Prostate Stem Cell Antigen as Therapy Target: Tissue Expression and in Vivo Efficacy of an Immunotoxin

Sarajane Ross, Susan D. Spencer, Ilona Holcomb, Christine Tan, JoAnne Hongo, Brigitte Devaux, Linda Rangell, Gilbert A. Keller, Peter Schow, Rita M. Steeves, Robert J. Lutz, Gretchen Frantz, Kenneth Hillan, Franklin Peale, Patti Tobin, David Eberhard, Laurence A. Lasky, and Hartmut Koeppen


ABSTRACT

We conducted an expression analysis of prostate stem cell antigen (PSCA) in normal urogenital tissues, benign prostatic hyperplasia (n = 21), prostatic intraepithelial neoplasia (n = 33), and primary (n = 137) and metastatic (n = 42) prostate adenocarcinoma, using isotopic in situ hybridization on tissue microarrays. In normal prostate, we observe PSCA expression in the terminal differentiated, secretary epithelium; strong expression was also seen in normal urothelium. Forty-eight percent of primary and 64% of metastatic prostatic adenocarcinomas expressed PSCA RNA. Our studies did not confirm a positive correlation between level of PSCA RNA expression and high Gleason grade. We characterized monoclonal anti-PSCA antibodies that recognize PSCA expressed on the surface of live cells, are efficiently internalized after antigen recognition, and kill tumor cells in vitro in an antigen-specific fashion upon conjugation with maytansinoid. Unconjugated anti-PSCA antibodies demonstrated efficacy against PSCA-positive tumors by delaying progressive tumor growth in vivo. Maytansinoid-conjugated antibodies caused complete regression of established tumors in a large proportion of animals. Our results strongly suggest that maytansinoid-conjugated anti-PSCA monoclonal antibodies should be evaluated as a therapeutic modality for patients with advanced prostate cancer.

INTRODUCTION

Prostate cancer is the most commonly diagnosed nondermatological malignancy in men, and according to the American Cancer Society accounts for 30,000 deaths annually in the United States.1 Whereas primary organ-confined cases can effectively be treated and cured by surgery and/or radiation therapy, therapeutic options are limited for metastatic, hormone-refractory disease (1). The lack of success with conventional therapeutic approaches and the existence of antigens expressed in an almost organ-specific pattern have prompted immunological approaches to the treatment of metastatic prostate cancer. Most of the studies designed to elicit active immunity in the patient are in their early stages and cannot be fully evaluated at this time (2–8).

Antibody-based therapy using unconjugated, toxin-conjugated, or radiolabeled reagents against tumor-associated target antigens has been beneficial for solid and hematolymphoid neoplasms (for a review, see Ref. 9). Recent clinical efforts have focused on the toxic natural compounds calicheamicin and maytansinoid DM1. Immunotoxins conjugates with these two toxins have shown efficacy with limited toxicity in preclinical studies and are now in various stages of clinical development for hematological and solid tumors (10, 11).

Received 9/27/01; accepted 3/1/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Sarajane Ross and Susan D. Spencer contributed equally to this work.

2 To whom requests for reprints should be addressed, at Genentech, Inc., Mail Stop 72B, 1 DNA Way, South San Francisco, CA 94080. Phone: (650) 225-8134; Fax: (650) 225-8989; E-mail: hkoeppen@gene.com.


The present study evaluates PSCA as a potential target for an antibody-based therapeutic approach against prostate cancer. We describe the expression pattern of PSCA in normal adult urogenital tissues and in primary and metastatic prostate cancer as well as the in vitro and in vivo characteristics of unconjugated and DM1-conjugated anti-PSCA antibodies. PSCA was originally identified as a glycosylated, glycosylphosphoinositol-linked cell surface antigen expressed in normal prostate, urinary bladder, kidney, and placenta. Expression has also been described in primary and metastatic prostate cancer as well as in neoplasms of the urinary bladder, esophagus, and pancreas by several groups using diverse methodologies (12–14). Here we conclusively show that PSCA is strongly expressed in differentiated luminal cells of the prostate and urothelium. PSCA RNA is present in 48% of primary and 64% of metastatic prostatic adenocarcinomas. In addition, we generated anti-PSCA monoclonal antibodies that specifically reacted with cell surface PSCA and, upon conjugation with maytansinoid DM1, demonstrated in vitro cytotoxicity and marked in vivo efficacy with complete tumor eradication in a large proportion of treated animals. To our knowledge, this is the first study showing eradication of established xenograft tumors using PSCA as a target in a passive immunotherapy approach.

