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Identification and Characterization of Prostein, a Novel Prostate-specific Protein[1]

Jiangchun Xu,[2] Michael Kalos, John A. Stolk, Eden J. Zasloff, Xinqun Zhang, Raymond L. Houghton, Aristides Maltez Filho, Marcos Nolasco, Roberto Badaró, and Steven G. Reed

Corixa Corp., Seattle, Washington 98104 [J. X., M. K., J. A. S., E. J. Z., X. Z., R. L. H., S. G. R.]; Hospital Aristides Maltez, Salvador 40125-001, Bahia, Brazil [A. M. F., M. N., R. B.]; and Department of Pathobiology, University of Washington, Seattle, Washington 98195 [S. G. R.]

ABSTRACT

In this report, we describe the application of a systematic, genome-based approach to identify prostein, a novel prostate-specific protein expressed in normal and malignant prostate tissues. Characterization of the prostein gene shows that prostein cDNA encodes a 553-amino acid protein. The protein is predicted to be a type IIIa plasma membrane protein with a cleavable signal peptide and 11 transmembrane-spanning regions. The prostein gene is located on chromosome 1 at the WI-9641 locus between q32 and q42. Prostein mRNA is shown to be uniquely expressed in normal and cancerous prostate tissues using Northern blot, cDNA microarray, and real-time PCR analyses. Furthermore, prostein mRNA expression does not appear to be prostate tumor grade related and is restricted exclusively to prostate cell lines. Immunohistochemical staining using a mouse monoclonal antibody generated against prostein demonstrates that this protein is specifically detected in prostate tissues both at the plasma membrane and in the cytoplasm. Prostein expression is androgen responsive because treatment of LNCaP cells with androgen up-regulates prostein message and protein expression levels. These results validate prostein as a prostate-specific marker with potential utility in the diagnosis and treatment of prostate cancer.

INTRODUCTION

Prostate cancer is the second leading cause of death among men in the United States. The American Cancer Society has estimated that approximately 179,000 new cases would be diagnosed with prostate cancer and 37,000 deaths from prostate cancer would occur in 1999 (1). Little is known about the genetic events associated with the malignant transformation of prostatic cells. This is due in large part to the cellular heterogeneity of the prostate and the lack of systematic analysis of prostate tissue gene expression. The identification of novel prostate-specific gene products that can be used as therapeutic and diagnostic reagents for prostate cancer is of critical importance for prevention and treatment of the disease. Molecules that are uniquely expressed or overexpressed in prostatic tumors and/or prostate tissues are potential candidates to serve as therapeutic vaccine antigens or as novel cancer markers.

Numerous approaches have been used to identify cancer-specific and cancer-associated markers, including expressed sequence tag sequencing (2, 3), serial analysis of gene expression (4, 5), and differential display PCR (6). Expression cloning using sera (7) or T cells (8) from cancer patients has been used to identify a panel of genes that are immunologically relevant and may be used as potential cancer vaccines and markers. Each of the above-mentioned approaches requires a tremendous amount of effort to identify a limited number of antigens. Furthermore, success depends on the availability of clinical reagents, and many antigens identified using these approaches are not cancer or tissue specific. Finally, none of the above-mentioned techniques provide a complete, systematic, and reliable comparison of the gene expression differences between two tissue types.

We have recently described a novel genome-based approach for the identification and characterization of tumor-specific proteins. This approach involves an initial cDNA library subtraction, followed by high-throughput microarray screening for tissue- and/or tumor-specific gene expression (9). In this report, we describe the application of this approach to isolate a novel prostate tissue-specific protein, prostein. The results presented in this report demonstrate that prostein is a prostate-specific marker with potential clinical utility for the diagnosis and treatment of prostate cancer.

MATERIALS AND METHODS

Tumor Samples, Cell Lines, Androgen Stimulation, and RNA Preparation. All clinical tissue samples used in this study were accompanied by clinical information and pathological reports and were histologically confirmed by pathologists. Prostate tumor cell lines LNCaP.FGC (metastatic adenocarcinoma), PC-3 (adenocarcinoma), and DU145 (carcinoma); breast tumor cell lines MDA-MB415, MCF7, SK-BR-3 (adenocarcinomas) MDA-MB453 (carcinoma), MDA-MB435S, T-47D, and BT474 (ductal carcinomas); colon tumor cell lines SW480 and COLO320 (adenocarcinomas); ovarian tumor cell lines SK-OV-3 (adenocarcinoma) and OV1063 (carcinoma); and lung tumor cell lines A549 (carcinoma), HTB-183, and HTB-177 (large cell lung carcinomas) were obtained from the American Type Culture Collection. Tumor cell lines 390T and 84T (squamous lung carcinomas) were kindly provided by Dr. Jill Siegfried (University of Pittsburgh Cancer Institute, Pittsburgh, PA); LT140-98 (lung adenocarcinoma) and TL1 (squamous lung carcinoma) were kindly provided by Dr. Elisabeth Repasky (Roswell Park Cancer Institute, Buffalo, NY); OT-391-73 (large cell endometrial carcinoma) was kindly provided by Dr. Heather Secrist (Corixa Corp.). 391-06 (large cell lung adenocarcinoma) was kindly provided by Dr. Rob Henderson (Corixa Corp.).

