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14. ABSTRACT Prostate cancer is a major cause of cancer-related mortality for men in the United States. The established treatment protocol for the advanced stage of the disease is by surgical castration and or chemotherapy. Although androgen ablation therapy is initially effective in inhibiting tumor growth in most patients, however with time, the tumors recurs with a more aggressive and metastatic phenotype, ultimately causing death. Thus limitation of treatment underscores the need of development of an alternative treatment approach. Several reports suggest that vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) induced angiogenesis is essential for the growth and metastasis of prostate cancer. As our recent reports indicate that the neurotransmitter dopamine (DA) can specifically and significantly inhibit VPF/VEGF induced angiogenesis, therefore, we investigated whether DA can inhibit angiogenesis, and thereby growth of prostate cancer. We here in this report demonstrate that DA can significantly inhibit PC3 human prostate cancer growth by inhibiting VPF/VEGF mediated angiogenesis by suppressing VEGFR-2 phosphorylation in tumor endothelial cells. Thus our results are significant because DA is already in clinical use with an established safety record; therefore our study may be rapidly translated to the clinics as a new and more effective therapy for prostate cancer.						
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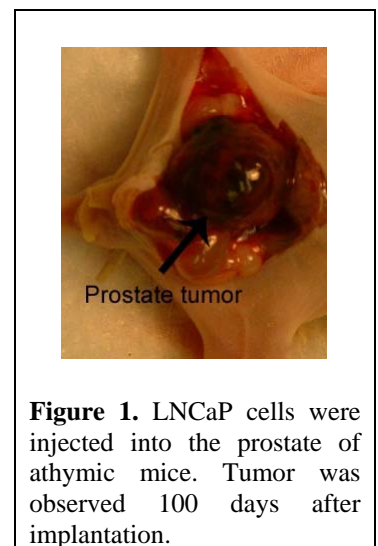
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Introduction: Metastatic prostate cancer is one of the major causes of cancer related death in American men. During 2007, an estimated 218,890 men will be diagnosed with prostate cancer, and 27,050 men will die of this disease in the United States (1). Because activation of the androgen receptor by androgens is required for cell proliferation in the prostate, androgen ablation therapy in the form of medical and or surgical castration is initially effective in inhibiting cancer cell growth in most patients (2). However, with time, the tumor recurs in an androgen-refractory manner, presenting with a more aggressive and metastatic phenotype and ultimately causing patient death (3-7). These limitations associated with the use of androgen ablation therapy for the treatment of advanced prostate cancer, underscore the need for the development of an alternative treatment approach (3-7). In this regard, angiogenesis represents an effective target since there are reports which indicate that transition of an androgen-dependent human prostate cancer into an androgen-independent subtype is associated with increased angiogenesis (3-7). Among the many stimulators of angiogenesis, vascular permeability factor/vascular endothelial growth factor-A (VPF/VEGF-A) is the most important cytokine responsible for angiogenesis, growth and metastasis of many solid tumors (8, 9). Based on the information presently available, it is likely that VPF/VEGF-A by inducing angiogenesis help the prostate cancer cells to survive in the absence of androgens either by turning on the anti-apoptotic machinery or helping these cells to enter quiescent state (3-7). This in turn allows the tumor cells the time necessary to undergo genetic changes to become more metastatic and androgen independent (3-7). Recently, it has been shown by us that the neurotransmitter dopamine (DA) can specifically and significantly inhibit VPF/VEGF-A induced angiogenesis by acting through the DA D₂ receptors present in endothelial cells (10-13). Therefore, it is rational to hypothesize that DA may also inhibit VPF/VEGF-A induced angiogenesis and growth of VPF/VEGF producing prostate cancer.

