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13. ABSTRACT (Maximum 200 Words)

Abundant evidence suggests that overexpression of TGFß by prostate cancer cells enhances their ability to grow and metastasize. TGFß is secreted by cells in a latent form that results from a noncovalent interaction between TGF-ß and its propeptide (latency-associated peptide, LAP). Mechanisms leading to active TGF-ß are poorly understood at present. Our lab discovered a mechanism of TGF-ß activation in which the integrin αVß6 binds to an RGD sequence near the C-terminus of LAP. αVß6 is only expressed in epithelial cells. We hypothesize that αVß6, by activating TGF-ß1, is an important regulator of normal prostate epithelial proliferation, and that overexpression of aVb6 by prostate tumor cells acts in concert with overexpression of its ligand latent TGF-ß1 to produce active TGF-ß1 and promote growth of the tumor. In this work, we are testing whether the ß6 integrin subunit is regulated by androgen, whether it is overexpressed in human prostate cancer, and whether it affects growth and metastasis of prostate cancer in an animal model. Our results to date indicate that ß6 expression is upregulated in the mouse in a delayed fashion after castration. We are now testing whether castration-induced prostate involution is affected by ß6 by comparing normaland ß6 KO animals, and producing mice that develop prostate cancer (TRAMP) that also lack ß6 gene expression.

14. SUBJECT TERMS
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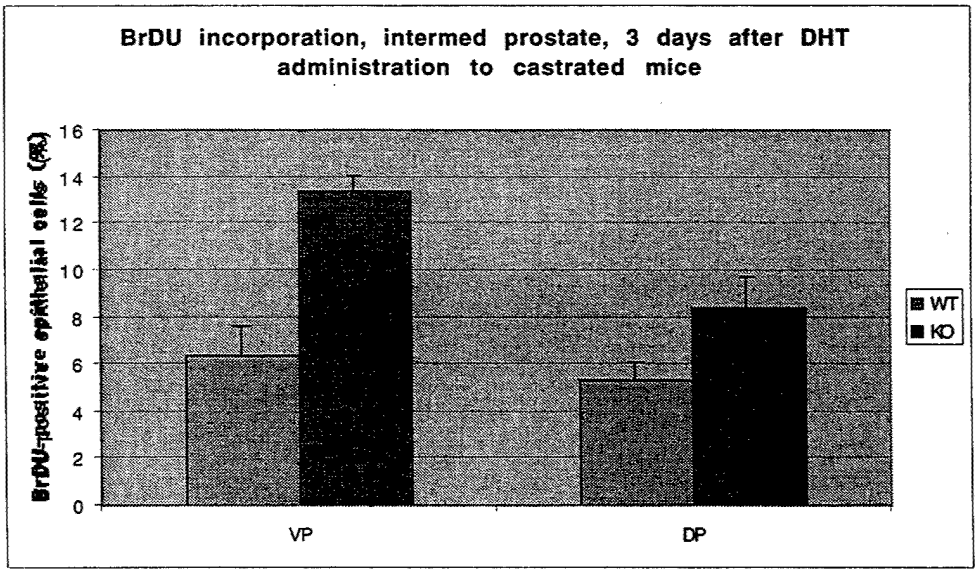
INTRODUCTION

The subject of this work is a system for the activation of latent TGFß in the prostate. The system consists of the αVβ6 integrin expressed on epithelial cells. Our previous work showed that this integrin can bind to an integrin recognition site (arg-gly-asp) on latent TGFß1 and effect its activation. TGFß is known to be important for regulating the growth and differentiation of various epithelia, and also to be important in cancer growth. Little is currently known about this system in the prostate: eg, what cells express αVβ6, how expression of the integrin is regulated, and if and when this system regulates prostate epithelial growth via production of active TGFß1. The purpose of the work is to demonstrate whether or not this system plays a role in prostate cancer. The scope of the work involves cell line and mouse experiments (to gauge the normal expression and regulation of αVβ6 in the prostate), and evaluation of human prostate cancer tissue and an *in vivo* mouse prostate cancer model (to address the question, does αVβ6-mediated activation of TGFß1 promote prostate cancer growth?).

