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TITLE: Thromboxane Synthase and Prostate Cancer Progression

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14. ABSTRACT The initiation and progression of prostate cancer remain not well understood to enable development of interventional therapy. Thromboxane synthase is an enzyme downstream of cyclooxygenase, utilizing prostaglandin H to form thromboxane A ₂ . Using immunohistochemistry analysis, we found that 25% of clinical prostate tumor specimens had strong expression of thromboxane synthase; 33% of cases had medium expression and 42% of cases had weak expression of thromboxane synthase. Prostate cancer cells isolated from lymph node metastasis had higher levels of thromboxane synthase expression and activity than those isolated from the primary tumor sites in an animal model. We cloned and sequenced full-length thromboxane synthase cDNA from PC-3 cells and constructed an expression vector. Increased expression of thromboxane synthase in DU145 cells was found to stimulate cell migration. Inhibition of thromboxane synthase or blockade of thromboxane A ₂ function inhibited prostate cancer cell migration. Further we found that PCa cells express receptors for TXA ₂ and stimulation with TXA ₂ mimetic led to the activation of RhoA and cell retraction. Our study suggest that thombxane synthase and its eicosanoid product play a contributory role in prostate cancer progression.					
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INTRODUCTION

The hemostatic system plays an important role in the regulation of tumor angiogenesis and progression. Prostate cancer is one of most common cancers affecting American men. The initiation and progression of prostate cancer remain not well understood to enable rational development of interventional therapy. In this proposal, we propose to study thromboxane synthase and its product, thromboxane A₂, in prostate cancer progression. Thromboxane synthase is an enzyme downstream cyclooxygenase, utilizing prostaglandin H to form thromboxane A₂. Thromboxane A₂ is a potent inducer of platelet aggregation which can subsequently lead to coagulation and thrombosis, a hematological complication affecting about 15% to 20% of cancer patients and the second leading cause of death in cancer patients. Platelet aggregation also release a plethora of angiogenesis regulators and fibrin deposition to facilitate angiogenesis and formation of tumor stroma. In addition, it has been demonstrated that thromboxane A₂ also can directly modulate endothelial cell angiogenic responses. It is our working hypothesis that TXA₂ may play an important role in prostate tumor progression and that this functional role of TXA₂ is achieved by modulating tumor cell motility, endothelial angiogenic responses, and platelet aggregation. TXA₂ produced by PCa cells can promote PCa cell migration as an autocoid and modulate endothelial cell angiogenic responses and stimulate platelet aggregation as a paracrine factor. Platelet aggregation release various angiogenesis regulators and cause coagulation, which subsequently leads to deposition of fibrin at tumor sites. These events, collectively, promote tumor angiogenesis and growth. The proposed work will provide significant insights into how prostate cancer cells regulate cell migration and hemostatic system to facilitate tumor angiogenesis, growth, and metastasis. The knowledge obtained from the proposed work will identify key targets (TX synthase and TXA₂ receptor) to develop interventional therapy for prostate cancer, advancing the program's eventual goal to eliminate prostate cancer.

BODY OF REPORT

List of Technical Objectives

Modified Task 2. Study the effect of increased expression of wild type TX synthase in DU145 cells on cell migration, proliferation, and apoptosis.

- a. Convert the mutated TX synthase expression construct to wild-type, through site directed mutagenesis approach.
- b. Transfect DU145 cells using the new, wild-type TX synthase expression construct and study the biosynthesis of TXA₂.
- c. Plate the transfected DU145 cells and vector control cells and study the proliferation rate using MTS proliferation assay kit.
- d. Plate TX synthase transfected DU145 cells, and their vector control, in 10 cm dishes and study whether TX synthase overexpression confers on DU145 cells resistance to apoptosis induced by COX inhibitor NS398, as assessed by DNA laddering and apoptosis induced by COX inhibitor NS398, as assessed by DNA laddering and apoptosis ELISA for DNA fragmentation.
- e. Plate TX synthase transfected DU145 cells and vector control cells in fibronectin-precoated Boyden chambers for migration assay as a standard procedure.

