Method of identifying and treating invasive carcinomas

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(54) METHOD OF IDENTIFYING AND TREATING INVASIVE CARCINOMAS

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(57) ABSTRACT

Prostasin protein has been found to be a useful marker for determination of the invasiveness of and as a means to treat human carcinomas. Using RT-PCR and western blot analyses, prostasin protein and mRNA expression were found in normal human prostate epithelial cells and the human prostate cancer cell line LNCaP, but not in the highly invasive human prostate cancer cell line DU-145. Immunohistochemistry studies of human prostate cancer specimens revealed a down-regulation of prostasin in high-grade tumors. Using RT-PCR and western blot analyses, prostasin protein and mRNA expression were found in a non-invasive human breast cancer cell line, MCF-7, while invasive human breast cancer cell lines MDA-MB-231 and MDA-MB-435s were found not to express either the prostasin protein or the mRNA.


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(57) ABSTRACT

Prostasin protein has been found to be a useful marker for determination of the invasiveness of and as a means to treat human carcinomas. Using RT-PCR and western blot analyses, prostasin protein and mRNA expression were found in normal human prostate epithelial cells and the human prostate cancer cell line LNCaP, but not in the highly invasive human prostate cancer cell lines DU-145 and PC-3. Immunohistochemistry studies of human prostate cancer specimens revealed a down-regulation of prostasin in high-grade tumors. Using RT-PCR and western blot analyses, prostasin protein and mRNA expression were found in a non-invasive human breast cancer cell line, MCF-7, while invasive human breast cancer cell lines MDA-MB-231 and MDA-MB-435s were found not to express either the prostasin protein or the mRNA. A non-invasive human breast cancer cell line, MDA-MB-433, was shown to express prostasin mRNA but not prostasin protein. Transfection of DU-145 and PC-3 cells with a full-length human prostasin cDNA restored prostasin expression and reduced the in vitro invasiveness by 68% and 42%, respectively. Transfection of MDA-MB-231 and MDA-MB-435s cells with a full-length human prostasin cDNA restored prostasin expression and reduced the in vitro invasiveness by 50% for each cell line.

6 Claims, 7 Drawing Sheets
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FIG. 1

Human Prostate Gland 20

Normal or Non-Invasive Cancer

YES! 22

NO! 24 or Reduced 26

Invasive Cancer

Prostasin Serine Protease 14

Normal

NO? 12

YES? 16

Cancer

Human Blood 10

FIG. 2

Human Mammary Gland 200

Non-Invasive Cancer

YES! 220

NO! 240 or Reduced 260

Invasive Cancer

Prostasin Serine Protease 14

Normal

NO? 120

YES? 160

Cancer

Human Blood 100
**FIG. 3**

- **Prostasin 40 kDa**
  - CC-2555
  - LNCaP
  - DU-145
  - PC-3

- **Prostasin 232 bp**

- **β-Actin 556 bp**
FIG. 6

Prostate

1,275 bp
951 bp

Breast

1,275 bp
951 bp

PC-3
DU-145
LNCaP
CC-2555
MDA-435
MDA-231
MDA-453
MCF-7

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FIG. 7

Vector Prostasin Vector Prostasin

40 kDa

DU-145 PC-3

Relative Invasiveness (percentage)

Vector Prostasin Vector Prostasin

p<0.01

p<0.001

FIG. 8

Vector Prostasin Vector Prostasin

40 kDa

MDA-MB-231 MDA-MB-435s

Relative Invasiveness (percentage)
METHOD OF IDENTIFYING AND TREATING INVASIVE CARCINOMAS

This invention relates to prostasin and its use in the diagnosis and treatment of prostate and breast cancers, and claims the priority to U.S. Provisional Application Serial No. 60/174,801 filed Jan. 6, 2000 and was supported in part by the Department of Defense Prostate Cancer Research Grant DAMD17-98-1-8590, and in part by grants to principal investigator K. X. CHAI from the Florida Hospital Gala Endowed Program for Oncologic Research.