For the androgen stimulation experiments, LNCaP cells were plated at 1.5×10^6 cells/T75 flask (for RNA isolation) or 3×10^5 cells/well of a 6-well plate (for FACS[3] analysis) and grown overnight in RPMI 1640 containing 10% charcoal-stripped FCS (Life Technologies, Inc.). Cell culture was continued for an additional 72 h in RPMI 1640 containing 10% charcoal-stripped FCS, with 1 nM of the synthetic androgen methyltrienolone (R1881; New England Nuclear) added at various time points. Cells were then harvested for RNA isolation and FACS analysis at 0, 1, 2, 4, 8, 16, 24, 48, and 72 h after androgen addition.

For tissue sample RNA isolation, tissues were frozen in liquid nitrogen and homogenized with a Polytron (Kinematica), and total RNA was prepared using Trizol reagent (Life Technologies, Inc.). Polyadenylated RNA was isolated using a Qiagen oligotex spin column mRNA purification kit. For cell line RNA isolation, total RNA was prepared using Trizol reagent (Life Technologies, Inc.).

cDNA Library Subtraction. cDNA library subtraction was performed as described by Hara *et al.* (10), with modifications (9).

Microarray. mRNA expression of prostein was determined using a microarray assay as described previously (9).

Northern Blot Analysis. Northern blot analysis was performed as described previously (9) using ^{32}P-labeled prostein cDNA probe.

Quantitative Real-Time PCR. Quantitative real-time PCR assay was performed as described previously (9) using 300 nM each of prostein forward

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[2] To whom requests for reprints should be addressed, at Corixa Corporation, 1124 Columbia Street, Suite 200, Seattle, WA 98104. Phone: (206) 754-5798; Fax: (206) 754-5715; E-mail: xu@corixa.com.

[3] The abbreviations used are: FACS, fluorescence-activated cell-sorting; ORF, open reading frame; BPH, benign prostate hyperplasia; PSA, prostate-specific antigen.

