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(54) Title: PROSTAPIN GENE AND PROTEIN AND USES THEREOF		
1 5' TAC TTC CCA ACC AGC AGC TGA AGA GCT GAA TCA CTA AGC GCA CAA ATG AAA AAG 6A 73 82 91 100 109 GAG GGG CAA AAT ATA AAG GCT TTA TAT TTC TTA GGG ACC CAC CTC CAG TCT 118 127 136 145 154 163 AAC TTG CCA CTC TGT GAA CTT CAC TAC TGA AAA ACA ACA AAA GCA ATC BOC ATA 172 181 190 199 208 217 AAA ATG GGT TGT CTC AAC AGC GCT AAC GTT GAA TTT TGC CTT GAT GTG TTC AAA H S L S T A R H Y E F C L D Y F R 225 235 245 253 262 271 GAG CTC AAC AGT AAC AAC ATA AGA GAT AAC ATC TTC TTT TCG CTA CTA GTC E L N S N H L G R R L F F S S L S L 280 289 298 307 316 325 CTT TAT GCT CTA GAT ATC ATC CTC CTT GGT GCG AGG GGA GAG ACT GCA GAG GAA L Y A L S H Y L G A R E G E T R E Q 334 343 352 361 370 379 TTG GAG AAG GTG CTT GAT TTT AGT CAT ACT GTA GAC TCA TTA AAA CCA GAG TTC L E X V L N F S H T V D S L S P G F 388 397 406 415 424 433 AAG GAC TCA CCA AAG TAC AGC CAA GAT GAA AAA ATT CAT TCT GAG TTT GGT GTC C D S P R C S Q A R R I N S E F R V 442 451 460 469 478 487 GAA TTC TCT CAA ATC AAC CAG CAG TCT AAC TCT AGC CTC AGC ATT GCG AAC E F S O I N O P D S R C T A S I A H 496 505 514 523 532 541 AAG CTC TAC GAG ACA AAG ACA ATG GCA TTT GAT CAA TAT TTA AGC TGT TCT R L Y G T Y N A F N Q V L S C S 550 559 568 577 586 595 GAG AAA TGC TAT CAG GCG AGG TTS CAA ACT GTG GAT TTT GAA CAG TCT ACA GAA E K M Y Q A R L Q T V D F O S T E 604 613 622 631 640 649 GAA AAC AAG AAA ATT AAT ACT TGT GTT GAA GAT AAA ACT AAT GAA AAA GTC E T R K N J H A H V E R K T H G E Y 658 667 676 685 694 703 GCA ATY CTC TTT GAA AAG AAC ACA ATY GAC CTC TCA TCT GTA ATG GTC GTG GTG 2 A H L F R E S T I D P S S Y N V L V 712 721 730 739 748 757 AAT ACC ATA TAT TTC AAA GGA CAA AAG CAA AAT AAA TTT CAA GGT AAA AAT GTA H T I T Y F E G R Q N K F O G K R V 766 775 784 793 802 811 ACT GTG GAA ATG TAT CAA ATG GGA ACA TTT AAA CTC GCT TTT GTA AAG GAG Y V E N H Y Q I E T F K L A F V K E 820 829 838 847 856 865 CCH CAG ATG CAA GTT CTT GAG CTC CCG TAC GTT AAC AAC AAA TTA AGC ATG ATT P Q N Q V L E L P Y V N K L S H I 874 883 892 901 910 919 ATT CTC CTT CCA GTA GAC ATA GCT AAT CTC AAA CAG ATA GAA AAG CAG CTC AAT I L L P V G I A H L K Q I E K Q L H 928 937 946 955 964 973 YGA GAG AGG TTT GAT GAG TGA ACA AGC TCT TCT AAC ATG ATG GAA AGA GAA GTT S G T F H E M Y S S S N H N E R E V 982 991 1000 1009 1018 1027 GAA GTA CAC CTC CCG AGA TTA AAG ATT AAG TAT GAG CTA AAT TCG CTC E V H L P R F K L E I X Y E L N S L 1036 1045 1054 1063 1072 1081 TTA AAA CTT CTA GCG CTA ACA GAT CTC TTC AAC GTC AAA GCT GAT CTT TCT L K P L G Y T D L F N Q Y R A D L S 1090 1099 1108 1117 1126 1135 GCA ATG TCA CCA ACC AAG BOC CTA TAT TTA TCA AAA BOC ATC CAC AAG TCA TAC G H S P T E G L V L S R A L H N S Y 1144 1153 1162 1171 1180 1189 CTG GAT GTT AGC GAA GAG GGC AGC GAG CAA GCA BOC ACT GAG GAG AGC ATC L D Y S E E T T A A A A A A A A A A 1198 1207 1216 1225 1234 1243 GCT GTA AAA AGC CTA CCA ATA AGA GCT CAG TTA AAG GCG AAC CAC CCC TTC CTA A Y L L R H R A G F R A H N P P L 1252 1261 1270 1279 1288 1297 TTC TTT ATA AAG CAC ACT CAC AGC AAC AGC ATC CTA TTC TTT GAG AAG CTT GCG F F R R T T H T L T L F C B K L A 1306 1315 1324 1333 1342 1351 TCT CCG TAA TCA GAT GAG GTT CAG TAA GCG TCA GAG TTG CAG ATG AGC TGC AGA 3 S P * 1360 1369 1378 1387 1396 1405 GAC AAT CCG GTG ACT TTC CCA GCG CCA AAA AGC TGT TCA CAC CTC ACA CAC CTC 1414 1423 1432 1441 1450 1459 TGT GCG TCA GTT TGC TCA TCT CCA AAA TAG GTC TAG GAT TTC CCA CCA TTT 1468 1477 1486 1495 1504 1513 CAT GAG TTG TGA AGC TAA GCG TTT GTT AAT CAT AGA AAA AGG TAG ACT TAT GCA 1522 1531 1540 1549 1558 1567 GAA AGC CTT TCT GCG TCT CTC ATC TGT GGT GTC TCA TTT GAG TGC TGT CCA GTG 1576 1585 1594 1603 1612 1621 ACA TGA TCA AAT CAA TGA GTA AAA TTT TAA GGG AAT AGA TTT TCT TGA CTT GTA 1630 1639 1648 1657 1666 1675 TGT ATC TGT GAG ATC TTA AAT AAG TGA CCG GAC ATC TCT GCT TAA AGA AAA CCA 1684 1693 1702 1711 1720 1729 GCT GAA GGG CTT CAA CTT TGC TGT TTT TAA ATA TTT TCC TTT CAT ATG TAA 1738 1747 1756 1765 1774 1783 ATA GAA TGT GGT CAG TTT TAG TTC AAA ATT CTT TGT TGA GAA TAA TAA ATG CAT 1792 1801 1810 1819 1828 1837 GAA ATA CTT TAA AGC TCT GTG AAG ACT TGT AAC ATG GCA GCA AAT AAA AAG AA 3' 1846 1855 1864 1873 1882 1891 ATA AAA GGA TAC TTT GAT GGT GAA TAA ATT GAA AAA AAA AAA AA 3'		
(57) Abstract Described are a novel gene and protein expressed in normal prostate and locally confined prostate cancer, termed PROSTAPIN. In advanced stage prostate cancers, PROSTAPIN expression is lost or attenuated. Provided are cDNA and amino acid sequences encoding PROSTAPIN, vectors and host cells containing PROSTAPIN polynucleotides, antibodies specifically reactive with PROSTAPIN, and various related compositions which are useful in detecting, diagnosing, prognosing, staging, monitoring, treating and determining susceptibility to prostate cancer, particularly advanced stage and metastatic prostate cancer.		

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PROSTAPIN GENE AND PROTEIN AND USES THEREOF**FIELD OF THE INVENTION**

- 5 The invention described herein relates to a novel gene, PROSTAPIN, and its expression product; to the expression of PROSTAPIN in normal and prostate cancer cells; and to diagnostic, prognostic, and therapeutic compositions and methods useful in the management of prostate cancer.

10 **BACKGROUND OF THE INVENTION**

- Prostate cancer is the most frequently diagnosed cancer and second leading cause of cancer death in men. Some 45,000 men die annually of this disease and only lung cancer has a higher mortality rate. In the United States, the chance of a man developing invasive
15 prostate cancer during his lifetime is approximately 1 in 6 or greater. Numerous fundamental limitations in the presently available methods used for the treatment, diagnosis and prognosis of prostate cancer have rendered it impossible to effectively manage.

- 20 Surgical prostatectomy, radiation therapy, hormone ablation therapy, and chemotherapy are the main components in the current arsenal for treating prostate cancer. However, these treatments are ineffective for the 45,000 prostate cancer patients who die of this disease every year. While some advances in the treatment of locally confined tumors have been achieved, prostate cancer is presently incurable once it has metastasized.

- 25 The major cause of morbidity and mortality from prostate cancer is the result of androgen-independent metastatic tumor growth. Patients with metastatic prostate cancer are treated by hormonal ablation therapy, but only with short-term success. Eventually, these patients develop an androgen-refractory state leading to aggressive
30 disease progression, ultimately resulting in the development of debilitating bone and other metastases causing death. The mechanism by which androgen-independent growth of prostate tumors occurs is not known. Moreover, there is no method available for predicting the emergence of metastatic disease. Metastatic prostate cancer is typically diagnosed by open or laparoscopic pelvic lymphadenectomy, whole body
35 radionuclide scans, skeletal radiography, and/or bone lesion biopsy. As a result, there is great interest in defining the molecular basis for advanced staged disease with the hope that these insights may improve the therapeutic options for these patients.

The primary sites of prostate cancer metastasis are the regional lymph nodes and bone. Bone metastases occur in sites of hematopoietically active red bone marrow, including lumbar vertebral column, ribs, pelvis, proximal long bones, sternum and skull. Bony metastases of prostate cancer differ from those of other tumors commonly colonizing bone in that they are characterized by a net gain in bone formation (osteoblastic) rather than resorption predominant in bone metastases of breast cancer and melanoma.

Until recently, bone metastasis was thought to be a late stage in disease progression. However, the recent development of highly sensitive techniques (such as RT-PCR for prostate specific genes) to detect prostate cancer cells has revised this notion. Prostate cancer cells have been detected in the peripheral blood and bone marrow of patients with advanced stage disease using RT-PCR assays for PSA mRNA (Ghossein et al., 1995; Seiden et al., 1994; Wood et al., 1994; Katz et al., 1994) or immunomagnetic bead selection for PSA protein (Brandt et al., 1996). When positive, these tests show that prostate cancer cells represent about 0.1-1.0% of the circulating blood cells. Moreover, it is now clear that small numbers of prostate cancer cells circulate in the peripheral blood and lodge in the bone marrow even in patients with early stage, low risk disease (Olsson et al., 1997; Deguchi et al., 1997; Katz et al., 1996). Interestingly, these cells tend to disappear in most patients following radical prostatectomy (Melchior et al., 1997). These results suggest that the primary tumor site is a constant source for seeding the marrow, and that only a small subset of these cells have the capacity to grow into a metastatic lesion. This concept is consistent with estimates from animal models for other tumor types that only about 1 in 10,000 circulating cancer cells are able to lodge in and productively colonize other organs (Fidler et al., 1990). The biological factors involved in advanced prostate cancer progression to bone metastasis are unknown. (See also, Lalani et al., 1997, *Cancer Metastasis Rev.* 16: 29-66).

A another factor complicating the management of prostate cancer is that reliable diagnostic and prognostic markers capable of accurately detecting early-stage tumors and/or predicting which patients will progress to advanced stages do not exist. Early detection and diagnosis of prostate cancer currently relies on digital rectal examinations (DRE), prostate specific antigen (PSA) measurements, transrectal ultrasonography, and transrectal needle biopsy. Serum PSA measurements in combination with DRE represent the leading diagnostic approach at present. Although a number of prostate cancer markers have been identified, and at least one, PSA, is in widespread clinical use, the ideal prostate tumor marker has been extremely elusive. Moreover, no marker capable of reliably predicting disease progression has been identified.

There is currently a tremendous worldwide effort aimed at the development of novel molecular approaches to prostate cancer diagnosis and treatment. For example, there is great interest in identifying truly prostate-specific genes and proteins that could be used as diagnostic, prognostic and/or therapeutic targets or reagents. Progress has been slow, and despite this intensive worldwide research effort, no effective biological approaches for treating prostate cancer have emerged in clinical practice. Similarly, the inability to reliably detect early-stage prostate cancer or predict which patients will progress to advanced disease persists.

Tumor suppressors are proteins which regulate cell growth. Absence of tumor suppressors by mutation, deletion, or loss of expression results in the malignant phenotype. Numerous tumor suppressor genes have been identified. Two of the more well known and studied tumor suppressor genes are the retinoblastoma (Rb) gene and the p53 gene, both of which are directly involved in influencing the cell cycle machinery. The expression of Rb inhibits cell cycle progression from G₁ into S phase. The p53 gene is the most frequently mutated gene in human cancers, with approximately half of all tumors containing abnormal p53 genes. p53 participates in a cell cycle checkpoint signal transduction pathway that causes either G₁ arrest or apoptosis following DNA damage. Loss of p53 function during tumorigenesis can result in progression through the cell cycle in the face of DNA damage and survival of a cell otherwise destined for death.

There is no known prostate-specific tumor suppressor. A class of serine protease inhibitors known as the serpins includes a protein, maspin, which may function as a tumor suppressor in both breast and prostate cancer. Maspin has been shown to be down-regulated in breast and prostate carcinoma (Zou et al., 1994, Science 263:526), and overexpression of maspin and/or exogenous addition of maspin has been shown to dramatically reduce the tumorigenic properties and metastatic potential of breast cancer cells (Zou et al., 1994, Science 263:526). Other members of the serpin family include leupin (SCCA2) (Suminami et al., 1991, BBRC 181:51), bomapin (Riewald and Schleef, 1995, JBC 270:26754), and leukocyte elastase inhibitor (Dubin et al., 1993, Biochem. J. 293:187). The latter, Leukocyte elastase inhibitor (LEI), appears to have a functional role in apoptosis. Specifically, LEI is converted to L-DNase II by digestion with elastase, whereupon it functions as an endonuclease in DNA degradation during apoptosis (Torriglia et al., 1998, MCB 18:3612). Several other serpins appear to have a role in apoptosis.

SUMMARY OF THE INVENTION

The present invention relates to a novel member of the serpin family, termed PROSTAPIN, which is expressed almost exclusively in the prostate. PROSTAPIN expression is greatly attenuated or completely lost in cells of advanced prostate tumors and metastases, while its expression is maintained at or near normal levels in locally confined prostate cancers. In addition to transcriptional loss of PROSTAPIN, there is initial evidence that the PROSTAPIN gene is substantially mutated in some advanced stage prostate tumors. Accordingly, PROSTAPIN may function as a prostate-specific tumor suppressor, apoptosis-inducer or apoptosis-modulator. The PROSTAPIN gene and protein, as well as factors capable of activating PROSTAPIN expression, may be useful as therapeutic agents capable of restoring critical tumor suppressor activity lost in advanced prostate cancer. In addition, PROSTAPIN may represent an ideal marker for predicting and identifying progression to advanced stage and metastatic prostate cancer, and may also be useful for determining susceptibility to advanced disease and for gauging prostate tumor aggressiveness.

The invention provides polynucleotides corresponding or complementary to all or part of the PROSTAPIN gene, mRNA, and/or coding sequence, preferably in isolated form, including polynucleotides encoding PROSTAPIN proteins and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to the PROSTAPIN gene or mRNA sequence or a part thereof, and polynucleotides or oligonucleotides which hybridize to the PROSTAPIN gene, mRNA, or to PROSTAPIN-encoding polynucleotides. Also provided are means for isolating cDNAs and the gene encoding PROSTAPIN, as well as those encoding mutated and other forms of PROSTAPIN. Additionally, functionally mutant PROSTAPIN polynucleotides are provided. Recombinant DNA molecules containing PROSTAPIN polynucleotides, cells transformed or transduced with such molecules, and host-vector systems for the expression of PROSTAPIN gene products are also provided. The invention further provides PROSTAPIN proteins and polypeptide fragments thereof. The invention further provides antibodies that bind to PROSTAPIN proteins and polypeptide fragments thereof, including polyclonal and monoclonal antibodies, murine and other mammalian antibodies, chimeric antibodies, humanized and fully human antibodies, and antibodies labeled with a detectable marker. The invention further provides methods for detecting the presence of PROSTAPIN polynucleotides and proteins in various biological samples, as well as methods for identifying cells that express PROSTAPIN. The invention further provides methods and assays for determining PROSTAPIN expression status, diagnosing advanced prostate cancer, gauging tumor aggressiveness, and predicting susceptibility to advanced prostate cancer. The invention further provides various therapeutic compositions and

strategies for treating prostate cancer by restoring functional PROSTAPIN to prostate tumor cells.

5 BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Nucleotide and amino acid sequences of PROSTAPIN (SEQ ID NOS. XX and XX, respectively), derived from the overlapping nucleotide sequences of SSH fragment cDNA clone 11P2A6 (5' 471 bp) (SEQ ID NO. XX) and cDNA clone 103 (SEQ ID NO. XX). Clones 10 11P2A6 and 103 overlap across 304 bp beginning at position 168. The 5' untranslated region indicates two translational STOP signals (indicated by asterisks) upstream of the START ATG. The sequence surrounding the start ATG (AAA ATG G) exhibits a Kozak sequence (A at position -3, and G at position +1). The underlined sequence in the carboxyl region indicates the putative highly exposed reactive site loop that is 15 characteristic of the serpin family.

FIG. 2. Amino acid sequence alignment of PROSTAPIN with several other Serpin family members. The alignment was performed using the PIMA1.4 alignment program of the Baylor College of Medicine Search Launcher Web site. The RSL sites are indicated in 20 bold. The protease cleavage site, indicated by the P1 and P1' residues, is boxed.

FIG. 3. Semi-quantitative RT-PCR analysis of PROSTAPIN expression in normal human tissue, prostate cancer xenograft tissue, and cell lines using primers derived from clone 11P2A6 cDNA (SEQ ID NO. XX). First strand cDNAs were prepared from 16 normal 25 tissues, the LAPC xenografts (4AD, 4AI and 9AD) and HeLa cells. Normalization was performed by PCR using primers to actin and GAPDH. Expression of PROSTAPIN is detected only in normal prostate and in the LAPC-9 AD xenograft.

FIG. 4. Northern blot analyses of PROSTAPIN expression in various normal human 30 tissues and prostate cancer xenografts. A and B: Multiple tissue northern blots probed with full length PROSTAPIN cDNA clone 103 (SEQ ID NO. XX). Size standards in kilobases (kb) are indicated. C: Multiple tissue RNA dot blot (Clontech, Human Master Blot cat# 7770-1) probed with PROSTAPIN CLONE 103 cDNA probe (SEQ ID NO. XX). D: Normal prostate and various prostate cancer xenograft Northern blot, showing lack of 35 expression in the LAPC-4 prostate cancer xenografts and down-regulated expression in the LAPC-9 xenograft relative to normal prostate expression levels.

FIG. 5. Loss of PROSTAPIN expression in metastatic prostate cancer. Semi-quantitative RT-PCR analysis on normalized first strand cDNAs derived from prostate cancer

xenografts, cell lines and human tissue specimens. The prostate pool (lane 3) comprises several normal prostate cDNAs and was obtained from Clontech (Palo Alto, California). Prostate 25 and Prostate 32 were derived from 25 and 32 year old individuals, respectively (BioChain). Gleason Grade and TMN Stage of the human prostate tumor specimens analyzed are indicated. For details, see Example 4.

FIG. 6. Mutant PROSTAPIN gene generated from prostate cancer xenograft LAPC-9 AD: LAPC-9 AD PROSTAPIN cDNA clone 2 nucleotide (SEQ ID NO. XX) and deduced amino acid (SEQ ID NO. XX) sequences. Point mutations in the nucleotide sequence and any resulting amino acid changes relative to the wild-type PROSTAPIN sequence of FIG. 1 (SEQ ID NO. XX) are indicated in boldface type and are underlined. A large insertion sequence (relative to wild-type PROSTAPIN) is indicated in bold and is underlined.

FIG. 7. Detection of PROSTAPIN protein in cell membrane fraction. The results show that PROSTAPIN is predominantly expressed in the light membrane fraction. See Example 6 for experimental details.