BACKGROUND AND PRIOR ART

For men in the U.S., prostate cancer is the most commonly diagnosed cancer, and the second leading cause of cancer-related death (Greenlee R. T., Murray T. Bolden S., Wingo Pa. Cancer statistics, 1999. Ca: a Cancer Journal for Clinicians 2000;50:7-33). Prostate cancers originate as localized lesions; some of these localized lesions will progress to become invasive, migratory and metastatic. Our current understanding of the mechanisms of the prostate cancer invasion process, however, is poor. Our ability to predict the acquisition of invasive potentials by a prostate cancer is limited.

The mechanisms leading to the development of a prostate cancer are complex. Currently, it is believed to be the result of multiple transformation steps from normal prostate glandular cells (Carter H. B. Piantadosi S. Isaacs J. T. Clinical evidence for and implications of the multistep development of prostate cancer. Journal of Urology. 143(4):742-6, 1990). The initial steps result in what are described as prostatic interepithelial neoplastic (PIN) lesions (Isaacs J. T. Molecular markers for prostate cancer metastasis. Developing diagnostic methods for predicting the aggressiveness of prostate cancer. [Review] [92 refs] American Journal of Pathology. 150(5):1511-21, 1997). These PIN lesions may then typically have three different fates based on an assessment of their impact to the patient. The PIN lesions can remain as such, not producing histologically detectable prostate cancer, or further transform into histologically detectable prostate cancer. Most of the histologically detectable prostate cancers will be asymptomatic in the patient and remain non-manifest clinically as many are discovered post-mortem (Carter H. Coffey D. Prostate Cancer: the magnitude of the problem in the United States. In a Multidisciplinary Analysis of Controversies in the Management of Prostate Cancer. (Eds. Coffey D. Rosnick M. Door R. et al.), ppl-9, Scardino P. (Eds. Coffey D. Resnick M. Door R. et al.), ppl-9, 1992), for which no effective treatment exists without the risk of life-threatening or life-altering. For the patients with non-organ-confined prostate cancers (discovered via either biopsy or surgery), undergoing systemic treatment early is essential to the management of their cancer (Yagoda A. Petrylak D. Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer [Review] [63 refs] Cancer. 71(3 Suppl): 1098-109, 1993). Consequently, it will be ideal, both medically and economically, if one could precisely upon early pathological examination of the tumor, which group of patients will have truly organ-confined disease versus which group will have invasive prostate cancer.

Clinical staging of prostate cancer generally depends on the results of three tests that are performed in the following order: a PSA (prostate-specific antigen) blood test as a screening method; DRE (digital rectal examination) for an initial indication of palpable disease; and, a biopsy to obtain samples for histological examination. Prostate cancers, removed either via biopsy or surgery, are graded histologically by the system of Gleason. (Gleason D F. Classification of prostatic carcinomas. Cancer Chemotherapy Reports Partl. 50(3):125-8, 1966), which is an evaluation of how aggressive and how poorly-differentiated the prostate cancers are. The aggressiveness of prostate tumors: of low Gleason scores (<5) is limited; of high Gleason scores (8–10) are highly aggressive; but, for the intermediate Gleason-score (5–7) prostate cancers (76% of prostate tumors), the accuracy of predicting their aggressiveness is poor (Gleason D F. Mellingor G T. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. Journal of Urology. 111 (1):58–64, 1974). Thus, the ability to accurately determine the aggressiveness of these intermediate Gleason-score prostate tumors has remained as a practical challenge to, and a primary goal for, prostate cancer research (Isaacs J. T. Molecular markers for prostate cancer metastasis. Developing diagnostic methods for predicting the aggressiveness of prostate cancer. [Review] [92 refs] American Journal of Pathology. 150(5):1511-21, 1997). Especially with regard to the number of patients (150,000) facing a decision of whether to undergo systemic treatment, the most urgent demand in prostate cancer care is the development of methods to enhance our ability to accurately predict the aggressiveness of the tumors with Gleason scores of 5–7.