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         10        20        30        40        50        60        70
MVQRLWVSRLLRHRKAQLLLVNLLTFGLEVCLAAGITYVPPLLLEVGVEEKFMTMVLGIGPVLGLVCVPL
         80        90       100       110       120       130       140
LGSASDHWRGRYGRRRPFIWALSLGILLSLFLIPRAGWLAGLLCPDPRPLELALLILGVGLLDFCGQVCF
        150       160       170       180       190       200       210
TPLEALLSDLFRDPDHCRQAYSVYAFMISLGGCLGYLLPAIDWDTSALAPYLGTQEECLFGLLTLIFLTC
        220       230       240       250       260       270       280
VAATLLVAEEAALGPTEPAEGLSAPSLSPHCCPCRARLAFRNLGALLPRLHQLCCRMPRTLRRLFVAELC
        290       300       310       320       330       340       350
SWMALMTFTLFYTDFVGEGLYQGVPRAEPGTEARRHYDEGVRMGSLGLFLQCAISLVFSLVMDRLVQRFG
        360       370       380       390       400       410       420
TRAVYLASVAAFPVAAGATCLSHSVAVVTASAALTGFTFSALQILPYTLASLYHREKQVFLPKYRGDTGG
        430       440       450       460       470       480       490
ASSEDSLMTSFLPGPKPGAPFPNGHVGAGGSGLLPPPPALCGASACDVSVRVVVGEPTEARVVPGRGICL
        500       510       520       530       540       550
DLAILDSAFLLSQVAPSLFMGSIVQLSQSVTAYMVSAAGLGLVAIYFATQVVFDKSDLAKYSA
```

Fig. 1. Amino acid sequence of prostein. Predicted signal sequences are *underlined*; predicted transmembrane regions are *boxed*.

(5′-CCCAGGACCTTGGAAATTCTACT-3′) and reverse (5′-ACCTTCCT-TCAACACCCTAACCT-3′) primers.

Bioinformatic Analysis. Transmembrane domains and protein localization of prostein were predicted by the PSORT algorithm using the prostein amino acid sequence.

FACS Analysis. A prostein-specific mouse monoclonal antibody 10E3-G4-D3 that recognizes an intracellular epitope of prostein was generated by standard hybridoma technologies and is described in detail elsewhere.[4] For FACS analysis, cells were harvested using trypsin (which was shown not to affect the antibody epitope), fixed in 2% formaldehyde, permeabilized in 0.5% saponin, and incubated with either the anti-prostein antibody 10E3-G4-D3 or an isotype-matched control antibody (purified anti-trinitrophenol murine IgG2a; PharMingen) at 1 μg/ml, followed by incubation with a FITC-conjugated antimouse immunoglobulin secondary antibody (PharMingen) at 5 μg/ml. After washes, the cells were analyzed using a FACSCalibur instrument (Becton Dickinson).

Immunohistochemical Studies. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissues by QualTek Molecular Laboratories using the prostein-specific mouse monoclonal antibody 10E3-G4-D3.

Chromosome Localization. The GeneBridge 4 Radiation Hybrid panel (Research Genetics) was used to determine the chromosomal location of prostein. Prostein primers 5′-ACTATGGTCCAGAGGCTGTG-3′ and 5′-AGAGGCGGCACATAGGTGAT-3′ were used in PCR reactions with DNA pools from the hybrid panel according to the manufacturer's instructions. After 38 cycles of amplification, the reaction products were separated on a 1.2% agarose gel, and the results were analyzed through the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research web server[5] to determine the probable chromosomal location.

RESULTS

Identification, Cloning, Sequence Analysis, and Chromosome Location of Prostein. A prostein cDNA fragment was isolated using the cDNA library subtraction method described previously (9). A full-length prostein cDNA clone was isolated by colony hybridization from a prostate tumor cDNA library using the fragment identified above. The full-length prostein cDNA is 3410 bp in length and contains an ORF of 553 amino acids (Fig. 1). Multiple stop codons in frame with the prostein ORF were identified in the prostein cDNA upstream of the presumptive initiator ATG, indicating that the entire prostein ORF had been obtained. The full-length prostein sequence was used to search GenBank DNA and protein databases, and no significant homologies were detected. The closest homology (28% identity and 44% similarity at the amino acid level) was with the human AIM-1 protein (GenBank ID, 5802879), a shared melanoma antigen recognized by HLA-A*0201-restricted T cells identified with the use of an *in vitro* immunoselected tumor line. Based on the PSORT algorithm, prostein is predicted to be a type IIIa plasma membrane protein with 11 potential transmembrane spans and a cleavable signal sequence. The putative transmembrane regions and signal peptide are indicated in Fig. 1.