FIG. 8. Chromosomal mapping of human PROSTAPIN, showing position within serpin gene cluster on chromosome 18q21.3.

FIG. 9. Southern blot analysis for PROSTAPIN gene. Ten micrograms of each DNA sample was digested with EcoRI, blotted onto nitrocellulose and probed with PROSTAPIN CLONE 103 cDNA probe (SEQ ID NO. XX). (A) Zooblot: Genomic DNAs prepared from several different organisms including human, monkey, dog, mouse, chicken and *Drosophila*. (B) Human BACs 2002H14, 2074J2, 2100H19, and PAC 152i22, containing the PROSTAPIN gene, a non-specific BAC (2116L1), and a non-specific PAC (40P22) (lanes 4 and 5, respectively). (C) Mouse BACs 74e14 (lanes 3 and 5), 213i3 (lanes 4 and 6) containing the mouse PROSTAPIN gene probed together with human positive (BAC 2074J2) and negative (BAC 40P22) controls.

FIG. 10. Intron/exon boundaries of the human wild-type PROSTAPIN gene. Sequence in capital letters designate exonic sequences (with the translation below) and sequence in lower case letters designate intronic sequences. A total of 6 introns and 7 exons were identified within the PROSTAPIN coding region.

FIG. 11. Amplification of PROSTAPIN exons from human genomic DNA: An example of the PCR products obtained from human genomic DNA using the primers described in Example 8. Human BAC DNA containing the PROSTAPIN gene was used as a positive control for PCR amplification.

FIG. 12. Western blot analysis of PROSTAPIN expression in lysates of cells transfected or transduced with PROSTAPIN using purified polyclonal antibody generated against a PROSTAPIN-GST fusion (see Example 9).

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FIG. 13. Western blot analysis of PROSTAPIN expression in lysates derived from LAPC xenografts (LAPC-4 AD, 9AD, and 9AI), prostate cancer cell lines (TsuPr1, LNCaP, PC-3) and a prostate tumor-normal matched patient sample cells (see Example 10).

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DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

As used herein, the terms "advanced prostate cancer", "locally advanced prostate cancer", "advanced disease" and "locally advanced disease" mean prostate cancers which have extended through the prostate capsule, and are meant to include stage C disease under the American Urological Association (AUA) system, stage C1 - C2 disease under the Whitmore-Jewett system, and stage T3 - T4 and N+ disease under the TNM (tumor, node, metastasis) system. In general, surgery is not recommended for patients with locally advanced disease, and these patients have substantially less favorable outcomes compared to patients having clinically localized (organ-confined) prostate cancer. Locally advanced disease is clinically identified by palpable evidence of induration beyond the lateral border of the prostate, or asymmetry or induration above the prostate base. Locally advanced prostate cancer is presently diagnosed pathologically following radical prostatectomy if the tumor invades or penetrates the prostatic capsule, extends into the surgical margin, or invades the seminal vesicles.

As used herein, the terms "metastatic prostate cancer" and "metastatic disease" mean prostate cancers which have spread to regional lymph nodes or to distant sites, and are meant to include stage D disease under the AUA system and stage TxNxM+ under the
5 TNM system. As is the case with locally advanced prostate cancer, surgery is generally not indicated for patients with metastatic disease, and hormonal (androgen ablation) therapy is the preferred treatment modality. Patients with metastatic prostate cancer eventually develop an androgen-refractory state within 12 to 18 months of treatment initiation, and approximately half of these patients die within 6 months thereafter. The
10 most common site for prostate cancer metastasis is bone. Prostate cancer bone metastases are, on balance, characteristically osteoblastic rather than osteolytic (i.e., resulting in net bone formation). Bone metastases are found most frequently in the spine, followed by the femur, pelvis, rib cage, skull and humerus. Other common sites for metastasis include lymph nodes, lung, liver and brain. Metastatic prostate cancer is
15 typically diagnosed by open or laparoscopic pelvic lymphadenectomy, whole body radionuclide scans, skeletal radiography, and/or bone lesion biopsy.

As used herein, the term "polynucleotide" means a polymeric form of nucleotides of at least 10 bases or base pairs in length, either ribonucleotides or deoxynucleotides or a
20 modified form of either type of nucleotide, and is meant to include single and double stranded forms of DNA.

As used herein, the term "polypeptide" means a polymer of at least 10 amino acids. Throughout the specification, standard three letter or single letter designations for amino
25 acids are used.

As used herein, the terms "hybridize", "hybridizing", "hybridizes" and the like, used in the context of polynucleotides, are meant to refer to conventional hybridization conditions, preferably such as hybridization in 50% formamide/6XSSC/0.1% SDS/100 µg/ml ssDNA, in
30 which temperatures for hybridization are above 37 degrees C and temperatures for washing in 0.1XSSC/0.1% SDS are above 55 degrees C, and most preferably to stringent hybridization conditions.

Additional definitions are provided throughout the subsections which follow.
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PROSTAPIN POLYNUCLEOTIDES

One aspect of the invention provides polynucleotides corresponding or complementary to all or part of the PROSTAPIN gene, mRNA, and/or coding sequence, preferably in isolated form, including polynucleotides encoding PROSTAPIN proteins and fragments

thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to the PROSTAPIN gene or mRNA sequence or a part thereof, and polynucleotides or oligonucleotides which hybridize to the PROSTAPIN gene, mRNA, or to PROSTAPIN-encoding polynucleotides (collectively, "PROSTAPIN polynucleotides").

A PROSTAPIN polynucleotide may comprise a polynucleotide having the sequence shown in FIG. 1 (SEQ ID NO. XX), a sequence complementary thereto, or a polynucleotide fragment thereof. Another embodiment comprises a polynucleotide which encodes the PROSTAPIN protein amino acid sequence shown in FIG. 1 (SEQ ID NO. XX) or a polynucleotide fragment thereof. Another embodiment comprises a polynucleotide which is capable of hybridizing under stringent hybridization conditions to the PROSTAPIN cDNA shown in FIG. 1 (SEQ ID NO. XX) or to a polynucleotide fragment thereof. In addition, the invention includes polypeptides derived from PROSTAPIN mutants, such as the LAPC-9 mutant PROSTAPIN described herein. Such mutant PROSTAPIN polynucleotides may comprise the sequence of the LAPC-9 PROSTAPIN mutant, as shown in FIG. 6 (SEQ ID NO. X), or a polypeptide fragment thereof. A related embodiment comprises a polynucleotide which is capable of hybridizing under stringent hybridization conditions to the PROSTAPIN mutant cDNA shown in FIG. 6 (SEQ ID NO. XX) or to a polynucleotide fragment thereof.

Specifically contemplated are genomic DNA, cDNAs, ribozymes, and antisense molecules, as well as nucleic acid molecules based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. For example, antisense molecules can be RNAs or other molecules, including peptide nucleic acids (PNAs) or non-nucleic acid molecules such as phosphorothioate derivatives, that specifically bind DNA or RNA in a base pair-dependent manner. A skilled artisan can readily obtain these classes of nucleic acid molecules using the PROSTAPIN polynucleotides and polynucleotide sequences disclosed herein.

Further specific embodiments of this aspect of the invention include primers and primer pairs, which allow the specific amplification of the polynucleotides of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. Probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers can be used to detect the presence of a PROSTAPIN polynucleotide in a sample and as a means for detecting a cell expressing a PROSTAPIN protein. Examples of such probes include polypeptides comprising all or part of the cDNA

sequence shown in FIG. 1 (SEQ ID NO. XX). Examples of primer pairs capable of specifically amplifying PROSTAPIN mRNA are described in the examples which follow. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on the sequences provided in FIG. 1 (SEQ ID NO. XX) and used effectively to amplify and/or detect PROSTAPIN.

As used herein, a polynucleotide is said to be "isolated" when it is substantially separated from contaminant polynucleotides which correspond or are complementary to genes other than the PROSTAPIN gene or which encode polypeptides other than PROSTAPIN gene product or fragments thereof. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated PROSTAPIN polynucleotide.

The PROSTAPIN polynucleotides of the invention are useful for a variety of purposes, including but not limited to their use as probes and primers for the amplification and/or detection of the PROSTAPIN gene(s), mRNA(s), or fragments thereof; as reagents for the diagnosis and/or prognosis of prostate cancer; as coding sequences capable of directing the expression of PROSTAPIN polypeptides; as tools for modulating or inhibiting the expression of the PROSTAPIN gene(s) and/or translation of the PROSTAPIN transcript(s); and as therapeutic agents.

METHODS FOR ISOLATING PROSTAPIN-ENCODING NUCLEIC ACID MOLECULES

The PROSTAPIN cDNA sequences described herein enables the isolation of other polynucleotides encoding the PROSTAPIN gene product(s), as well as the isolation of polynucleotides encoding PROSTAPIN gene product homologues, alternatively sliced isoforms, allelic variants, and mutant forms of the PROSTAPIN gene product. Various molecular cloning methods that can be employed to isolate full length cDNAs encoding the PROSTAPIN gene are well known (See, for example, Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2d edition., Cold Spring Harbor Press, New York, 1989; Current Protocols in Molecular Biology. Ausubel et al., Eds., Wiley and Sons, 1995). For example, lambda phage cloning methodologies may be conveniently employed, using commercially available cloning systems (e.g., Lambda ZAP Express, Stratagene). Preferably, cDNA libraries may be generated from normal testis tissue, placental tissue, prostate cancer cell lines, prostate cancer xenografts or another PROSTAPIN-expressing source. Phage clones containing PROSTAPIN gene cDNAs may be identified by probing with labeled PROSTAPIN cDNA or a fragment thereof. For example, in one embodiment, the PROSTAPIN cDNA of FIG. 1 or a portion thereof can be synthesized and used as a probe to retrieve overlapping and full length cDNAs corresponding to the PROSTAPIN-1 gene. The PROSTAPIN gene itself may be isolated by screening genomic DNA libraries, bacterial

artificial chromosome libraries (BACs), yeast artificial chromosome libraries (YACs), and the like, with PROSTAPIN DNA probes or primers.

RECOMBINANT DNA MOLECULES AND HOST-VECTOR SYSTEMS

5 The invention also provides recombinant DNA or RNA molecules containing a PROSTAPIN polynucleotide, including but not limited to phages, plasmids, phagemids, cosmids, YACs, BACs, as well as various viral and non-viral vectors well known in the art, and cells transformed or transfected with such recombinant DNA or RNA molecules. As used herein, a recombinant DNA or RNA molecule is a DNA or RNA molecule that has been subjected to
10 molecular manipulation in vitro. Methods for generating such molecules are well known (see, for example, Sambrook et al, 1989, supra).

The invention further provides a host-vector system comprising a recombinant DNA molecule containing a PROSTAPIN polynucleotide within a suitable prokaryotic or
15 eukaryotic host cell. Examples of suitable eukaryotic host cells include a yeast cell, a plant cell, or an animal cell, such as a mammalian cell. Examples of suitable mammalian cells include various prostate cancer cell lines such as LnCaP, PC-3, DU145, LAPC-4, TsuPr1, other transfectable or transducible prostate cancer cell lines, as well as a number of mammalian cells routinely used for the expression of recombinant proteins
20 (e.g., COS, CHO, 293, 293T cells). More particularly, a polynucleotide comprising the coding sequence of PROSTAPIN may be used to generate PROSTAPIN proteins or fragments thereof using any number of host-vector systems routinely used and widely known in the art.

25 A wide range of host-vector systems suitable for the expression of PROSTAPIN proteins or fragments thereof are available, see for example, Sambrook et al., 1989, supra; Current Protocols in Molecular Biology, 1995, supra). Preferred vectors for mammalian expression include but are not limited to pcDNA 3.1 myc-His-tag (Invitrogen) and the retroviral vector pSR α tkneo (Muller et al., 1991, MCB 11:1785). Using these expression vectors,
30 PROSTAPIN may be preferably expressed in several prostate cancer cell lines, including for example PC-3, LNCaP and TsuPr1. The host-vector systems of the invention are useful for the production of a PROSTAPIN protein or fragment thereof. Such host-vector systems may be employed to study the functional properties of PROSTAPIN and PROSTAPIN mutations.

35

Proteins encoded by the PROSTAPIN gene, or by fragments thereof, will have a variety of uses, including but not limited to generating antibodies, as therapeutic agents, and in methods for identifying ligands and other agents and cellular constituents that bind to a PROSTAPIN gene product. Antibodies raised against PROSTAPIN proteins or fragments

thereof may be useful in diagnostic and prognostic assays, imaging methodologies, and therapeutic methods in the management of prostate cancer. Various immunological assays useful for the detection of PROSTAPIN proteins are contemplated, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), immunocytochemical methods, and the like. Such antibodies may be labeled and used as immunological imaging reagents capable of detecting prostate cells (e.g., in radioscintigraphic imaging methods).

10 PROSTAPIN PROTEINS

Another aspect of the present invention provides various PROSTAPIN proteins and polypeptide fragments thereof. As used herein, PROSTAPIN refers to a protein that has or includes the amino acid sequence of human PROSTAPIN as provided in FIG. 1 (SEQ ID NO. XX), the amino acid sequence of other mammalian PROSTAPIN homologues, as well as allelic variants and conservative substitution mutants of these proteins that have PROSTAPIN activity. The PROSTAPIN proteins of the invention include those specifically identified herein, as well as allelic variants, conservative substitution variants and homologs that can be isolated/generated and characterized without undue experimentation following the methods outlined below. Such PROSTAPIN proteins will be collectively referred to as the PROSTAPIN proteins, the proteins of the invention, or PROSTAPIN. As used herein, the term "PROSTAPIN polypeptide" refers to a polypeptide fragment or a PROSTAPIN protein of at least 10 amino acids, preferably at least 15 amino acids, and more preferably at least 20 amino acids.

25 A specific embodiment of a PROSTAPIN protein comprises a polypeptide having the amino acid sequence shown in FIG. 1 (SEQ ID NO. XX). As used herein, the term "wild-type PROSTAPIN" is meant to refer to a protein having the amino acid sequence depicted in FIG. 1 (SEQ ID NO. XX). In general, for example, naturally occurring allelic variants of human PROSTAPIN will share significant homology (e.g., 70 - 90%) to the PROSTAPIN amino acid sequence provided in FIG. 1. Typically, allelic variants of the PROSTAPIN protein will contain conservative amino acid substitutions from the PROSTAPIN sequence herein described or will contain a substitution of an amino acid from a corresponding position in a PROSTAPIN homologue.

35 One class of PROSTAPIN allelic variants will be proteins that share a high degree of homology with at least a small region of the PROSTAPIN amino acid sequence, but will further contain a radical departure from the sequence, such as a non-conservative substitution, truncation, insertion or frame shift. Such alleles are termed mutant alleles of PROSTAPIN and represent proteins that typically do not perform the same biological

functions. Mutant PROSTAPIN proteins having altered biological function are also included within the scope of the invention. As used herein, the term "functional mutant", when used to modify the term PROSTAPIN, is meant to refer to a PROSTAPIN polypeptide which contains one or more mutations that alter or eliminate PROSTAPIN biological activity, including the LAPC-9 mutant described herein (FIG. 6; SEQ ID NO. XX). Thus, the invention also provides mutant PROSTAPIN proteins and mutant PROSTAPIN polypeptides, such as those corresponding to the amino acid sequences encoded by the LAPC-9 PROSTAPIN mutant as shown in FIG. 6 (SEQ ID NO. XX).

Conservative amino acid substitutions can frequently be made in a protein without altering either the conformation or the function of the protein. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

PROSTAPIN proteins may be embodied in many forms, preferably in isolated form. As used herein, a protein is said to be "isolated" when physical, mechanical or chemical methods are employed to remove the PROSTAPIN protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated PROSTAPIN protein. A purified PROSTAPIN protein molecule will be substantially free of other proteins or molecules which impair the binding of PROSTAPIN to antibody or other ligand. The nature and degree of isolation and purification will depend on the intended use. Embodiments of the PROSTAPIN protein include a purified PROSTAPIN protein and a functional, soluble PROSTAPIN protein. In one form, such functional, soluble PROSTAPIN proteins or fragments thereof retain the ability to bind antibody or other ligand.

The invention also provides PROSTAPIN polypeptides comprising biologically active fragments of the PROSTAPIN amino acid sequence, such as a polypeptide corresponding to part of the amino acid sequence shown in FIG. 1 (SEQ ID NO. XX). Such polypeptides

of the invention exhibit properties of PROSTAPIN, such as the ability to elicit the generation of antibodies which specifically bind an epitope associated with PROSTAPIN.

PROSTAPIN polypeptides can be generated using standard peptide synthesis technology and the amino acid sequences of the human PROSTAPIN protein disclosed herein. Alternatively, recombinant methods can be used to generate nucleic acid molecules that encode a polypeptide fragment of the PROSTAPIN protein. In this regard, the PROSTAPIN-encoding nucleic acid molecules described herein provide means for generating defined fragments of PROSTAPIN. PROSTAPIN polypeptides are particularly useful in generating domain specific antibodies, identifying agents or cellular factors that bind to PROSTAPIN or a PROSTAPIN domain, and in prostate cancer therapeutic strategies which comprise the restoration of PROSTAPIN functionality. PROSTAPIN polypeptides containing particularly interesting structures can be predicted and/or identified using various analytical techniques well known in the art, including, for example, the methods of Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis, or on the basis of immunogenicity. Fragments containing such structures are particularly useful in generating subunit specific anti-PROSTAPIN antibodies or in identifying cellular factors that bind to PROSTAPIN.

PROSTAPIN ANTIBODIES

Another aspect of the invention provides antibodies that bind to PROSTAPIN proteins and polypeptides. The most preferred antibodies will selectively bind to PROSTAPIN and will not bind (or will bind weakly) to non-PROSTAPIN proteins and polypeptides. Anti-PROSTAPIN antibodies that are particularly contemplated include monoclonal and polyclonal antibodies as well as fragments containing the antigen binding domain and/or one or more complement determining regions of these antibodies. As used herein, an antibody fragment is defined as at least a portion of the variable region of the immunoglobulin molecule which binds to its target, i.e., the antigen binding region.

As used herein, a PROSTAPIN antibody is an antibody which (1) was raised against a preparation comprising a PROSTAPIN protein, a PROSTAPIN polypeptide, a mutant PROSTAPIN protein or polypeptide, a fusion protein comprising any of the foregoing, a cell preparation containing PROSTAPIN protein or polypeptide, a cell engineered to express a PROSTAPIN protein or polypeptide, or a similar PROSTAPIN immunogen, and/or (2) binds to a PROSTAPIN and/or mutant PROSTAPIN protein or polypeptide.

PROSTAPIN antibodies of the invention may be particularly useful in prostate cancer diagnostic and prognostic assays, imaging methodologies, and therapeutic strategies. The invention provides various immunological assays useful for the detection and

quantification of PROSTAPIN and mutant PROSTAPIN proteins and polypeptides. Such assays generally comprise one or more PROSTAPIN antibodies capable of recognizing and binding a PROSTAPIN or mutant PROSTAPIN protein, as appropriate, and may be performed within various immunological assay formats well known in the art, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like. In addition, immunological imaging methods capable of detecting prostate cancer are also provided by the invention, including but limited to radioscintigraphic imaging methods using labeled PROSTAPIN antibodies. Such assays may be clinically useful in the detection, monitoring, and prognosis of prostate cancer, particularly advanced prostate cancer.

PROSTAPIN antibodies may also be used in methods for purifying PROSTAPIN and mutant PROSTAPIN proteins and polypeptides and for isolating PROSTAPIN homologues and related molecules. For example, in one embodiment, the method of purifying a PROSTAPIN protein comprises incubating a PROSTAPIN antibody, which has been coupled to a solid matrix, with a lysate or other solution containing PROSTAPIN under conditions which permit the PROSTAPIN antibody to bind to PROSTAPIN; washing the solid matrix to eliminate impurities; and eluting the PROSTAPIN from the coupled antibody. Other uses of the PROSTAPIN antibodies of the invention include generating anti-idiotypic antibodies that mimic the PROSTAPIN protein.

