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CONTENTS

(Contents continued on next page)

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Antiangiogenic Activity of Prostate-Specific Antigen

Anne H. Fortier, Barbara J. Nelson, Davida K. Grella, John W. Holaday

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."

Materials and Methods

Proliferation

Single-donor human umbilical vein endothelial cells (HUVEC) were obtained frozen at passage 1 from Clonetics (San Diego, CA). The cells were maintained in endothelial cell growth medium (Clonetics) supplemented with bovine brain extract (Clonetics), cultured at 37 °C in 5% CO_2 in moist air and used at passages 2–5 in all experiments. For proliferation assays, HUVEC were resuspended in endothelial cell basal medium-2 (EBM-2; Clonetics) supplemented with 2% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories, Inc., Logan, UT) and 2 m*M* L-glutamine (BioWhittaker, Inc., Walkersville, MD) and cultured overnight. Cells were incubated with various concentrations, at least three concentrations (0.1–10 μ*M*) in several repeat experiments, of purified human PSA (Vitro Diagnostics, Littleton, CO) or media alone for 30 minutes, and then stimulated with 10 ng/mL of FGF-2 (R&D Systems, Inc., Minneapolis, MN) or media for an additional 48 hours. Cell proliferation was assessed with a colorimetric enzyme-linked immunosorbent assay (ELISA) kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) that measured the amount of bromodeoxyuridine (BrdU) incorporated during DNA synthesis. Results are expressed as the mean absorbance of triplicate cultures measured at 370 nm (reference wavelength, 492 nm).

Bovine adrenal capillary endothelial cells (BCEC) were obtained at passage 9 from J. Folkman (Children's Hospital, Harvard Medical School, Boston, MA). The cells were cultured and maintained as described previously *(11)*. For evaluation of PSA inhibition of BCEC proliferation, cells were exposed to five different concentrations of purified PSA or media, in triplicate wells, for 30 minutes at 37 °C in 10% CO_2 prior to stimulation with FGF-2. Cell proliferation was assessed by counting the number of cells per well with a Coulter Z1 particle counter (Coulter Corp., Hialeah, FL).

Single-donor adult human microvascular dermal cells (HMVEC-d) were obtained frozen at passage 4 from Clonetics. The cells were maintained in microvascular endothelial cell growth medium-2 (EGM-2-MV; Clonetics). Cells were cultured as for HUVEC described above and were used at passages 5–8 in all experiments. For proliferation assays, HMVEC-d were resuspended in endothelial cell basal medium-2 (Clonetics) supplemented with 2% FBS and 2 m*M* L-glutamine. Cells were plated as described above for BCEC, and after 30 minutes' preincubation with at least five different concentrations of PSA in triplicate wells, cultured with FGF-2 (10 ng/mL) for an additional 48 hours. Cell proliferation was assessed as above by counting the number of cells per well.

B16BL6, a murine melanoma, obtained from the National Cancer Institute-Frederick Cancer Research and Development Center (Frederick, MD) cell repository and human prostate cancer cell line, PC3, a gift from J. Folkman, were maintained in Dulbecco's modified Eagle medium (BioWhittaker, Inc.), supplemented with 5% FBS and 2 m*M* L-glutamine. The ability of PSA to inhibit proliferation of B16BL6 was assessed with a colorimetric ELISA kit (Boehringer Mannheim Biochemicals) for BrdU incorporation as described above for HUVEC, and inhibition of PC3 proliferation was assessed by cell counts from triplicate cultures; each experiment was performed with five doses of PSA.

Migration

To determine the ability of PSA to block HUVEC migration induced by recombinant FGF-2 or recombinant VEGF 165 (R&D Systems, Inc.), we performed a wound-migration assay as previously described *(20)*. To date, the migration assay as described here has been established only with HUVEC; migration assays with bovine (BCEC) and human (HMVEC-d) endothelial cells are currently under development in our laboratory. In brief, HUVEC in endothelial cell growth medium were plated onto 1.5% gelatin-coated tissue culture dishes (Corning Costar, Inc., Cambridge, MA) and incubated for 72 hours at 37 °C in 5% CO_2 in moist air. After incubation, confluent monolayers were wounded with a sterile, single-edged No. 9 razor blade (VWR Scientific, Media, PA), washed with phosphate-buffered saline (PBS; BioWhittaker, Inc.), and further incubated in endothelial cell basal medium supplemented with 1% FBS, 2 m*M* L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL fungizone. Wounded monolayers were exposed to 2 ng/mL FGF-2 or 10 ng/mL VEGF in the presence or absence of PSA or to media alone for 16–20 hours. The monolayers were fixed with absolute methanol and stained with hematoxylin solution, Gill No. 3 (Sigma Diagnostics, St. Louis, MO). Migration was quantified by counting the number of cells that migrated from the wound edge into the denuded area along a 1-cm distance in duplicate cultures. Dose-dependent inhibition of migration was assessed in several repeat experiments (greater than five experiments with FGF-2-stimulated cells and two separate experiments with VEGF-stimulated cells) using at least five different concentrations of PSA in duplicate cultures.