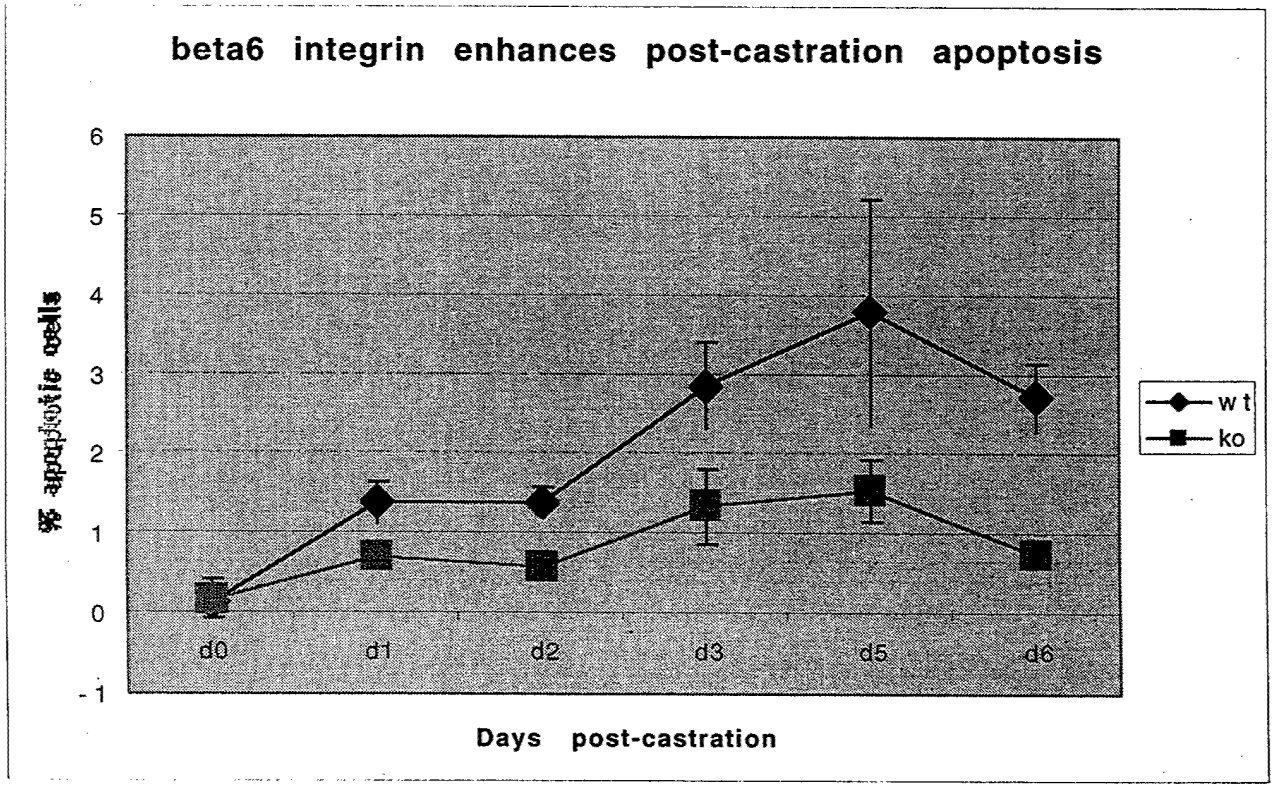
Body

1. We determined avb6 intregrin expression in mouse and human prostate. avb6 is not expressed in resting mouse prostate except for low levels in proximal ducts. After castration, avb6 is readily detectable at 48 h in a patchy distribution - ie, some lumens are stained and others are negative. By 7 days post-castration, avb6 expression is more uniform. (We looked at functional differences between b6+ and b6- ducts as discussed below). In human prostate, avb6 is expressed uniformly in basal cells and in atrophic epithlium. However, it is virtually absent in all cancers we examined.
2. Although avb6 is upregulated after castration, we did not find that androgen altered the level of avb6 expression in 2 prostate epithlial cell lines examined (from Dr. EL Wilson). A luminal cell line expresses avb6 (and activates TGFb), and a basal cell line does not express avb6. Neither pattern of avb6 expression in the presence or absence of DHT inmthe culture medium. Our hypothesis is that androgen withdrawal leads indirectly to avb6 upregulation in the prostate.