To determine the chromosome localization of prostein, PCR analysis was performed using the GeneBridge 4 radiation hybrid panel. Prostein was mapped to the long arm of chromosome 1 at the WI-9641 locus between q32 and q42.

The Prostein Transcript Is Uniquely Expressed in Normal Prostate and Prostate Tumor Tissues. The tissue expression profile of the prostein transcript was determined using three independent approaches. Northern blot analysis was initially used to determine the expression of prostein in prostate tumors, normal prostate, BPH, and a panel of normal tissues including colon, kidney, liver, lung, pancreas, skeletal muscle, brain, stomach, testis, small intestine, and bone marrow. As shown in Fig. 2, a 3.8-kb transcript was detected in four of four prostate tumors, one normal prostate, and one BPH sample; expression was undetectable in the other normal tissues tested. The prostate-specific expression was further analyzed by microarray analysis on a larger panel of tissues (Fig. 3). Similar to the results from Northern blot analysis, prostein mRNA expression was shown to be restricted to prostate tissues. Prostein expression levels in prostate tissues ranged from 2- to 66.5-fold higher than that of the paired normal tissues, with an average overexpression of 23.3-fold in prostate tissues compared to other tissues tested. Quantitative real-time PCR, a more sensitive and quantitative assay, was also used to examine prostein mRNA expression. As shown in Fig. 4*A*, prostein mRNA could be detected in 23 of 23 prostate tumors, 3 of 3 BPH samples, and 3 of 3 normal prostate samples. No significant expression was detected in any of the other normal tissues tested. In summary, the data generated from each of these three independent methodologies demonstrate that the prostein transcript is expressed in a prostate-specific manner by the majority of normal and malignant prostate tissues.

As shown in Figs. 2, 3, and 4*A*, prostein expression is not tumor specific because normal prostate, BPH, and prostate tumors all express prostein message. Prostein mRNA expression was further examined on a Gleason panel consisting of six normal prostate tissues, six BPH samples, and a panel of prostate tumors of various Gleason grades. As shown in Fig. 4*B*, prostein mRNA was expressed in all normal prostate, BPH, and prostate tumors of various Gleason grades. Additionally, no correlation was observed between the level of prostein mRNA expression and the Gleason grade of the tumors. PSA mRNA expression levels were also assessed on the same Gleason panel; similar to prostein, PSA expression levels were not Gleason grade dependent (data not shown).

We have also examined the expression of prostein in a cell line panel consisting of *in vitro* established tumor cell lines and pooled normal and tumor tissues. Prostein expression is specific for prostate

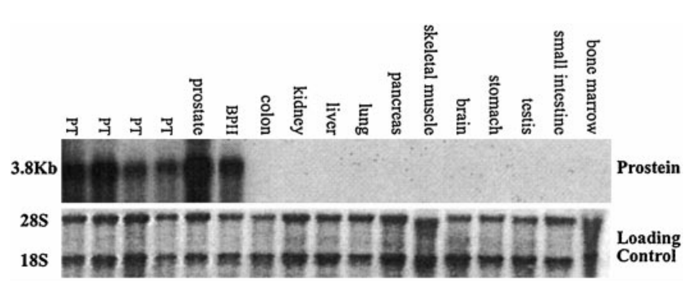

Fig. 2. Northern blot analysis of prostein gene expression in prostate and other tissues. Total RNAs (10 μg/lane) were run on a formaldehyde denaturing gel, blotted, and probed with a random primed prostein cDNA probe. The loading control is the RNA stained with methylene blue showing 18S and 28S rRNA bands.

[4] Michael Kalos, Thomas S. Vedvick, Steven G. Reed, David H. Devsing, and Gary R. Fouger. Characterization of 10E3-G4-D3, a murine monoclonal antibody specific for prostein. Manuscript in preparation.