Biocoat 8-μm invasion chambers (Collaborative Biomedical Products, Bedford, MA) were precoated with Matrigel (Collaborative Biomedical Products). The lower chambers were filled with assay media containing 5 ng/mL FGF-2 or assay media alone, and the upper chamber had HUVEC pretreated for 30 minutes with PSA (5 μ*M*) or media. Cells were incubated in the upper chambers for 24 hours at 37 °C in 5% CO_2. After incubation, the noninvading cells were removed with a cotton swab, and cells on the lower surface of the membrane were stained with Diff-Quik (Dade Diagnostics, Aquado, Puerto Rico). The membrane was removed and mounted on a microscope slide, and the number of cells invaded was determined by counting the cells at ×150 magnification in the central field of the membrane from triplicate cultures.

Endothelial Tube Formation

For induction of endothelial tube formation, the following procedure was adapted from the protocol of Kubota et al. *(21)*. In brief, Matrigel is aliquoted into a 96-well tissue culture plate and allowed to gel. PSA or 2-methoxyestradiol, as a positive control for inhibition *(22)*, was added to Matrigel, followed by the addition of HUVEC. After 16 hours, endothelial cells were microscopically evaluated for tube formation.

In Vivo Metastatic Model

B16BL6 murine melanoma cells (5×10^4) were inoculated into C57BL/6J male mice (The Jackson Laboratory, Bar Harbor, ME) via the lateral tail vein on day 0. In two separate experiments, beginning on day 3, three groups of five mice each were treated subcutaneously for 11 consecutive days with either PBS (0.1 mL) or PSA (9 μ*M*) or positive control Endostatin™ protein (15 μ*M*). On day 14, mice were killed, lungs were removed and fixed in formalin (Sigma Chemical Co., St. Louis, MO), and melanoma lesions were counted with the aid of a dissecting microscope. Histologic examination of the fixed lung tissues confirmed the presence of tumor cells containing melanin. Animal care and use procedures were performed in accordance with standards described in the National Institutes of Health *Guide for Care and Use of Laboratory Animals*.

Inhibition of PSA Enzymatic Activity

The ability of α_1-antichymotrypsin (ACT) to inhibit the proteolytic activity of PSA was measured by use of the synthetic substrate S-2586 (MeO–Suc–Arg–Pro–Tyr–pNA, where MeO = methoxy, Suc = succinyl, and pNA = paranitroaniline). The rate of hydrolysis of S-2586 (1.3 m*M*) by 6 μg PSA (0.89 μ*M*) with and without pretreatment with an equimolar concentration of ACT (Sigma Chemical Co.) was monitored at 405 nm in 50 m*M* Tris–HCl, 0.1 *M* NaCl (pH 7.8). Stable complexes of PSA and ACT formed after a 4-hour incubation at 37 °C and were confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The results were plotted as an increase in absorbance versus time in minutes. The ability of ACT to inhibit the antimigratory activity of PSA was measured by preincubating PSA (5 μ*M*) with an equimolar concentration of ACT for 4 hours at 37 °C prior to addition to the HUVEC migration assay.

Statistical Analysis

Means were compared by use of the Student's *t* test (two-tailed). Differences were considered to be statistically significant at $P<.05$.