It is now commonly believed that cancers occur via multiple transformation steps by accumulating mutations in three classes of genes: proto-oncogenes (Park M. Oncogenes. In The Genetic Basis of Human Cancer (Eds. Vogelstein B. and Kinzler K W), pp205–28, McGraw-Hill Health Professions Divisions, 1998); tumor-suppressor genes (Knuttila S. Aalto Y. Bjorkqvist A M. EL-Rifai W. Hemmer
van Moorselaar R J, Giroldi L A, Ichikawa T, Isaacs J T, Taki­


As for the treatment options of prostate cancer, patients with truly organ-confined prostate cancer can be cured by radical prostatectomy. Patients with non-organ-confined prostate cancers, however, have very low survival rates and the current treatments are largely ineffective (Yagoda A, Perylak D. Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer. [Review] 63 refs Cancer. 71(3 Suppl): 1098–109, 1993).


SUMMARY OF THE INVENTION

The first objective of the present invention is to reduce deficiencies in the prior art with specific regard to differen-
tial diagnosis of invasive prostate and breast cancers and to treatment of invasive and metastatic prostate and breast cancers.

The second objective of the present invention is to provide a new marker for prostate and breast cancer. The third objective of the invention is to provide as a drug to patients with carcinoma of the prostate via delivery of a functional prostasin gene. The fourth objective of the invention is to provide as a drug to patients with carcinoma of the prostate via delivery of a functional prostasin cDNA. The fifth objective of the invention is to provide as a drug to patients with carcinoma of the breast via delivery of a functional prostasin gene. The sixth objective of the invention is to provide as a drug to patients with carcinoma of the breast via delivery of a functional prostasin cDNA.

This invention identifies prostasin serine protease as a potential marker, and as a tumor invasion suppressor for prostate and breast cancers and thus provides methods (a), (b), and (c) of determining invasiveness levels of human carcinomas:

(a) using prostasin protein levels, comprising the steps of: sampling a human carcinoma tissue; determining prostasin protein levels in the human carcinoma tissue; preferably by applying an immunological reagent-antibody to said tissue wherein the reagent-antibody becomes bound to prostasin protein in said tissue, and determining invasiveness of the human carcinoma tissue based on the prostasin protein levels: or

(b) using prostasin mRNA levels, comprising the steps of: sampling a human carcinoma tissue; determining prostasin mRNA levels in the human carcinoma tissue preferably by applying prostasin-specific anti-sense RNA probes in an in situ hybridization to determine the prostasin mRNA levels in the separated human carcinoma tissue the determination of the prostasin mRNA levels in the separated human carcinoma sample tissue; to make possible and determining invasiveness of the human carcinoma tissue based on the prostasin mRNA levels; or

(c) using prostasin gene promoter DNA methylation levels, comprising the steps of: sampling a human carcinoma tissue; determining prostasin gene promoter DNA methylation levels in the human carcinoma tissue preferably by applying prostasin-promoter-specific oligonucleotide primers in a PCR to determine the prostasin gene promoter DNA methylation levels in the sampled human carcinoma tissue and determining invasiveness of the human carcinoma tissue based on the prostasin promoter DNA methylation levels.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Before explaining the disclosed embodiment of the present invention in detail it is to be understood that the invention is not limited in its application to the details of the particular arrangement shown since the invention is capable of other embodiments. Also, the terminology used herein is for the purpose of description and not of limitation.

Using RT-PCR and western blot analyses, prostasin protein and mRNA expression were found in human prostate epithelial cells and the human prostate cancer cell line LNCaP, but discovered not present in the highly invasive human prostate cancer cell lines DU-145 and PC-3.

FIG. 2 is a diagrammatic representation of the analysis of prostasin indicative of the absence or existence, or of the invasiveness of human mammary carcinoma.

FIG. 3 shows human prostasin expression in prostate epithelial cells.

FIG. 4A shows immunohistochemical detection of prostasin protein in benign human prostate tissues.

FIG. 4B is an enlarged view of the boxed region of FIG. 4A.

FIG. 4C shows immunohistochemistry of benign human prostate tissues with prostasin antibody omitted in the procedures, no epithelial cells displayed any staining.