Body: Task 1 described in the Statement of Work of our proposed application was as follows:

a. Develop a prostate cancer orthotopic mouse model

We successfully accomplished the goal by generating the orthotopic prostate cancer mouse models. Preclinical prostate cancer models were generated by us using 8-week old male athymic BALB/c nude mice (NCI). Exponentially growing human prostate cancer cell lines, LNCaP and PC-3 (ATCC) were used for implantation. Mice were anesthetized and a transverse incision was made in the lower abdomen. Thereafter, the urinary bladder and seminal vesicles were delivered through the incision to expose the dorsal prostate. LNCaP cells (2x10⁶ cells/50 μ l medium) or PC3 cells (~3x10⁵ cells/50 μ l medium) were injected under the prostate capsule with a 26.5 gauge needle. Finally, incision was closed with a running suture 5-0 silk. For LNCaP cells, tumor formation (Figure 1) and lymph node metastasis were observed. The incidence of tumor formation in this model was 70% and almost 50% of the tumor bearing animals showed para-aortic lymph node metastasis. For the PC-3 model, we observed 100% incidence of tumor formation and para-aortic lymph node metastasis.



b. In vivo experiments for examining the effects of dopamine and other drugs

c. Detection of cell proliferation, angiogenesis, apoptosis in prostate cancer

Because the objective of our proposal was to determine the effect of dopamine in androgen-independent prostate cancer (Reviewer B also suggested this valid experiment in his or her review), we therefore undertook initial experiments in orthotopic human PC3 bearing nude mice as follows:

(a) *Experimental Procedures:* PC3 bearing mice with high volume of tumor in the prostate and para-aortic lymph node metastasis (as verified by laparotomy before the start of the experiments) were selected. These animals were divided into two groups (Groups A, B). Group A animals were treated with 50mg/kg of dopamine i.p. for 7 continuous days and Group B animals received vehicle. Finally, the tumor bearing animals were sacrificed on Day 8 after completion of treatment and thereafter, the tumor growth, angiogenesis and tumor cell proliferation were determined.

(b) *Results:* DA significantly inhibited tumor growth, angiogenesis i.e. microvessel density (MVD/CD31) and tumor cell proliferation in PC3 bearing mice when compared with vehicle treated controls (Figures 2, 3, 4).

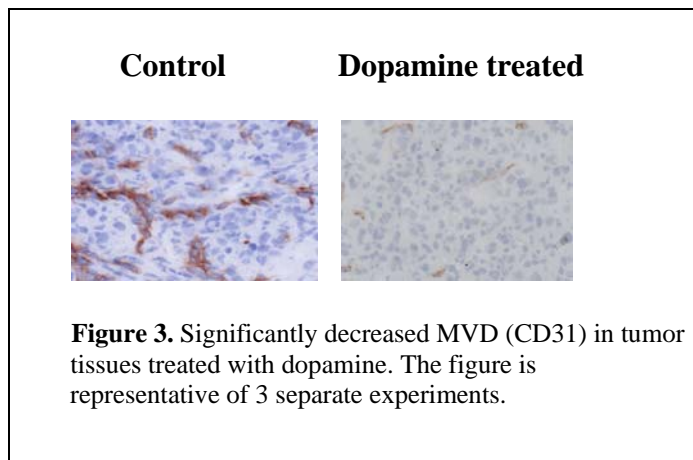
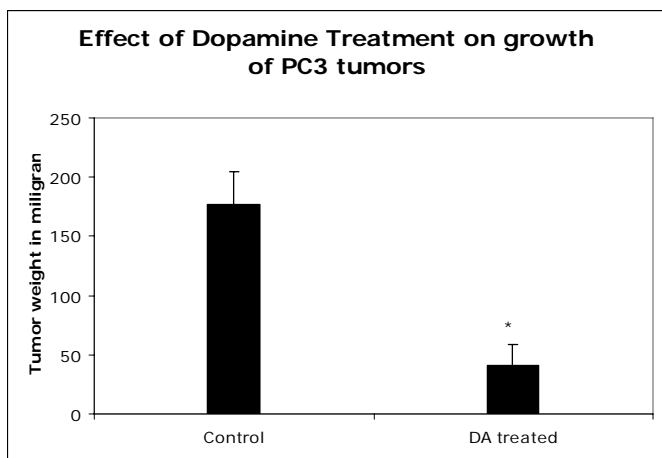


Figure 3. Significantly decreased MVD (CD31) in tumor tissues treated with dopamine. The figure is representative of 3 separate experiments.

Figure 2. Tumor growth of PC3 bearing mice. DA: dopamine. Results are mean \pm SEM of 3 separate experiments. n=8 for each experimental group (* $<p$ 0.05).