Results

We first observed the antiproliferative effects of PSA in HUVEC (Fig. 1, A). Purified human PSA demonstrated potent and dose-related inhibitory activity on FGF-2-stimulated proliferation of HUVEC, with an IC_{50} (i.e., the concentration at which inhibition was 50%) of 4 μ*M*. To determine whether PSA inhibited a variety of endothelial cells or simply displayed specificity for HUVEC, we tested the ability of PSA to inhibit proliferation of BCEC and HMVEC-d (Fig. 1, B and C). PSA once again potently inhibited FGF-2-stimulated endothelial cell pro-

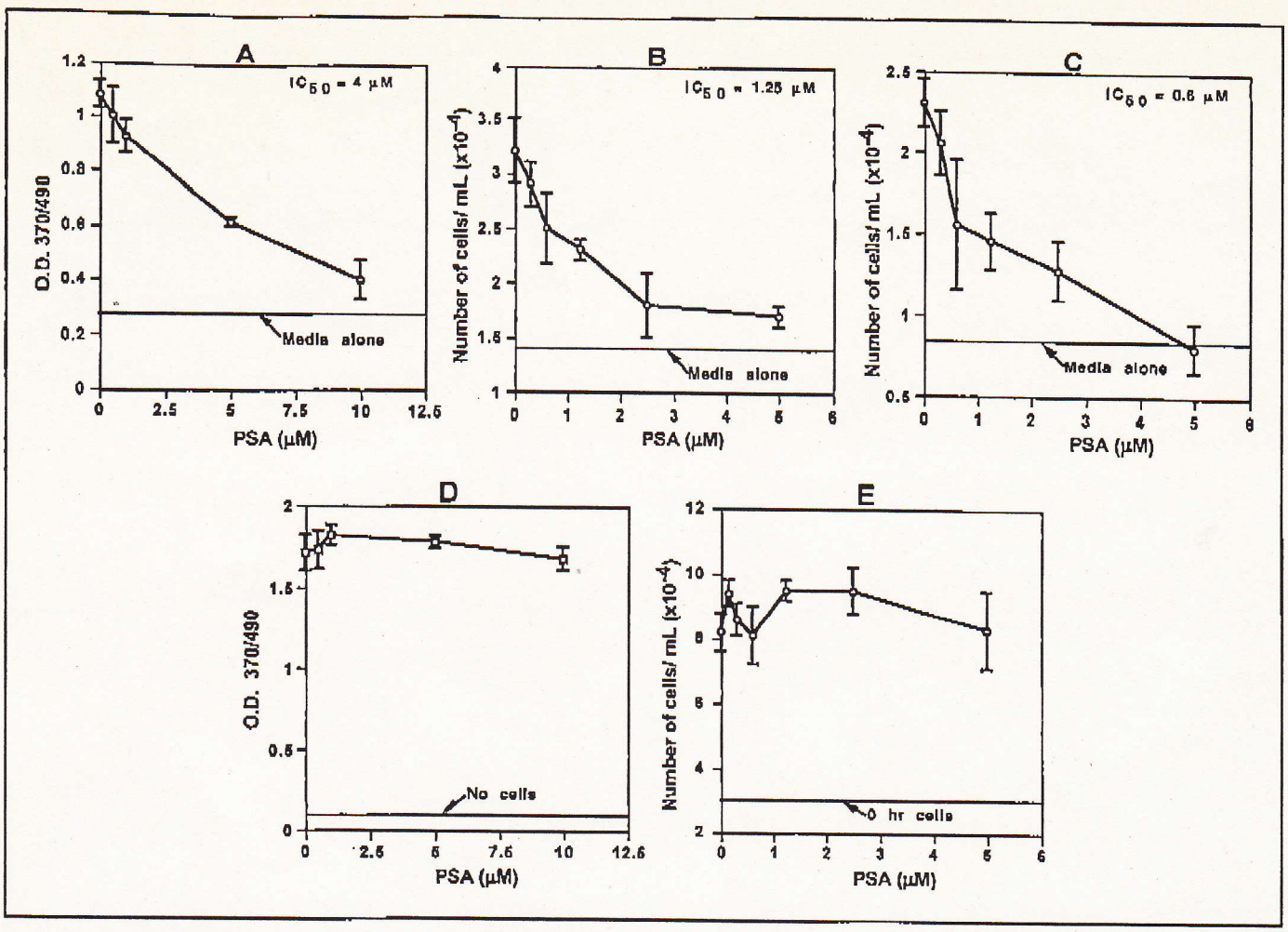

Fig. 1. Effects of prostate-specific antigen (PSA) on proliferation of endothelial and tumor cells *in vitro*. Endothelial cells—human umbilical vein epithelial cells (HUVEC) (**A**), bovine adrenal capillary endothelial cells (**B**), and human microvascular dermal cells (HMVEC-d) (**C**)—were cultured with fibroblast growth factor-2 (FGF-2) in the presence or absence of the indicated concentrations of purified human PSA for 3 days at 37 °C in 5% CO_2 with humidity. Tumor cell lines—murine melanoma B16BL6 (**D**) and human prostate carcinoma PC3 (**E**)—were cultured without FGF-2 (these cells do not require exogenous FGF-2 for proliferation) in the presence or absence of purified human PSA. Cell proliferation was estimated by measurement of the amount of incorporated bromodeoxyuridine (**A** and **D**) or by performing cell counts (**B, C,** and **E**) as described in the "Materials and Methods" section. Each data point is the mean of observations from triplicate experiments ± 1 standard deviation. O.D. = optical density; IC_{50} = concentration at which inhibition was 50%.

liferation, with an IC_{50} for BCEC cells of 1.25 μ*M* and an IC_{50} for HMVEC-d of 0.6 μ*M*.

PSA does not appear to have a direct stimulatory or inhibitory effect on the proliferation of cancer cells. The growth of murine melanoma cells (B16BL6) or human prostate cancer cells (PC3) was unaffected by the addition of purified human PSA (Fig. 1, D and E, respectively).