Research Progress

When we submitted our request for a one year no-cost extension we listed Modified Task 2 above as our stated objective. However shortly after we receiving the extension we initiated a collaboration with Dr. Anthony Ashton of the Albert Einstein Medical School. Dr. Ashton had developed antibodies individually recognizing the two thromboxane receptors, i.e. TP α and TP β . These antibodies which were useful in immunohistochemistry gave us the unique opportunity to conduct a clinical study on expression of TP α and TP β in human prostate tissues. Therefore we modified our objectives to the analysis of TP α and TP β expression. The results of which are presented below.

Expression of TP-beta and TP-alpha in human prostate

Expression of TP-beta and TP-alpha was analyzed by immunohistochemistry in 51 radical prostatectomy specimens, which included 25 organ-confined (pT2) and 26 locally advanced diseases (16 pT3a and 10 pT3b). Of those 25 organ-confined prostate cancers, 10, 14 and 1 had a Gleason score 6, 7 and 8 respectively, whereas of those 26 locally advanced diseases, 21, 4 and 1 had a Gleason score 7, 8 and 9 respectively. Immunoreactivity was evaluated semiquantitatively according to staining intensity and percentage of cells stained. A staining score, which was determined by the percentage of tumor cells with moderate and strong staining intensity, was assigned to each tumor. Mann-Whitney test was used for statistical analysis and a p value of <0.05 was considered significant.

In normal prostatic glands, TP-beta was mainly expressed in the basal cells. Increased expression in luminal cells was found in areas of atrophy, basal cell hyperplasia, inflammation and transitional cell metaplasia. Significantly increased TP-beta expression was also found in high-grade prostatic intraepithelial neoplasia (Figure 1). The expression of TP-alpha was very focal and weak in non-neoplastic prostate. In high-grade prostatic intraepithelial neoplasia, the expression of TP-alpha was variably increased (Figure 2). Both markers were heterogeneously expressed in prostatic adenocarcinomas. When the cases was separated into 2 groups according to pathologic stage (pT), a tendency of increased expression of both markers was noted in the advanced tumors (Table 1). However, these differences were not statistically significant. Interestingly, when the tumors were categorized according to Gleason score, expression of both markers was significantly increased in moderately to poorly differentiated tumors (Gleason score ≥ 7) compared to moderately well differentiated tumors (Gleason score 6) (Table 2, Figure 1 and 2). There was no further significant differences

in the expression of either marker between moderately poorly differentiated tumors (Gleason score 7) and poorly differentiated tumors (Gleason score 8 and higher). These results suggest that TP-alpha and TP-beta may play important roles in the initiation and early progression of prostate cancer.

