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PRINCIPAL INVESTIGATOR:  John S. Munger, M.D.

CONTRACTING ORGANIZATION:  New York University School of Medicine
New York City, New York  10016

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The Role of αvβ6-mediated latent TGF-β Activation in Prostate Cancer

John S. Munger, M.D.

New York University School of Medicine
New York City, New York 10016

E-Mail: mungej01@med.nyu.edu

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

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 is expressed in prostate luminal epithelial cells, which activate TGF-β1 in a β6-dependent manner, and that β6 expression is upregulated in the mouse in a delayed fashion after castration.
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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: 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 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 first 12 months of the project addressed tasks 1 (determine β6 expression in normal prostate and prostate cancer cells) and 2 (determine the effect of androgen on β6 expression).

We began by looking at well-established prostate cell lines established by Dr. EL Wilson of NYU [2]. One line has characteristics of prostate luminal epithelial (LE) cells, the other of prostate basal epithelial (BE) cells. Because the β6 integrin is expressed only in epithelial cells, we did not look at other cell types (e.g., prostate smooth muscle cells).

We did immunostaining using a rabbit monoclonal Ab (designated B1) obtained from D Sheppard at UCSF. We found that the LE cells stained intensely for β6 integrin, whereas the BE cells were negative. We confirmed these results functionally in 2 ways. First, we did adhesion assays using recombinant LAP-β1, the ligand for αvβ6. LE cells, but not BE cells, were able to adhere and spread on plastic coated with LAP-β1 at coating concentrations similar to that needed for other cell types expressing β6 integrin (e.g., transfected SW-480 colon carcinoma cells). Second, we found that LE cells in culture were able to activate TGFβ1 in an αvβ6-dependent manner. This is shown in Figure 1.

![TGF-β activation by prostate luminal epithelial cells](image)

**Figure 1.** LE cells were cocultured with reporter cells (TMLC) that respond to TGF-β by producing luciferase. Compared to reporter cells cultured alone (black bars), reporter cells cultured with LE cells detect active TGFβ (white bars); the signal is abrogated by a reagent that inhibits active TGFβ (LAP) and by an anti-β6 Ab (10D5), demonstrating that the active TGFβ is produced via αvβ6.

We next examined the effect of androgen on β6 expression by these cells. Both cell types express androgen receptor (AR), and both cell types are normally cultured in the presence of testosterone. Dihydrotestosterone (DHT) is added to the serum-free culture medium of the LE cells, and is present in the 10% serum included in the BE cell medium. Therefore, we set
up subconfluent cultures of LE and BE cells in medium without DHT (for the BE cells this was done by using charcoal-treated serum). After DHT-free growth for 24 or 48 hours, cells were immunostained for β6 integrin. The staining patterns were unchanged: LE cells still expressed a high level of β6 and BE cells were negative. Thus, at least in cell culture, we find no evidence of androgen regulation of β6 expression.

We also examined TGFβ activation by LE and BE cells in the presence and absence of DHT. As expected, based on the immunostaining results, the BE cells did not activate TGFβ, regardless of DHT status. However, when LE cells are cultured for 24 h in the absence of DHT and then cocultured with TGFβ-sensitive reporter cells, we find increased active TGFβ. This is shown in Figure 2.

![Activation of TGFβ by luminal epithelial cells cultured with or without DHT](image)

**Figure 2.** Luminal epithelial cells were cultured with or without DHT for 24 h, then cocultured for an additional 24 hours with TGFβ-responsive reporter cells to measure production of active TGFβ. For each condition, cells were cultured with and without a reagent that inhibits active TGFβ (LAP); in each case, the difference between luciferase activity with and without LAP is proportional to the amount of active TGFβ. SW480 colon Ca cells mock- or β6-transfected are shown as negative and positive controls for TGFβ activation, respectively.

Thus, in short-term cell culture experiments we find that β6 expression is not androgen-regulated, although the β6-expressing LE cells are able to activate greater amounts of TGFβ in the absence of DHT. This latter effect might be due to increased TGFβ expression or to some other effect, such as enhanced interaction of αVβ6 with the cytoskeleton.