Various methods for the preparation of antibodies are well known in the art. For example, antibodies may be prepared by immunizing a suitable mammalian host using a PROSTAPIN protein, peptide, or fragment, in isolated or immunoconjugated form (Antibodies: A Laboratory Manual, CSH Press, Eds., Harlow, and Lane (1988); Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). In addition, fusion proteins of PROSTAPIN may also be used, such as a PROSTAPIN GST-fusion protein. In a particular embodiment, a GST fusion protein comprising all or most of the open reading frame amino acid sequence of FIG. 1 may be produced and used as an immunogen to generate appropriate antibodies. Cells expressing or overexpressing PROSTAPIN may also be used for immunizations. Similarly, any cell engineered to express PROSTAPIN may be used. This strategy may result in the production of monoclonal antibodies with enhanced capacities for recognizing endogenous PROSTAPIN.

The amino acid sequence of PROSTAPIN as shown in FIG. 1 (SEQ ID NO. XX) may be used to select specific regions of the PROSTAPIN protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the PROSTAPIN amino acid sequence may be used to identify hydrophilic regions in the PROSTAPIN structure. Regions of the PROSTAPIN protein that show immunogenic structure, as well as other

regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. For the generation of antibodies which specifically recognize a mutant PROSTAPIN protein, amino acid sequences unique to the mutant (relative to wild type PROSTAPIN) are preferable. For example, for generating antibodies to the LAPC-9 mutant PROSTAPIN protein, the inserted or unique amino acid sequences shown in FIG. 6 (SEQ ID NO. XX) may be used to select specific regions.

Methods for preparing a protein or polypeptide for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be used; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be effective. Administration of a PROSTAPIN immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

PROSTAPIN monoclonal antibodies are preferred and may be produced by various means well known in the art. For example, immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the PROSTAPIN protein or PROSTAPIN fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either in vitro or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antiserum which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Scfy, or F(ab')₂ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the PROSTAPIN protein can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin. Humanized or human PROSTAPIN antibodies may also be produced and are preferred for use in therapeutic contexts. Various approaches

for producing such humanized antibodies are known, and include chimeric and CDR grafting methods; methods for producing fully human monoclonal antibodies include phage display and transgenic methods (for review, see Vaughan et al., 1998, Nature Biotechnology 16: 535-539).

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Fully human PROSTAPIN monoclonal antibodies may be generated using cloning technologies employing large human Ig gene combinatorial libraries (i.e., phage display)(Griffiths and Hoogenboom, Building an in vitro immune system: human antibodies from phage display libraries. In: Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man. Clark, M. (Ed.), Nottingham Academic, pp 45-64 (1993); Burton and Barbas, Human Antibodies from combinatorial libraries. *Id.*, pp 65-82). Fully human PROSTAPIN monoclonal antibodies may also be produced using transgenic mice engineered to contain human immunoglobulin gene loci as described in PCT Patent Application WO98/24893, Jakobovits et al., published December 3, 1997 (see also, Jakobovits, 1998, Exp. Opin. Invest. Drugs 7(4): 607-614). This method avoids the in vitro manipulation required with phage display technology and efficiently produces high affinity authentic human antibodies.

Reactivity of PROSTAPIN antibodies with PROSTAPIN protein or mutant PROSTAPIN protein, as appropriate, may be established by a number of well known means, including Western blot, immunoprecipitation, ELISA, and FACS analyses using, as appropriate, PROSTAPIN proteins, peptides, PROSTAPIN-expressing cells or extracts thereof.

A PROSTAPIN antibody or a fragment thereof may be labeled with a detectable marker and used for targeting the detectable marker to a PROSTAPIN positive cell (Vitetta, E.S. et al., 1993, Immunotoxin therapy, in DeVita, Jr., V.T. et al., eds, Cancer: Principles and Practice of Oncology, 4th ed., J.B. Lippincott Co., Philadelphia, 2624-2636). Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme.

METHODS FOR THE DETECTION OF PROSTAPIN

Another aspect of the present invention relates to methods for detecting PROSTAPIN polynucleotides and PROSTAPIN proteins, as well as methods for identifying a cell which expresses PROSTAPIN.

More particularly, the invention provides assays for the detection of PROSTAPIN polynucleotides in a biological sample, such as serum, bone, prostate, and other tissues, urine, cell preparations, and the like. Detectable PROSTAPIN polynucleotides include, for

example, a PROSTAPIN gene or fragments thereof, PROSTAPIN mRNA, alternative splice variant PROSTAPIN mRNAs, and recombinant DNA or RNA molecules containing a PROSTAPIN polynucleotide. A number of methods for amplifying and/or detecting the presence of PROSTAPIN polynucleotides are well known in the art and may be employed in
5 the practice of this aspect of the invention.

In one embodiment, a method for detecting PROSTAPIN mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using PROSTAPIN polynucleotides as sense
10 and antisense primers to amplify PROSTAPIN cDNAs therein; and detecting the presence of the amplified PROSTAPIN cDNA. In another embodiment, a method of detecting the PROSTAPIN gene in a biological sample comprises first isolating genomic DNA from the sample; amplifying the isolated genomic DNA using PROSTAPIN polynucleotides as sense and antisense primers to amplify the PROSTAPIN gene therein; and detecting the
15 presence of the amplified PROSTAPIN gene. Any number of appropriate sense and antisense probe combinations may be designed from the nucleotide sequence provided in FIG. 1 (SEQ ID NO. XX) and used for this purpose, as will be understood by those skilled in the art.

20 The invention also provides assays for detecting the presence of a PROSTAPIN protein in a tissue of other biological sample such as serum, bone, prostate, and other tissues, urine, cell preparations, and the like. Methods for detecting a PROSTAPIN protein are also well known and include, for example, immunoprecipitation, immunohistochemical analysis, Western Blot analysis, molecular binding assays, ELISA, ELIFA and the like.

25 For example, in one embodiment, a method of detecting the presence of a PROSTAPIN protein in a biological sample comprises first contacting the sample with a PROSTAPIN antibody, a PROSTAPIN-reactive fragment thereof, or a recombinant protein containing an antigen binding region of a PROSTAPIN antibody; and then detecting the binding of
30 PROSTAPIN protein in the sample thereto.

Methods for identifying a cell which expresses PROSTAPIN are also provided. In one embodiment, an assay for identifying a cell which expresses a PROSTAPIN gene comprises detecting the presence of PROSTAPIN mRNA in the cell. Methods for the detection of
35 particular mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as in situ hybridization using labeled PROSTAPIN riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for PROSTAPIN, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA

and the like). Alternatively, an assay for identifying a cell which expresses a PROSTAPIN gene comprises detecting the presence of PROSTAPIN protein in the cell or secreted by the cell. Various methods for the detection of proteins are well known in the art and may be employed for the detection of PROSTAPIN proteins and PROSTAPIN expressing cells.

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PROSTAPIN expression analysis may also be useful as a tool for identifying and evaluating agents which modulate PROSTAPIN gene expression. PROSTAPIN expression is significantly reduced in prostate cancer samples, prostate cancer xenografts and cell lines. The mechanism of inactivation is unclear, since southern blotting of DNA derived from the xenografts (LAPC-4 AD, 4AI, 9AD), prostate cancer cell lines (PC-3, DU145, LNCaP) and normal human DNA show no remarkable differences in intensity or banding pattern. Similar observations were made for the tumor suppressor gene PTEN/MMAC1, which encodes a dual-specificity phosphatase (Whang et al., 1998, PNAS 95: 5246). PTEN/MMAC1 mRNA expression was restored in nonexpressing prostate cancer cells by in vitro treatment with the demethylating agent 5-azadeoxycytidine (Whang et al., 1998, PNAS 95: 5246). This suggests that methylation was responsible for silencing of the PTEN/MMAC1 gene. A similar mechanism of transcriptional inactivation may explain loss of PROSTAPIN expression in some of the prostate cancer specimens. Identification of a molecule or biological agent that could reactivate PROSTAPIN expression may be of therapeutic value in the treatment of prostate cancer. Such an agent may be identified by using a screen that allows for recognizing the acquisition of PROSTAPIN expression by RT-PCR, nucleic acid hybridization or antibody binding.

As will be appreciated, the foregoing methods may be applied to the detection of mutant PROSTAPIN polynucleotides and proteins using, as appropriate, probes, primers, antibodies and other binding agents capable of detecting such mutant forms.

ASSAYS FOR DETERMINING PROSTAPIN EXPRESSION STATUS

PROSTAPIN gene expression appears to be lost or greatly attenuated in advanced prostate cancers. Thus, determining the status of PROSTAPIN expression in an individual may be used to diagnose advanced stage prostate cancer as well as provide prognostic information useful in defining appropriate therapeutic options. Similarly, the expression status of PROSTAPIN may provide information useful for predicting susceptibility to advanced stage disease, rate of progression, and/or tumor aggressiveness. The invention provides methods and assays for determining PROSTAPIN expression status, diagnosing advanced prostate cancer, and predicting susceptibility to advanced prostate cancer. PROSTAPIN expression status is meant to include quantitative and/or qualitative aspects, i.e., the level of wild type PROSTAPIN expression as well as the presence of functional PROSTAPIN mutations.

In one aspect, the invention provides assays useful in determining the presence of advanced stage prostate cancer in an individual. Presently, advanced stage prostate cancer is commonly diagnosed by pathological examination of prostate and surrounding tissues surgically removed during radical prostatectomy. Unfortunately, in most cases of advanced stage prostate cancer, surgery is not desirable, but is generally performed because there is no method of reliably distinguishing between advanced and localized prostate cancer other than pathological examination of surgically removed tissues. In other words, for most patients who learn that they have advanced prostate cancer, the undesirable surgical option has already been performed. The invention provides a means of distinguishing between advanced prostate cancer and locally confined prostate cancer by assaying for PROSTAPIN expression. The means comprises detecting a marked loss or absence of wild type PROSTAPIN expression in prostate tumor tissues and cells relative to expression levels in normal prostate tissue and cells. As demonstrated in the Examples which follow, PROSTAPIN mRNA is expressed at easily detectable levels in all normal prostate tissues and all locally confined prostate cancer tissues tested. In contrast, wild type PROSTAPIN expression is either completely undetectable or greatly attenuated in all advanced stage prostate tumor specimens, cell lines derived from prostate cancer metastases, and SCID mouse xenografts derived from human prostate cancer metastases.

In one embodiment, a method or assay for identifying the presence of advanced prostate cancer comprises determining the level of PROSTAPIN mRNA expressed by cells in a test sample, preferably a prostate, prostate tumor, lymph, bone or peripheral blood sample; and comparing the level so determined to the level of PROSTAPIN expressed in normal prostate, preferably a comparable known normal prostate tissue sample. The absence or substantial attenuation of PROSTAPIN mRNA expression in the test sample relative to normal prostate indicates the presence of advanced prostate cancer. Attenuation of PROSTAPIN mRNA expression is "substantial" when expression is reduced by at least about 10%, and preferably by about 30-50% or more, relative to PROSTAPIN mRNA expression levels detectable in normal prostate.

In a related embodiment, PROSTAPIN expression status may be determined at the protein level rather than at the nucleic acid level. For example, such a method or assay would comprise determining the level of PROSTAPIN protein expressed by cells in a test sample, preferably a prostate, prostate tumor, lymph, bone or peripheral blood sample; and comparing the level so determined to the level of PROSTAPIN expressed in normal prostate, preferably a comparable known normal prostate tissue sample. The absence or substantial attenuation of PROSTAPIN protein expression in the test sample relative to

normal prostate indicates the presence of advanced prostate cancer. PROSTAPIN antibodies or binding partners capable of detecting PROSTAPIN protein expression may be used in a variety of assay formats well known in the art for this purpose.

- 5 A specific, preferred embodiment comprises determining the expression status of a patient's PROSTAPIN mRNA or PROTEIN in the cells of a known prostate tumor sample and comparing the level of PROSTAPIN expression so determined to the level expressed by normal prostate cells, the presence of comparable expression levels being indicative of a locally confined or less advanced stage. In this regard, prostate tumor cells may be
10 "known" by virtue of their origin, e.g., biopsied from a tumor mass, or by the presence of one or more molecular markers of prostate cancer cells. A number of such molecular markers are known, including for example PSCA and PSMA.

Assaying the expression status of a prostate cancer marker and PROSTAPIN in the same
15 tissue sample, preferably simultaneously, may be particularly useful where tumor origin of the sample cannot be assured. In such cases, the presence of a known prostate cancer molecular marker in the sample can be used to identify the sample as prostate cancer, while the level of PROSTAPIN expressed in the same sample may be used as a tool for determining the presence of advanced prostate cancer (as well as susceptibility to
20 advanced prostate cancer and tumor aggressiveness). In a specific embodiment, expression of PSCA and PROSTAPIN in a sample tissue are assayed together. PSCA is widely over-expressed across all stages and grades of prostate cancer. Thus the presence of PSCA over-expression relative to expression levels in normal prostate may be used to reliably identify samples which comprise prostate cancer cells.

25 Depending on the method of detection used, a single sample may be heterogeneous for the expression of the known tumor marker. For example, a sample may be shown to contain some regions of cells expressing (or over-expressing, as appropriate) the marker while other regions do not express the marker (or expressing normal levels of the marker). In
30 such cases, it may be most appropriate to use the level of PROSTAPIN expression in the regions showing expression or over-expression of the marker in order to reliably determine that patient's prostate cancer stage. In other cases, the tissue sampled is inherently heterogeneous for a number of cell types, such as, for example, blood. Here, the presence of the known prostate cancer marker may be used to identify and/or isolate the prostate
35 cancer cells from other cells present in the sample. The PROSTAPIN expression status in the known prostate cancer marker positive cells should be used for staging purposes. This type of combined analysis may be used not only for determining locally confined cancers, but also for determining advanced stage cancers, aggressiveness and susceptibility to

advanced stage cancer, by assaying PROSTAPIN expression as described above together with a known prostate cancer marker.

5 Peripheral blood may be conveniently assayed by the combined analysis described above using RT-PCR to detect and quantify the expression of PROSTAPIN and known prostate tumor marker mRNAs. RT-PCR amplification of a known tumor marker mRNA combined with the absence or attenuation of RT-PCR amplifiable PROSTAPIN mRNA (relative to normal prostate expression levels) provides an indication of the presence of advanced prostate cancer and may provide information concerning the aggressiveness of the
10 originating tumor. RT-PCR detection assays for tumor cells in peripheral blood are currently being evaluated for use in the diagnosis and management of a number of human solid tumors. In the prostate cancer field, these include RT-PCR assays for the detection of cells expressing PSA and PSM (Verkaik et al., 1997, Urol. Res. 25: 373-384; Ghossein et al., 1995, J. Clin. Oncol. 13: 1195-2000; Heston et al., 1995, Clin. Chem. 41: 1687-1688). RT-PCR assays are well known in the art. Semi-quantitative RT-PCR assays for PROSTAPIN
15 expression are described in greater detail by way of the examples which follow. Such assays may also be employed for the detection (and quantitation) of a known prostate tumor marker.

20 A related aspect of the invention is directed to predicting susceptibility to developing advanced prostate cancer in an individual. In one embodiment, a method for predicting susceptibility to advanced prostate cancer comprises determining the level of PROSTAPIN mRNA or PROSTAPIN protein expressed by cells in a first prostate or prostate tumor sample, comparing the level so determined to the level of PROSTAPIN mRNA or
25 PROSTAPIN protein expressed in a second normal prostate tissue, the absence or substantial attenuation of PROSTAPIN mRNA or PROSTAPIN protein expression in the first sample relative to the second sample indicating susceptibility to advanced prostate cancer, wherein the degree of attenuated PROSTAPIN expression relative to normal prostate is proportional to the degree of susceptibility to advanced prostate cancer.

30 Yet another related aspect of the invention is directed to methods for gauging prostate tumor aggressiveness. In one embodiment, a method for gauging aggressiveness of a prostate tumor comprises determining the level of PROSTAPIN mRNA or PROSTAPIN protein expressed by cells in a sample of the prostate tumor, comparing the level so
35 determined to the level of PROSTAPIN mRNA or PROSTAPIN protein expressed in a normal prostate tissue taken from the same individual or a normal prostate tissue reference sample, wherein the degree of PROSTAPIN mRNA or PROSTAPIN protein expression loss in the prostate tumor sample relative to the normal prostate sample proportionally indicating degree of aggressiveness.

Methods for detecting and quantifying the expression of PROSTAPIN mRNA or protein are described herein and use standard nucleic acid and protein detection and quantification technologies well known in the art. Standard methods for the detection and quantification of PROSTAPIN mRNA include in situ hybridization using labeled PROSTAPIN riboprobes, Northern blot and related techniques using PROSTAPIN polynucleotide probes, RT-PCR analysis using primers specific for PROSTAPIN, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like. In a specific embodiment, semi-quantitative RT-PCR may be used to detect and quantify PROSTAPIN mRNA expression as described in the Examples which follow. Any number of primers capable of amplifying PROSTAPIN may be used for this purpose, including but not limited to the various primer sets specifically described herein. Standard methods for the detection and quantification of protein may be used for this purpose. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type PROSTAPIN protein may be used in an immunohistochemical assay of biopsied tissue.

Since loss of PROSTAPIN expression appears to correlate with advanced disease stage, the expression of normal levels of PROSTAPIN by prostate cancer cells may also be useful in identifying locally confined prostate cancer. The invention provides methods and assays for identifying locally confined prostate cancer comprising determining the expression status of a patient's PROSTAPIN mRNA or PROTEIN in the cells of a known prostate tumor sample and comparing the level of PROSTAPIN expression so determined to the level expressed by normal prostate cells, the presence of comparable expression levels being indicative of a locally confined or less advanced stage.

In addition to the methods and assays described above, wherein the expression levels of PROSTAPIN are determined and evaluated, the invention also provides methods and assays capable of detecting functional mutations of the PROSTAPIN gene. Similar to the loss or attenuation of PROSTAPIN expression, the presence of a functional PROSTAPIN mutation also correlates with advanced prostate cancer and may be used to distinguish advanced from locally confined prostate cancers, predict aggressiveness, and determine susceptibility to advanced prostate cancer. The general molecular diagnostic methods described above may be used for this purpose, provided that the means used to detect expression are capable of specifically identifying a PROSTAPIN mutation expected to result in a loss of PROSTAPIN function. In this regard, for detection of PROSTAPIN mutant mRNAs, molecular probes or primers specifically designed to hybridize to or amplify the mutant PROSTAPIN, but not wild-type PROSTAPIN are used. In a specific embodiment, a hybridization probe comprising the nucleotide sequence of the LAPC-9 PROSTAPIN mutant as shown in FIG. 6 (SEQ ID NO. XX) may be used. Alternatively, a probe comprising a

fragment of the sequence shown in FIG. 6 (SEQ ID NO. XX) which contains enough of the mutant sequence to render it capable of specifically hybridizing to mutant but not wild type PROSTAPIN may be used. In another embodiment, primers designed to PCR amplify polynucleotides containing the sequences specific to the LAPC-9 mutant PROSTAPIN sequence shown in FIG. 6 (SEQ ID NO. XX), may be used to detect the expression of a functional PROSTAPIN mutant. In another embodiment, primers designed to amplify polynucleotides corresponding to either wild type or mutant PROSTAPIN may be used to amplify PROSTAPIN sequences which may then be sequenced and analyzed for the presence of mutations. Functional PROSTAPIN mutants may also be identified at the genomic level, by direct sequencing or by SSCP analysis of genomic DNA to identify PROSTAPIN mutations or polymorphisms that correlate with prostate cancer. Mutant or polymorphic exons can be sequenced and compared to wild type PROSTAPIN using standard technologies. In one embodiment, the primer pairs described in Example 8 may be used to sequence particular PROSTAPIN exons.

THERAPEUTIC APPLICATIONS OF PROSTAPIN

Loss of wild type PROSTAPIN expression or the expression of functionally mutant PROSTAPIN correlates with advanced and metastatic prostate cancer. Structurally, PROSTAPIN is a member of a family of proteins which contain both tumor suppressors (e.g., maspin) and proteins involved in apoptosis (e.g., LEI). Accordingly, the PROSTAPIN protein may function as a prostate-specific tumor suppressor, apoptosis-inducer or apoptosis-modulator, or may have another biological activity involved in modulating prostate cancer progression. Therapeutic strategies which restore functional PROSTAPIN to prostate tumor cells may result in inhibition of primary prostate tumors and prostate cancer metastasis, tumor regression, and/or an inhibition in the rate or extent of disease progression.