MATERIALS AND METHODS

TMAs. We used paraffin blocks of normal and neoplastic human tissues from two separate institutions (University of Sheffield, Sheffield, United Kingdom, and University of Virginia, Charlottesville VA) to build two microarrays of prostatic tissue as described previously (15). These two TMAs contained 240 and 294 tissue elements, respectively. Six additional arrays consisting of 144 elements each of normal, hyperplastic, and neoplastic prostate tissues were built at the University of Michigan (Ann Arbor, MI). Tissues were represented in triplicate cores 600 μm in diameter. A detailed description of our TMA and ISH technology has recently been published (16). All cases of prostate cancer had been assigned a Gleason score by the pathologist at the original institution based on histological evaluation of a prostatectomy specimen. The total numbers of cases with normal prostate, PIN, and primary and metastatic prostate cancer are summarized on Table 1. In the category of PIN, we considered only high-grade lesions with the typical architectural and cytological features (17). The sites of metastatic prostate cancer were the lymph nodes (n = 24), liver (n = 9), lung (n = 2), and 1 case each of brain, meninges, diaphragm, kidney, stomach, testis, and soft tissue. We were unable to evaluate skeletal metastases because the decalcification procedure used for bone-containing tissues damages the RNA to an extent that it becomes unsuitable for ISH.5 The age range for the patients was 39–82 years.

ISH. PCR primers were designed to amplify a 751-bp fragment of human PSCA corresponding to nucleotides 1–751 of the GenBank sequence (NM_005672). A probe for PSCA corresponding to nucleotides 784–1074 of the human PSCA GenBank sequence (NC_005672). A probe for PSCA corresponding to nucleotides 1–751 of the GenBank sequence (NM_005672). A probe for PSCA corresponding to nucleotides 784–1074 of the human PSCA GenBank sequence (BC004251). Primers

4 The abbreviations used are: PSCA, prostate stem cell antigen; TMA, tissue microarray; ISH, in situ hybridization; PIN, prostatic intraepithelial neoplasia; BPH, benign prostatic hyperplasia; ATCC, American Type Culture Collection; FBS, fetal bovine serum; CHO, Chinese hamster ovary; EM, electron microscopy.

5 S. Ross and H. Koeppen, personal observation.
Proteinase K at 37 °C for 15 min, TMA sections were processed for ISH as described previously (18). Sense control probes showed no signal above background (data not shown).

**Scoring and Analysis of TMA PSA Expression.** The intensity of PSA expression evaluated microscopically was given a score of 0–4, with 4 being the highest expression observed. The percentage of epithelial cells positive for PSA within a TMA element was estimated as <10%, 10–50%, or >50%. Patients with expression of PSA in <10% of epithelial cells were considered negative. The intensity score and percentage of positive cells in elements from the same patient were averaged. Pathological diagnoses and ISH results were tabulated using Microsoft Excel.

**Immunohistochemistry.** Immunohistochemical staining for Ki-67 was performed with a rabbit polyclonal antibody (DAKO Corporation, Carpinteria, CA) on the two in-house-built prostate TMA sections according to standard procedures and the manufacturer’s guidelines. Represented on these two TMAs were 22 cases of normal prostate tissues, 4 cases of BPH, 7 cases of PIN, and 90 primary and metastatic PC, Gleason grade 4 or 5. The primary antibody used was a rabbit polyclonal antibody (DAKO Corporation) at a dilution of 1:100. Cases of prostate cancer were scored according to intensity of expression (%).