FIG. 4D is an enlarged view of the boxed region of FIG. 4C.

FIG. 4E shows immunohistochemistry of human prostate tumor with prostasin antibody omitted in the procedures, no epithelial cells displayed any staining.

FIG. 4F shows immunohistochemical detection of prostasin protein in Gleason grade 2 prostate tumor.

FIG. 4G shows immunohistochemical detection of prostasin protein in Gleason grade 4 prostate tumor.

FIG. 4H is an enlarged view of the boxed region of FIG. 4G.

FIG. 4I shows immunohistochemical detection of prostasin protein in Gleason grade 3 prostate tumor.

FIG. 4J is an enlarged view of the boxed region of FIG. 4I.

FIG. 4K shows immunohistochemical detection of prostasin protein in Gleason grade 4 prostate tumor.

FIG. 4L shows immunohistochemical detection of prostasin protein in Gleason grade 4 prostate tumor.

FIG. 4M is an enlarged view of the boxed region of FIG. 4K.

FIG. 5 shows human prostasin expression in human breast cancer cell lines.

FIG. 6 shows promoter hypermethylation in human breast cancer cell lines.

FIG. 7 shows prostasin protein expression and in vitro invasive properties of the DU-145 and the PC-3 transfectants.

FIG. 8 shows prostasin protein expression and in vitro invasive properties of the MDA-MB-231 and the MDA-MB-435s transfectants.

BRIEF DESCRIPTION OF THE FIGURE

FIG. 1 is a diagrammatic representation of the analysis of prostasin indicative of the absence or existence, or of the invasiveness of human prostate carcinoma.
were also discovered not to express either the prostasin protein or the mRNA. A non-invasive human breast cancer cell line, MDA-MB-453, was shown to express prostasin mRNA but not prostasin protein. Examination of the prostasin gene promoter in the human prostate and breast cancer cell lines by Southern blot analysis revealed heterogeneous methylation of the promoter in DU-145, PC-3 and MDA-MB-453 cells, and homogeneous methylation of the promoter in MDA-MB-231 and MDA-MB-435s cells. The prostasin gene promoter in normal human prostate epithelial cells, the LNCaP and the prostasin gene promoter in the human prostate and breast cancer cell lines by Southern blot analysis revealed heterogeneous methylation of the promoter in DU-145, PC-3 and MDA-MB-453 cells, and homogeneous methylation of the promoter in MDA-MB-231 and MDA-MB-435s cells. The prostasin gene promoter in normal human prostate epithelial cells, the LNCaP and the prostasin gene promoter in the human prostate and breast cancer cell lines by Southern blot analysis revealed heterogeneous methylation of the promoter in DU-145, PC-3 and MDA-MB-453 cells, and homogeneous methylation of the promoter in MDA-MB-231 and MDA-MB-435s cells. The prostasin gene promoter in normal human prostate epithelial cells, the LNCaP and the MCF-7 cells was shown to be unmethylated. Transfection of MDA-MB-231 and MDA-MB-435s cells with a full-length human prostasin cDNA restored prostasin expression and reduced the in vitro invasiveness by 68% and 42%, respectively. Transfection of MDA-MB-231 and MDA-MB-435s cells with a full-length human prostasin cDNA restored prostasin expression and reduced the in vitro invasiveness by 50% for either cell line. Cell proliferation was unaffected by re-expression of prostasin. Our data indicate that prostasin is implicated in normal prostate biology and its down-regulation in prostate cancer, and its absence in invasive prostate and breast cancer cell lines indicates increased invasiveness. Our results also indicate that delivering a functional human prostasin gene to invasive prostate and breast cancers can reduce the invasiveness.

For a facile understanding of the invention embodied herein, reference should now be made to FIGS. 1 and 2. FIG. 1 sets forth a schematic for determining if a male has prostate cancer. Human blood 100 is taken from the male and analyzed for the presence of prostasin serine protease 14. If NO 12 prostasin is found, there is probably little or no cancer. If there is a presence YES 16 of prostasin serine protease 14, there is prostate cancer.

