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

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The Role of αVβ6-mediated latent TGF-B1 Activation in Prostate Cancer

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Abundant evidence suggests that overexpression of TGF-β by prostate cancer cells enhances their ability to grow and metastasize. TGF-β is secreted by cells in a latent form that results from a noncovalent interaction between TGF-β and its propeptide (latency-associated peptide, LAP). Mechanisms leading to active TGF-β are poorly understood at present. Our lab discovered a mechanism of TGF-β activation in which the integrin αVβ6 binds to an RGD sequence near the C-terminus of LAP. αVβ6 is only expressed in epithelial cells. We hypothesize that αVβ6, by activating TGF-β1, is an important regulator of normal prostate epithelial proliferation, and that overexpression of αVβ6 by prostate tumor cells acts in concert with overexpression of its ligand latent TGF-β1 to produce active TGF-β1 and promote growth of the tumor. In this work, we are testing whether the β6 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 β6 expression is upregulated in the mouse in a delayed fashion after castration. We are now testing whether castration-induced prostate involution is affected by β6 by comparing normal and β6 KO animals, and producing mice that develop prostate cancer (TRAMP) that also lack β6 gene expression.

transforming growth factor-B, integrin, TGFβ activation, PTEN, phosphatase, cell proliferation, epithelium
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INTRODUCTION

The subject of this work is a system for the activation of latent TGFβ 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 TGFβ1 and effect its activation [1]. TGFβ 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: e.g., 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 TGFβ1. 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 TGFβ1 promote prostate cancer growth?).
BODY

The second 12 months of the project addressed tasks 1 (determine β6 expression in normal prostate and prostate cancer cells), 2 (determine the effect of androgen on β6 expression), 3 (effect of β6 on prostate epithelial proliferation) and 5 (effect of β6 on cancer in a mouse model of prostate cancer).

We have castrated control and β6 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 β6 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 β6 KO mice. Once we generate combined β6KO/TRAMP (estimate: 2-3 months) mice we will assess their tumor growth and metastasis.

We have looked at β6 expression in human prostate cancer tissue. In normal areas, β6 is expressed in basaol cells. In the cancer areas, we see no β6 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

- β6 is expressed in basal cells of human prostate. This may be important for growth control of these cells (via TGFβ 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 TGFβ, or because they are of luminal origin.
REPORTABLE OUTCOMES

The human prostate data are reportable.
CONCLUSIONS

These experiments are the first to describe β6 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 β6 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 TGFβ by tumor cells. However, to my knowledge there has never been an analysis of the role of a TGFβ activator in tumor cells. Yet, our results with αvβ6 and lung fibrosis [1] point out the critical role that a TGFβ activator can play in a TGFβ-dependent process. If a specific TGFβ 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.
REFERENCES