3. We use b6-/- mice to measure the effect of avb6 (and resulting TGFb activation) on prostate epithelium proliferation. At baseline in adult animals, BrDU incorporation is very low and no difference was seen between wild type and b6-/- animals. Additional animals were castrated (to induce high avb6 expression). Ten days after castration, animals were treated with androgen (DHT) to stimulate rapid growth of prostate. At 3 days post androgen replacement, BrDU incorporation was markedly increased, as expected, and was about 2-fold higher in b6-/- than in wild type. This result is consistent with endogenously activated TGFb being present in the wildtype mice and acting as a brake on proliferation. This is the first example of avb6 regulating epithelial cell growth via TGFb activation.

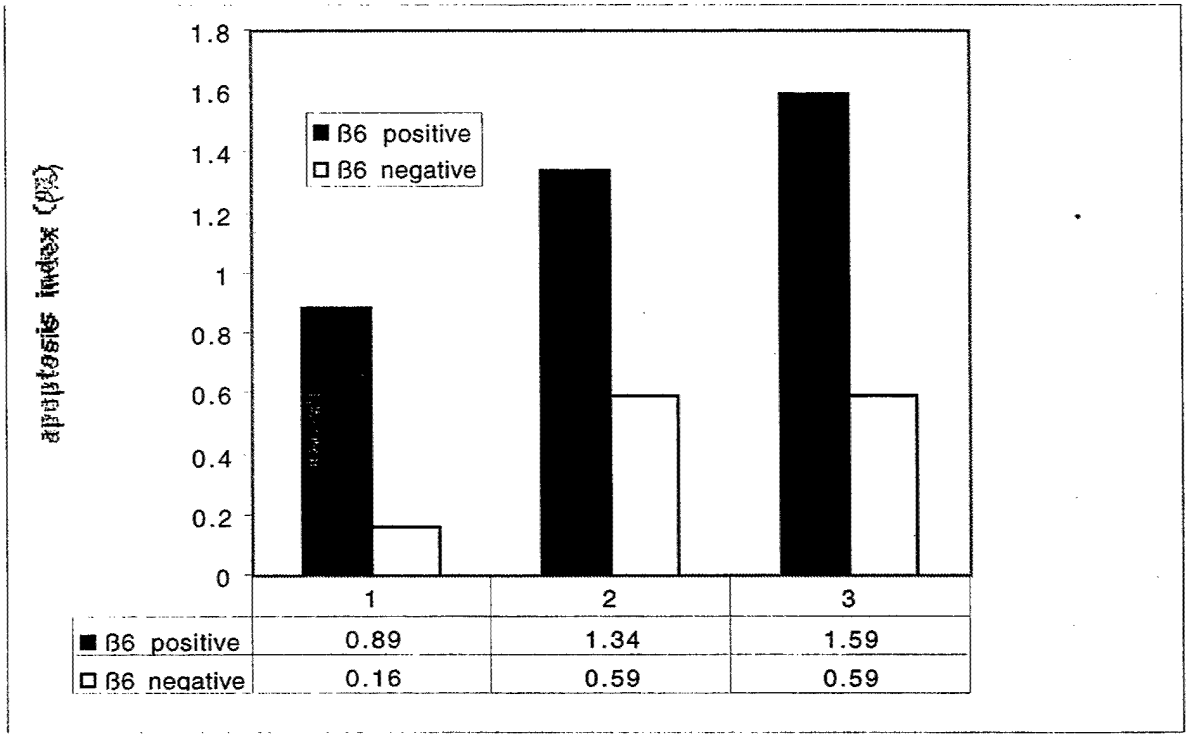


4. PTEN is reported to influence integrin function by acting as a phosphatase for integrin-interacting proteins like FAK. However, in experiments in which we transfected cells with sense and antisense PTEN sequences, we found no evidence that PTEN expression alters TGFb activation by avb6.