**Preparation of Anti-PSA-DM1 Immunconjugate.** The conjugation of the anti-PSA antibody 8D11 and the control anti-ragweed antibody 10D9 with the maytansinoid toxin DM1 was performed according to the procedure described previously for the antibody C242 (11). 8D11-DM1 contains an average of 2.5 DM1 molecules/antibody molecule. Indicating that the conjugation did not affect the affinity of 8D11, the binding of the conjugated antibody to His-tagged PSA protein in an ELISA assay was identical to that of the unconjugated antibody (data not shown).

**Immunogold Labeling and EM.** Transfected cell lines expressing gD-PSA were grown in 6-well plates and incubated at 4°C with 10 μg/ml anti-gD or anti-PSA antibody for 1 h. After incubation with primary antibodies, the cells were treated with 10-nm gold adducts of goat antimouse IgG for 1 h. The cells were then switched to 37°C for 1.25 h before fixation in Karnovsky’s fixative and processed for EM. For autoradiography, transfected cells were incubated with iodinated anti-PSA antibodies at 37°C, fixed, washed, and prepared for EM autoradiography as described elsewhere (24). Thin sections were observed in a Philips CM12 equipped with a digitizing GATAN camera.

**Antibody Internalization Assay.** MCF7.Her2 cells stably transfected with gD-PSA were grown in 6-well dishes and then incubated at 37°C with 20 μM 125I-labeled anti-PSA and anti-HER2 monoclonal antibodies for 5 h in DMEM supplemented with 10% FBS. To determine the cellular distribution of radiolabeled antibody, the cells were first washed extensively with DMEM and then incubated for 5 min in 2 m urea-50 mM glycine (pH 2.4)-150 mM NaCl. The released radioactivity was considered to represent cell surface-associated antibody. Cells were then solubilized in 8 m urea-150 mM NaCl, and the radioactivity within the lysate was considered to represent internalized antibody. Assays were performed in duplicate wells. The number of antibody molecules bound and internalized per cell was determined on the basis of the specific activity of the iodinated antibody.

**In Vitro Cytotoxicity Assay.** Tumor cells lines stably transfected with gD-PSA or with vector alone were plated in 96-well microtiter plates at the following densities in complete medium: 10^3 cells/well for HCT116.gD.PSCA, 2 × 10^3 cells/well for MCF7.Her2.gD.PSCA, 10^3 cells/well for PC3.gD.PSCA, and 1.5 × 10^5 cells/well for PC3.gD.PSCA. After the cells had been allowed to adhere for 16 h, they were exposed to various concentrations of immunoconjugate 8D11-DM1 (equivalent to 1200 to 0.06 ng/ml DM1). After incubation at 37°C for 7 days, the monolayers were washed twice with PBS and stained with crystal violet dye (0.5% in methanol). The stained cells were solubilized in 50 mM sodium citrate in 50% ethanol for 20 min with shaking. The absorbance at 450 nm (A_450) of the solubilized cells was measured with a spectrophotometer, and the fraction of surviving cells determined by dividing the A_450 of treated cells by the A_450 of nontreated cells. To determine the cytotoxicity of the immunoconjugate on quiescent cells, the assay was performed in serum-free medium.

**In Vivo Tumor Growth Assays.** Female NCR nude mice (6–8 weeks of age; Taconic, Inc., Germantown, NY) were inoculated s.c. with 5 × 10^6 PC3.gD.PSCA or 1.5 × 10^7 SW780 cells. Tumor volume was calculated based on two dimensions, measured using calipers, and was expressed in mm^3 according to the formula: \( V = 0.5a \times b^2 \), where \( a \) and \( b \) are the long and short diameters of the tumor, respectively. Antibody injections were started either 24 h before tumor inoculation or after tumors were established. In the latter type of study, animals were randomized according to tumor volume (mean tumor volume between 100 and 200 mm^3) before antibody injections.}