[5] http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl.

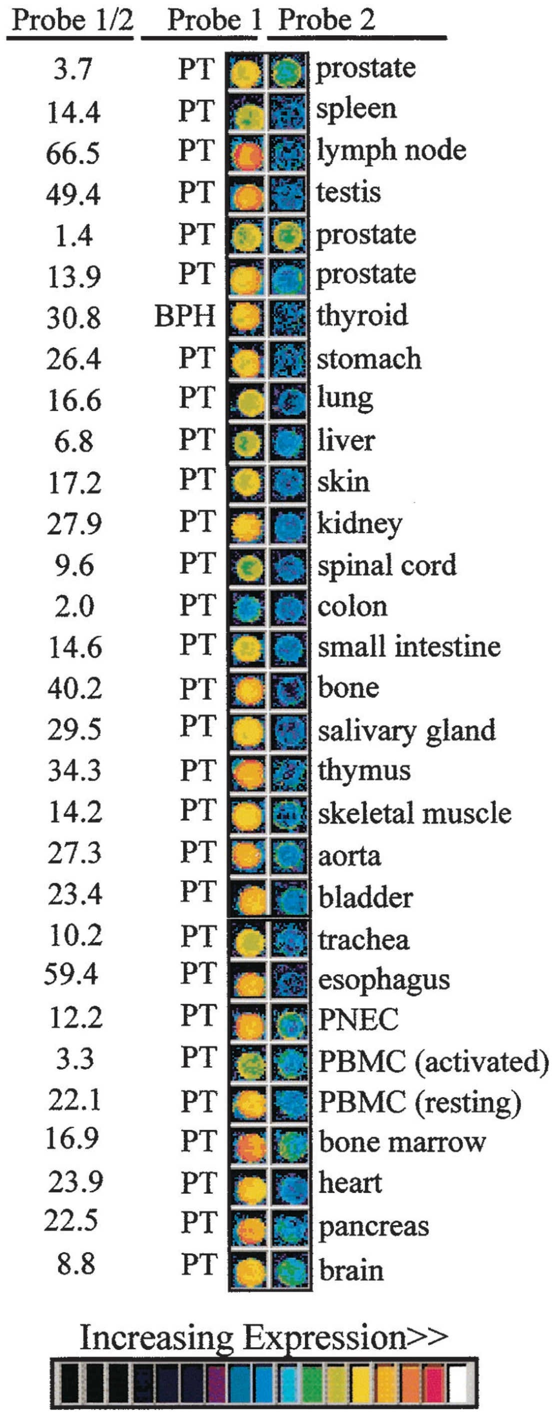

Fig. 3. Prostein gene expression determined by microarray. Prostein cDNA was PCR amplified, arrayed onto glass slides, and probed with a 1:1 mixture of Cy3-labeled probe 1 and Cy5-labeled probe 2. Fluorescent scans represented in pseudocolor correspond to hybridization intensities. Ratios represent the fluorescence intensity of probe 1:probe 2.

and is not detected in other tumor cell lines, including breast, lung, ovarian, and colon tumor cell lines. As shown in Fig. 4*C*, with the exception of a single breast tumor cell line, MDA-MB-415, which expresses a low level of prostein, all other tumor cell lines tested did not express significant amounts of prostein. Furthermore, prostein was not detected in pooled breast, lung, ovary, and colon tumor and normal tissues.

Prostein mRNA and Protein Are Expressed in Prostate Tumor Cell Lines. The expression of prostein mRNA and protein in prostate tumor cell lines was determined using quantitative reverse transcription-PCR and FACS analysis. As shown in Fig. 5*A*, prostein-specific transcripts could be detected in normal prostate and prostate tumors as well as in LNCaP cells and were detected at a very low level in PC-3 but not in DU-145 prostate tumor cells. These data also demonstrate that normal prostate and prostate tumor tissues express significantly higher levels of prostein transcript than the prostate tumor cell lines tested. The FACS analysis shown in Fig. 5*B* confirmed the real-time PCR analysis because the prostein-specific monoclonal antibody 10E3-G4-D3 specifically stained LNCaP cells and also stained PC-3 cells at a low level but failed to react with DU-145 cells. Because LNCaP cells expressed higher transcript levels and stained more intensely with the anti-prostein monoclonal antibody when compared with PC-3 and DU145 cells, these results demonstrate that prostein mRNA levels correlate with protein expression levels.

Prostein Protein Expression Is Restricted to Prostate Tissues. To characterize the protein expression of prostein in tissues, immunohistochemical analysis was performed using the monoclonal antibody 10E3-G4-D3. The specificity of 10E3-G4-D3 for prostein was demonstrated initially by ELISA assays using prostein-coated plates, and the 10E3-G4-D3 epitope was subsequently mapped to a peptide that is represented by prostein sequence. Furthermore, FACS and Western analyses have shown that 10E3-G4-D3 reacted specifically with cell lines transduced to express prostein (data not shown).[4] Using 10E3-G4-D3, prostein protein was shown to be specifically expressed in prostate tumors and normal prostate (Fig. 6). Expression was detected in all prostatic glandular cells. Furthermore, expression levels were not tumor grade related because similar staining intensity was seen in tumors of different grades. Prostate tumor tissues metastatic to lymph node, bone, and liver also expressed high levels of prostein (data not shown). Protein expression was not detected in normal heart, kidney, liver, lung, or colon. The immunohistochemistry analysis also showed a punctate plasma membrane staining pattern. In many areas, the staining appeared to be clustered within the cytoplasm in a perinuclear location. The staining was typically polarized and located between the nucleus and the lumen in normal and benign prostate epithelial cells. In prostate carcinomas, the polarity of the staining was completely random from cell to cell but usually remained polarized within each cell.

To comprehensively examine prostein expression in tissues, multitissue arrays were performed on human specimens representing major human normal organs as well as a wide range of human neoplasias. Approximately 4700 specimens were tested, including a total of 65 specimens from normal prostate and prostate carcinomas. All 65 prostate specimens stained positive using the prostein-specific antibody 10E3-G4-D3, whereas the remainder of approximately 4635 non-prostatic tissue specimens were all negative (data not shown).