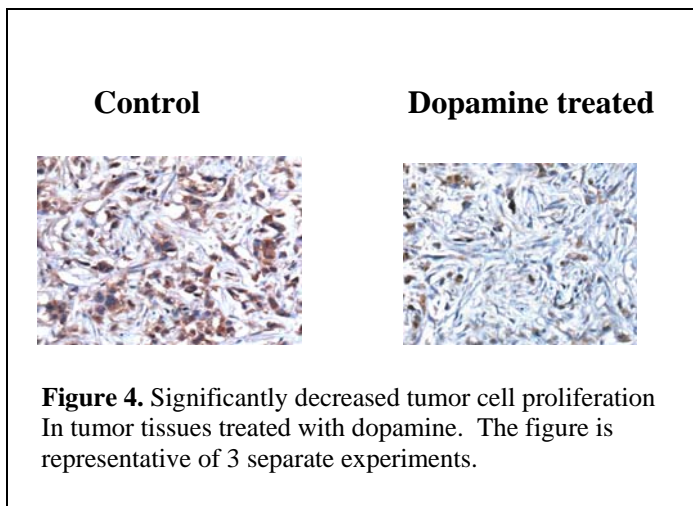


Figure 4. Significantly decreased tumor cell proliferation in tumor tissues treated with dopamine. The figure is representative of 3 separate experiments.

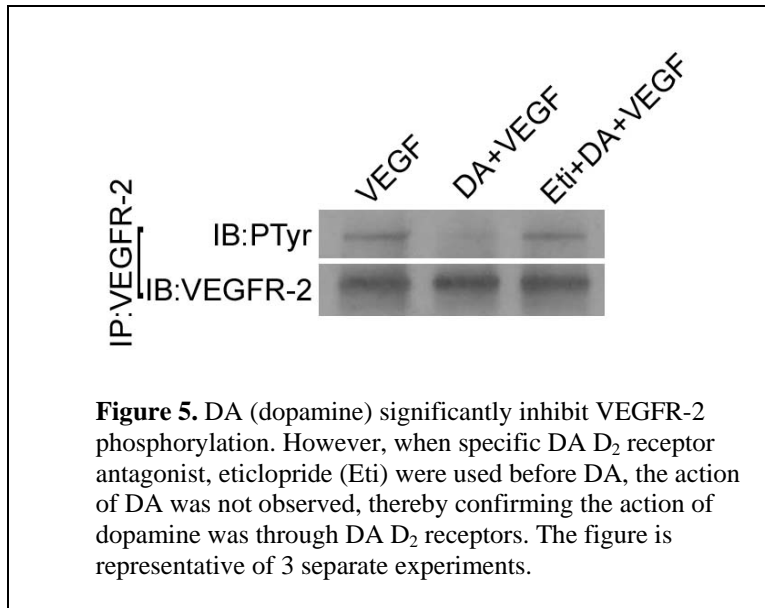
Further experiments with either dopamine alone or in combination with conventional anti-cancer drugs are in progress in PC3, LNCaP and TRAMP.

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Task 2. To elucidate the inhibitory mechanism of dopamine on VPF/VEGF-A mediated tumor angiogenesis.

a. Signaling and biochemical experiments with tumor endothelial cells

We successfully isolated tumor endothelial cells from PC3 prostate tumor tissues and demonstrated that dopamine by acting through its D₂ receptors present on tumor endothelial cells (10, 13) significantly inhibit the phosphorylation of VEGFR-2, the most important receptor of VEGF-A, through which VEGF-A mediates its angiogenic action (9) (Figure 5).



Key Research Accomplishments:

1. We have successfully developed orthotopic mouse models of preclinical human prostate cancer that will enable us to study the role of the neurotransmitter dopamine as an anti-angiogenic in prostate cancer.
2. We have successfully isolated tumor endothelial cells from PC3 prostate cancer tissues.
3. We have elucidated a molecular pathway that is responsible for DA mediated inhibition of VPF/VEGF-A actions in prostate cancer.

Reportable Outcome:

Developed orthotopic mouse model of human prostate cancer in immunocompromised mice.

Dopamine is an effective anti-angiogenic agent in PC3 human prostate cancer bearing mice.

Conclusion: DA is an effective anti-angiogenic agent in a preclinical human prostate cancer model and its action is through inhibition of VPF/VEGF-A actions.

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