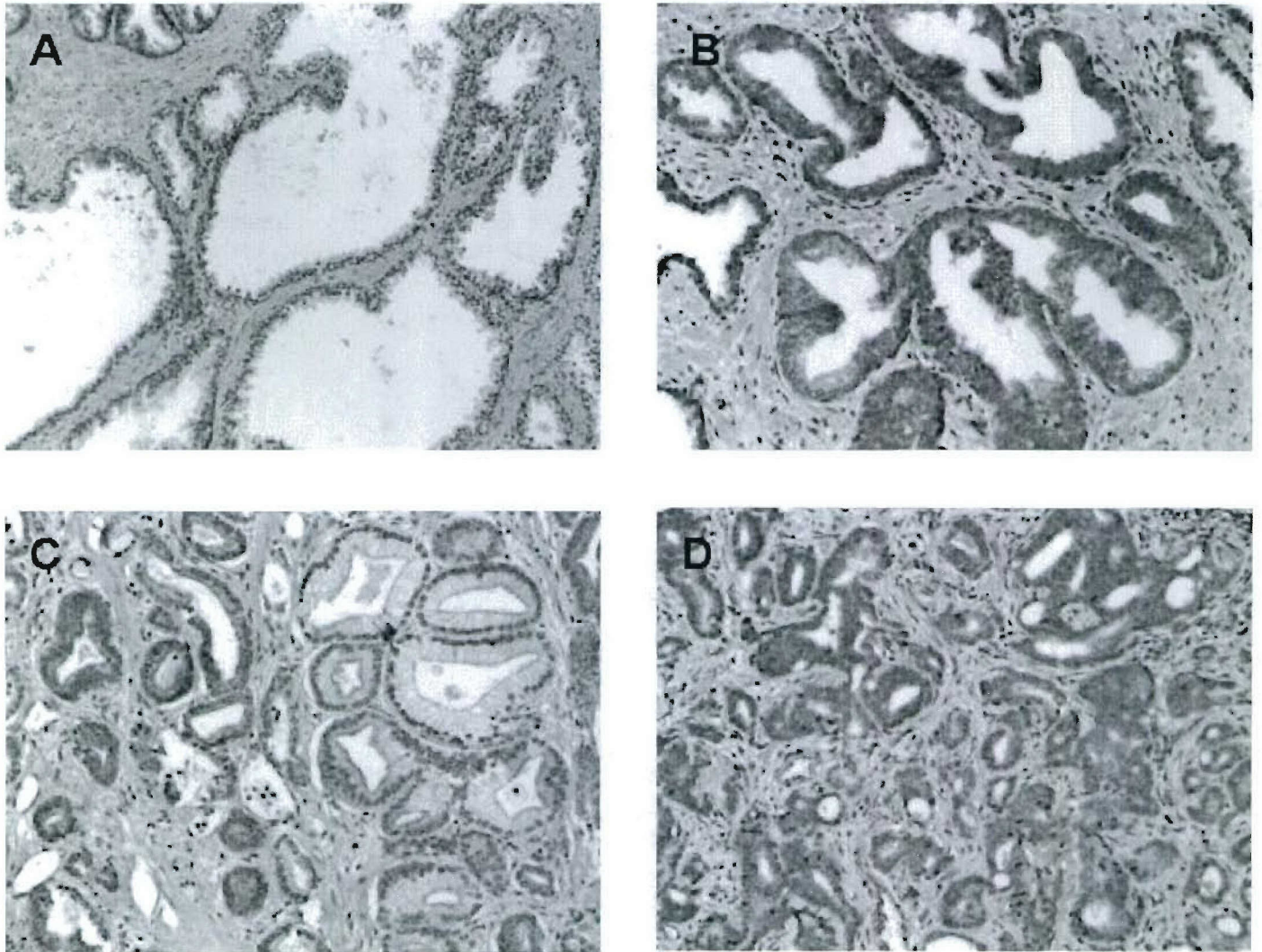


Figure 1. TP-beta expression in human prostate.

A, normal; B, high-grade prostatic intraepithelial neoplasia; C, Gleason score 6 prostatic adenocarcinoma; and D, Gleason score 7 prostatic adenocarcinoma.