If a biopsy of the prostate gland 20 is analyzed for the presence of a normal amount YES 22 of prostasin serine protease 14, there is no cancer or there is non-invasive cancer. If there is NO 24 or Reduced 26 amount of prostasin serine protease 14, there is invasive cancer.

FIG. 2 sets forth a schematic for determining if a female has breast cancer. Human blood 100 is taken from the female and analyzed for the presence of prostasin serine protease 140. If NO 120 prostasin is found, is there is prospectively no cancer. If there is a presence YES 160 of prostasin serine protease 140, there is breast cancer. If a biopsy of the breast 200 is analyzed for the presence of YES 220 of prostasin serine protease 140, there is no invasive cancer but may be non-invasive cancer. If there is NO 240 or Reduced 260 amount of prostasin serine protease 140, there is invasive cancer.

The levels of prostasin protein in the epithelial cells of the human prostate gland can be used as a diagnostic marker for the potential invasiveness of prostate tumors. The supporting evidence came from our findings that two invasive human prostate cancer cell lines DU-145 and PC-3 do not express prostasin while normal prostate epithelial cells and a non-invasive prostate cancer cell line LNCaP express both the prostasin mRNA and protein.

Refer now to FIG. 3 which shows human prostasin expression in prostate epithelial cells. By means of western blot analysis (upper panel), prostasin (as a 40-kDa band) was detected in normal human prostate epithelial cells (CC-2555) and the LNCaP cells, but not in the DU-145 or PC-3 cells. An equal amount of total protein (100 µg) was loaded for each sample. At the mRNA level, human prostasin mRNA (via a 232-bp amplified DNA band) was detected in normal prostate epithelial cells (CC-2555) and the LNCaP cells, but not in the DU145 or PC-3 cells as analyzed by RT-PCR/Southern blot hybridization (middle panel). Co-amplification of a 556-bp human β-actin message (as shown in the gel photograph in the lower panel) confirmed the quality and the quantity of the RNA applied in each RT-PCR.

Expression of prostasin protein is reduced in high-grade human prostate tumor Prostatectomy specimens from 39 patients (128 sections) were subjected to immunohistochemistry using a prostasin-specific antibody. Overall, in non-tumor or benign prostate epithelia, 89.0% of the examined areas demonstrated positive staining for prostasin protein and 11.0% were considered negative (based on the scoring system used for HercepTest™, DAKO Corporation, Carpinteria, Calif.). In all tumor specimens that were examined, prostasin was detected in 93.3% of the low Gleason grade areas (≤grade 2), 44.4% of Gleason grade 3 areas, 21.1% of Gleason grade 4 areas, but not in Gleason grade 5 areas (data summarized in Table 1). The mean prostasin immunostaining score was found significantly decreased in high-grade prostate tumors as compared to non-tumor areas (ANOVA, p<0.0001).

Representative staining images of non-tumor (benign) areas and prostate tumor areas are shown in FIGS. 4A–4I, which provides immunohistochemical detection of prostasin in tissue. Paraffin-embedded human prostate sections were stained for prostasin protein expression evaluation using a specific antibody as described (Yu J X. Chao L. Chao J. Prostasin is a novel human serine protease from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994). Prostasin positive staining (brown color) was detected in the cytoplasm and apical membrane in non-tumor or benign epithelial cells.