For evaluation of the *in vitro* effects of PSA on endothelial cell migration in response to FGF-2 or VEGF, confluent monolayers of HUVEC were scraped to remove a section of monolayer and cultured for 24 hours with FGF-2 or VEGF in the presence or absence of purified human PSA. The endothelial cell response was assessed by counting the number of cells that migrated into the denuded area of the cell monolayer. The data in Fig. 2, A and B, demonstrate the dose–response of HUVEC to the inhibitory effects of PSA on FGF-2- and VEGF-stimulated migration, respectively, with an IC_{50} for PSA versus FGF-2 of 1.2 μ*M* and for PSA versus VEGF of 4 μ*M*. On a molar basis, the inhibitory activity of purified PSA on both endothelial cell proliferation and migration was approximately fivefold to 10-fold less potent than that of the antiangiogenic proteins Angiostatin® protein and Endostatin™ protein (data not shown).

Assays to measure migration of endothelial cells can be coupled with measurement of another parameter of angiogenesis, invasion, by performing the assay in a two-chamber environment where the chambers are separated with a membrane filter coated with Matrigel. In this assay, PSA (5 μ*M*) inhibited FGF-2-stimulated HUVEC invasion through Matrigel by 77%. In addition, at concentrations ranging from 0.3 to 3 μ*M*, purified human PSA inhibited tube formation of HUVEC in Matrigel by approximately 50%. This inhibition appeared to be dose dependent and not the result of toxicity because endothelial cells appeared viable. Although some elongation of the endothelial cells was noted, there were no junctions and, thus, no clear indication of functional tubes made by the endothelial cells.

We have been using the murine B16BL6 melanoma cell line in a model of experimental metastasis to assess the antitumor effects of the angiogenesis inhibitor Endostatin™ protein. In this model, mice were inoculated intravenously with B16BL6 cells on day 0. On day 3, the mice were treated subcutaneously once