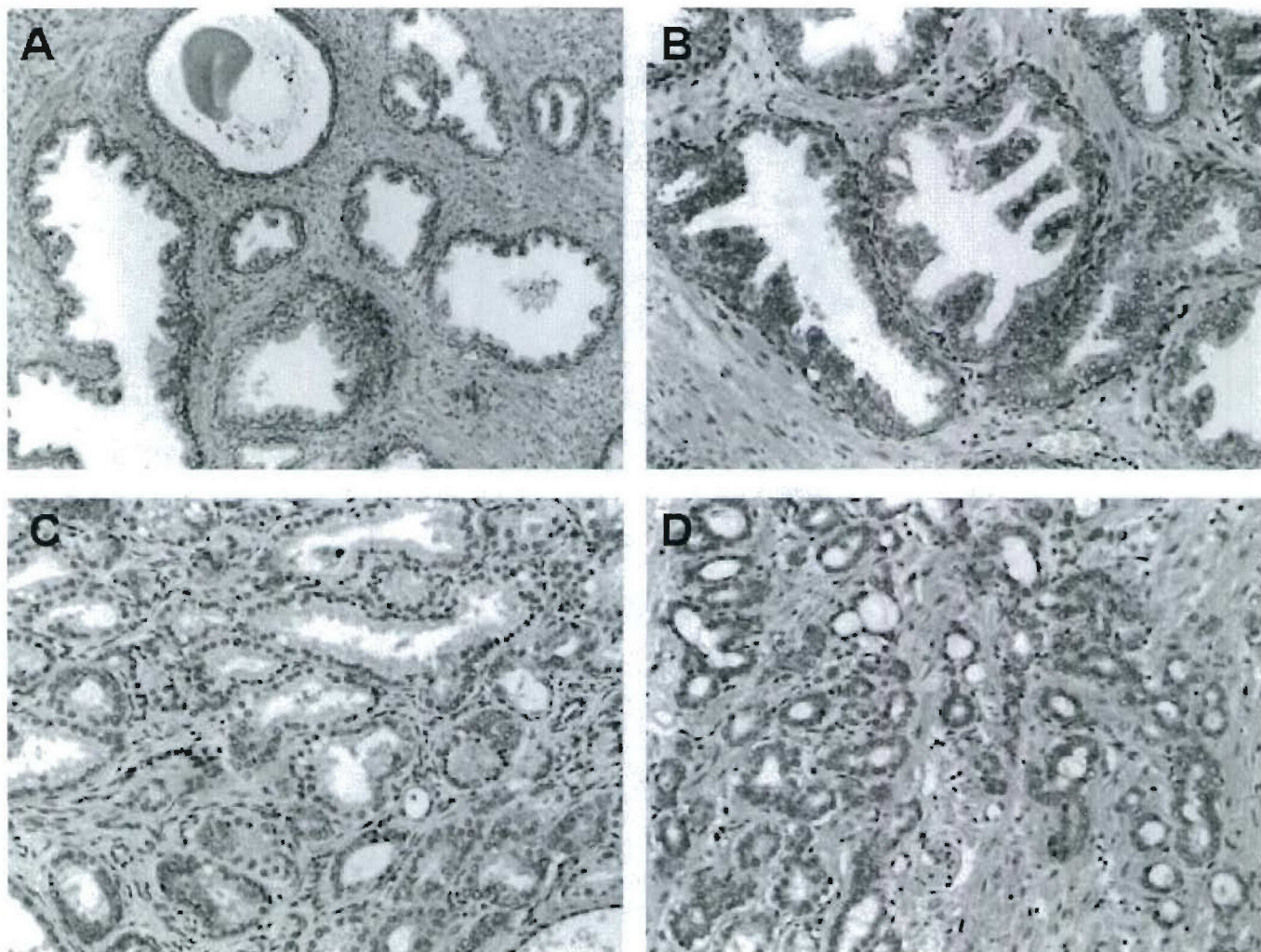


Figure 2. TP-alpha expression in human prostate.

A, normal; B, high-grade prostatic intraepithelial neoplasia; C, Gleason score 6 prostatic adenocarcinoma; and D, Gleason score 7 prostatic adenocarcinoma.

Table 1. TP expression in prostate according to pathologic stage

Pathologic Stage	N	TP-BETA Median (range)	TP-ALPHA Median (range)
pT2	25	50 (5-100)	20 (5-70)
pT3	26	70 (5-100)	30 (5-80)
P Value		0.1171	0.2443

TABLE 2. TP EXPRESSION IN PROSTATE ACCORDING TO GLEASON SCORE

GLEASON SCORE	N	TP-BETA Median (range)	TP-ALPHA Median (range)
6	10	30 (5-80)	15 (5-70)
≥7	41	70 (5-100)	30 (5-80)
P Value		0.0082	0.0109

Key Research Accomplishments

We were able to demonstrate that antibodies individually recognizing TP α and TP β were suitable for immunohistochemical studies on human prostate tissue. Further we were able to show a significant increase in expression of TP α and TP β with increased Gleason grade.