The prostasin protein was detected in the cytoplasm and on the plasma membrane (apical) of benign epithelial cells lining the secretory lumen as well as in the secretion inside the lumen (FIGS. 4A and 4B, score 3, or ++), confirming the results of (Yu J X. Chao L. Chao J. Prostasin is a novel human serine protease from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994). When a pre-immune rabbit serum was used in place of the prostasin antiserum, no staining was observed in either the non-tumor epithelia (FIGS. 4C and 4D) or tumor epithelia (FIG. 4E). Tumor epithelia displayed various degrees of prostasin immunostaining as shown in FIGS. 4F–4I. In Gleason grade 1–2 tumors, moderate prostasin staining is seen in the cytoplasm and on the plasma membrane of some epithelial cells, as well as in the secretion in the lumen (FIGS. 4G and 4H, score 2, or ++). In Gleason grade 3 tumors, a lesser number of epithelial cells displayed the moderate level prostasin staining (FIGS. 4I and 4J). In Gleason grade 4 tumors, most epithelial cells did not show any prostasin staining, while some prostasin staining can be seen in rare, sporadic tumor cells (FIGS. 4K and 4L, as indicated by the arrow, score 0). Genetically, prostate tumors are heterogeneous and multi-focal in nature, in that one patient's gross-anatomy tumor comes from multiple initial lesions which are caused by different initial transformation events and
progress to different stages by different ensuing transformations (Isaacs J T, Bova G S, Prostate Cancer. In The Genetic Basis of Human Cancer (Eds. Vogelstein B and Kinzler K W), pp653-60, McGraw-Hill Health Professions Division, 1998). The Gleason grading, when used as a percentage of each cancer occupied by Gleason grade 4/5 areas, is independent ly associated with prostate cancer progression (Stamey T A, McNeal J E, Yemoto C M, Sigal B M, Johnstone I M. Biological determinants of cancer progression in men with prostate cancer. JAMA 281:1395-1400, 1999). We found a significant decrease of prostasin expression in the high-grade, i.e., the more progressively transformed tumors.

As earlier indicated, FIG. 5 shows human prostasin expression in human breast cancer cell lines. By means of western blot analysis (upper panel), prostasin (as a 40-kDa band) was detected in MCF-7 cells, but not in MDA-MB-453, MDA-MB-231, or MDA-MB-435s cells. An equal amount of total protein (100 µg) was loaded for each sample. At the mRNA level, human prostasin mRNA (via a 232-bp amplified DNA band) was detected in MCF-7 and MDA-MB-453 cells, but not in the MDA-MB-231 or MDA-MB-435s cells as analyzed by RT-PCR/Southern blot hybridization (lower panel).

Prostasin mRNA expression is seen absent in two invasive human breast cancer cell lines while two non-invasive breast cancer cell lines express prostasin mRNA or protein. Analysis of prostasin expression in human breast cancer cell lines showed that the non-invasive MCF-7 and MDA-MB-453 cells express the prostasin mRNA while the highly invasive MDA-MB-231 and MDA-MB-435s cells do not express the prostasin mRNA (see FIG. 5). Expression of prostasin mRNA in normal human breast can be demonstrated by the presence of two GenBank™ normal human breast EST sequences coding for prostasin (Accession numbers R48653, and R48557). The MCF-7 cells also express the prostasin protein as determined by western blot analysis (again see FIG. 5).

Prostasin down-regulation in prostate or breast cancer can be caused by promoter methylation or gene-specific mutation. Prostasin expression decreases with increasing prostate cancer grade and is absent in invasive prostate and breast cancer cell lines. The chromosomal locus where the human prostasin gene is, 16pll.2, however, is not known to be an LOH hot-spot in prostate cancer or in breast cancer. Epigenetic events (such as DNA methylation) may be an alternative mechanism of loss of expression for tumor suppressors or invasion suppressors. Refer now to FIG. 6 which shows the expression of human prostasin in human prostate and breast cancer cell lines. Genomic DNA (5 µg) from the various cell lines (as indicated in the figure) were digested with the following restriction enzyme combinations, Xho I/BamH I (X/B), flanking cuts of the methylation-sensitive site), Xho I/BamH I/Msp I (X/B/M), or Xho I/BamH I/Hpa II (X/B/H). The digests were resolved in 0.8% agarose gel and transferred to an Immobilon-N membrane for hybridization with a nick-translated prostasin promoter probe (bases 703-1469 of the prostasin gene sequence U33446). The probe detects a fragment of 1,275 bp, which is cut by the methylation-insensitive enzyme Msp I to yield a 1,052-bp fragment for all DNA samples. The methylation-sensitive isoschizomere Hpa II yields the 1,052-bp fragment in the CC-2555 (normal prostate epithelial), the LNCaP, and the MCF-7 samples, indicating the hypomethylated or unmethylated state of the prostasin promoter in these cells. For DU-145, PC-3, and MDA-MB-453 DNA, both the 1,052-bp and the 1,275-bp fragments are generated in the methylation-sensitive digestion, suggesting incomplete methylation (one of two or more chromosomes) or clonal methylation in a sub-population of cells. For the MDA-MB-231 and MDA-MB-435, however, the Hpa II digestion did not yield the 1,052 bp but rather gave the 1,275-bp fragment. This homogeneous methylation pattern indicates that the Msp I/Hpa II site, a promoter methylation pattern (see FIG. 5). Expression of prostasin in our RT-PCR/Southern blot analysis in the DU-145 and PC-3 cells argues against this possibility.