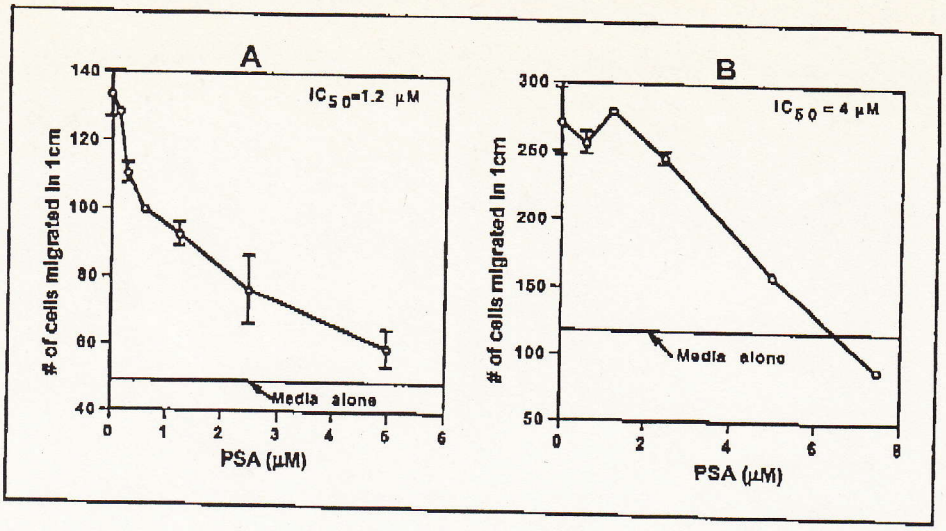

Fig. 2. Effects of prostate-specific antigen (PSA) on migration of endothelial cells. Human umbilical vein endothelial cells were stimulated with fibroblast growth factor-2 (2 ng/mL) (A) or vascular endothelial growth factor (10 ng/mL) (B) to migrate either in the absence of PSA or in the presence of the indicated concentrations of PSA. Migration was assessed by counting the number of cells that migrated into the denuded portion of the monolayer over a 1-cm distance along the wounded edge. Results reported here are the mean number of cells ± 1 standard deviation counted in duplicate cultures. IC_{50} = concentration at which inhibition was 50%.

a day for 11 consecutive days with purified human PSA (9 μ*M*). On day 14, the mice were killed, their lungs were removed, and the number of melanoma tumor nodules on the surface of the lungs were counted. The number of lung metastases in control mice treated with PBS was 115 ± 16 (mean ± standard deviation) and in PSA-treated mice 70 ± 8 (mean ± standard deviation), representing a 40% reduction in tumor number (P = .003). While the inhibition of B16BL6 development in the lung induced by PSA was not as great as that observed in Endostatin™ protein-treated mice (86% inhibition in the same experiment; mean number of lung metastasis was 16 ± 8; P = .0002), we also observed a fivefold to 10-fold difference in potency between the two proteins in our *in vitro* assays. Further studies in tumor models in which both vessel density and tumor growth can be assessed are needed to characterize the inhibition observed after PSA treatment.

PSA has serine protease activity and, in serum, is predominantly bound to the protease inhibitor ACT *(23)*. We tested the ability of ACT to inhibit both serine protease activity of our purified PSA (Fig. 3, A) as well as the antimigratory effects of PSA on FGF-2-stimulated HUVEC (Fig. 3, B). By use of equimolar concentrations of ACT and PSA, preincubation of PSA with ACT completely blocked serine protease activity (Fig. 3, A) and significantly blocked migration inhibition (Fig. 3, B; P = .0038) when compared with cells treated with PSA alone.

Discussion

In this report, we provide evidence that PSA is an endothelial cell-specific inhibitor of angiogenesis that exhibits potent antiproliferative activities on a variety of cultured endothelial cells. In addition, we show that PSA blocked migration of FGF-2-stimulated HUVEC. Furthermore, in a murine model of metastatic disease, purified human PSA inhibited the number of surface lung metastases by 40%.

As Folkman *(24)* noted, there are two prominent compartments in growing tumors: the cancer cells and the endothelial cells making up the blood vessels that provide the cancer cells with oxygen and nutrients. The balance between the positive and