Various strategies for restoring normal PROSTAPIN function in vivo are available, including protein therapy and gene therapy methods. For gene therapy, a vector comprising a polynucleotide encoding wild type PROSTAPIN or a peptide mimetic with PROSTAPIN biological activity may be administered to the prostate cancer patient such that the vector makes contact with the prostate tumor cells. Preferably, the vector will be capable of integrating the PROSTAPIN gene into the patient's tumor cells (e.g., retroviral vectors) and/or is capable of highly efficient in vivo transduction (e.g., adenoviral vectors). The vector may be delivered via any route which results in the vector making contact with the tumor cells. A preferred route of administration is by intraprostatic injection. Multiple injections may be required to account for clearance of the initial dose and achieve more uniform distribution of the vector to the tumor. Alternatively, compositions comprising the wild type PROSTAPIN protein or a peptide mimetic or a small molecule mimetic may be

administered to a patient such that the composition makes contact with the tumor cells. In addition, methods capable of inducing transcription of functional PROSTAPIN in vivo may be employed.

- 5 Preferably, functional PROSTAPIN restoration is accomplished via gene transfer methods, such as those further described below. For example, if PROSTAPIN functions as an apoptosis-inducing gene, gene therapy transfer of PROSTAPIN into prostate tumor cells may be used to trigger apoptosis of the tumor cells. If PROSTAPIN functions as a prostate-specific tumor suppressor gene, in vivo PROSTAPIN gene restoration therapy may be
10 useful to slow or reverse prostate cancer cell growth.

- A PROSTAPIN polynucleotide encoding wild type PROSTAPIN may be operably linked to a promoter capable of driving the expression of functional PROSTAPIN within the cells of the target tumor and utilized for gene therapy. Preferably, expression of the PROSTAPIN
15 gene will be regulated by a prostate-specific promoter is utilized. An example of a preferred promoter is the PSA promoter.

- Various gene therapy vectors may be used to deliver the PROSTAPIN gene into the cells of the target tissue (e.g., prostate, prostate tumors, prostate metastasis), wherein
20 PROSTAPIN protein is expressed and exerts PROSTAPIN functionality. There are a great many viral vectors well known in the gene therapy field which may be utilized, including but not limited to adenoviral, retroviral, and vaccinia vectors. See, for example, Jolly, D. Cancer Gene Therapy, vol. 1, pages 51-64 (1994).

- 25 Preferred viral vectors include adenovirus, more preferably in non-replicating or replication defective forms. For example, replication defective adenovirus vectors in which the E1A and E1B regions of the adenovirus genome have been deleted may be used. Adenovirus type 5 of subgroup C is most preferred for generating replication-defective adenovirus vectors for PROSTAPIN gene therapy, although adenoviruses of
30 any of the 42 different serotypes or subgroups A-F may be employed.

- As is generally known, various cell lines may be used to propagate recombinant adenoviruses, so long as they complement any replication defect which may be present. A preferred cell line is the human 293 cell line, although other replication permissive cell
35 lines may be employed as appropriate. Other complementary combinations of viruses and host cells may be employed in connection with the present invention; for example adenovirus lacking functional E2 in combination with E2-expressing cells, adenovirus lacking functional E4 in combination with E4-expressing cells, and the like.

For additional information concerning construction, propagation, purification, and use of adenoviruses, see, for example, Horwitz, M. S. Adenoviridae and their Replication, In: Fields, B. N. and Knipe, D. M., eds., Fundamental Virology, 2nd ed. New York, N.Y., Raven Press, Ltd., pages 771-813 (1991); and Howley, P. M. Papillomavirinae and their
5 Replication, In: Fields, B. N. and Knipe, D. M., eds., Fundamental Virology, 2nd ed. New York, N.Y., Raven Press, Ltd., pages 743-767 (1991).

In one embodiment, the PROSTAPIN clone 103 cDNA (SEQ ID NO. XX) is inserted into a replication defective adenovirus in which the E1A and E1B regions have been deleted.
10 Recombinant adenovirus containing the PROSTAPIN cDNA is then propagated in 293 cells and purified according to standard methods. Purified recombinant adenovirus may then be delivered to the target tissue via an appropriate route which will result in delivery of the recombinant adenovirus to the cells of the target tissue. Where the target tissue is the prostate or a locally confined primary prostate tumor, recombinant adenovirus may
15 be injected intraprostatically, preferably in multiple doses. Where the tissue target is one or more tumors in an individual with advanced prostate cancer, a more systemic route of administration, either alone or in combination with a direct delivery method (e.g., intraprostatic injection), may be used. For example, recombinant adenovirus may be injected directly into the lymph and/or vascular system in order to target tumors within
20 lymphatic system or bone marrow as appropriate.

In another embodiment, a polynucleotide encoding a PROSTAPIN protein in which the RSL site is deleted may be used to construct an adenovirus. The resulting recombinant adenovirus may be used to study PROSTAPIN function and, specifically, the function of
25 the RSL, by comparing the activities of the RSL-deleted PROSTAPIN and wild type PROSTAPIN proteins expressed in prostate cancer and other cell lines or in appropriate animal models. As an example, adenoviruses encoding wild type PROSTAPIN and RSL-deleted PROSTAPIN may be used to compare the effects of the encoded proteins on tumor cell growth by expressing these two forms of PROSTAPIN in prostate cancer
30 xenograft models. Examples of prostate cancer cells into which these forms of PROSTAPIN may be introduced by the recombinant adenoviruses include LAPC-4, LAPC-9, LnCap, PC-3. Xenograft tumors may be conveniently generated by subcutaneous, orthotopic or intraosseous injection of the vector-transduced cells into SCID or other immune deficient mice.

35 Examples of retroviral vectors in which the PROSTAPIN gene may be inserted include, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia

virus (GaLV). In addition, a number of other retroviral vectors capable of incorporating multiple genes, including selectable markers and target-specific factors, may be employed. Retroviral vectors may be engineered to include a polynucleotide encoding a protein which is specifically reactive with prostate cancer cells, such as, for example, polynucleotides encoding prostate cancer cell specific antibodies or fragments thereof.

In addition to viral vectors, PROSTAPIN polynucleotides may be delivered to target tumor and surrounding tissue via liposomes. For example, liposomes comprised of DOTMA, such as the Lipofectin™ products available from Vical, Inc. (San Diego, CA) may be used. A variety of transfection techniques are known and may be used. For delivering liposomes containing PROSTAPIN polynucleotides, injection into the site of the target tumor or systemic injection methods may be used. Where, for example, the target tumor is a primary prostate tumor, direct injection into the prostate is preferred. As another example, where the target comprises lymph and/or bone metastases, injection into the lymphatic system and/or arterial system, respectively, may be preferred. Liposomes may be enhanced to increase their tissue specificity by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization. In one embodiment, the liposome may be coupled to a monoclonal antibody which recognizes a cell surface prostate tumor antigen, such as PSCA. Methods for covalently attaching antibodies or fragments thereof to a liposome bilayer are known.

IDENTIFICATION OF PROSTAPIN TARGET PROTEASE

The target of PROSTAPIN is likely to be a protease that plays a functional role in prostate cancer metastasis. The PROSTAPIN gene and/or protein may be used as tools to identify this protease. One method involves screening a yeast two-hybrid cDNA library with the prostapin gene as a bait, or by screening a cDNA expression library using prostapin protein as a probe. Alternatively, prostapin protein may be used to study the biochemical interaction with a panel of known proteases, such as: Prostate Specific Antigen, human Kallikrein 2, urokinase type plasminogen activator, tissue plasminogen activator, plasmin, granzyme B, thrombin, cathepsins B, L and D, and human neutrophil elastase.

KITS

For use in the diagnostic and therapeutic applications described or suggested above, kits are also provided by the invention. Such kits may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may

comprise a probe which is or can be detectably labeled. Such probe may be an antibody or polynucleotide specific for a PROSTAPIN protein or a PROSTAPIN gene or message, respectively. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radionucleotide label.

10 **EXAMPLES**

Various aspects of the invention are further described and illustrated by way of the several examples which follow, none of which are intended to limit the scope of the invention.

15 **EXAMPLE 1:**

ISOLATION OF PROSTAPIN cDNA FRAGMENT AND EXPRESSION ANALYSIS

20 **MATERIALS AND METHODS**

LAPC Xenografts:

LAPC xenografts were obtained from Dr. Charles Sawyers (UCLA) and generated as described (Klein et al, 1997, Nature Med. 3: 402-408). Androgen dependent and independent LAPC-4 xenografts (LAPC-4 AD and AI, respectively) and LAPC-9 AD xenografts were grown in male SCID mice and were passaged as small tissue chunks in recipient males. LAPC-4 AI xenografts were derived from LAPC-4 AD tumors. Male mice bearing LAPC-4 AD tumors were castrated and maintained for 2-3 months. After the LAPC-4 tumors re-grew, the tumors were harvested and passaged in castrated males or in female SCID mice.

30 **Cell Lines:**

The human cell lines HeLa (cervical carcinoma), 293 (embryonic kidney), A431 (epidermoid carcinoma), Colo205 (colon carcinoma), KCL22 (lymphoid blast crisis of chronic myelogenous leukemia), LnCaP (prostate cancer), DU145 (prostate cancer) and PC-3 (prostate cancer) were obtained from the ATCC. The LAPC-4 cell line, derived from the LAPC-4 AD xenograft, was generated as described (Klein et al., 1997, supra) and obtained from Dr. Robert Reiter (UCLA). All cell lines were maintained in DMEM with 5% fetal calf serum.

RNA Isolation:

Tumor tissue and cell lines were homogenized in Trizol reagent (Life Technologies, Gibco BRL) using 10 ml/ g tissue or 10 ml/ 10⁸ cells to isolate total RNA. Poly A RNA was purified from total RNA using Qiagen's Oligotex mRNA Mini and Midi kits. Total and mRNA were
 5 quantified by spectrophotometric analysis (O.D. 260/280 nm) and analyzed by gel electrophoresis.

Oligonucleotides:

The following HPLC purified oligonucleotides were used.

10

RSACDN (cDNA synthesis primer):

5'TTTTGTACAAGCTT₃₀3'

Adaptor 1:

15

5'CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT3'
 3'GGCCCGTCCA5'

Adaptor 2:

5'GTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAGGT3'
 3'CGGCTCCA5'

20

PCR primer 1:

5'CTAATACGACTCACTATAGGGC3'

Nested primer (NP)1:

5'TCGAGCGGCCGCCCGGGCAGGT3'

25

Nested primer (NP)2:

5'AGCGTGGTCGCGGCCGAGGT3'

Suppression Subtractive Hybridization:

30

Suppression Subtractive Hybridization (SSH) was used to identify cDNAs corresponding to genes which may be down-regulated in androgen independent prostate cancer compared to androgen dependent prostate cancer.

35

Double stranded cDNAs corresponding to the LAPC-4 AD xenograft (tester) and the LAPC-4 AI xenograft (driver) were synthesized from 2 µg of poly(A)⁺ RNA isolated from xenograft tissue, as described above, using CLONTECH's PCR-Select cDNA Subtraction Kit and 1 ng of oligonucleotide RSACDN as primer. First- and second-strand synthesis were carried out as described in the Kit's user manual protocol (CLONTECH Protocol No. PT1117-1, Catalog No. K1804-1). The resulting cDNA was digested with Rsa I for 3 hrs. at

37°C. Digested cDNA was extracted with phenol/chloroform (1:1) and ethanol precipitated.

Driver cDNA (LAPC-4 AI) was generated by combining in a 1:1 ratio Rsa I digested LAPC-4 AI cDNA with a mix of digested cDNAs derived from human benign prostatic hyperplasia (BPH), the human cell lines HeLA, 293, A431, Colo205, and mouse liver.

Tester cDNA (LAPC-4 AD) was generated by diluting 1 µl of Rsa I digested LAPC-4 AD cDNA (400 ng) in 5 µl of water. The diluted cDNA (2 µl, 160 ng) was then ligated to 2 µl of adaptor 1 and adaptor 2 (10 µM), in separate ligation reactions, in a total volume of 10 µl at 16°C overnight, using 400 u of T4 DNA ligase (CLONTECH). Ligation was terminated with 1 µl of 0.2 M EDTA and heating at 72°C for 5 min.

The first hybridization was performed by adding 1.5 µl (600 ng) of driver cDNA to each of two tubes containing 1.5 µl (20 ng) adaptor 1- and adaptor 2- ligated tester cDNA. In a final volume of 4 µl, the samples were overlaid with mineral oil, denatured in an MJ Research thermal cycler at 98°C for 1.5 minutes, and then were allowed to hybridize for 8 hrs at 68°C. The two hybridizations were then mixed together with an additional 1 µl of fresh denatured driver cDNA and were allowed to hybridize overnight at 68°C. The second hybridization was then diluted in 200 µl of 20 mM Hepes, pH 8.3, 50 mM NaCl, 0.2 mM EDTA, heated at 70°C for 7 min. and stored at -20°C.

PCR Amplification, Cloning and Sequencing of Gene Fragments Generated from SSH:

To amplify gene fragments resulting from SSH reactions, two PCR amplifications were performed. In the primary PCR reaction 1 µl of the diluted final hybridization mix was added to 1 µl of PCR primer 1 (10 µM), 0.5 µl dNTP mix (10 µM), 2.5 µl 10 x reaction buffer (CLONTECH) and 0.5 µl 50 x Advantage cDNA polymerase Mix (CLONTECH) in a final volume of 25 µl. PCR 1 was conducted using the following conditions: 75°C for 5 min., 94°C for 25 sec., then 27 cycles of 94°C for 10 sec, 66°C for 30 sec, 72°C for 1.5 min. Five separate primary PCR reactions were performed for each experiment. The products were pooled and diluted 1:10 with water. For the secondary PCR reaction, 1 µl from the pooled and diluted primary PCR reaction was added to the same reaction mix as used for PCR 1, except that primers NP1 and NP2 (10 µM) were used instead of PCR primer 1. PCR 2 was performed using 10-12 cycles of 94°C for 10 sec, 68°C for 30 sec, 72°C for 1.5 minutes. The PCR products were analyzed using 2% agarose gel electrophoresis.

The PCR products were inserted into pCR2.1 using the T/A vector cloning kit (Invitrogen). Transformed E. coli were subjected to blue/white and ampicillin selection. White colonies

were picked and arrayed into 96 well plates and were grown in liquid culture overnight. To identify inserts, PCR amplification was performed on 1 ml of bacterial culture using the conditions of PCR1 and NP1 and NP2 as primers. PCR products were analyzed using 2% agarose gel electrophoresis.

5

Bacterial clones were stored in 20% glycerol in a 96 well format. Plasmid DNA was prepared, sequenced, and subjected to nucleic acid homology searches of the GenBank, dBest, and NCI-CGAP databases.

10 **RT-PCR Expression Analysis:**

First strand cDNAs were generated from 1 µg of mRNA with oligo (dT)12-18 priming using the Gibco-BRL Superscript Preamplification system. The manufacturers protocol was used and included an incubation for 50 min at 42°C with reverse transcriptase followed by RNase H treatment at 37°C for 20 min. After completing the reaction, the volume was increased to 200 µl with water prior to normalization. First strand cDNAs from 16 different normal human tissues were obtained from Clontech.

20 Normalization of the first strand cDNAs from multiple tissues was performed by using the primers 5'atatcgccgcgctcgtcgtcgacaa3' and 5'agccacacgcagctcattgtagaagg 3' to amplify β-actin. First strand cDNA (5 µl) was amplified in a total volume of 50 µl containing 0.4 µM primers, 0.2 µM each dNTPs, 1XPCR buffer (Clontech, 10 mM Tris-HCL, 1.5 mM MgCl₂, 50 mM KCl, pH8.3) and 1X Klentaq DNA polymerase (Clontech). Five µl of the PCR reaction was removed at 18, 20, and 22 cycles and used for agarose gel electrophoresis. PCR was performed using an MJ Research thermal cycler under the following conditions: initial denaturation was at 94°C for 15 sec, followed by a 18, 20, and 22 cycles of 94°C for 15, 65°C for 2 min, 72°C for 5 sec. A final extension at 72°C was carried out for 2 min. After agarose gel electrophoresis, the band intensities of the 283 bp β-actin bands from multiple tissues were compared by visual inspection. Dilution factors for the first strand cDNAs were calculated to result in equal β-actin band intensities in all tissues after 22 cycles of PCR. Three rounds of normalization were required to achieve equal band intensities in all tissues after 22 cycles of PCR.

35 To determine expression levels of the 11P2A6 gene, 5 µl of normalized first strand cDNA was analyzed by PCR using 25, 30, and 35 cycles of amplification using the following primer pairs, which were designed with the assistance of (MIT; for details, see, www.genome.wi.mit.edu):

5'- GAG TCT GGC TGG TTG ATT TGA GAG -3' (SEQ ID NO. XX)
5'- CCA GTC TAA CTT GCC ACT CTG TGA -3' (SEQ ID NO. XX)

Semi quantitative expression analysis was achieved by comparing the PCR products at cycle numbers that give light band intensities.

5

RESULTS:

Several SSH experiments were conducted as described in the Materials and Methods, supra, and led to the isolation of numerous candidate gene fragment clones. All candidate clones were sequenced and subjected to homology analysis against all sequences in the major public gene and EST databases in order to provide information on the identity of the corresponding gene and to help guide the decision to analyze a particular gene for differential expression. In general, gene fragments which had no homology to any known sequence in any of the searched databases, and thus considered to represent novel genes, as well as gene fragments showing homology to previously sequenced expressed sequence tags (ESTs), were subjected to differential expression analysis by RT-PCR and/or Northern analysis.

One of the gene fragment cDNA clones showing no homology to any known gene or EST sequence was designated 11P2A6. The isolated 11P2A6 cDNA (SEQ ID NO. XX) was 471 bp in length and has the nucleotide sequence of nucleotide residues 1 through 471 in the PROSTAPIN cDNA sequence shown in FIG. 1 (SEQ ID NO. XX).

Differential expression analysis by RT-PCR showed that the 11P2A6 (PROSTAPIN) gene is expressed at approximately equal levels in the LAPC-9 AD xenograft and in normal prostate tissue, but at greatly reduced levels in the LAPC-4 AI xenograft and at undetectable levels in the LAPC-4 AD xenograft (FIG. 3, panel A). RT-PCR expression analysis of first strand cDNAs from 16 normal tissues detected expression of the 11P2A6 (PROSTAPIN) gene only in prostate tissue after 30 cycles of PCR amplification, while lower level expression was detected in lung and placenta after 35 cycles (FIG. 3, panels B and C).

30

EXAMPLE 2:

ISOLATION AND STRUCTURAL ANALYSIS OF FULL LENGTH cDNA ENCODING HUMAN PROSTAPIN

35

The full length cDNA encoding the gene corresponding to the 11P2A6 clone (Example 1, above) was isolated as follows. A normal human prostate cDNA library (Clontech) was screened with a probe comprising the 11P2A6 cDNA (SEQ ID NO. XX). Several positive clones were identified, and the largest of these, clone 103, was sequenced. Clone 103

(SEQ ID NO XX) contains an open reading frame encoding a 379 amino acid protein (see FIG. 1). Amino acid homology analysis of the clone 103 sequence revealed 30- 40% homology to a class of serine protease inhibitors known as serpins. Accordingly, the gene corresponding to clones 11P2A6 and clone 103 (and the encoded protein) were
5 named "PROSTAPIN" (PROSTate serine Protease INhibitor). PROSTAPIN is most closely associated with the serpin family member human LEI, which may have a role in apoptosis (Torriglia et al., 1998, MCB 18:3612).

The nucleotide sequences of PROSTAPIN clones 103 (SEQ ID NO. XX) and 11P2A6 (SEQ
10 ID NO. XX), which overlap in the 5' non-coding region of the PROSTAPIN gene, were combined to form the contiguous PROSTAPIN nucleotide sequence depicted in FIG. 1 (SEQ ID NO. XX). The 5' untranslated region contains two translational stop signals (indicated by asterisks in FIG. 1A) upstream of the start ATG, which falls within the Kozak sequence 5'-AAA ATG G-3'. A highly conserved reactive site loop characteristic of the
15 serpin family is located in the carboxy-terminal region of PROSTAPIN (underlined sequence in FIG. 1).