We have also looked at the regulation of β6 within the murine prostate as a function of androgen. We do this by immunostaining frozen sections of mouse prostate obtained from normal or castrated mice. In normal mouse prostate, we find minimal β6 expression in prostate epithelium. Similarly, in mice that are 2 days and 5 days post-castration we detect only trace amounts of epithelial β6 expression. However, when we
examined prostates from mice 8 weeks post-castration, we found high levels of β6 expression in prostate epithelium. β6 was clearly expressed in luminal cells; in addition, in many areas there was increased staining adjacent to the basement membrane that might represent β6-expressing basal cells.

Thus, our in vivo data suggest that prostate β6 expression is affected by androgen, but in a delayed and therefore perhaps indirect manner. One can speculate that active TGFβ generated by αVβ6 in the involuted prostate contributes to the growth arrest observed in that state.

An obvious test of this idea is to examine β6 knockout mice after castration. This is planned as part of task 3 in the coming year. In anticipation of this, we have acquired the β6 KO mice (on a C57Bl/6 background). Because these mice will also be crossed with a transgenic mouse line (TRAMP mice, which develop prostate cancer), we plan to house the β6 mice in our transgenic facility. Doing so requires that the mice be rederived (superovulated WT females are impregnated by β6 KO males in another facility; the fertilized eggs are removed and implanted in a recipient female within the transgenic facility). Therefore, over the last 4 months we have been working to generate these rederived mice and currently have 5 heterozygous pups and two more pregnant recipient females. Once these mice are available in sufficient numbers, we will examine the histology of β6 KO prostates and compare prostate proliferation (by BrdU labeling) in wild type and β6 KO mice (in the normal state, during prostate involution after castration and then during prostate regeneration following DHT pellet implantation).

In other work, we have made a recent observation that may bear on this work. Work by S Nishimura (UCSF), in which our lab collaborated, has shown that the integrin αVβ8 can activate latent TGFβ1. This has now been reported in abstract form at the ASCB meeting in December 2000, and a manuscript (on which I am an author) is in preparation. β8 is expressed more widely than is β6 – it is expressed not only in epithelial cells but also in smooth muscle cells and the CNS. There is no published work on β8 expression in the prostate. The importance to this project is that β8 is therefore another potential player as an activator of TGFβ3 in the prostate. Currently I do not plan to modify the proposed work to examine β8; however, if as we progress observations suggest a major role for β8 in the prostate I may discuss a possible change/addition to our plan with the US Army Medical Research and Materiel Command staff.
KEY RESEARCH ACCOMPLISHMENTS

- In cell lines, αVβ6 is expressed at high levels in luminal but not basal prostate epithelial cells.
- In short-term experiments, β6 is not regulated by androgen in either the murine prostate or in cell lines. However, in long-term experiments, β6 expression is upregulated in the involuted prostate of castrated mice. This is consistent with the idea that β6-mediated TGFβ activation is kept at low levels in the normal prostate, but is important in maintaining the involuted state (TGFβ inhibits epithelial cell growth).
- Although androgen removal does not increase αVβ6 expression in luminal prostate cell lines, it does increase TGFβ activation.
REPORTABLE OUTCOMES

None yet. We need data from β6 knockout mice (task 3, year 2) to complement the current results.
CONCLUSIONS

These experiments are the first to examine the role of αVβ6-mediated activation of latent TGFβ in the prostate. Our results suggest that normally αVβ6 expression in the prostate epithelium is kept at a low level, which (we theorize) is one factor allowing normal expression of the prostate epithelium. Interestingly, there is a delayed expression of β6 integrin after castration, which may contribute to the continued inhibition of epithelial growth in that state.

Our results also show that prostate cell lines (specifically, a luminal cell line) can express αVβ6 and activate TGFβ1 in an αVβ6-dependent manner. Although this particular cell line is not tumorigenic, it is immortalized and p53-null and therefore presumably has some characteristics of prostate cancer cells. Work in the next 2 years will directly examine the expression and influence of αVβ6 in malignant prostate cells.

The first year of this project has also been important as a training period in the techniques needed for analyzing murine prostate. We are now in a strong position to extend the work to the subsequent tasks.

“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