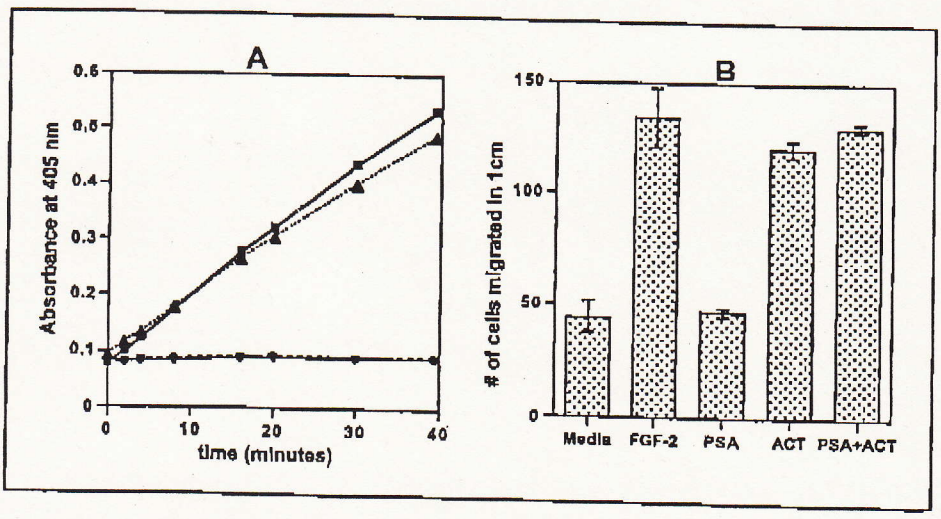

Fig. 3. Inhibition of prostate-specific antigen (PSA) activities by α-antichymotrypsin (ACT). Proteolytic activity of PSA was measured (A) by use of the synthetic substrate S-2586 (MeO–Suc–Arg–Pro–Tyr–pNA, where MeO = methoxy, Suc = succinyl, and pNA = para-nitroaniline), and the rate of hydrolysis was monitored at 405 nm. PSA (0.89 μ*M*) (■) or ACT (0.92 μ*M*) (●) was incubated alone, with substrate, and hydrolysis was measured over a 40-minute period. For analysis of an inhibitory effect of ACT on PSA, PSA was preincubated with (▼) or without (▲) equimolar amounts of ACT at 37 °C for 4 hours before the addition of substrate. When substrate was added, hydrolysis was measured over a 40-minute period. Mean results of triplicate cultures are plotted as an increase in absorbance versus time in minutes; errors for each time point are not shown but were less than or equal to 5% of the mean. Inhibitory activity of PSA on human umbilical vein epithelial cells migration was assessed in the presence or absence of ACT. (B) For comparison, the number of cells that migrated in response to media alone and fibroblast growth factor-2 (FGF-2) is shown. Active PSA was preincubated with an equimolar concentration of ACT at 37 °C for 4 hours (PSA + ACT). Preincubation of PSA for 4 hours at 37 °C did not alter the inhibitory capacity of PSA, and ACT alone did not inhibit FGF-2-stimulated migration of HUVEC. Preincubation of cells with PSA and ACT significantly blocked inhibition of migration compared with treatment of cells with PSA alone (P = .0038). Results reported here are the mean number of cells ± 1 standard deviation counted in duplicate cultures.

negative regulators produced by these two compartments determines the ultimate growth rate of the tumor. Many reports *(25)* have used morphimetric analysis to establish a predictive association between microvessel density (angiogenesis) and prostate cancer progression. Our results suggest that, in addition to its role as an indicator of prostate cancer, PSA may also inhibit the growth of blood vessels associated with cancer progression; however, when prostate cancer progression occurs in spite of elevations of PSA, the local angiogenic stimulators overcome the effects of PSA and dominate. Our observations are therefore consistent with other data indicating a "paradoxical" increase in PSA associated with disease cure in certain circumstances *(26)*. Furthermore, in patients with prostate cancer, PSA values range between 4 and 15 000 ng/mL (equivalent to 0.1 n*M*–0.5 μ*M*), with the highest concentrations in the range of our *in vitro* inhibitory results (0.3–5 μ*M*). These data suggest that the production of PSA by prostate cancers is one reason for their characteristically slow growth and that PSA may help to maintain homeostasis in the face of progressing angiogenesis and cancer.

With the exception of data indicating a potential role as an IGFBP-3 protease *(27)*, surprisingly little is known about the role of PSA in cancer. The antiangiogenic and antitumor effects of PSA described in this report may result from its actions as a serine protease, since ACT blocked both its enzymatic activity and its antiangiogenic activity *in vitro*. These data may point to a generalized action of serine proteases and suggest that other serine proteases and members of the kallikrein multigene family of enzymes should be evaluated for potential antiangiogenic actions.

Cohen et al. *(27)* have speculated that lowering PSA production or decreasing PSA enzymatic activity may inhibit the progression of prostate cancer. Likewise, a number of initiatives are under way to develop a vaccine against PSA *(5,6)*. Our findings indicate that these strategies should be rethought in view of the evidence that PSA acts as an antiangiogenic agent that may play a physiologic role in slowing the progression of cancer.

Published reports indicating that PSA is not prostate specific and that higher PSA concentrations are associated with improved outcome in breast cancer are consistent with our findings. Furthermore, the antiangiogenic drugs thalidomide and TNP470 have recently been reported to produce statistically significant increases in PSA concentrations *in vitro (28)*. We believe that such an effect may partially account for the antiangiogenic properties of these drugs *in vivo*. Taken together, our data may indicate that elevations of PSA in a variety of malignancies are part of a normal homeostatic process to fight cancer progression. Furthermore, the administration of PSA as a drug to augment endogenous concentrations could provide a rational therapeutic approach in the treatment of cancer.

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NOTES

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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 ?*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 ?*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 ?*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.