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Award Number: DAMD17-00-1-0036

TITLE: The Role of aVb6-mediated latent TGF-B1 Activation in Prostate Cancer

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REPORT DATE: January 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 2002	Annual (1 Jan 01 - 31 Dec 2001)

4. TITLE AND SUBTITLE
The Role of aVb6-mediated latent TGF-B1 Activation in Prostate Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0036

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Abundant evidence suggests that overexpression of TGF-ß by prostate cancer cells enhances their ability to grow and metastasize. TGF-ß 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
transforming growth factor-B, integrin, TGFB activation, PTEN, phosphatase, cell proliferation, epithelium

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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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 [1]. 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

The second 12 months of the project addressed tasks 1 (determine ß6 expression in normal prostate and prostate cancer cells), 2 (determine the effect of androgen on ß6 expression), 3 (effect of ß6 on prostate epithlial proliferation) and 5 (effect of ß6 on cancer in a mouse model of prostate cancer).

We have castrated control and ß6 KO mice and sacrificed them at multiple time points. We have also BrDU-labeled control and KO mice. We now have sufficient samples to analyze and are systematically analyzing the samples. We are doing TUNEL staining of the involuting prostate specimens to see if ß6 expression alters the rate of apoptosis. Preliminary results suggest that at the day 3 time point there is less apoptosis in the KO mice. The only remaining samples to generate are exvoluting prostates in normal and KO mice (castrated mice are given DHT pellets to induce regrowth of the prostate).

We have obtained TRAMP mice and are currently crossing them with the ß6 KO mice. Once we generate combined ß6KO/TRAMP (estimate: 2-3 months) mice we will assess their tumor growth and metastasis.

We have looked at ß6 expression in human prostate cancer tissue. In normal areas, ß6 is expressed in basaol cells. In the cancer areas, we see no ß6 expression except in occasional intensely stained cells. The tumors are of intermediate grades and we are trying to get samples of higher grade tumors as well as of metastases to see if s6 expression is a marker of more aggressive cancer.

KEY RESEARCH ACCOMPLISHMENTS

- ß6 is expressed in basal cells of human prostate. This may be important for growth control of these cells (via TGfß activation) and might be involved in growth control of prostate stem cells, which may exist in this compartment.
- Human prostate cancer, at least of intermediate grade, does not express s6 except for rare positive cells. This may be because these lower grade tumors are still growth-inhibited by TGFß, or because they are of luminal origin.

REPORTABLE OUTCOMES

The human prostate data are reportable.

CONCLUSIONS

These experiments are the first to describe ß6 expression in human prostate cancer, and in normal prostate. The remaining work done has not yet been fully analyzed but should give insights into the role of ß6 integrin on prostate epithelial cell proliferation during androgen withdrawal and replacement.

"So what." There are many reports in the literature relating cancer outcomes and tumor cell behavior to increased expression of TGFß by tumor cells. However, to my knowledge there has never been an analysis of the role of a TGFß activator in tumor cells. Yet, our results with $\alpha v\beta 6$ and lung fibrosis [1] point out the critical role that a TGFß activator can play in a TGFß-dependent process. If a specific TGFß activator can be identified as important in a cancer, this knowledge might be important for determining prognosis and for developing therapies in which the activator is a target.

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