Expression of Prostein Is Up-Regulated by Androgen. To determine whether prostein expression was affected by androgen, LNCaP cells were grown overnight in androgen-depleted media and then supplemented with the synthetic androgen methyltrienolone. Cells were harvested after androgen stimulation for various time periods, and prostein expression was measured both at the mRNA level using Northern blot analysis and at the protein level using FACS analysis. As shown in Fig. 7*A*, culture of LNCaP cells in the absence of androgen for 72 h (0 h time point) reduced prostein mRNA levels. Culture of LNCaP cells in the presence of androgen resulted in the induction of prostein mRNA, which was detectable as early as 2 h after androgen treatment and increased through 48–72 h of culture in the presence of androgen. As shown in Fig. 7*B*, prostein protein levels were also affected by the presence of androgen. Culture of LNCaP in the absence of androgen for 72 h (0 h time point) resulted in a loss of detectable prostein protein expression. Culture of LNCaP cells in the presence of androgen resulted in the induction of prostein protein,

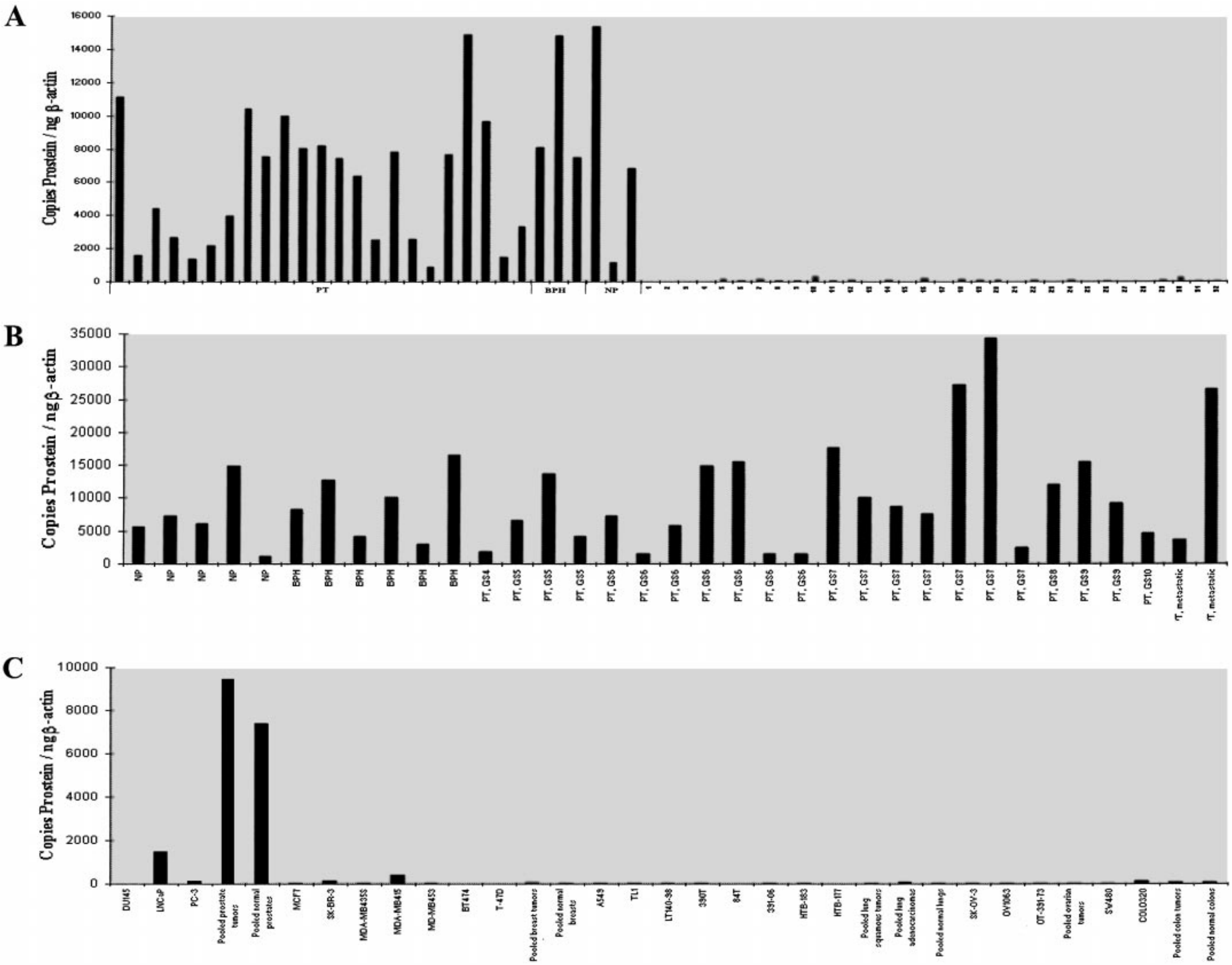

Fig. 4. Real-time PCR analysis of prostein mRNA expression. *A*, expression of prostein in prostate tumors, BPH, normal prostate, and a panel of other normal tissues. *PT*, prostate tumor; *NP*, normal prostate; *1*, activated peripheral blood mononuclear cells (PBMC); *2*, resting PBMC; *3*, normal bone marrow; *4*, normal heart; *5*, normal brain; *6*, normal brain (cerebellum); *7*, normal brain (cerebrum); *8*, normal pancreas; *9*, normal lung; *10*, normal liver; *11*, normal skin; *12*, normal kidney; *13*, normal spinal cord; *14*, normal colon; *15*, normal small intestine; *16*, normal stomach; *17*, normal prostate epithelial cells; *18*, normal bronchus; *19*, normal trachea; *20*, normal adrenal gland; *21*, normal aorta; *22*, normal skeletal muscle; *23*, normal thymus; *24*, normal salivary gland; *25*, normal bone; *26*, normal esophagus; *27*, normal bladder; *28*, normal thyroid gland; *29*, normal spleen; *30*, normal pituitary gland; *31*, normal retina; *32*, normal cartilage. *B*, expression of prostein in normal prostate, BPH, and prostate tumors of various Gleason scores (*GS*). *C*, expression of prostein in prostate, breast, lung, ovarian, and colon tumor cell lines. Prostate tumor cell lines, DU145, LNCaP, and PC-3; breast tumor cell lines, MCF7, SK-BR-3, MDA-MB435S, MDA-MB415, MDA-MB453, BT474, and T-47D; lung tumor cell lines, A549, TL1, LT140-98, 390T, 84T, 391-06, HTB-183, and HTB-177; ovarian tumor cell lines, SK-OV-3, OV1063, and OT-391-73; colon tumor cell lines, SW480 and COLO320. PCR was performed on a Perkin-Elmer/Applied Biosystems 5700 Prism instrument with the SYBR green assay using cDNA reverse transcribed from RNA of various tissues. β-Actin was used as an internal reference control. Results are representative of five experiments.

which was detectable as early as 8 h after androgen treatment and increased through 72 h of culture in the presence of androgen.

DISCUSSION

We have previously described a rapid, efficient, and systematic approach to identify prostate cancer- or tissue-specific genes (9). Here, we report the application of this approach to identify and characterize a novel prostate-specific protein, prostein. In this report, we demonstrate that prostein is abundantly and specifically expressed in prostate tumors and normal prostate tissues. Indeed, prostein was recovered multiple times from prostate libraries using the approach described in this report. Despite the relative abundance of prostein, it appears not to have been identified by other cloning methodologies. This observation may provide further evidence for the utility of our approach in identifying tumor-specific proteins not readily discovered by other methods.