The serpin protease inhibitory domain is known as the reactive-site loop (RSL) and is located 30-50 residues from the carboxyl-terminus. The RSL is about 15-20 amino acids
20 in length and contains a hinge region and a variable region. The hinge region confers stability to the serpin-protease complex. As is evident from the amino acid alignment of various serpin family members shown in FIG. 2, the RSL hinge region is highly conserved among the serpins. The RSL variable region contains the reactive site amino acid P1, which determines specificity of inhibition. During inhibition, the RSL binds to the
25 protease active site, undergoes nucleophilic attack by the catalytic serine residue, resulting in cleavage of the serpin at P1-P1'. The PROSTAPIN RSL hinge region amino acid sequence (GTEAAAATG) is highly homologous to all other serpins analyzed, with the exception of maspin (FIG. 2). The PROSTAPIN RSL variable region amino acid sequence is distinct, with a lysine at P1 and a serine at P1'. This indicates that PROSTAPIN most
30 likely targets a different protease than do the other serpins.

PROSTAPIN clone 103 (SEQ ID NO. XX) has been deposited with the American Type Culture Collection ("ATCC") (Mannassas, VA) as plasmid pProstapin on May 15, 1998 as
35 ATCC Accession Number 98757.

EXAMPLE 3:**NORTHERN BLOT ANALYSIS OF PROSTAPIN EXPRESSION**

Northern blot analysis on panels of normal human and prostate tumor xenograft tissues using a labeled PROSTAPIN clone 103 (SEQ ID NO. XX) probe were conducted to confirm the prostate specificity of PROSTAPIN expression initially established by RT-PCR expression analysis (see Example 1). Further Northern blot analysis of PROSTAPIN expression is described in Example 4.

Two panels of normal human tissues were evaluated. The results from one of the panels, which contained 16 normal human tissues, are shown in FIG. 4 (Panels A & B). In this first panel, PROSTAPIN RNA was only detected in prostate, expressed as two distinct transcripts of about 2.3 and 3.0 kb. To extend this analysis, the clone 103 probe was used to analyze a second normal tissue panel, comprising an RNA dot blot matrix of 37 normal human tissues (Clontech, Palo Alto, CA; Human Master Blot™). The results, shown in FIG. 4 (Panel C), show PROSTAPIN expression only in prostate and trachea. No expression signal was detected in brain, spinal cord, heart, aorta, skeletal muscle, colon, bladder, uterus, stomach, testis, ovary, pancreas, pituitary gland, adrenal gland, thyroid gland, salivary gland, mammary gland, kidney, liver, small intestine, spleen, thymus, peripheral leukocytes, lymph node, bone marrow, appendix, lung, placenta, fetal brain, fetal heart, fetal kidney, fetal liver, fetal spleen, fetal thymus, or fetal lung.

Northern blot analysis of PROSTAPIN expression in the LAPC-4 AD, LAPC-4 AI and LAPC-9 AD prostate tumor xenografts was conducted with the labeled clone 103 probe (SEQ ID NO. XX). The results, shown in FIG. 4, Panel D, show detectable expression only in the LAPC-9 AD xenograft (as well as a concurrently analyzed normal prostate). Interestingly, although two distinct transcripts of about 2.3 and 3.0 kb are detected in normal prostate tissue, only the larger transcript is expressed in the LAPC-9 xenograft. Additionally, although no PROSTAPIN expression was detected in the LAPC-4 AI subline by Northern blotting, RT-PCR analysis of first-strand cDNA derived from the LAPC-4 AI xenograft showed very low level expression (see Example 1 and FIG. 3).

Further Northern blot analysis of three prostate cancer cell lines derived from patient's with advanced stage prostate cancer (LNCaP, PC-3 and DU145) showed no detectable expression of PROSTAPIN. The absence of expression in these cell lines was confirmed by semi-quantitative RT-PCR (see Example 4, below).

EXAMPLE 4:**PROSTAPIN EXPRESSION IN CLINICAL TISSUE SAMPLES,
PROSTATE CANCER CELL LINES AND XENOGRAPHS**

5

Further analysis of PROSTAPIN expression in various normal and prostate cancer clinical tissue samples was conducted by semi-quantitative RT-PCR in order to further examine the question of whether loss of PROSTAPIN expression correlates with advanced prostate cancer grade and clinical stage. Concurrent analysis of PROSTAPIN expression
10 in the LAPC xenografts and several prostate cancer cell lines was conducted.

Graded (Gleason Grade) and staged (according to TMN system) human tissues were obtained from the Human Tissue Resource Center at the University of California Los Angeles. PrEC cells were obtained from Clonetics. The prostate cancer cell lines were
15 obtained from readily available sources. For reference, the TMN staging of the human prostate tumor tissues used in this analysis was as follows: (1) Stage T2c, tumor is confined to the prostate and involves both lobes; (2) Stage T3a, tumor extends through the prostate capsule; (3) Stage T3c, extracapsular extension with invasion of seminal vesicles.

20

The human tissue samples included normal prostate, prostate cancer, and 4 examples of prostate cancer together with their matched normal controls (i.e., from the same patient). First strand cDNA was prepared from these tissues as well as the LAPC xenografts, prostate cancer cell lines (LNCaP, PC3, DU145) and normal prostate mRNA (obtained
25 from Clontech and BioChain). PCR analysis was performed on the normalized cDNAs using the primers described in Example 1 (i.e., SEQ ID NOS. XX and XX).

The results show that PROSTAPIN is expressed in all normal prostate tissues and the prostate tumor specimens derived from patients with localized disease, but that
30 PROSTAPIN expression is lost or dramatically attenuated in all tumor specimens from patients with extracapsular extension and advanced disease (FIG. 5, lanes 20 and 22). In addition, the results show complete absence of PROSTAPIN expression in all of the prostate cancer cell lines and the LAPC-4 xenografts (FIG. 5, lanes 6, 7, 9, 10, 11). All three of the prostate cancer cell lines originated from advanced prostate cancer patient
35 metastasis tumors. Specifically, LNCaP was derived from a prostate cancer lymph node metastasis, PC-3 was derived from a prostate cancer bone metastasis, and DU-145 was derived from a prostate cancer brain metastasis. The LAPC-4 xenograft was derived from a lymph node metastasis.

Consistent with the RT-PCR and Northern blot results obtained in Examples 1 and 3, respectively, a PROSTAPIN transcript was detected in the LAPC-9 xenograft. However, as shown in Example 5, below, the PROSTAPIN transcript expressed in the LAPC-9 xenograft is a substantially mutated, partially unspliced variant which includes point mutations, a stop codon in what would be the center of the wild type PROSTAPIN sequence, and an unspliced intron. Although the biological activity of this PROSTAPIN mutant (if any) has not been characterized, it is likely that wild type PROSTAPIN functionality is either substantially altered or, more likely, completely lost. Like the LAPC-4 xenografts, LAPC-9 also represents advanced stage disease, as it was generated from a bone tumor biopsy of a patient with hormone-refractory metastatic prostate cancer.

The data obtained from this combined analysis indicates that loss of wild type PROSTAPIN expression and/or expression of a functional mutation correlates with advanced stage and metastatic prostate cancer. All prostate cancer cell lines, xenografts and patient samples derived from advanced stage tumors or metastasis show a complete lack or sharp attenuation of wild type PROSTAPIN expression. Moreover, it appears that loss of PROSTAPIN expression may coincide with the development of metastatic disease, since expression is lost even at the lower grade/stage of extracapsular disease. Loss of functional PROSTAPIN may be one of the first molecular events coinciding with and/or leading to the development of metastasis.

EXAMPLE 5:

ISOLATION AND ANALYSIS OF cDNA ENCODING PROSTAPIN MUTATION

As evident from the Northern blot analyses of PROSTAPIN expression described in Example 3, only one of the two distinct PROSTAPIN transcripts expressed in normal prostate is detectable in the LAPC-9 xenograft. In order to examine the possibility that the LAPC-9 xenograft expresses an aberrant version of the PROSTAPIN gene, a full length PROSTAPIN cDNA was isolated from LAPC-9. Briefly, an LAPC-9 AD cDNA library was constructed in lambda ZAP Express (Stratagene). The library was screened using PROSTAPIN clone 103 (SEQ ID NO. XX) as a probe. A positive clone (clone 2) was identified and sequenced. Clone 2 cDNA (SEQ ID NO XX) comprises 2472 bp and has the nucleotide and deduced amino acid sequences shown in (FIG. 6). Further analysis determined that clone 2 represents a partially unspliced version of the PROSTAPIN message with an intron of 714 bp and point mutations at positions 298 (G/T), 474 (T/C), 572 (A/G), 593 (A/T), 1567 (A/C), 1584 (T/C), 1613 (C/T), 1822 (G/T), 2085 (C/T) in FIG. 6 (clone 2 – SEQ ID NO. XX). In addition, clone 2 contains several point mutations, one of which results in a stop codon at amino acid residue 90 in the open reading frame (FIG. 6).

All point mutations were confirmed by sequencing of RT-PCR products of first strand cDNA derived from LAPC-9 AD.

EXAMPLE 6:

5 SUBCELLULAR LOCALIZATION OF PROSTAPIN PROTEIN

MATERIALS AND METHODS:

To initially characterize the PROSTAPIN protein, cDNA clone 103 (SEQ ID NO. XX) was cloned into the pcDNA 3.1 Myc-His plasmid (Invitrogen) (which encodes a 6His tag at the carboxyl-terminus), transfected into 293T cells, and analyzed by subcellular fractionation. More specifically, the sequence encoding the PROSTAPIN ORF from PROSTAPIN clone 103 cDNA (SEQ ID NO. XX) was tagged with a 6His tag at the carboxyl-terminus and was transfected into 293T cells. Cell lysates were prepared by dounce homogenization of cells in a hypotonic buffer. Cellular debris was removed by low speed centrifugation (10,000 X g) and the supernatant containing the cytosol and light membrane fraction was separated by a 100,000 X g centrifugation. Equal amounts of protein from each fraction were analyzed using an anti-His antibody (Santa Cruz) that recognizes recombinant PROSTAPIN or an anti-SV 40 large T antibody (Santa Cruz). Cell conditioned media (Cell sup) was also analyzed to identify any secreted PROSTAPIN protein.

RESULTS:

Western blot analysis of cytosol and light membrane fractions using an anti-His antibody demonstrated that 90% of PROSTAPIN protein was present in the membrane fraction (FIG. 7). As a control, SV40 large T antigen, an endogenous cytosolic protein, was shown to be largely cytosolic. This suggests that PROSTAPIN is associated with plasma membrane and/or endoplasmic reticulum.

EXAMPLE 7:

30 CHROMOSOMAL LOCALIZATION OF THE PROSTAPIN GENE

The chromosomal localization of PROSTAPIN was determined using the GeneBridge 4 Human/Hamster radiation hybrid (RH) panel (Walter et al., 1994, Nat. Genetics 7:22) (Research Genetics, Huntsville Al). The following PCR primers, which amplify a 148 bp PROSTAPIN product, were employed:

11P2A6.9	ata cct gga tgt cag cga aga g	(SEQ ID NO. XX)
11P2A6.10	tag gat cgt gtt ggt atg agt gtg	(SEQ ID NO. XX)

The resulting mapping vector for the 93 radiation hybrid panel DNAs was:

0000101010000012012001000000101001010100110111002010000100000100001010001
01000000101001000010.

5

This vector and the mapping program at <http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl> placed PROSTAPIN on chromosome 18q21.3 between D18S983 and D18S537 with an LOD>15. The chromosomal location of PROSTAPIN is schematically depicted in FIG. 8.

10

The PROSTAPIN gene co-localizes to the 500 kb 18q21.3 region along with 6 other serpin family members, including maspin, leupin and bomapin (Bartuski et al., 1997, Genomics 43:321). The region of 18q21.3 has been associated with loss of heterozygosity (LOH) in advanced metastatic prostate cancer and in recurrent disease (Brothman et al., 1999, 15 The Prostate 38:303). Loss of expression of PROSTAPIN alone or in combination with another serpin may contribute to the growth characteristics and invasiveness of aggressive prostate cancer. It is interesting to note that maspin expression in the prostate and in the LAPC xenografts (determined by RT-PCR) mimics the expression of PROSTAPIN. It may be that some of the genes in this serpin cluster at 18q21 may exhibit 20 coordinate regulation of expression.

EXAMPLE 8:

IDENTIFICATION OF INTRON-EXON BOUNDARIES OF PROSTAPIN GENE

25 Genomic PROSTAPIN clones were isolated by screening the human BAC library CID (Cal. Tech. library D, purchased from Research Genetics) and a human PAC library (release I, Peter deJong, University of New York, Buffalo). Four positive clones were obtained: 2062H14, 2074J2, 2100K19 from the BAC library; 152i22 from the PAC library. These clones were confirmed by Southern blot analysis (FIG. 9) using PROSTAPIN clone 103 30 cDNA probe (SEQ ID NO. XX).

Clone 2074J2 was used to identify the intron-exon boundaries for the PROSTAPIN gene. The PROSTAPIN gene was found to contain seven exons and six introns within the region of the coding sequence (FIG 10).

35

The following seven pairs of primers were designed within introns to amplify exonic sequences from genomic DNA for sequencing or for single-stranded conformational polymorphism (SSCP) analysis:

	Exon 1:		
	ProsEi	atgggttctctcagcacagctaacg	(SEQ ID NO. XX)
		(within exon, begins at start site)	
	Pros2	aattaatttgctgacccagagcg	(SEQ ID NO. XX)
5			
	Exon 2:		
	Pros3	gtagctatcactactgatcttgatc	(SEQ ID NO. XX)
	Pros4	atgggcaaaagaaggagcttttctac	(SEQ ID NO. XX)
10			
	Exon 3:		
	Pros5	tttattcagaggcaaacaccttgct	(SEQ ID NO. XX)
	Pros6	atgtcatgtgactcttctcactcttc	(SEQ ID NO. XX)
	Exon 4:		
15	Pros7	gaattttagaatacattgagctgtag	(SEQ ID NO. XX)
	Pros8	atctgcctatgtcagggtgcagacttc	(SEQ ID NO. XX)
	Exon 5:		
	Pros9	taaatttctcatgactcttcacct	(SEQ ID NO. XX)
20	Pros10	tatcctccaacatttgatgagctctg	(SEQ ID NO. XX)
	Exon 6:		
	Pros11	tagagtgttcatgcagatatccgtgt	(SEQ ID NO. XX)
	Pros12	aatcaatgactacgctaataatgcatgag	(SEQ ID NO. XX)
25			
	Exon 7:		
	Pros13	gaagttgaaccactcacactgagaatt	(SEQ ID NO. XX)
	ProsEi6	attgtctctgcacctcatctgcaa	(SEQ ID NO. XX)
		(5'untranslated region)	

An example of genomic amplification of PROSTAPIN exonic sequences using these primer sets is shown in FIG. 11.

35

EXAMPLE 9:**GENERATION OF PROSTAPIN POLYCLONAL ANTIBODY**

5 Sheep polyclonal anti-PROSTAPIN antibodies were generated using a purified GST-PROSTAPIN fusion protein. The fusion protein contains amino acid residues 1-106 from the PROSTAPIN sequence and was generated by PCR using the following primers:

GST-pros5': gtggatccatgggttctctcagca (underlined sequence: BamHI site)
(SEQ ID NO. XX)

10

GST-pros3': ataccccgggtggaatgctgagg (underlined sequence: SmaI site)
(SEQ ID NO. XX)

15 The PCR product was inserted directionally into a pGEX-4T (GST-fusion) vector (Pharmacia). Fusion protein was produced in bacteria and purified using a Glutathione-Sepharose column (Pharmacia).

20 Sheep were immunized with 200 µg/injection every two weeks for an eight week period. Immune serum was affinity purified using a GST-PROSTAPIN column. Purified antibody was used to probe western blots of lysates from several cell lines including: 293T cells transfected with pcDNA vector with or without His-tagged PROSTAPIN, TsuPr1 cells infected with retrovirus generated with the retroviral expression vector pSR α tkneo with or without PROSTAPIN (FIG. 12). The results show that the anti-PROSTAPIN antibody
25 recognizes His-tagged PROSTAPIN with similar intensity as an anti-His antibody. In addition, untagged PROSTAPIN expressed in TsuPr1 cells is also recognized with high efficiency and specificity.

30

EXAMPLE 10**WESTERN BLOT ANALYSIS OF PROSTAPIN EXPRESSION IN PROSTATE
CANCER**

35 Western blotting of lysates derived from LAPC xenografts (LAPC-4 AD, 9AD, and 9AI), prostate cancer cell lines (TsuPr1, LNCaP, PC-3) and a prostate tumor-normal matched patient sample using the purified antibody described in Example 9 showed PROSTAPIN expression only in the normal prostate tissue (FIG. 13). Significantly lower levels of prostapin were detected in the matched prostate cancer sample. PROSTAPIN expression was undetectable in the xenografts and

the prostate cancer cell lines. These results confirm loss or down-regulation of PROSTAPIN in prostate cancer.

5

10 Throughout this application, various publications are referenced within parentheses. The disclosures of these publications are hereby incorporated by reference herein in their entireties.

15 The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any which are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such
20 modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

CLAIMS

1. An isolated PROSTAPIN protein having an amino acid sequence shown in FIG. 1 (SEQ ID NO. XX) or a polypeptide fragment thereof.
3. A pharmaceutical composition comprising the PROSTAPIN protein of claim 1.
4. An isolated PROSTAPIN polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid sequence shown in FIG. 1 (SEQ ID NO. XX) over its entire length.
5. An isolated polynucleotide encoding the PROSTAPIN protein of claim 1.
6. An isolated polynucleotide encoding a PROSTAPIN polypeptide whose sequence is encoded by the cDNA contained in plasmid pProstapin as deposited with American Type Culture Collection as Accession No. 98757.
7. An isolated polynucleotide selected from the group consisting of (a) a polynucleotide having the sequence as shown in FIG. 1 (SEQ ID NO. XX), wherein T can also be U; (b) a polynucleotide fully complementary to (a); and (c) a polynucleotide which hybridizes under stringent conditions to (a) or (b).
8. A recombinant expression vector which contains a polynucleotide according to claim 7.
9. A host cell which contains an expression vector according to claim 8.
10. A process for producing a PROSTAPIN protein comprising culturing a host cell of claim 9 under conditions sufficient for the production of the polypeptide and recovering the polypeptide from the culture.
11. A PROSTAPIN polypeptide produced by the method of claim 10.
12. An antibody which binds to the PROSTAPIN protein of claim 1.
13. A monoclonal antibody according to claim 12.
14. A fragment of the antibody of claim 13 which binds to the PROSTAPIN protein of claim 1.

15. A recombinant protein comprising the antigen binding domain of the antibody of claim 13.
- 5 16. The antibody of claim 12 or 13 which is labeled with a detectable marker.
17. The antibody fragment of claim 14 which is labeled with a detectable marker.
18. The recombinant protein of claim 15 which is labeled with a detectable marker.
- 10 19. A method of detecting the presence of a PROSTAPIN protein in a biological sample comprising contacting the sample with an antibody of claim 12 or 13, an antibody fragment of claim 14, or a recombinant protein of claim 15, and detecting the binding of PROSTAPIN protein in the sample thereto.
- 15 20. A method of detecting the presence of a PROSTAPIN polynucleotide in a biological sample, comprising
- 20 (a) contacting the sample with a polynucleotide probe which specifically hybridizes to the PROSTAPIN cDNA contained within plasmid pProstapin as deposited with American Type Culture Collection as Accession No. 98757 or the polynucleotide as shown in FIG. 1 (SEQ ID NO. XX) or the complements thereof; and
- 25 (b) detecting the presence of a hybridization complex formed by the hybridization of the probe with PROSTAPIN polynucleotide in the sample, wherein the presence of the hybridization complex indicates the presence of PROSTAPIN polynucleotide within the sample.
- 30 21. A method of detecting the presence of PROSTAPIN mRNA in a biological sample comprising:
- (a) producing cDNA from the sample by reverse transcription using at least one primer;
- 35 (b) amplifying the cDNA so produced using PROSTAPIN polynucleotides as sense and antisense primers to amplify PROSTAPIN cDNAs therein;
- (c) detecting the presence of the amplified PROSTAPIN cDNA,

wherein the PROSTAPIN polynucleotides used as the sense and antisense probes are selected from the group consisting of the polynucleotide shown in FIG. 1 (SEQ ID NO. XX), fragments thereof, and complements thereof.