Reportable Outcomes

- *Presentations*

- Thromboxane Synthase and TP Receptors In Tumor Progression*

- Kenneth V. Honn

- 9th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Related Diseases. San Francisco, CA . September 11-14, 2005. (Podium Presentation)

- Abstracts Published

- Thromboxane Synthase and TP Receptors In Tumor Progression*

- Kenneth V. Honn

- 9th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Related Diseases. San Francisco, CA . September 11-14, 2005.

- Thromboxane Synthase in Human Breast Cancer Cells*

- Yande Guo, Keqin Tang, Yinlong Cai, Yan Qiao, Daotai Nie, and Kenneth V. Honn

- 9th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Related Diseases. San Francisco, CA. September 11-14, 2005.

- Patents applied: None
- Degrees obtained that are supported by this award: None
- Development of cell lines, tissue or serum repositories: None
- Funding applied or obtained: Yes

- Based on the above described findings as well other findings previously reported we submitted an application to the National Institutes of Health entitled "Role of Thromboxane in Prostate Cancer Progression " this grant received a sufficient priority score and will be funded next month.

Conclusions:

Our studies over the past one year extension of this grant demonstrated that thromboxane receptors $TP\alpha$ and $TP\beta$ were both expressed in human prostate tissues. The expression was weak in the non-neoplastic prostate. Expression was found in high grade prostatic intraepithelial neoplasia and both markers increased expression in high Gleason grade tumors versus low Gleason tumors.