---

### Table 1: Incidence and intensity of expression of PSCA in human prostate tissue

<table>
<thead>
<tr>
<th>Type of prostate tissue (No. of samples analyzed)</th>
<th>Incidence according to intensity of expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/BPH (n = 78)</td>
<td>1+  2+  3+  4+  Total</td>
</tr>
<tr>
<td>PIN (n = 33)</td>
<td>13  17  13  1  47</td>
</tr>
<tr>
<td>PC* Gleason grade ≤7 (n = 76)</td>
<td>19  16  13  1  49</td>
</tr>
<tr>
<td>PC, Gleason grade &gt;7 (n = 61)</td>
<td>20  12  13  3  48</td>
</tr>
<tr>
<td>Metastatic PC (n = 42)</td>
<td>29  9   19  7   64</td>
</tr>
</tbody>
</table>

*PC, prostate cancer.
doses for most studies unless otherwise indicated. Tumors were measured twice a week throughout the experiment. Mice were euthanized before mean tumor volumes reached 2000 mm³ or when tumors showed signs of impending ulceration. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, published by the NIH (NIH Publication 85-23, revised 1985)

RESULTS

PSCA Expression in Normal Tissues and Prostate Cancer. PSCA was expressed to various degrees in individual samples of normal prostate. Some areas of prostate expressed very high levels, whereas other areas were completely negative. There was no obvious morphological correlation with level of expression and prostatic region or secretory state. Expression was localized exclusively to the secretory epithelium (Fig. 1, A and B). Basal epithelial cells and prostatic stroma were negative for PSCA RNA in all cases examined (Fig. 1C). Neuroendocrine cells were identified by an immunohistochemical stain for chromogranin A and were negative for PSCA; the distribution and prevalence of chromogranin A-positive cells was quite distinct from that of PSCA-positive cells (Fig. 1D). The RNA integrity of all tissues was confirmed by isotopic ISH with an actin probe (data not shown).

The strongest expression of PSCA RNA in normal urogenital tissues was detected in the urothelium, with equal levels in the renal pelvis, ureter, urinary bladder, and urethra (Fig. 1, C–E). In most areas, the strongest expression was observed in the most superficially located umbrella cells, and the level of expression decreased progressively toward the deeper sections of the mucosa with the lowest expression in the most basally located cells. The stromal tissues of the urinary tract were completely negative for PSCA RNA. In the kidney, scattered PSCA-positive epithelial cells were observed in the distal portions of the collecting system; no expression was noted in the glomerular tufts or in epithelial cells of proximal collecting ducts and Bowman’s capsule.

PSCA Expression in Abnormal Prostate. Expression of PSCA RNA was seen in BPH, PIN, and invasive prostatic adenocarcinoma (Table 1). The phosphorimager scan and image of a representative TMA examined are shown in Fig. 2, A and B, respectively. This TMA included 5 cases of normal urothelium, 4 cases of normal prostate, 4 cases of BPH, 7 cases of PIN, 35 cases of well- and poorly differentiated primary prostate cancer, and 27 cases of metastatic prostate cancer.

As in normal prostate, PSCA expression was restricted to epithelial cells and was focally distributed. The level of expression in the neoplastic prostatic tissues varied from negative to very high. Cases of primary adenocarcinoma were separated into two categories according to Gleason grade: cases with a Gleason grade ≤7, and those with a Gleason grade ≥8. Separation into these two categories has previously been shown to correlate with clinical outcome (25). Cases with metastatic prostate cancer represented a third category. Although expression was variable and no overt correlation with Gleason grade or stage of the disease could be found, the percentage of metastatic prostate cancer cases positive for PSCA was higher (64%) compared with nonmalignant prostate disease and organ-confined prostate cancer (48%; see Table 1). Each case was assigned an intensity score, and patients were grouped according to the level of PSCA expression (Fig. 2, C–J). To determine whether PSCA expression correlated with proliferative activity, we performed immunohistochemical staining for Ki-67 on a subset of cases of primary and metastatic prostate cancer. No correlation was observed between expression of PSCA and expression of Ki-67 (data not shown).