5 22. A method of detecting the PROSTAPIN gene in a biological sample comprising:

(a) isolating genomic DNA from the sample;

10 (b) amplifying the isolated genomic DNA using PROSTAPIN polynucleotides as sense and antisense primers to amplify the PROSTAPIN gene therein;

(c) detecting the presence of the amplified PROSTAPIN gene,

15 wherein the PROSTAPIN polynucleotides used as the sense and antisense probes are selected from the group consisting of the polynucleotides shown in FIG. 1 (SEQ ID NO. XX), fragments thereof, and complements thereof.

20 23. An assay for identifying a cell which expresses a PROSTAPIN gene comprising detecting the presence of PROSTAPIN mRNA in the cell.

24. An assay for identifying a cell which expresses a PROSTAPIN gene comprising detecting the presence of PROSTAPIN protein in the cell.

25 25. A method of diagnosing the presence of advanced prostate cancer in an individual comprising:

(a) obtaining a test sample of tissue from the individual;

30 (b) determining the level of PROSTAPIN mRNA expressed in the test sample;

(c) comparing the level so determined to the level of PROSTAPIN mRNA expressed in a comparable known normal tissue sample,

35 the absence or substantial attenuation of PROSTAPIN mRNA expression in the test sample relative to the normal tissue sample indicating the presence of advanced prostate cancer.

26. The method of claim 25, wherein the test sample is prostate tissue and the normal tissue sample is normal prostate tissue.

27. The method of claim 25, wherein the test sample is prostate tumor tissue and the normal tissue sample is normal prostate tissue.
- 5 28. The method of claim 25, wherein the test and normal tissue samples are peripheral blood.
29. A method of diagnosing the presence of advanced prostate cancer in an individual comprising:
- 10 (a) obtaining a test sample of tissue from the individual;
- (b) determining the level of PROSTAPIN protein expressed in the test sample;
- 15 (c) comparing the level so determined to the level of PROSTAPIN protein expressed in a comparable known normal tissue sample,
- the absence or substantial attenuation of PROSTAPIN protein expression in the test sample relative to the normal tissue sample indicating the presence of advanced prostate cancer.
- 20 30. The method of claim 29, wherein the test sample is prostate tissue and the normal tissue sample is normal prostate tissue.
- 25 31. The method of claim 29, wherein the test sample is prostate tumor tissue and the normal tissue sample is normal prostate tissue.
32. A recombinant viral vector which contains a polynucleotide encoding the PROSTAPIN protein of claim 1.
- 30 33. The recombinant vector of claim 32 which is an adenoviral vector.
34. The recombinant vector of claim 32 which is a retroviral vector.
- 35 35. A composition for gene therapy of prostate cancer comprising the recombinant viral vector of claim 32, 33 or 34.
36. A liposome containing a PROSTAPIN protein according to claim 1.

37. A liposome containing a PROSTAPIN polynucleotide encoding the PROSTAPIN protein of claim 1.
- 5 38. A method of treating a patient with advanced prostate cancer comprising administering a PROSTAPIN protein having the amino acid sequence of FIG. 1 (SEQ ID NO. XX) to the patient in an amount sufficient to restore normal levels of PROSTAPIN to the patient's prostate tumor cells.
- 10 39. A method of treating a patient with advanced prostate cancer comprising administering a composition according to claim 35 to the patient in an amount sufficient to result in restoring normal levels of PROSTAPIN expression within the patient's prostate tumor cells.
- 15 40. An isolated polynucleotide selected from the group consisting of (a) a polynucleotide having the sequence as shown in FIG. 6 (SEQ ID NO. XX), wherein T can also be U; (b) a polynucleotide fully complementary to (a); and (c) a polynucleotide which hybridizes under stringent conditions to (a) or (b).

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FIG. 1-1

		10		19		28		37		46		55						
5'	TAC	TTT	CCA	ACC	AGC	AGG	TGA	AGA	GCT	GAA	TCA	CTA	AGG	GCA	CAA	ATG	AAA	AGG
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		64		73		82		91		100		109						
	GAG	GGG	CAA	AAT	ATG	AAG	GCT	TTA	TAT	TTC	TTC	TGA	GGG	ACG	CAG	CTC	CAG	TCT
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		118		127		136		145		154		163						
	AAC	TTG	CCA	CTC	TGT	GAA	CTT	CAC	TAC	TGG	AAA	GCA	ACA	AAG	GCA	GTC	GGC	ATA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		172		181		190		199		208		217						
	AAA	ATG	GGT	TCT	CTC	AGC	ACA	GCT	AAC	GTT	GAA	TTT	TGC	CTT	GAT	GTG	TTC	AAA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	M	G	S	L	S	T	A	N	V	E	F	C	L	D	V	F	K	
		226		235		244		253		262		271						
	GAG	CTG	AAC	AGT	AAC	ATA	GGA	GAT	AAC	ATC	TTC	TTT	TCT	TCG	CTG	AGT	CTG	
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	E	L	N	S	N	I	G	D	N	I	F	F	S	S	L	S	L	
		280		289		298		307		316		325						
	CTT	TAT	GCT	CTA	AGC	ATG	GTC	CTC	CTT	GGT	GCC	AGG	GGA	GAG	ACT	GCA	GAG	CAA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	L	Y	A	L	S	M	V	L	L	G	A	R	G	E	T	A	E	Q
		334		343		352		361		370		379						
	TTG	GAG	AAG	GTG	CTT	CAT	TTT	AGT	CAT	ACT	GTA	GAC	TCA	TTA	AAA	CCA	GGG	TTC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	L	E	K	V	L	H	F	S	H	T	V	D	S	L	K	P	G	F
		388		397		406		415		424		433						
	AAG	GAC	TCA	CCT	AAG	TGC	AGC	CAA	GCT	GGA	AGA	ATT	CAT	TCC	GAG	TTT	GGT	GTC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	K	D	S	P	K	C	S	Q	A	G	R	I	H	S	E	F	G	V
		442		451		460		469		478		487						
	GAA	TTC	TCT	CAA	ATC	AAC	CAG	CCA	GAC	TCT	AAC	TGT	ACC	CTC	AGC	ATT	GCC	AAC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	E	F	S	Q	I	N	Q	P	D	S	N	C	T	L	S	I	A	N
		496		505		514		523		532		541						
	AGG	CTC	TAC	GGG	ACA	AAG	ACG	ATG	GCA	TTT	CAT	CAG	CAA	TAT	TTA	AGC	TGT	TCT
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	R	L	Y	G	T	K	T	M	A	F	H	Q	Q	Y	L	S	C	S
		550		559		568		577		586		595						
	GAG	AAA	TGG	TAT	CAA	GCC	AGG	TTG	CAA	ACT	GTG	GAT	TTT	GAA	CAG	TCT	ACA	GAA
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	E	K	W	Y	Q	A	R	L	Q	T	V	D	F	E	Q	S	T	E
		604		613		622		631		640		649						
	GAA	ACG	AGG	AAA	ATG	ATT	AAT	GCT	TGG	GTT	GAA	AAT	AAA	ACT	AAT	GGA	AAA	GTC
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	E	T	R	K	M	I	N	A	W	V	E	N	K	T	N	G	K	V
		658		667		676		685		694		703						
	GCA	AAT	CTC	TTT	GGA	AAG	AGC	ACA	ATT	GAC	CCT	TCA	TCT	GTA	ATG	GTC	CTG	GTG

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FIG. 1-2

A	N	L	F	G	K	S	T	I	D	P	S	S	V	M	V	L	V
		712			721			730			739			748			757
AAT	ACC	ATA	TAT	TTC	AAA	GGA	CAA	AGG	CAA	AAT	AAA	TTT	CAA	GGT	AAA	AAT	GTA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	T	I	Y	F	K	G	Q	R	Q	N	K	F	Q	G	K	N	V
		766			775			784			793			802			811
ACT	GTG	GAA	ATG	ATG	TAT	CAA	ATT	GGA	ACA	TTT	AAA	CTG	GCC	TTT	GTA	AAG	GAG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
T	V	E	M	M	Y	Q	I	G	T	F	K	L	A	F	V	K	E
		820			829			838			847			856			865
CCG	CAG	ATG	CAA	GTT	CTT	GAG	CTG	CCC	TAC	GTT	AAC	AAC	AAA	TTA	AGC	ATG	ATT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
P	Q	M	Q	V	L	E	L	P	Y	V	N	N	K	L	S	M	I
		874			883			892			901			910			919
ATT	CTG	CTT	CCA	GTA	GGC	ATA	GCT	AAT	CTG	AAA	CAG	ATA	GAA	AAG	CAG	CTG	AAT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
I	L	L	P	V	G	I	A	N	L	K	Q	I	E	K	Q	L	N
		928			937			946			955			964			973
TCG	GGG	ACG	TTT	CAT	GAG	TGG	ACA	AGC	TCT	TCT	AAC	ATG	ATG	GAA	AGA	GAA	GTT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
S	G	T	F	H	E	W	T	S	S	S	N	M	M	E	R	E	V
		982			991			1000			1009			1018			1027
GAA	GTA	CAC	CTC	CCC	AGA	TTC	AAA	CTT	GAA	ATT	AAG	TAT	GAG	CTA	AAT	TCC	CTG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
E	V	H	L	P	R	F	K	L	E	I	K	Y	E	L	N	S	L
		1036			1045			1054			1063			1072			1081
TTA	AAA	CCT	CTA	GGG	GTG	ACA	GAT	CTC	TTC	AAC	CAG	GTC	AAA	GCT	GAT	CTT	TCT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
L	K	P	L	G	V	T	D	L	F	N	Q	V	K	A	D	L	S
		1090			1099			1108			1117			1126			1135
GGA	ATG	TCA	CCA	ACC	AAG	GGC	CTA	TAT	TTA	TCA	AAA	GCC	ATC	CAC	AAG	TCA	TAC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
G	M	S	P	T	K	G	L	Y	L	S	K	A	I	H	K	S	Y
		1144			1153			1162			1171			1180			1189
CTG	GAT	GTC	AGC	GAA	GAG	GGC	ACG	GAG	GCA	GCA	GCA	GCC	ACT	GGG	GAC	AGC	ATC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
L	D	V	S	E	E	<u>G</u>	<u>T</u>	<u>E</u>	<u>A</u>	<u>A</u>	<u>A</u>	<u>A</u>	<u>T</u>	<u>G</u>	<u>D</u>	<u>S</u>	<u>I</u>
		1198			1207			1216			1225			1234			1243
GCT	GTA	AAA	AGC	CTA	CCA	ATG	AGA	GCT	CAG	TTC	AAG	GCG	AAC	CAC	CCC	TTC	CTG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
<u>A</u>	<u>V</u>	<u>K</u>	<u>S</u>	<u>L</u>	<u>P</u>	M	R	A	Q	F	K	A	N	H	P	F	L
		1252			1261			1270			1279			1288			1297
TTC	TTT	ATA	AGG	CAC	ACT	CAT	ACC	AAC	ACG	ATC	CTA	TTC	TGT	GGC	AAG	CTT	GCC
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
F	F	I	R	H	T	H	T	N	T	I	L	F	C	G	K	L	A
		1306			1315			1324			1333			1342			1351
TCT	CCC	TAA	TCA	GAT	GGG	GTT	GAG	TAA	GGC	TCA	GAG	TTG	CAG	ATG	AGG	TGC	AGA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

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FIG. 1-3

S	P	*																
	1360		1369		1378		1387		1396		1405							
GAC	AAT	CCT	GTG	ACT	TTC	CCA	CGG	CCA	AAA	AGC	TGT	TCA	CAC	CTC	ACA	CAC	CTC	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1414		1423		1432		1441		1450		1459							
TGT	GCC	TCA	GTT	TGC	TCA	TCT	GCA	AAA	TAG	GTC	TAG	GAT	TTC	TTC	CAA	CCA	TTT	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1468		1477		1486		1495		1504		1513							
CAT	GAG	TTG	TGA	AGC	TAA	GGC	TTT	GTT	AAT	CAT	GGA	AAA	AGG	TAG	ACT	TAT	GCA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1522		1531		1540		1549		1558		1567							
GAA	AGC	CTT	TCT	GGC	TTT	CTT	ATC	TGT	GGT	GTC	TCA	TTT	GAG	TGC	TGT	CCA	GTG	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1576		1585		1594		1603		1612		1621							
ACA	TGA	TCA	AGT	CAA	TGA	GTA	AAA	TTT	TAA	GGG	ATT	AGA	TTT	TCT	TGA	CTT	GTA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1630		1639		1648		1657		1666		1675							
TGT	ATC	TGT	GAG	ATC	TTG	AAT	AAG	TGA	CCT	GAC	ATC	TCT	GCT	TAA	AGA	AAA	CCA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1684		1693		1702		1711		1720		1729							
GCT	GAA	GGG	CTT	CAA	CTT	TGC	TTG	GAT	TTT	TAA	ATA	TTT	TCC	TTG	CAT	ATG	TAA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1738		1747		1756		1765		1774		1783							
ATA	GAA	TGT	GGT	GAG	TTT	TAG	TTC	AAA	ATT	CTC	TGT	TGA	GAA	TAA	TAA	ATG	CAT	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1792		1801		1810		1819		1828		1837							
GAA	ATA	CCT	TAA	AGC	TCT	GTG	AAG	ACT	TGT	AAC	ATG	GCA	GCA	ATC	AAA	TGG	CTT	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	1846		1855		1864		1873		1882									
ATA	AAA	GGA	TAC	TTT	GAA	TGT	GGA	TAA	ATT	GAA	AAA	AAA	AAA	AAA	AAA	AA	3'	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

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FIG. 2-1

1	15	16	30	31	45	46	60	61	75	76	90
1 Bomapin	MDSLATSINQFALEL	SKKLAESAQGNKIFF	SSWSISTSLTIVYLG	AKGTTAAQMAQVLQF	NRDQG---	VKCDPE-					
2 PAI-2	MEDLCVANTLFALNL	FKHLAKASPTQNLFL	SPWSISSAMVYMG	SRGSTEDQMAKVLQF	NEVGANAVTPMTPEN	FTSCGFMQIQKGSY					77
3 SCCA1	MNSLSEANTKFMFDL	FQQFR-KSKENNIFY	SPISITSALGMVLLG	AKDNTAQIKKVLHF	DQVTEN----	TT-G-					70
4 SCCA2	MNSLSEANTKFMFDL	FQQFR-KSKENNIFY	SPISITSALGMVLLG	AKDNTAQISKVLHF	DQVTEN----	TT-E-					70
5 Prostatin	MGSLSTANVEFCLDV	FKELNSNNIGDNIFF	SSLSLLYALSMVLLG	ARGETAELQEKVLHF	SHTVDS----	LKPG-					72
6 CAP-2	MDDLCEANGTFAISL	FKILGEEDNSRNVFF	SPMSISSALAMVFMG	AKGSTAAQMSQALCL	YKDG-----	D---					65
7 PTI-6	MDVLAEANGTFALNL	LKTLGK-DNSKNVFF	SPMSMSCALAMVYMG	AKGNTAAQMAQILSF	NKSGGG----	GD---					67
8 LEI	MEQLSSANTRFALDL	FLALSENPNAGNIFI	SPFSISSAMAMVFLG	TRGNTAAQLSKTFHF	NTVEE-----						65
9 Maspin	MDALQLANSFAVDL	FKQLCEKEPLGNLFF	SPICLSTLSLAQVG	AKGDTANEIGQVLHF	ENVKD-----						65
	91	105	106	120	121	135	136	150	151	165	166
1 Bomapin	--MEFNLSNSEEIHS	DFQTLISEILKPND	YLLKTANAIYGEKTY	AFHNKYLEDMKTYFG	AEPQPVNFVEASDQI	RKDINSWVERQTEGK					165
2 PAI-2	PDAILQAQAADKIHS	SFRSLSSAINASTGN	YLLESVNKLFGKSA	SFREEYIRLCQKYYS	SEPQAVDFLECAEEA	RKKINSWVKQTGKG					180
3 SCCA1	--ATYHVDRSGNVHH	QFQKLLTEFNKSTDA	YELKIANKLFGKTY	LFLQEYLDATKKFYQ	TSVESVDFAPEES	RKKINSWVESQTNEK					158
4 SCCA2	--ATYHVDRSGNVHH	QFQKLLTEFNKSTDA	YELKIANKLFGKTY	QFLQEYLDATKKFYQ	TSVESTDFANAPES	RKKINSWVESQTNEK					158
5 Prostatin	--DSPKCSQAGRIHS	EFGVEFSQINQPDNS	CTLSIANRLYGTMT	AFHQQYLSCEKMYQ	ARLQTVDFEQSTEET	RKMINAWVENKTNGK					160
6 CAP-2	-----IHR	GFQSLSEVNRTGTQ	YLLRTANRLFGKTC	DFLPDFKEYCQKFYQ	AEELELSFAEDTEEC	RKHINDWVAEKTGK					143
7 PTI-6	-----IHQ	GFQSLLTEVNKTGTQ	YLLRVANRLFGKSC	DFLSSFRDSCQKFYQ	AEMEELDFISAVEKS	RKHINTWVAEKTGK					145
8 LEI	-----VHS	RFQSLNADINKRGAS	YILKLANRLYGEKTY	NELPEFLVSTQKTYG	ADLASVDFQHASEDA	RKTINQWVKGQTEGK					143
9 Maspin	-----IPF	GFQTVTSDVNKLSSF	YSLKLIKRLYVDKSL	NLSTEFISSTKRPIYA	KELETVDKFCKLEET	KGQINNSIKDLTDGH					143
	181	195	196	210	211	225	226	240	241	255	256
1 Bomapin	IQNLLPDDSDVSDTTR	MILVNALYFKGIWEH	QFLVQNTTEKPFRLN	ETTSKPQVMMFMKKK	LHIFHIEKPKAVGLQ	LYYKSRDLSLLILLP					270
2 PAI-2	IPNLLPEGSVDGDTTR	MVLVNAVYFKGKWK	PFEKKLNGLYPFRVN	SAQRTPVQMMYLREK	LNIGYIEDLKAQILE	LPYAG-DVSMFLLLP					269
3 SCCA1	IKNLIPEGNIGSNTT	LVLVNAIYFKGQWEK	KFNKEDTKEEFWPN	KNTYKSIQMMRQYTS	FHFASLEDVQAKVLE	IPYKGDLSMIVLLP					248
4 SCCA2	IKNLFDPDGTIGNOTT	LVLVNAIYFKGQWEN	KFKKENTKEEFWPN	KNTYKSVQMMRQYNS	FNFALLEDVQAKVLE	IPYKGDLSMIVLLP					248
5 Prostatin	VANLFGKSTIDPSSV	MVLVNTIYFKGQRQN	KFQ-----	KN--VTVEMMYQIGT	FKLAFVKEPQMQVLE	LPYVNNKLSMIILLP					237
6 CAP-2	ISEVLDAGTVDPLTK	LVLVNAIYFKGKWE	QFDRKYTRGMLFKTN	E-EKKTQVQMMFKEAK	FKMGYADEVHTQVLE	LPYVEEELSMVILLP					232
7 PTI-6	IAELLSPGSVDPLTR	LVLVNAVYFRGNWDG	QFDKENTEERLFKVS	KNEEKPVQMMFKQST	FKKTYIGEITQILV	LPYVGKELNMIIMLP					235
8 LEI	IPELLASGMVDNMTK	LVLVNAIYFKGNWGD	KFMKEATTNAPFRLN	KKDRKTVKMMYQKKK	FAYGYIEDLKCRVLE	LPYQGEELSMVILLP					233
9 Maspin	FENILADNSVNDQTK	ILVVNAAYFVGKWMK	KFPESETKECPFRLN	KTDTKPVQMMNMEAT	FCMGNIDSINCKIE	LPFQNKHLSMFILLP					233

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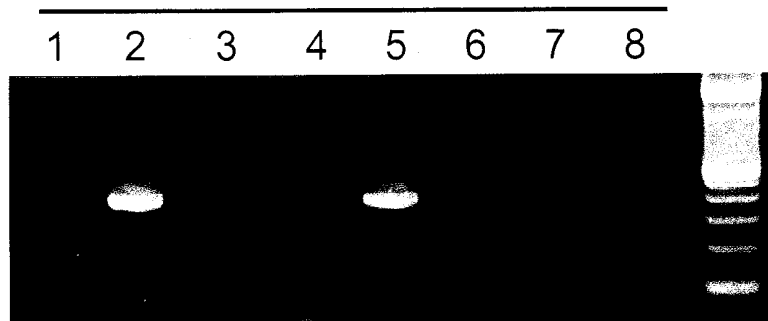
FIG. 2-2