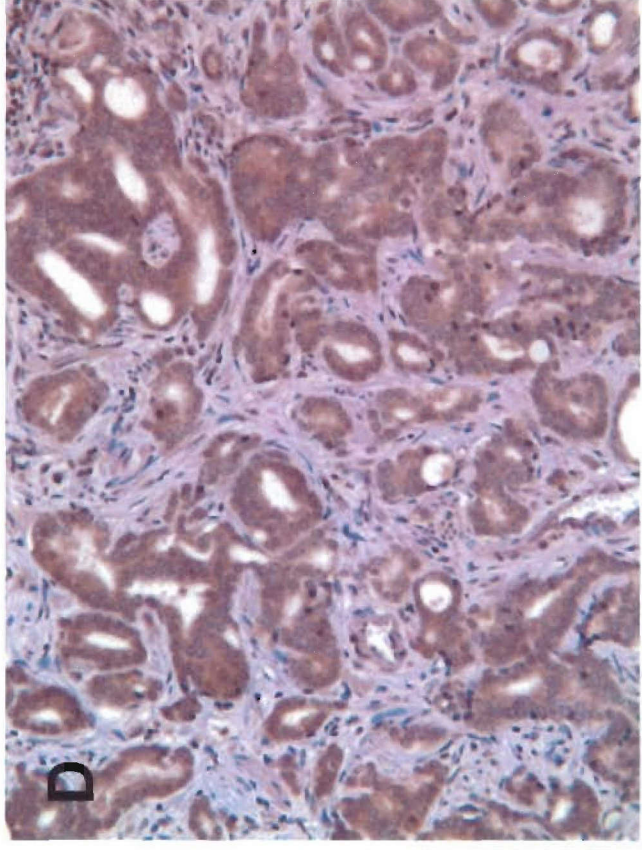
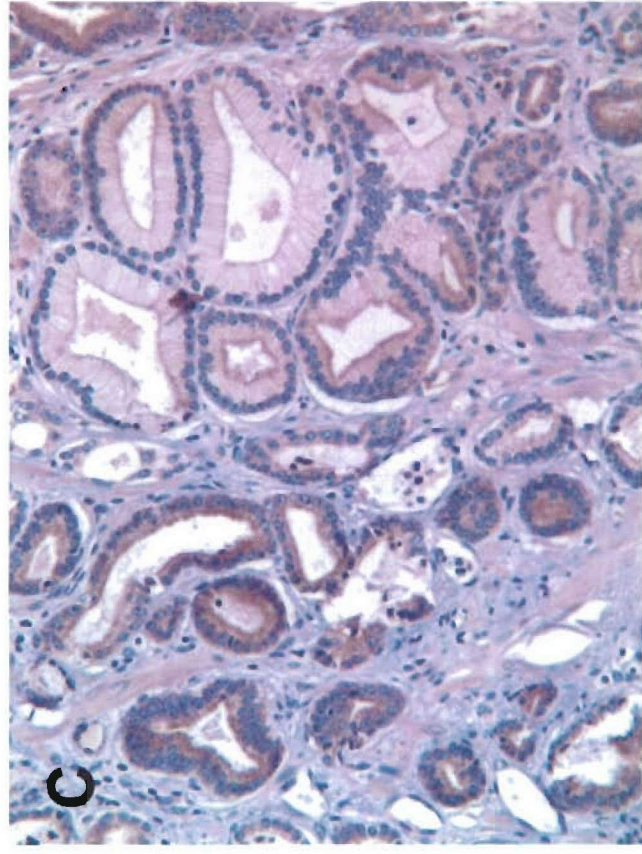
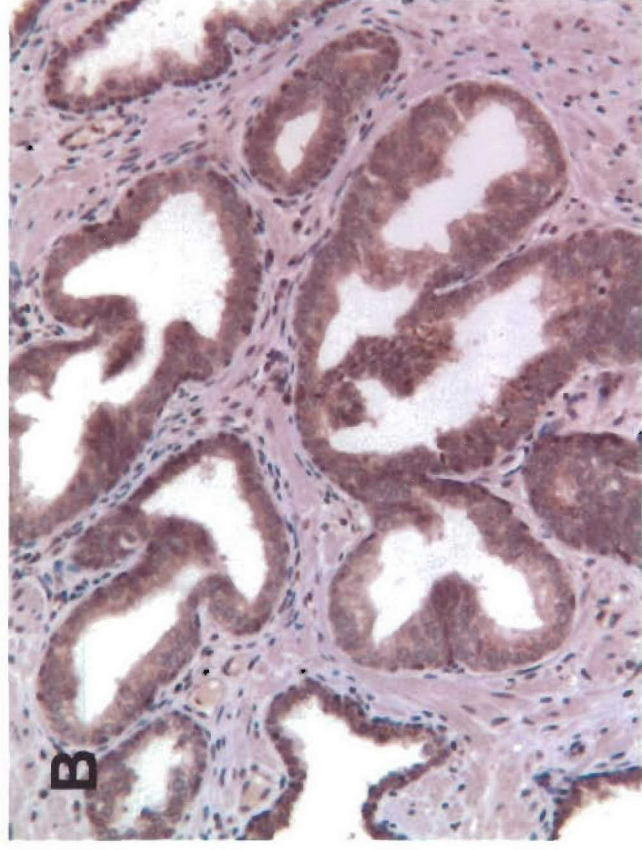
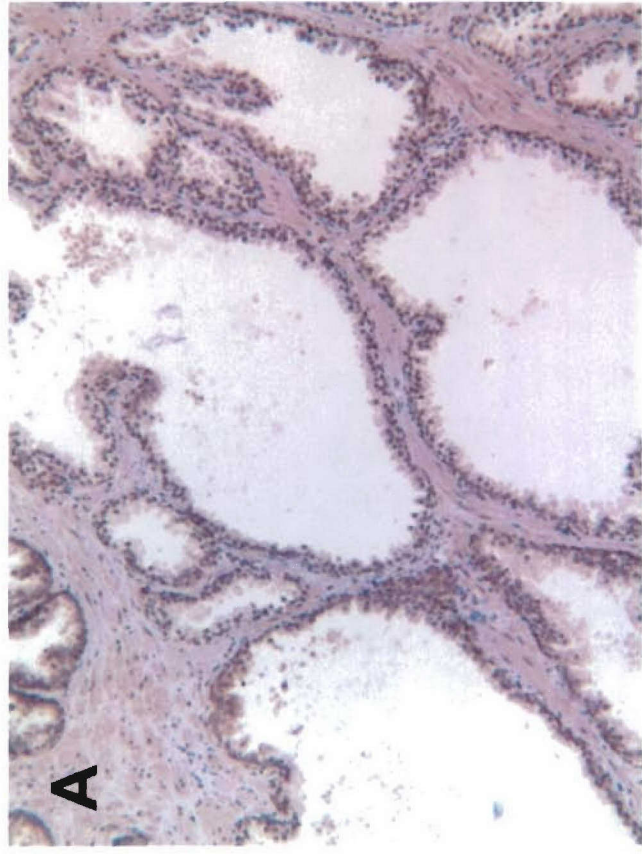