Tumors positive for PSCA RNA were stratified according to signal intensity (1+, 2+, 3+, and 4+) and according to percentage of positive tumor cells (<50% versus ≥50%). Most tumors that scored

![Fig. 1. A–C. PSCA expression in the adult prostate is limited to differentiated, secretory cells. Shown are bright- (A) and darkfield (B) images at ×200 magnification. C, ×400 magnification of the brightfield image in A shows negative basal cells (arrowhead) adjacent to positive secretory cells (arrow). D, immunohistochemical staining for chromogranin A outlines occasional positive neuroendocrine cells within the epithelial cell population (magnification, ×400). E and F, PSCA expression in normal transitional cell mucosa is strongest in superficial cells (magnification, ×200). Bright- (A and E) and darkfield images (B and F) are shown for the same microscopic field.](image-url)
positive for PSCA showed expression in the majority of malignant cells (>50%). We did not observe statistically significant differences in the pattern or intensity of PSCA expression between cases with lower and higher Gleason grade or between cases of organ-confined and metastatic disease (data not shown).

Generation and Characterization of Monoclonal Antibodies to PSCA. After an initial screen by ELISA, we identified three monoclonal antibodies (6F8, 8D11, and 5F2) that reacted strongly with PSCA protein on the surface of live cells by flow cytometry using a CHO cell line transfected to express PSCA (data not shown). The affinities of 6F8, 8D11, and 5F2 for His-tagged PSCA were $2.8 \times 10^{-9}$ M, $3.6 \times 10^{-9}$ M, and $2.6 \times 10^{-9}$ M, respectively, as calculated from association and dissociation rate constants measured using a BIAcore system. Cross-competition experiments demonstrated that these antibodies recognized the same or overlapping epitopes (data not shown).

Additional cell lines that stably expressed human PSCA after transfection were generated for further in vitro and in vivo studies. Flow cytometry analysis demonstrated that the anti-PSCA antibody 8D11 specifically recognized PSCA expressed on the surface of four of these cell lines (Fig. 3). None of our antihuman PSCA monoclonal antibodies cross-reacted with murine PSCA (data not shown).

Internalization of Anti-PSCA Antibodies. Two complementary EM techniques, ultrastructural autoradiography and immunogold labeling, were used to identify the internalization pathway of anti-PSCA antibodies. Autoradiographic silver grains were first detected over the villi of transfected cells. Within minutes, the silver grains were observed over flask-shaped invaginations ~50–100 nm in diameter that resembled caveolae (Fig. 4, A and C). Silver grains were not associated with coated pits of vesicles. At later time points, the autoradiographic grains were visualized in different organelles of the endosomal compartment (Fig. 4, E–G). Because of the low resolution of the autoradiographic method, we used an immunogold labeling technique to unambiguously discriminate between different cell surface microdomains (see “Materials and Methods” for details). Gold
Antibody-DM1 conjugate was internalized via the caveolae pathway and accumulated within the endolysosomal compartments.

In Vivo Efficacy of Anti-PSCA Antibody against PSCA-positive Tumors. The growth of PSCA-positive tumors was significantly delayed (P < 0.05) in animals treated with unconjugated anti-PSCA antibody before tumor cell inoculation compared with animals treated with control antibody (Fig. 6A). Tumors eventually grew, although at a much slower rate than the control treated tumors. In addition, we observed a statistically significant retardation of tumor growth in animals treated with unconjugated anti-PSCA antibodies after tumor establishment compared with animals treated with control antibody (P < 0.05). The growth rates of the tumors in the different experimental groups appeared similar after day 20 of the experiment (Fig. 6B).