Sequence homology comparisons suggest that the prostein gene product does not belong to a known family of proteins. Unlike a number of previously described prostate-specific genes such as PSA, glandular kallikrein, and the newly discovered serine protease prostase (11), prostein does not appear to be a serine protease and shows no homology to kallikrein family members. The closest identifiable homology to a known protein is to the human AIM-1 protein. Although the mechanisms for prostate-specific expression and the biological function of prostein are unknown, localization of the prostein gene to a region of chromosome 1 reported to be linked to prostate cancer susceptibility in hereditary prostate cancer (12, 13) suggests that the prostein gene may play a role in prostate cancer malignancy. The observation that the strongest homology of prostein was with a previously identified tumor antigen also provides the possibility that these molecules may be involved in the malignant phenotype.

The data presented in this report do not demonstrate correlation of

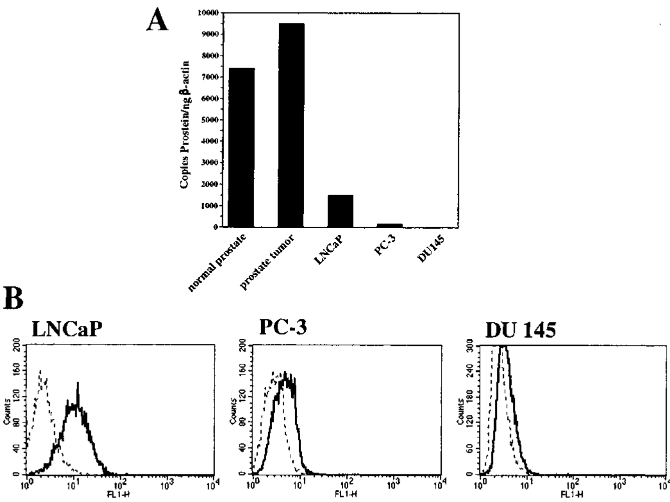

Fig. 5. Expression of prostein message and protein in prostate cancer cell lines. *A*, real-time PCR analysis. Analysis was performed as described in the Fig. 4 legend. Each value is the average of duplicate measurements, with a SD of less than 10%. *B*, FACS analysis. *Thick line*, 10E3-G4-D3; *thin line*, isotype-matched murine IgG2a. The data are representative of multiple experiments.

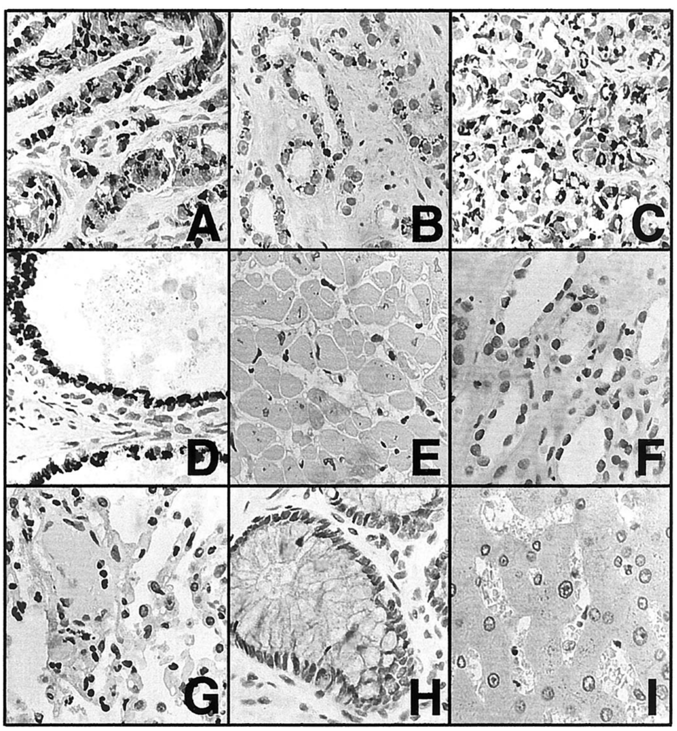

Fig. 6. Immunohistochemical staining of prostein. Prostate tumors (*A–C*), normal prostate (*D*), normal heart (*E*), kidney (*F*), lung (*G*), colon (*H*), and liver (*I*) were stained with mouse monoclonal antibody 10E3-G4-D3 against prostein. This experiment is a representative of multiple experiments.

prostein gene expression and prostate tumor Gleason grade. However, due to the cellular heterogeneity of prostate tumor tissue samples, it is likely that the percentage of actual cancer cells in each sample varies, and this may affect the levels of prostein gene expression detected. Immunohistochemical staining of a large panel of prostate tumors of various Gleason grades will further address the issue of prostein gene expression and Gleason grade.

Prostein appears to be expressed in an exclusively prostate-specific pattern because prostein mRNA and protein were not detected in any of the non-prostatic tissues tested. Recent reports have demonstrated similarities in gene expression between prostate and breast tissues (14). However, as shown in this manuscript, prostein mRNA expression could not be detected in normal and cancer breast tissues. Therefore, prostein could be useful in clinical diagnosis as an additional marker to both enhance the accuracy and reduce the false positive and negative rates of the currently used PSA test. Use of prostein in this setting may reduce or eliminate the issues of PSA cross-reactivity with other closely related serine proteases such as glandular kallikreins 1 and 2. Both protein-based assays (FACS, cytostain) and mRNA-based assays (reverse transcription-PCR) are currently being evaluated to determine the potential of prostein as a marker to detect prostate cancer cells circulating in the blood stream, which in turn may provide stage-related diagnostic and prognostic information. We anticipate that further characterization of the prostein genomic sequence will allow us to determine whether prostein is amplified in prostate cancers. The potential amplification of the prostein genomic sequence may be an important tool for prostate cancer diagnosis. Furthermore, the identification and characterization of the prostein promoter sequence may provide important information for the development of gene therapy approaches.