Figure 1

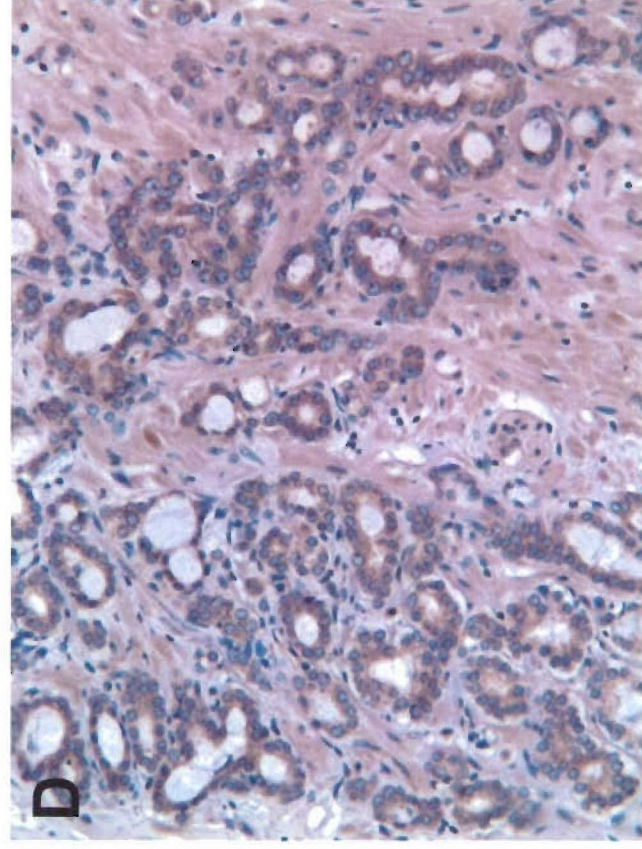
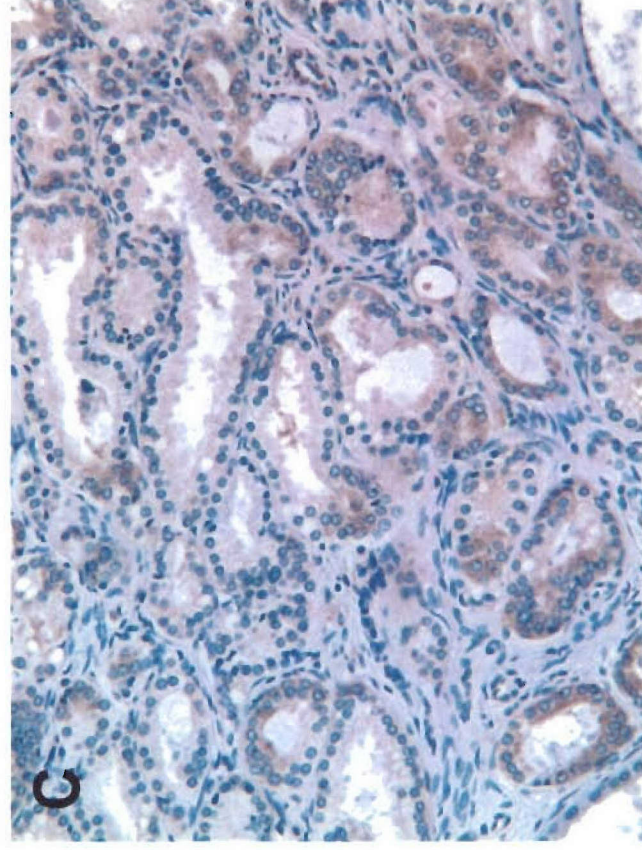