In subsequent studies we evaluated the efficacy of DM1-conjugated antibodies against established xenograft tumors (average tumor volume at the initiation of therapy, 200 mm³). Tumor volumes in animals treated with anti-PSCA-DM1 declined after the start of therapy to the extent of complete tumor regression (Fig. 7A). Histological evaluation of the tumor inoculation site at the conclusion of the study, i.e., 30 days after the last injection of antibody, confirmed the absence of viable tumor cells in all eight animals (not shown). As in the study shown, we observed a decrease in tumor volume in the group treated with conjugated control antibody ~15–25 days after initiation of treatment in several experiments. Fig. 7B shows a study in which efficacy in animals with much larger tumors (average tumor volume, 520 mm³) was evaluated. Unlike animals treated with conjugated control antibody, the animals treated with the anti-PSCA immunoconjugate showed a continuous decline of tumor volume during the course of therapy (Fig. 7B).

We also evaluated the cytotoxic potential of 8D11-DM1 in MCF7.Her2.gD.PSCA and PC3.neo as target cells. As demonstrated in a cell viability assay after a 7-day exposure, the IC₅₀ of 8D11-DM1 on PC3.gD.PSCA cells was ~1 ng/ml DM1 (equivalent to 3.8 × 10⁻¹⁰ m antibody; Fig. 5C). In comparison, the nonspecific cytotoxicity of 8D11-DM1 on antigen-negative cells was 75-fold lower (IC₅₀ > 75 ng/ml). In addition, the IC₅₀ of the irrelevant control antibody-DM1 conjugate was ~75 ng/ml DM1 in both the PC3.gD.PSCA cells and the antigen-negative PC3.neo cells (data not shown).

Fig. 5. In vitro cytotoxicity of the anti-PSCA immunoconjugate 8D11-DM1. Shown is cell survival as a function of toxin concentration. Assays were performed in serum-containing (A–C) or serum-free (D) tissue culture medium. A, MCF7.Her2.gD.PSCA and MCF7.Her2 (○), B, HCT116.gD.PSCA clone 6 (●), HCT116.gD.PSCA clone 8 (▲), and HCT116 (□); C and D, PC3.gD.PSCA (●) and PC3.neo (○). Data shown are the mean values of quadruplicate analyses.

We also evaluated the cytotoxic potential of 8D11-DM1 in MCF7.Her2.gD.PSCA cells (Fig. 5A) as well as in two subclones of HCT116.gD.PSCA (Fig. 5B) that express different levels of PSCA. 8D11-DM1 killed MCF7.Her2.gD.PSCA with an IC₅₀ of 1 ng/ml DM1 and the parental cell line MCF7.Her2 with an IC₅₀ of 75 ng/ml DM1. The IC₅₀ of 8D11-DM1 in HCT116.gD.psc clone 6 (~1.6 × 10⁶ receptors/cell as determined by Scatchard analysis; data not shown) was ~6 ng/ml DM1; in clone 8 (~400,000 receptors/cell), the IC₅₀ was 42 ng/ml DM1. These data show that the cytotoxic effects of the immunoconjugate are observed in an antigen-specific pattern and that the degree of cytotoxicity is dependent on the number of PSCA molecules on the cell surface.