1	Bomapin	271	285	300	315	330	345	360
2	PAI-2	EDIN-----GLEQLEK	AITYEKLNEWTSADM	MELYEVQLHLPKFKL	EDSYDLKSTLSSMG	SDAFSQSKADFSGMS	SARNLFLSNVFKAF	341
3	SCCA1	DEIADVSTGLELLES	EITYDKLNKWTSDK	MAEDEVEVYIPQFKL	EEHYELRSILRSMGM	EDAFNKGRANFSGMS	ERNDLFLSEVFHQAM	359
4	SCCA2	NEID-----GLQKLEE	KLTAEKLMEWTSQN	MRETRVDLHLPREFKV	EESYDLKDTLRTMG	VDIFN-GDADLSG	GSRGLVLSGVLHKAF	333
5	Prostapin	NEID-----GLQKLEE	KLTAEKLMEWTSQN	MRETCVDLHLPREFKM	EESYDLKDTLRTMG	VNIFN-GDADLSG	WSHGLSVSKVLHKAF	333
6	CAP-2	VGIA-----NLKQIEK	QLNSGTFHEWTSASN	MMEREVEVHLPRFKL	EIKYELNSLLKPLGV	TDLFNQVKADLSGMS	PTKGLYLSKAIHKS	323
7	PTI-6	DDNT-----DLAVVEK	ALTYEKFKAWTNSK	LTKSKVQVFLPRKL	EESYDLEPFLRRLGM	IDAFDEAKADFSGMS	TEKNVPLSKVAHKCF	318
8	LEI	DETT-----DLRTVEK	ELTYEKFVEWTRLDM	MDEEEVEVSLPRFKL	EESYDMESVLRNLGM	TDAFELGKADFSGMS	-QTDLSLSKVVKHKS	320
9	Maspin	DDIEDESTGLKKIEE	QLTLEKLHEWTKPEN	LDFIEVNVSLPRFKL	EESYTLNSDLARLGV	QDLFNSSKADLSGMS	GARDIFISKIVHKSF	323
		KDVEDESTGLEKIEK	QLNSESLSQWTNPST	MANAKVKLSIPKFKV	EKMIDPKACLENLGL	KHIFSEDTSDFS	ETKGVALSNNVIHKVC	323
		361	375	390	405	420	435	450
1	Bomapin	VEINEQGTEAAAGSG	SEIDIRIRV-PSIEF	NANHPFLFFIRHNKT	NTILFYGRLCSP	397		
2	PAI-2	VDVNEEGTEAAAGTG	GVMTGRIGH-GGPQF	VADHPFLFLIMHKIT	NCILFFGRFSSP	415		
3	SCCA1	VEVTEEGAEAAAATA	VVGFGSSPASTNEEF	HCNHPFLFFIRQNK	NSILFYGRFSSP	390		
4	SCCA2	VEVTEEGVEAAAATA	VVVVELSPSTNEEF	CCNHPFLFFIRQNK	NSILFYGRFSSP	390		
5	Prostapin	LDVSEEGTEAAAATG	DSIAVKSILP-MRAQF	KANHPFLFFIRHTHT	NTILFCGKLASP	379		
6	CAP-2	VEVNEEGTEAAAATA	VVRNSRCSR-MEPRF	CADHPFLFFIRRHKT	NCILFCGRFSSP	374		
7	PTI-6	VEVNEEGTEAAAATA	AIMMRCAR-FVPRF	CADHPFLFFIQHRKT	NGILFCGRFSSP	376		
8	LEI	VEVNEEGTEAAAATA	G-IATFCMLMPEENF	TADHPFLFFIRHNSS	GSILFLGRFSSP	379		
9	Maspin	LEITEDGGDSIEVPG	ARILQH-----KDEL	NADHPFIYIIRHNKT	RNIIFFGKFCSP	375		

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FIG. 3A

27x

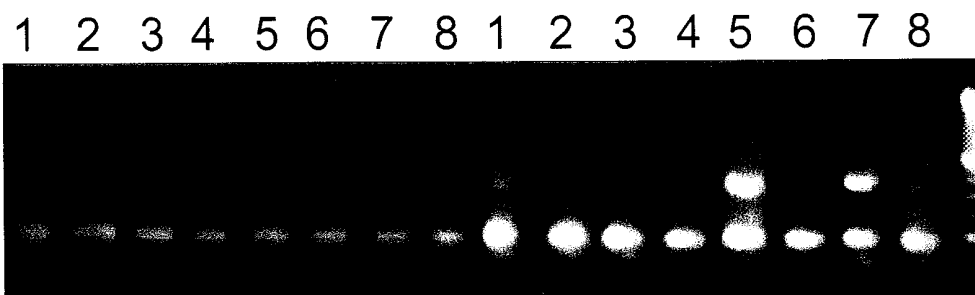
**Panels**

1. Brain
2. Prostate
3. LAPC-4 AD
4. LAPC-4 AI
5. LAPC-9 AD
6. HeLa
7. Murine cDNA
8. Neg. control

FIG. 3B

30x

35x

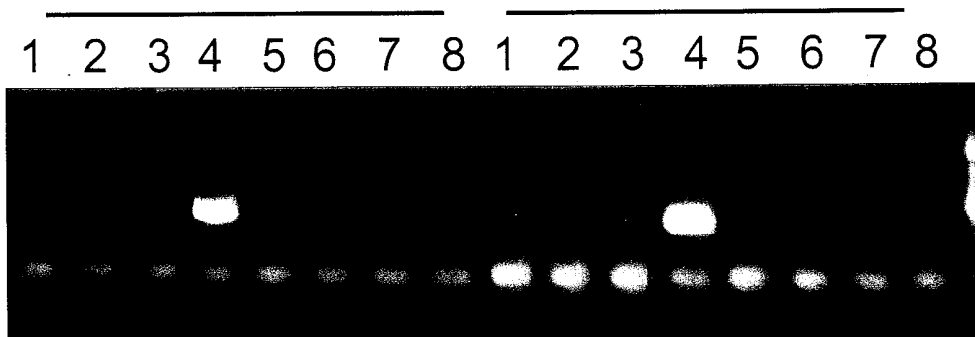
**Panels**

- | | |
|-----------|--------------------|
| 1. Brain | 5. Lung |
| 2. Heart | 6. Pancreas |
| 3. Kidney | 7. Placenta |
| 4. Liver | 8. Skeletal Muscle |

FIG. 3C

30x

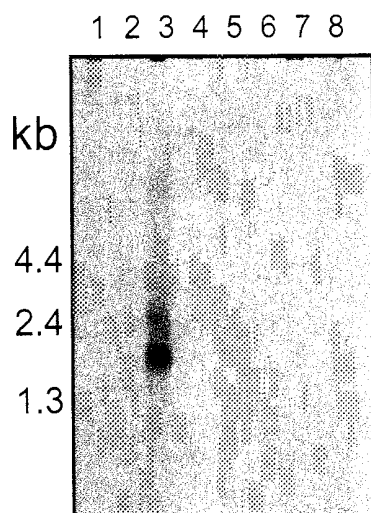
35x

**Panels**

- | | |
|---------------|--------------------|
| 1. Colon | 5. Small Intestine |
| 2. Ovary | 6. Spleen |
| 3. Leukocytes | 7. Testis |
| 4. Prostate | 8. Thymus |

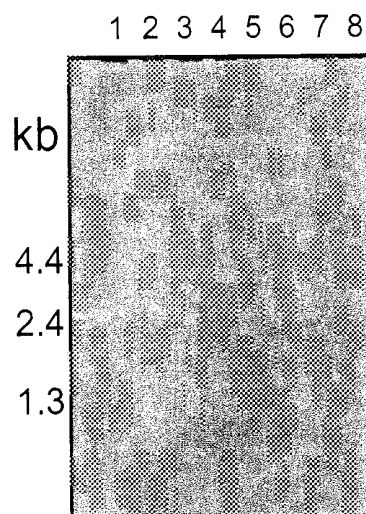
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FIG. 4A

**Panels**

1. Spleen
2. Thymus
3. Prostate
4. Testis
5. Ovary
6. Small Intestine
7. Colon
8. Leukocytes

FIG. 4B

**Panels**

1. Heart
2. Brain
3. Placenta
4. Lung
5. Liver
6. Skeletal Muscle
7. Kidney
8. Pancreas

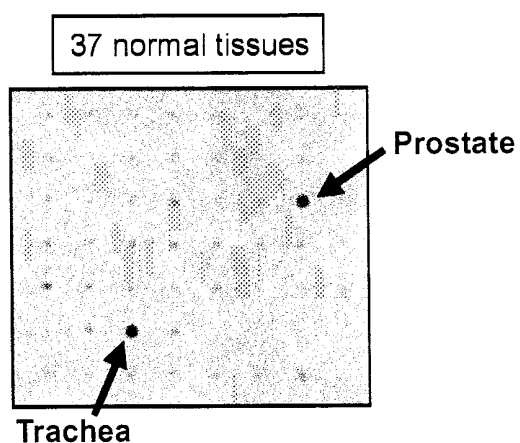
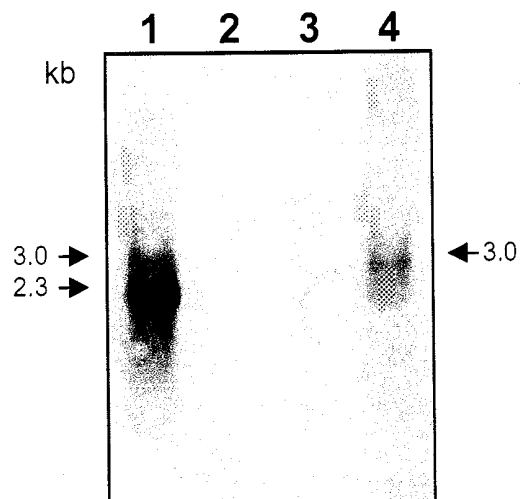


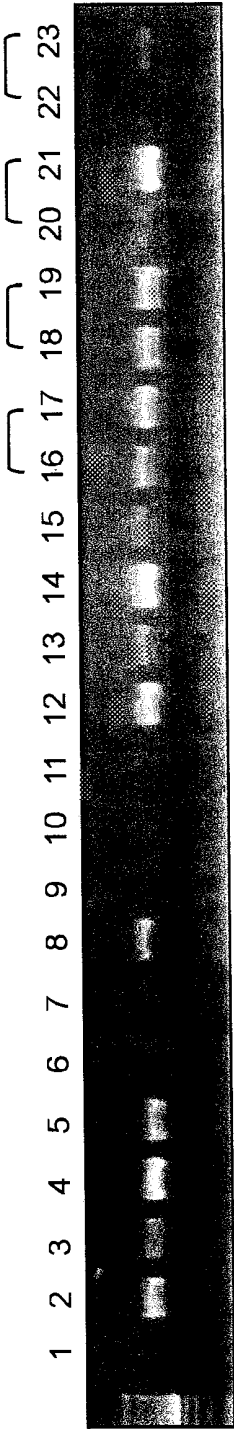
FIG. 4C



1. prostate
2. LAPC-4 AD
3. LAPC-4 AI
4. LAPC-9 AD

FIG. 4D

FIG. 5



Clinical Tissues			
Tissues and Cell Lines	Patients	Gleason Grade and Stage	Extracapsular disease
1. Small Intestine	12. PrCa 711	3+4	No (localized)
2. PrEC	13. PrCa 745	2+3	No (localized)
3. Prostate (pool)	14. PrN 811		
4. Prostate 25	15. PrN 980		
5. Prostate 32	16. PrCa 863	4+5	No (localized)
6. LAPC-4 AD	17. PrN 864	T2c	
7. LAPC-4 AI	18. PrCa 996	PIN	No (localized)
8. LAPC-9 AD	19. PrN 997		
9. LNCaP	20. PrCa 1026	4+3	Yes (extensive invasion, including seminal vesicles)
10. PC3	21. PrN 1027	T3c	Yes
11. DU145	22. PrCa 696	3+5	
	23. PrN 697	T3a	

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FIG. 6-1

5'	10				19				28				37				46				55			
	TGG	AAA	GCA	ACA	AAG	GCA	GTC	GGC	ATA	AAA	ATG	GGT	TCT	CTC	AGC	ACA	GCT	AAC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
											M	G	S	L	S	T	A	N						
			64			73			82			91			100			109						
	GTT	GAA	TTT	TGC	CTT	GAT	GTG	TTC	AAA	GAG	CTG	AAC	AGT	AAC	AAC	ATA	GGA	GAT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	V	E	F	C	L	D	V	F	K	E	L	N	S	N	N	I	G	D						
			118			127			136			145			154			163						
	AAC	ATC	TTC	TTT	TCT	TCG	CTG	AGT	CTG	CTT	TAT	GCT	CTA	AGC	ATG	GTC	CTC	CTT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	N	I	F	F	S	S	L	S	L	L	Y	A	L	S	M	V	L	L						
			172			181			190			199			208			217						
	GGT	GCC	AGG	GGA	GAG	ACT	GCA	GAG	CAA	TTG	GAG	AAG	GTG	CTT	CAT	TTT	AGT	CAT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	G	A	R	G	E	T	A	E	Q	L	E	K	V	L	H	F	S	H						
			226			235			244			253			262			271						
	ACT	GTA	GAC	TCA	TTA	AAA	CCA	GGG	TTC	AAG	GAC	TCA	CCT	AAG	TGC	AGC	CAA	GCT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	T	V	D	S	L	K	P	G	F	K	D	S	P	K	C	S	Q	A						
			280			289			298			307			316			325						
	GGA	AGA	ATT	CAT	TCC	GAG	TTT	GGT	GTC	TAA	TTC	TCT	CAA	ATC	AAC	CAG	CCA	GAC						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	G	R	I	H	S	E	F	G	V	*	F	S	Q	I	N	Q	P	D						
			334			343			352			361			370			379						
	TCT	AAC	TGT	ACC	CTC	AGC	ATT	GCC	AAC	AGG	CTC	TAC	GGG	ACA	AAG	ACG	ATG	GCA						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	S	N	C	T	L	S	I	A	N	R	L	Y	G	T	K	T	M	A						
			388			397			406			415			424			433						
	TTT	CAT	CAG	CAA	TAT	TTA	AGC	TGT	TCT	GAG	AAA	TGG	TAT	CAA	GCC	AGG	TTG	CAA						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	F	H	Q	Q	Y	L	S	C	S	E	K	W	Y	Q	A	R	L	Q						
			442			451			460			469			478			487						
	ACT	GTG	GAT	TTT	GAA	CAG	TCT	ACA	GAA	GAA	ACG	AGG	AAA	ACG	ATT	AAT	GCT	TGG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	T	V	D	F	E	Q	S	T	E	E	T	R	K	<u>I</u>	I	N	A	W						
			496			505			514			523			532			541						
	GTT	GAA	AAT	AAA	ACT	AAT	GGA	AAA	GTC	GCA	AAT	CTC	TTT	GGA	AAG	AGC	ACA	ATT						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	V	E	N	K	T	N	G	K	V	A	N	L	F	G	K	S	T	I						
			550			559			568			577			586			595						
	GAC	CCT	TCA	TCT	GTA	ATG	GTC	CTG	GTG	AAT	GCC	ATA	TAT	TTC	AAA	GGA	CAA	TGG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	D	P	S	S	V	M	V	L	V	N	<u>A</u>	I	Y	F	K	G	Q	W						
			604			613			622			631			640			649						
	CAA	AAT	AAA	TTT	CAA	GTA	AGA	GAG	ACA	GTT	AAA	AGT	CCT	TTT	CAG	CTA	AGT	GAG						
	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
	Q	N	K	F	Q	V	R	E	T	V	K	S	P	F	Q	L	S	E						

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FIG. 6-2

658	667	676	685	694	703
GTA AGT ATT TTA TTT TCA GAC TCA TGA CAA ATG TTG GAG GAT ACA ATA ATC ATT					
V S I L F S D S * Q M L E D T I I I					
712	721	730	739	748	757
TAA GGA CAA TTT AGA AAG ATG TAG TGA TTT AGT GAA AAT ATT GGT CTA GGT TTC					
* G Q F R K M * * F S E N I G L G F					
766	775	784	793	802	811
TGT TGG TTC TTT TTA TTG TAT TTT CTA CAG ATT TTC ATT TTT CCT TTA TTA AGT					
C W F F L L Y F L Q I F I F P L L S					
820	829	838	847	856	865
GAC AAT AAC TTT TAT CAC AGA GCA CCT AAC TGG AGA CTT GGG ATA CTA AGA TTC					
D N N F Y H R A P N W R L G I L R F					
874	883	892	901	910	919
TCT GGC AGA GGA GAA AAC CCA TTC TTT TCT TAA CGC TCT CTC GGC TTA TTC TTT					
S G R G E N P F F S * R S L G L F F					
928	937	946	955	964	973
CCA TAT ATA CTG TGG TTA TGT TCT CCA GCA GCA CAC TAA GGC TAT CTG TGT TAT					
P Y I L W L C S P A A H * G Y L C Y					
982	991	1000	1009	1018	1027
TTT TTT TTC TAG AGA GTT TCT TGA GGA AAG ATT AAA AAG AAA ATG ATT TAA TGA					
F F F * R V S * G K I K K K M I * *					
1036	1045	1054	1063	1072	1081
TAT ATC CTT TTT CTT CCC ACT AAA ATC ATG TTA GCT AAA AAT CCT GAC TTT GTT					
Y I L F L P T K I M L A K N P D F V					
1090	1099	1108	1117	1126	1135
TTT GGT AGA CCA AGC TAT CTC TAT ATA TTA TTA GAA CAA TTC AGT CTC TAA CCA					
F G R P S Y L Y I L L E Q F S L * P					
1144	1153	1162	1171	1180	1189
TGA CTT ATT TTA AAT TAA AAA AAT GGA TAA CCT TTG CAA AGG GAA GTA ATA AGG					
* L I L N * K N G * P L Q R E V I R					
1198	1207	1216	1225	1234	1243
AAT CTA CTT TGT AGT TTC TAT TTT ACC CAT GCC TTT AGA GTG TTC ATG CAG ATA					
N L L C S F Y F T H A F R V F M Q I					
1252	1261	1270	1279	1288	1297
TCC GTG TTA CGC AAG GTA ATC AGT ACA CAC ACA TGT GCA CTC ACA TAT GTA AGT					
S V L R K V I S T H T C A L T Y V S					

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FIG. 6-3

1306	1315	1324	1333	1342	1351
ATA CTA TAA TCT TTT TCT TCT TAA CAG GGT AAA AAT GTA ACT GTG GAA ATG ATG					
I L * S F S S * Q G	K N V T V E M M				
1360	1369	1378	1387	1396	1405
TAT CAA ATT GGA ACA TTT AAA CTG GCC TTT GTA AAG GAG CCG CAG ATG CAA GTT					
Y Q I G T F K L A F V K E P Q M Q V					
1414	1423	1432	1441	1450	1459
CTT GAG CTG CCC TAC GTT AAC AAC AAA TTA AGC ATG ATT ATT CTG CTT CCA GTA					
L E L P Y V N N K L S M I I L L P V					
1468	1477	1486	1495	1504	1513
GGC ATA GCT AAT CTG AAA CAG ATA GAA AAG CAG CTG AAT TCG GGG ACG TTT CAT					
G I A N L K Q I E K Q L N S G T F H					
1522	1531	1540	1549	1558	1567
GAG TGG ACA AGC TCT TCT AAC ATG ATG GAA AGA GAA GTT GAA GTA CAC CTC CCC					
E W T S S S N M M E R E V E V H L P					
1576	1585	1594	1603	1612	1621
CGA TTC AAA CTT GAA ACT AAG TAT GAG CTA AAT TCC CTG TTA AAA TCT CTA GGG					
R F K L E I K Y E L N S L L K S L G					
1630	1639	1648	1657	1666	1675
GTG ACA GAT CTC TTC AAC CAG GTC AAA GCT GAT CTT TCT GGA ATG TCA CCA ACC					
V T D L F N Q V K A D L S G M S P T					
1684	1693	1702	1711	1720	1729
AAG GGC CTA TAT TTA TCA AAA GCC ATC CAC AAG TCA TAC CTG GAT GTC AGC GAA					
K G L Y L S K A I H K S Y L D V S E					
1738	1747	1756	1765	1774	1783
GAG GGC ACG GAG GCA GCA GCA GCC ACT GGG GAC AGC ATC GCT GTA AAA AGC CTA					
E G T E A A A A T G D S I A V K S L					
1792	1801	1810	1819	1828	1837
CCA ATG AGA GCT CAG TTC AAG GCG AAC CAC CCC TTC CTT TTC TTT ATA AGG CAC					
P M R A Q F K A N H P F L F F I R H					
1846	1855	1864	1873	1882	1891
ACT CAT ACC AAC ACG ATC CTA TTC TGT GGC AAG CTT GCC TCT CCC TAA TCA GAT					
T H T N T I L F C G K L A S P *					
1900	1909	1918	1927	1936	1945
GGG GTT GAG CAA GGC TCA GAG TTG CAG ATG AGG TGC AGA GAC AAT CCT GTG ACT					
1954	1963	1972	1981	1990	1999