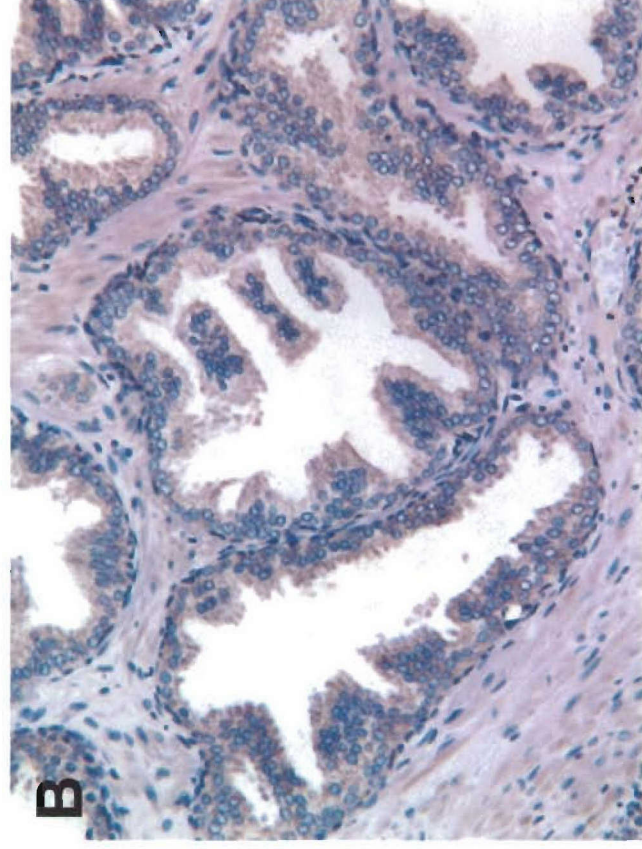
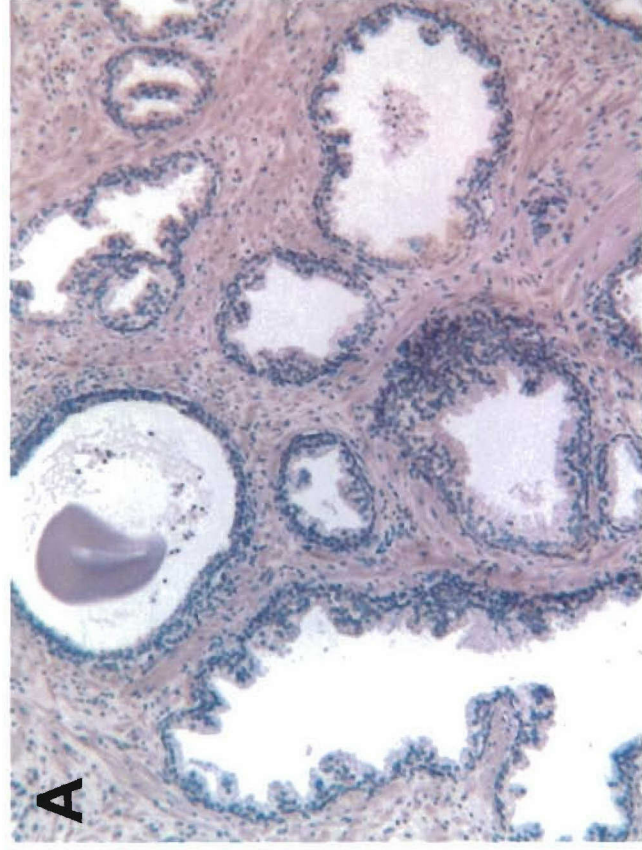


Figure 2