5. We failed in our attempt to determine the influence of avb6 on a mouse model of cancer. The idea was to cross TRAMP mice, which express an oncogene in the prostate and develop prostate cancer, with b6-/- and see if the absence of avb6 affects growth of the primary tumor and/or metastases. The problem was that the TRAMP/b6-/- mice bred very poorly. Littermate wildtype TRAMP mice that we bred did well. However, although we managed to generate about 8 breeding pairs of b6-/- and TRAMP/b6-/- mice, litters were rarely produced and when pups appeared they generally wasted and died. One problem may have been pathogens. At the start of the experiment, the mice were housed in a specific pathogen-free facility. However, the facility developed a murine hepatitis virus infection and breeding was halted in the facility for ~ 6 months. To continue our experiment, we were forced to transfer our mice to another non-pathogen free facility where we could continue to breed. The cross may have been unexpectedly susceptible to pathogens there; wild type, TRAMP and b6-/- mice continued to breed well, but TRAMP/b6-/- mice bred with b6-/- mice did not. We are still hoping to complete this experiment but will have to rebreed from the beginning in a clean facility. It is also possible that an unexpected genetic abnormality in TRAMP/b6-/- mice prevents breeding regardless of pathogens.
6. We did additional characterization of the effects of avb6-mediated TGFb activation in mouse prostate not originally proposed. Since TGFb is alleged to be an inducer of apoptosis in prostate after castration, we measured the rate of apoptosis as a function of time after castration in WT and b6-/- mice. Apoptosis was measured by TUNEL by a blinded observer. We found that the rate of apoptosis in b6-/- is consistently only ~half that of wildtype mice. This is consistent with avb6-mediated TGFb activation in post-castration prostate influencing apoptosis. Differences are significant at all time points except day 5. We also



compared the rate of apoptosis in lumens expressing avb6 and lumens not expressing avb6, at 2 days post-castration in wild-type mice. We did this by using serial sections for b6 immunohistochemistry and for TUNEL. (Recall that early after castration we found that avb6 expression is heterogeneous). In this case, apoptosis rate was ca. 3-fold higher in b6+ lumens than in b6- lumens. This results strengthens the previous result because it eliminates the possibility of an irrelevant difference in b6-/- and b6+/+ mice accounting for the difference in apoptosis. Finally, we directly measured active TGFb in supernatants of homogenized prostate tissue 4 days post-castration and found a significant increase in wild type compared to b6-/- mice.



	1	2	3
ß6 positive	0.89	1.34	1.59
ß6 negative	0.16	0.59	0.59

Key Research Accomplishments

-First description of b6 expression in normal and malignant human prostate and in murine prostate.
-Evidence that b6 expression in prostate is indirectly regulated by androgen.
-Evidence that avb6 in prostate activates TGFb that regulates prostate epithelial proliferation and apoptosis.
-PTEN is not involved in regulating TGFb activation by avb6

Reportable Outcomes

1. Two manuscripts are being prepared (on the effects of avb6 on murine prostate apotosis and proliferation, and on b6 expression in normal and malignant human prostate).
2. Animal model: TGFb effects quantifiable by measurements of post-castration apoptosis and androgen-induced exvolution.
3. I plan to apply for additional grant funds to study mechanisms involved in regulating b6 gene expression in prostate.

Conclusions

Avb6-mediated TGFb activation in the prostate can regulate epithelial cell proliferation and apoptosis in murine prostate. In humans, the presence of avb6 in basal cells (not seen in mice) suggests that TGFb activation might be important in suppressing the proliferation of these cells. The results in mice suggest that hormonal therapy in humans might increase avb6 expression and result in increased TGFb activation. This might have positive or negative effects on tumor growth and metastasis.