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FIG. 6-4

TTC	CCA	CGG	CCA	AAA	AGC	TGT	TCA	CAC	CTC	ACA	CAC	CTC	TGT	GCC	TCA	GTT	TGC				

2008				2017				2026				2035				2044				2053	
TCA	TCT	GCA	AAA	TAG	GTC	TAG	GAT	TTC	TTC	CAA	CCA	TTT	CAT	GAG	TTG	TGA	AGC				

2062				2071				2080				2089				2098				2107	
TAA	GGC	TTT	GTT	AAT	CAT	GGA	AAA	AGG	TAG	ATT	TAT	GCA	GAA	AGC	CTT	TCT	GGC				

2116				2125				2134				2143				2152				2161	
TTT	CTT	ATC	TGT	GGT	GTC	TCA	TTT	GAG	TGC	TGT	CCA	GTG	ACA	TGA	TCA	AGT	CAA				

2170				2179				2188				2197				2206				2215	
TGA	GTA	AAA	TTT	TAA	GGG	ATT	AGA	TTT	TCT	TGA	CTT	GTA	TGT	ATC	TGT	GAG	ATC				

2224				2233				2242				2251				2260				2269	
TTG	AAT	AAG	TGA	CCT	GAC	ATC	TCT	GCT	TAA	AGA	AAA	CCA	GCT	GAA	GGG	CTT	CAA				

2278				2287				2296				2305				2314				2323	
CTT	TGC	TTG	GAT	TTT	TAA	ATA	TTT	TCC	TTG	CAT	ATG	TAA	ATA	GAA	TGT	GGT	GAG				

2332				2341				2350				2359				2368				2377	
TTT	TAG	TTC	AAA	ATT	CTC	TGT	TGA	GAA	TAA	TAA	ATG	CAT	GAA	ATA	CCT	TAA	AGC				

2386				2395				2404				2413				2422				2431	
TCT	GTG	AAG	ACT	TGT	AAC	ATG	GCA	GCA	ATC	AAA	TGG	CTT	ATA	AAA	GGA	TAC	TTT				

2440				2449				2458				2467									
GAA	TGT	GGA	TAA	ATT	GAA	TTC	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AA	3'						

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FIG. 7

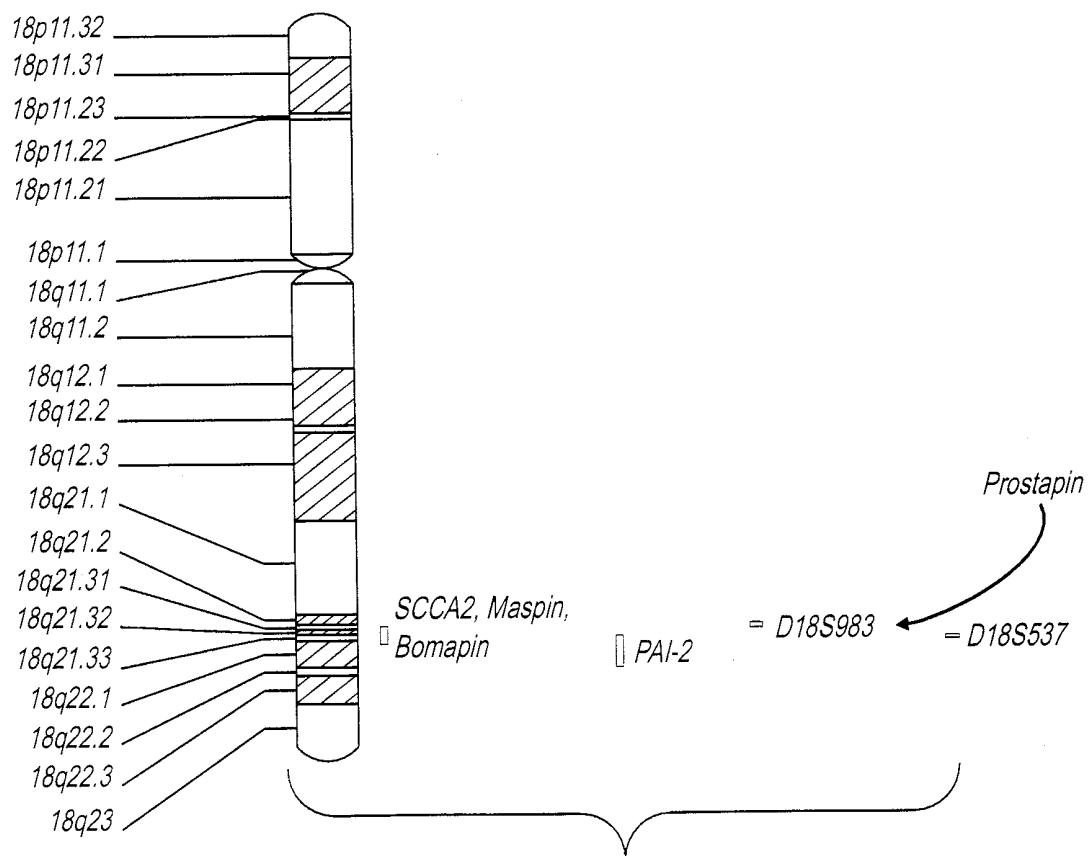
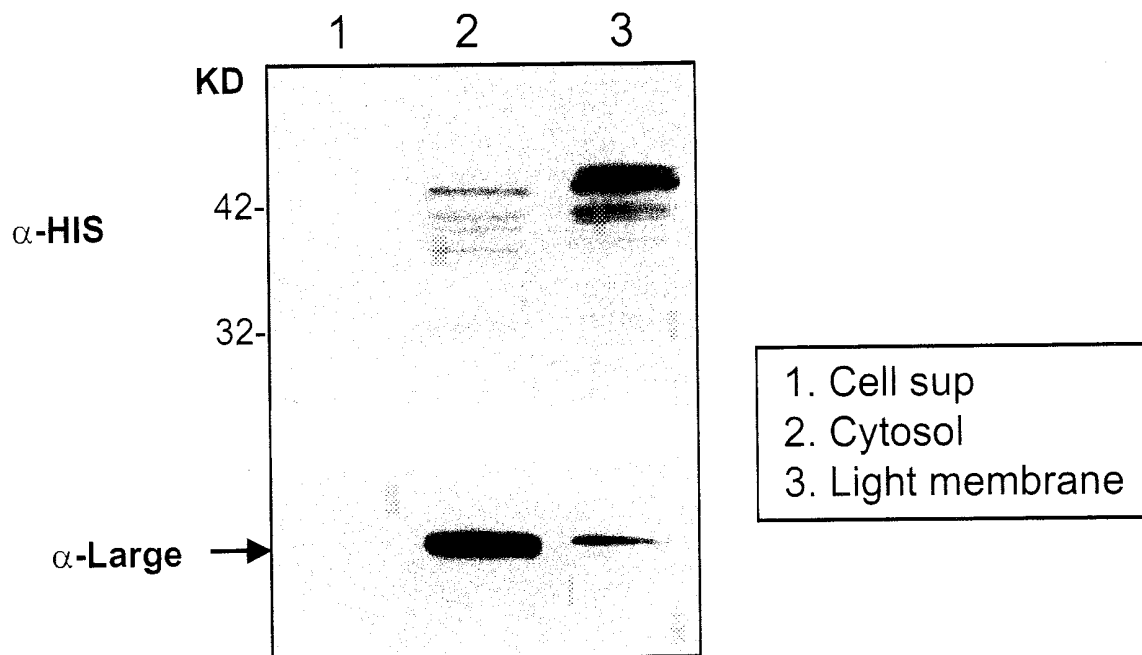


FIG. 8

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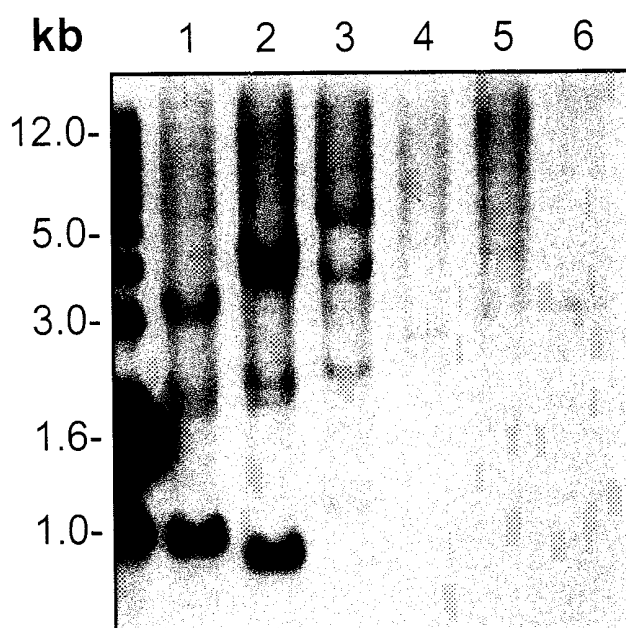
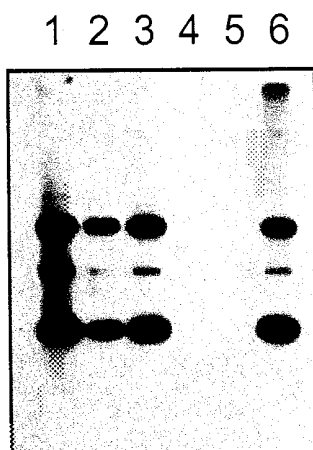


FIG. 9A

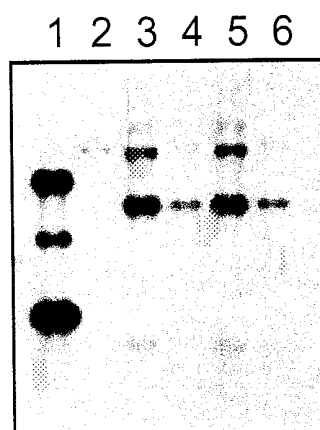
1. Human
2. Monkey
3. Dog
4. Mouse
5. Chicken
6. Drosophila

FIG. 9B



1. 2002H14
2. 2074J2
3. 2100H19
4. hu BAC
5. hu BAC
6. 152i22

FIG. 9C



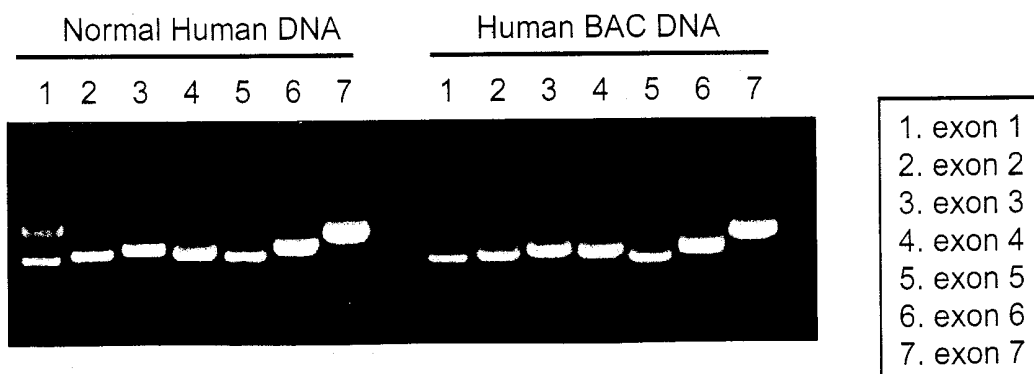
1. 2074J2 (human)
2. 40P22 (human)
3. 74e14 (mouse)
4. 213i3 (mouse)
5. 74e14 (mouse)
6. 213i3 (mouse)

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FIG. 10

	Intron	5' boundary	3' boundary
1.		¹⁶⁸ TTGGAGAAG gtatggaatt.....tcttttctag	¹⁶⁹ GTGCTTCAT Val Leu His
		Leu Glu Lys	
2.		²²⁸ TCACCTAAG gtatgataat.....ctcacctaag	²²⁹ TGCAGCCAA Cys Ser Gln
		Ser Pro Lys	
3.		³⁵⁷ TTTCATCAG gtaagtccat.....tctttcatag	³⁵⁸ CAATATTTA Gln Tyr Leu
		Phe His Gln	
4.		⁴⁷⁵ AAACTAATG gtaaggataa.....tttatacaag	⁴⁷⁶ GAAAAGTC Gln Tyr Leu
		Lys Thr Asn G	
5.		⁵⁷⁹ AAATTTCAA gtaagagaga.....ttcttaacag	⁵⁸⁰ GGTAAAAAT Gly Lys Asn
		Lys Phe Gln	
6.		⁷³⁵ CTGAAACAG gtaaaattat.....tgtctcttag	⁷³⁶ ATAGAAAAG Ile Glu Lys
		Leu Lys Gln	

FIG. 11



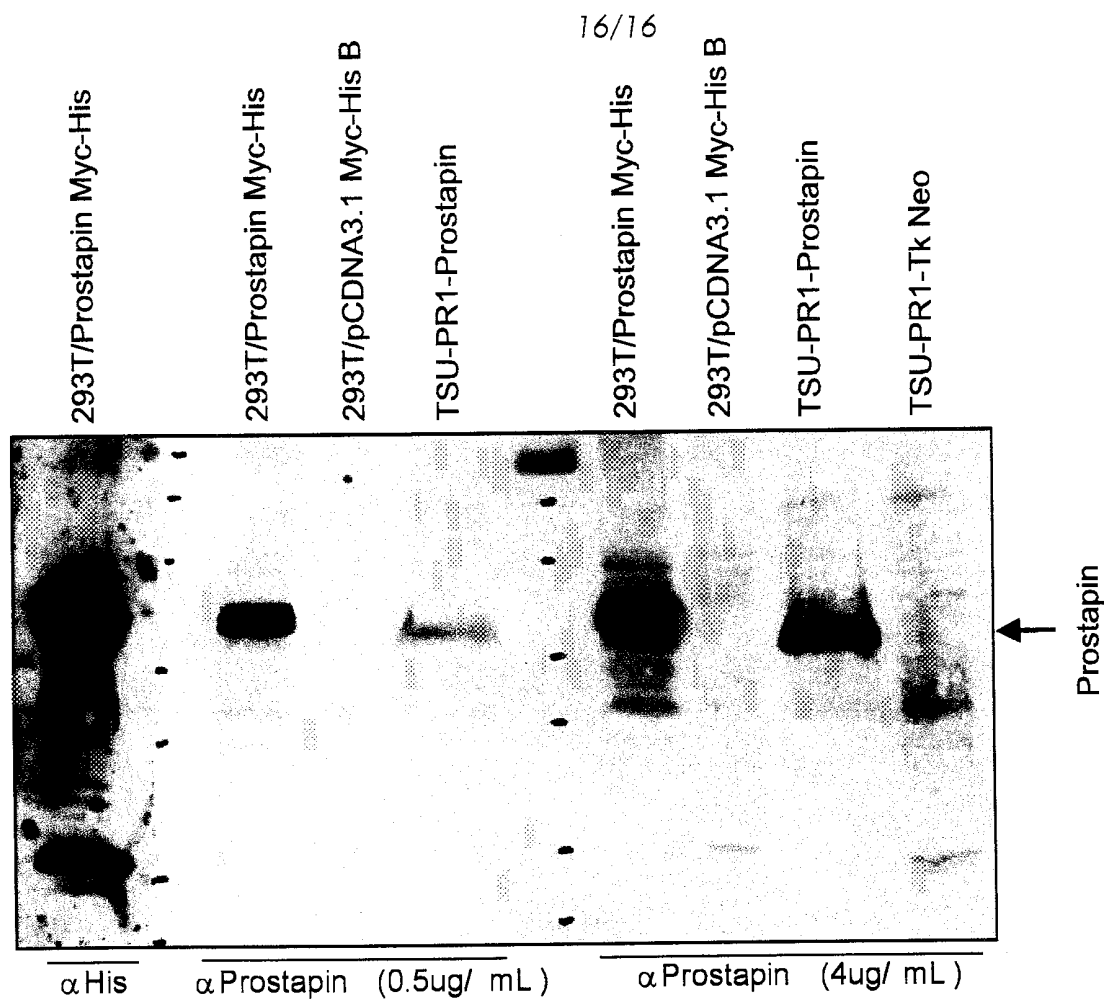


FIG. 12

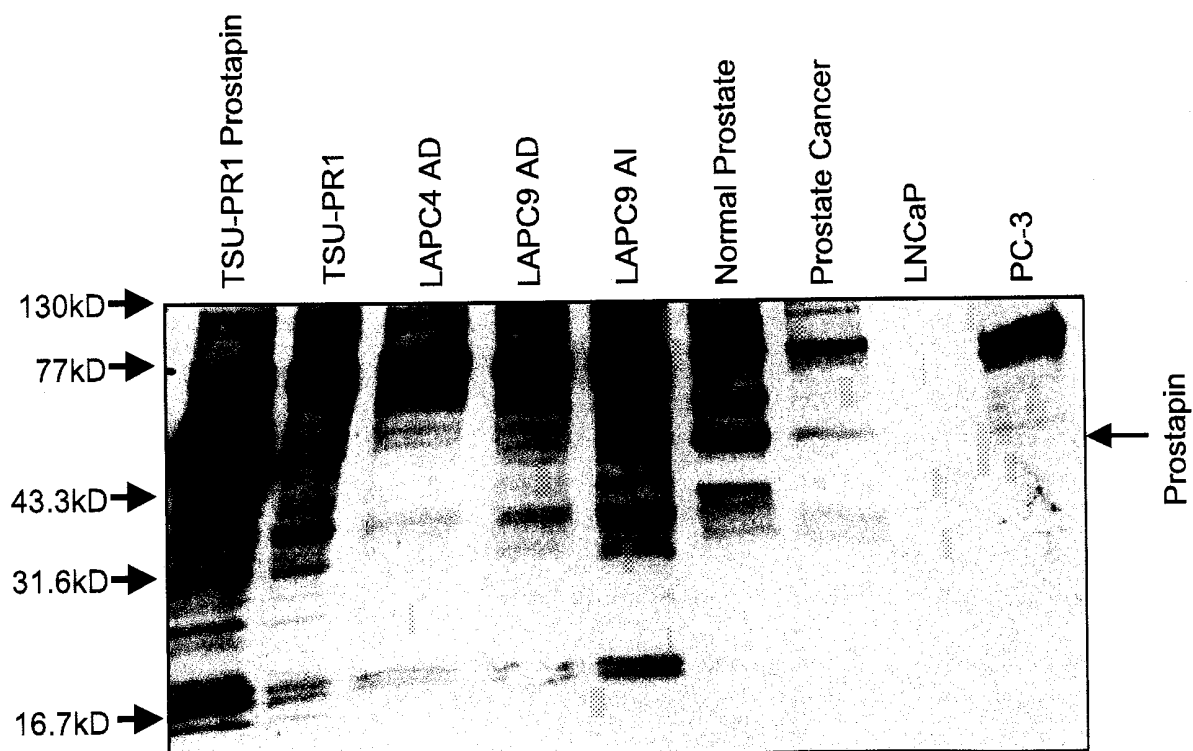


FIG. 13