In Vitro Cytotoxicity of Maytansinoid-conjugated Anti-PSCA Antibodies. The cytotoxicity of the DM1-conjugated antibodies was examined using PC3.gD.PSCA and PC3.neo as target cells. As demonstrated in a cell viability assay after a 7-day exposure, the IC₅₀ of 8D11-DM1 on PC3.gD.PSCA cells was ~1 ng/ml DM1 (equivalent to 3.8 × 10⁻¹⁰ m antibody; Fig. 5C). In comparison, the nonspecific cytotoxicity of 8D11-DM1 on antigen-negative cells was 75-fold lower (IC₅₀ > 75 ng/ml). In addition, the IC₅₀ of the irrelevant control antibody-DM1 conjugate was ~75 ng/ml DM1 in both the PC3.gD.PSCA cells and the antigen-negative PC3.neo cells (data not shown).
course of therapy. Therapy was discontinued before macroscopic resolution of the tumor, and all animals evaluated histologically showed residual tumor. However, the in vivo measurement of the tumor volume represents an overestimate of the true tumor mass, because the lesions consisted of abundant fibrosis with infiltrates of inflammatory cells as well as nests of viable tumor cells (data not shown). Transfected cells with high levels of PSCA expression were used in the in vivo studies described above. To address the issue of whether cells with lower levels of expression are sensitive to anti-PSCA-DM1 therapy, we performed a tumor efficacy study with the bladder cancer cell line SW780. This cell line endogenously expresses PSCA protein at a 10-fold lower level than the PC3.gD.PSCA cell line (*H1100500,000 PSCA molecules/cell by Scatchard analysis; data not shown) and exhibits minimal sensitivity in the in vitro cytotoxicity assay (data not shown). Treatment of SW780 tumor-bearing animals with DM1-conjugated anti-PSCA antibody resulted in marked retardation of tumor growth, whereas animals treated with control antibody-DM1 showed progressive tumor growth (Fig. 7C). Residual tumors isolated from anti-PSCA-DM1-treated animals still expressed PSCA RNA at the original level as determined by ISH (data not shown).

DISCUSSION

In parallel with our previous characterization of the expression pattern of murine PSCA in normal and neoplastic tissue (18), we performed an expression analysis of the human orthologue in benign and malignant urogenital tissues. Our observations confirm previous reports (26–28) describing expression in normal and neoplastic prostate tissue, kidney, and bladder. Unlike the original description of expression in the prostatic basal cell compartment, we conclusively show that the terminally differentiated, secretory epithelial cell is the site of expression of PSCA in the prostate. Similarly, in transitional cell mucosa of the urinary tract, expression was strongest in the differentiated, superficial cell population. Whether PSCA should still be considered a “stem cell” antigen based on its distant sequence homology to members of the Ly-6 gene family, including the stem cell antigens sca-1 and sca-2 (29), should be re-evaluated once the function of this protein has been determined.
As our data show, PSCA is not a gene that is uniformly expressed within the secretory epithelium of normal prostate or within the malignant cell population of a prostatic adenocarcinoma. It might be argued that expression analysis using the TMA technology might not be the appropriate tool to determine the incidence of expression of PSCA given its sometimes focal expression pattern. Although only a very small portion of the original tissue specimen is available for review, the TMA technology has been valuable for expression profiling of numerous indications (30). Adequate assessment of gene expression can typically be accomplished by sampling three representative areas of a given specimen (30). Essentially all of our cases had been sampled at least three times. Furthermore, we observed a very high concordance in a subset of cases, in which elements of normal or neoplastic tissues had been represented in multiple sets of three (data not shown).

A previous report described up-regulation of PSCA in prostate cancer metastatic to bone (28). Although there was a trend toward overexpression in metastatic cases in our series, the differences between the individual groups were not statistically significant. Because decalcification techniques lead to the loss of RNA integrity, we were not able to examine PSCA expression by our ISH technique in any bone marrow metastases. The differences in metastatic sites may contribute to any discrepancies in PSCA expression between the two studies.

The expression profile of PSCA together with the in vivo efficacy data suggests that PSCA is a promising therapeutic target for PSCA-expressing tumors. The two most important aspects in the consideration of toxin-conjugated therapeutic antibodies are potential toxicity and antitumor efficacy. Issues of toxicity can be addressed in our model only to a limited extent because the anti-PSCA antibody does not recognize the murine orthologue. Toxin-conjugated antimurine PSCA antibodies or a human PSCA transgenic or knock-in mouse model would be appropriate tools to evaluate toxicity in a mouse model. The efficacy of the toxin-conjugated anti-PSCA antibodies observed in the in vivo model should be interpreted in the context of our in vitro cytotoxicity studies, which demonstrate the broad concentration range in which DM1-conjugated anti-PSCA antibody exhibits antigen-specific cytotoxicity. This observation is consistent with previous studies using the same toxin conjugated to an antibody of different specificity (11) against a different tumor target. Because the therapeutic index for free DM1 is too small for clinical use (31), it is necessary for the intact immunoconjugate to be internalized into the target cell. Using ultrastructural autoradiography, we could show that cell surface bound anti-PSCA antibody is internalized. PSCA is anchored to the cell surface through a glycosylphosphoinositol linkage, and proteins with this particular linkage have been described as being internalized after engagement with ligand or antibody (32, 33).

