# ACCELERATED DISCOVERY

## Antiangiogenic Activity of Prostate-Specific Antigen

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***Background:*** **Measurement of serum levels of prostate-specific antigen (PSA) is widely used as a screening tool for prostate cancer. However, PSA is not prostate specific, having been detected in breast, lung, and uterine cancers. In one study, patients whose breast tumors had higher levels of PSA had a better prognosis than patients whose tumors had lower PSA levels. To test the hypothesis that PSA may have antiangiogenic properties, we evaluated the effects of PSA on endothelial cell proliferation, migration, and invasion, which are key steps in angiogenesis, the process by which tumors develop a blood supply.** ***Methods:*** **To assess the antiproliferative effects of PSA, we treated bovine endothelial cells and human endothelial cell lines (HUVEC and HMVEC-d) with purified human PSA (0.1–10 μ*M*) and then stimulated them with 10 ng/mL fibroblast growth factor-2 (FGF-2). Effects on FGF-2- or vascular endothelial growth factor (VEGF)-stimulated endothelial cell migration, invasion, and tube formation were measured by use of one cell line only (HUVEC). PSA was administered to mice at 9 μ*M* for 11 consecutive days after intravenous inoculation of B16BL6 melanoma cells to assess its ability to inhibit the formation of lung colonies (i.e., metastatic tumors).** ***Results:*** **PSA inhibited endothelial cell proliferation, migration, and invasion at $IC_{50}$ (i.e., the concentration at which inhibition was 50%) values ranging from 0.3–5 μ*M*. In addition, PSA inhibited endothelial cell responses to both angiogenic stimulators tested, FGF-2 and VEGF. In a mouse model of metastatic disease, daily PSA treatment resulted in a 40% reduction in the mean number of lung tumor nodules compared with phosphate-buffered saline treatment (two-sided *P* = .003).** ***Conclusion:*** **To our knowledge, this is the first report that PSA may function in tumors as an endogenous antiangiogenic protein. This function may explain, in part, the naturally slow progression of prostate cancer. Our findings call into question various strategies to inhibit the expression of PSA in the treatment of prostate cancer. [J Natl Cancer Inst 1999; 91:1635–40]**

With the exception of skin cancers, prostate cancer is the most frequently diagnosed cancer among U.S. men, with an estimated 179 000 new cases and 37 000 deaths expected by the American Cancer Society in 1999 *(1)*. Over the past decade, the measurement of circulating levels of prostate-specific antigen (PSA) in the serum to screen for prostate cancer resulted in an increase in the reported incidence of this disease *(2)*. Despite the importance of PSA as a surrogate marker for prostate cancer, relatively little is known about the biologic function of this molecule. PSA is a serine protease and a member of the human kallikrein multigene family of enzymes *(3)*. Studies *(4)* have suggested that PSA may serve to modulate insulin-like growth factor (IGF) function in prostate cancer by blocking the interaction between IGF and its binding protein, insulin-like growth factor-binding protein (IGFBP). Although, to our knowledge, there are no reports to indicate any direct effect of PSA on the proliferation or metastasis of prostate cancer cells, efforts to design and manufacture anti-PSA vaccines are under way under the assumption that PSA itself may adversely affect the outcome of prostate cancer *(5,6)*.

PSA is neither prostate specific nor made exclusively by prostate epithelium. PSA has been found in patients with breast, lung, and uterine cancers *(7,8)*. Circulating serum concentrations of PSA have been documented in healthy women and in women with benign and malignant breast diseases *(9–11)*. Furthermore, as with men, the PSA gene in female breast tissues is regulated by androgens and progestins *(12)*. In one study of particular interest, patients with breast tumors with high levels of PSA had a better prognosis than those patients whose tumors had lower PSA levels *(13)*.

In cancer, the growth of the tumor is dependent on the angiogenic growth of new blood vessels *(14)*. Angiogenesis is a tightly regulated process, modulated by the dynamic interplay between angiogenic stimulators and inhibitors that control endothelial cell proliferation, migration, and invasion. This concept is reinforced by the earlier discovery of endogenous stimulators of angiogenesis, such as fibroblast growth factors (FGF) and vascular endothelial growth factors (VEGF), and, more recently, by the discovery of endogenous inhibitors of angiogenesis, including the Angiostatin® and Endostatin™ proteins *(15–17)*. Preliminary results indicate that increased concentrations of the antiangiogenic Endostatin™ protein may occur in animals and patients with growing tumors, which may indicate that angiogenesis is taking place *(18)*. These and other observations prompted our speculation that increasing PSA concentrations may not be a harbinger of bad news and prostate cancer progression but, rather, may indicate that the body is attempting to fight cancer by producing its own antiangiogenic proteins. If so, then PSA would be expected to demonstrate an inhibitory effect on the key elements of angiogenesis.

The process of angiogenesis is complex and involves a number of orchestrated steps that can be studied separately *in vitro*, such as FGF-2- and/or VEGF-stimulated endothelial cell proliferation and migration. For example, the Angiostatin® and Endostatin™ proteins inhibit these processes *(15,19)*. We hypothesized that PSA may have antiangiogenic properties. To test this hypothesis, we systematically evaluated the effects of PSA on endothelial cell proliferation, migration, and invasion.

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*See* "Notes" following "References."