Expression of prostein on the cell surface was initially predicted by the bioinformatic algorithm PSORT. In support of this observation, immunohistochemical staining revealed a punctate surface-staining pattern. FACS analysis using prostein peptide-specific polyclonal antibodies to a predicted surface-expressed epitope showed surface staining of LNCaP cells.[4] FACS analysis using the monoclonal antibody 10E3-G4-D3 only stained permeabilized prostate tumor cell lines, suggesting that the epitope recognized by 10E3-G4-D3 is intracellular. The potential expression of prostein on the cell surface suggests that prostein may be an excellent target for therapeutic antibodies. Antibody-mediated therapies toward cell surface proteins such as CD20 and HER2/neu are being used as treatments for non-Hodgkin's lymphoma (15) and breast cancer (16), respectively. In prostate, antibodies against prostate-specific membrane antigens are being evaluated as a therapeutic approach to treat prostate cancers (17). Recently, a new prostate-specific cell surface antigen, STEAP, has been described (18). However, the expression of STEAP is not entirely specific for prostate because it has been found both at message and at protein levels in other tissues, such as normal bladder and colon and other types of tumors. In contrast, prostein is very specif-

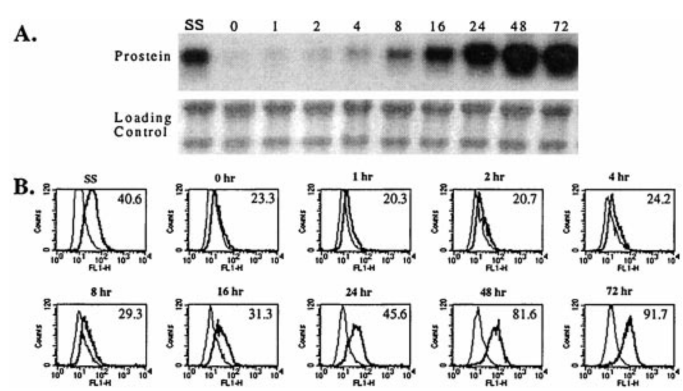

Fig. 7. Regulation of prostein expression by androgen. Expression of prostein mRNA (*A*) and protein (*B*) in LNCaP cells at steady state (*SS*), after 72 h of androgen deprivation (*0*), and at specified time periods (1, 2, 4, 8, 16, 24, 48, and 72 h) after androgen treatment. *A*, Northern blot analysis. Total RNAs (5 μg/lane) were run on a formaldehyde denaturing gel, blotted, and probed with a random primed prostein cDNA probe. The loading control is the RNA stained with methylene blue showing 18S and 28S rRNA bands. *B*, FACS analysis. *Thick line*, FACS histogram profile on permeabilized LNCaP cells using the murine anti-prostein monoclonal antibody 10E3-G4-D3; *thin line*, FACS histogram profile on permeabilized LNCaP cells using an isotype-matched murine anti-TNP monoclonal antibody. Numbers in the *top right corner* of each histogram indicate the mean fluorescence intensity of 10E3-G4-D3 staining.

ically and highly expressed in almost all prostate tumors and normal prostate tissues. It is highly expressed in metastatic prostate cancers and is likely to be expressed on the cell surface. These features of prostein make it an ideal target for prostate cancer antibody therapy. Furthermore, the homogeneous expression pattern of prostein on all prostate glandular cells indicates that therapeutic regimens that use prostein could be very efficient and powerful because all prostatic cells can be targeted. Finally, prostein expression could potentially be used as a marker for *in vivo* imaging diagnosis to detect metastatic prostate cancer cells.

In addition to diagnostic and antibody therapeutic uses, immunotherapy strategies involving a vaccine targeting prostein are being evaluated. The immunological relevance of prostein and its potential as a vaccine candidate have been demonstrated by *in vitro* T-cell priming and stimulation experiments.[4] The homology of prostein with a shared melanoma antigen demonstrated to elicit immune response provides further evidence for the potential of prostein to serve as a tumor antigen. The development of adjuvant and delivery systems to generate and expand strong immune responses to prostein could allow this protein to be developed as an effective prostate cancer-specific vaccine.

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