DM1 inhibits the polymerization of tubulin into filaments and thus interferes with the formation of the mitotic spindle in mitotically active cells and with axonal transport in neuronal cells. This mechanism of action of DM1 would indicate that epithelial tissues with a rapid cellular turnover are particularly prone to potential side effects. Although free DM1 toxin may account for the temporary growth inhibition of control tumors (see Fig. 7, A and B) because of its effect on mitotically active cells, we did not observe any in vivo toxicity related to free DM1 in normal host tissues. Normal urothelium, which shows the highest levels of PSCA RNA in postmitotic, terminally differentiated cells (18), would be an obvious concern in this context. However, we attempted to mimic cellular quiescence in our in vitro cytotoxicity assay by performing the assay in serum-free medium. Under those circumstances, cytotoxicity by the immunoconjugate on PSCA-positive cells was greatly reduced.

Although the in vitro cytotoxicity data are quite informative, they may lead to underestimation of the in vivo efficacy of the immunoconjugate. For example, the immunoconjugate shows limited in vitro cytotoxicity on the bladder cancer cell line SW780, which expresses ~500,000 PSCA molecules on the cell surface. Nevertheless, there was marked in vivo efficacy with the same cell line and the same immunoconjugate. The increased length of exposure in the in vivo studies may explain this discrepancy, although additional mechanisms cannot be ruled out.

The efficacy of unconjugated anti-PSCA antibodies has now been convincingly demonstrated in two separate studies using independently derived monoclonal antibodies (Ref. 34 and this study). If treatment is started before tumor inoculation, retardation of tumor growth, inhibition of metastatic spread, and prolonged survival are observed (34). Similar, although less dramatic, effects have been observed in models using established tumors (34). The use of an antibody-toxin immunoconjugate in our studies markedly increased the in vivo efficacy and caused complete eradication of established tumors in the majority of animals when challenged with a cell line expressing high levels of PSCA. We observed significant growth retardation in animals challenged with cells expressing at least 10-fold lower levels of PSCA. The inability to achieve tumor eradication in the latter model may be attributable to several possible reasons. We know from ISH experiments that the residual tumors in animals treated with the anti-PSCA immunoconjugate still express high levels of PSCA RNA (data not shown), excluding the possibility of emerging antigen-loss tumor variants. Length of treatment and antibody dose could be manipulated to achieve increased efficacy in the treatment of tumors with low-level expression of PSCA. There is no evidence from our in vitro experiments that tumor cells acquire resistance to DM1. Tumor dormancy after continued treatment with the immunoconjugate is another possibility, although we observed mitotic activity in the residual microscopic tumors. Generation of neutralizing antibodies against the therapeutic antibody or against the toxin conjugate is not a consideration in this nude mouse xenograft model.

In summary, our studies illustrate a promising approach for the treatment of PSCA-expressing tumors. Advanced hormone-refractory prostate cancer, for which no effective therapies are available at this point, would be an obvious indication. Other tumor types that have been reported to express PSCA (35, 36) should be considered as well.

ACKNOWLEDGMENTS

We thank Barbara Wright for help with the immunohistochemistry for Ki-67 and chromogranin A, and the Genentech Histology Laboratory for sectioning of TMA blocks.

REFERENCES


Prostate Stem Cell Antigen as Therapy Target: Tissue Expression and in Vivo Efficacy of an Immunoconjugate

Sarajane Ross, Susan D. Spencer, Ilona Holcomb, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/9/2546

Cited articles
This article cites 31 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/9/2546.full#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/9/2546.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.