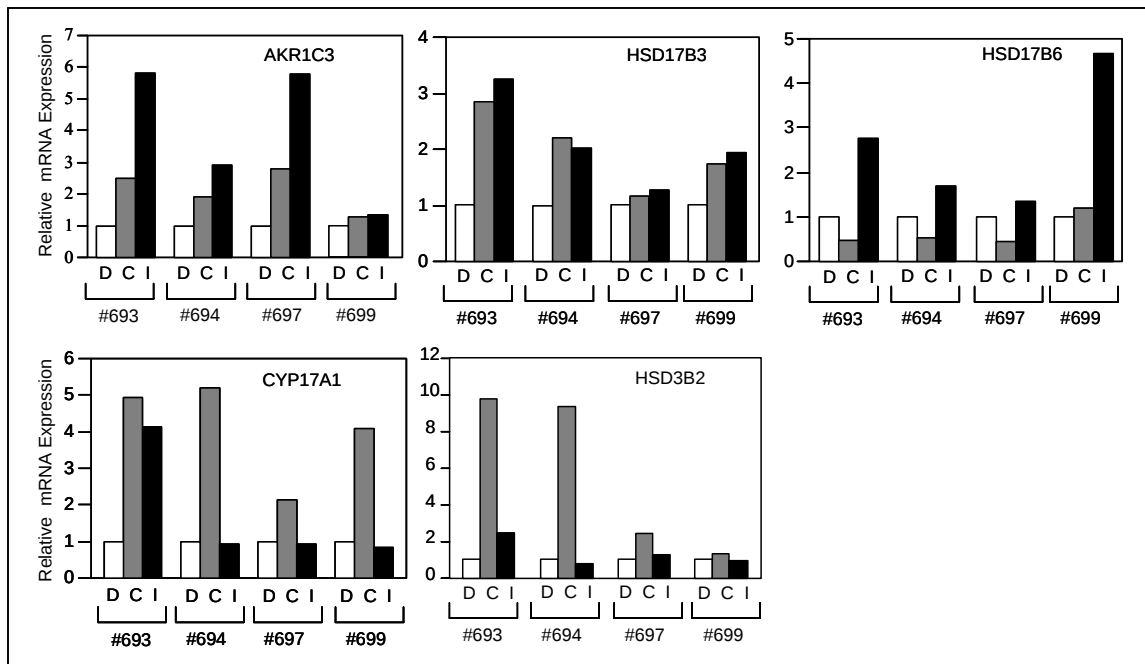


Figure 4. Quantitative RT-PCR analysis of enzymes mediating androgen synthesis in VCaP xenografts. RNA from VCaP xenografts prior to castration (D), ~5 days after castration (C), and at progression (I) was isolated and analyzed for expression of the indicated enzymes.

Aim 2. Test the hypothesis that serum levels of androgen metabolites are increased in patients with progression to androgen independence (months 1-36)

2a. Determine in a cross-sectional study whether the progression to AIPCa is associated with increased levels of DHT metabolites (months 1-18)

Research findings: Archived serum samples from men who had undergone androgen deprivation therapy and were either in remission or had progressed to CRPC (relapse) were collected and analyzed by RIA for a series of androgens and androgen metabolites. Figure 5 shows the expression of a presumed major metabolite, 3alpha-diolG versus DHEA-S. As expected, there is a slight increase in 3alpha-diolG with increasing levels of DHEA-S. However, there appears to be a subset of men in the relapse group with increased 3alpha-diolG (3aD), although the difference between the groups is not statistically significant.

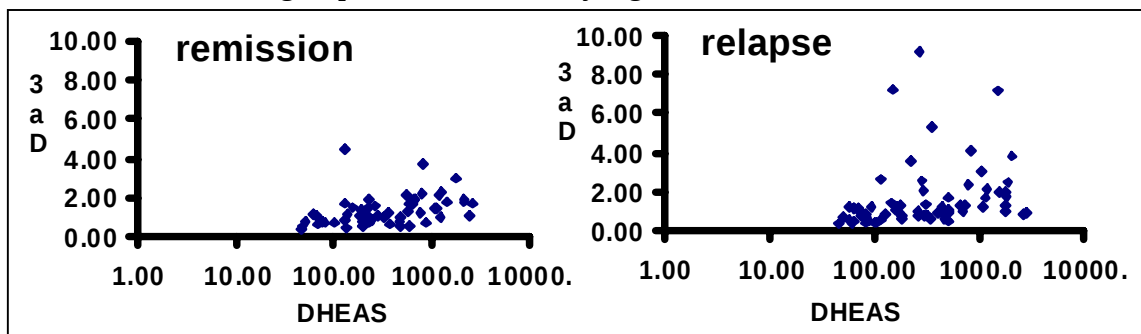


Figure 5. DHEA-S versus 3alpha-diolG levels in men with prostate cancer who are androgen ablated and in remission or relapsed.

2b. Determine in a longitudinal study whether the progression to AIPCa is associated with increased levels of DHT metabolites (months 1-36)

Research findings: Serum samples are currently being collected for this study. We are also analyzing serum samples from additional patients with relapsed CRPC who are being treated with agents to suppress androgen synthesis (ketoconazole and dutasteride), which will both expand our cohort of samples and address whether hormone levels correlate with responses.

KEY RESEARCH ACCOMPLISHMENTS

- xenograft model for increased androgen synthesis in prostate cancer progression
- evaluation of androgen production in clinical samples with prostate cancer progression

REPORTABLE OUTCOMES

Taplin,M.E., Manola,J., Oh,W.K., Kantoff,P.W., Bubley,G.J., Smith,M., Barb,D., Mantzoros,C., Gelmann,E.P., and Balk,S.P. (2008). A phase II study of mifepristone (RU-486) in castration-resistant prostate cancer, with a correlative assessment of androgen-related hormones. BJU. Int. 101, 1084-1089.

Bao,B.Y., Chuang,B.F., Wang,Q., Sartor,O., Balk,S.P., Brown,M., Kantoff,P.W., and Lee,G.S. (2008). Androgen receptor mediates the expression of UDP-glucuronosyltransferase 2 B15 and B17 genes. Prostate 68, 839-848.

CONCLUSION

The recent work further supports the conclusion that a mechanism for tumor progression after androgen deprivation is increased intratumoral androgen synthesis. Further xenograft studies will focus on the VCaP model to determine the efficacy of inhibiting key enzymes in the process. Studies in clinical material will focus on collection of more samples and preclinical efforts to develop therapies that suppress androgen synthesis.

REFERENCES

Taplin,M.E., Manola,J., Oh,W.K., Kantoff,P.W., Bubley,G.J., Smith,M., Barb,D., Mantzoros,C., Gelmann,E.P., and Balk,S.P. (2008). A phase II study of mifepristone (RU-486) in castration-resistant prostate cancer, with a correlative assessment of androgen-related hormones. BJU. Int. 101, 1084-1089.

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APPENDICES

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