The significance of the finding on prostasin gene promoter hypermethylation in prostate or breast cancer is that the measurement of prostasin down-regulation as a cancer marker may be achieved by using a binary assay (yes-or-no), instead of a gradually decreasing quantity in the immuno-histochemistry assay (which is quite arbitrary).

Re-expression of human prostasin protein in invasive human prostate and breast cancer cells reduces invasiveness in vitro. At this point, reference should be made to FIG. 7 which shows the expression of human prostasin protein in two (or more) chromosome 16's of these cell lines is methylated at the prostasin gene locus. The prostasin gene on the unmethylated chromosome may contain mutations that silenced the expression. An alternative explanation for the heterogeneous methylation pattern is that the methylation occurs in clonal cell populations, however, the lack of detectable prostasin mRNA in our RT-PCR-Southern blot analysis in the DU-145 and PC-3 cells argues against this possibility.

It can be seen that Polygonal DU-145 and PC-3 cells transfected with the human prostasin cDNA (designated
DU-145/Pro, and PC-3/Pro, respectively) were confirmed to express the human prostasin protein, as shown in the western blot analysis of the cell lysate (FIG. 7, upper panel). The vector-transfected cells, designated DU-145/Vect or PC-3/Vect, respectively, were used as negative control in the western blot. A further examination of the DU-145/Pro and the PC-3/Pro cells by immunocytochemistry confirmed that 100% of the cells expressed the prostasin protein (data not shown). In vitro Matrigel chemoinvasion assays (FIG. 7, lower panel), the invasiveness of DU-145/Pro cells was determined to be at 32% of that of DU-145/Vect cells (or, the reduction of invasiveness was at 68%). The invasiveness of PC-3/Pro cells was determined to be at 58% of that of PC-3/Vect cells (or, the reduction of invasiveness was at 42%). We performed in vitro cell proliferation assays on DU-145/Pro vs. DU-145/Vect cells, and on PC-3/Pro vs. PC-3/Vect cells, but did not observe any difference between the growth rates of the prostasin cDNA-transfected or the vector-transfected cells over an 8-day period (data not shown).

Forced re-expression of human prostasin in two invasive human breast cancer cell lines reduced invasiveness. A full-length human prostasin cDNA under the control of an RSV promoter was transfected into the invasive breast cancer MDA-MB-231 and MDA-MB-435s cells. Reference should be made to FIG. 8 which shows prostasin protein expression and in vitro invasive properties of the MDA-MB-231 and the MDA-MB-435s transfectants. MDA-MB-231 and MDA-MB-435s cells transfected with either a vector DNA (labeled as “vector”) or a prostasin cDNA construct (labeled as “prostasin”) were analyzed by a western blot analysis using a prostasin-specific antibody (upper panel) or subjected to an in vitro Matrigel chemoinvasion assay (lower panel) as referenced in (Liu D F. Rabbanl S A. Induction of urinary plasminogen activator by retin acid results in increased invasiveness of human prostate cancer cells PC-3. Prostate. 27(5):269-76, 1995). The expressed human prostasin protein (a 40-kDa band) was detected in the prostasin cDNA-transfected MDA-MB-231 and MDA-MB-435s, but not in the vector-transfected cells. In the Matrigel chemoinvasion assay, the vector-transfected cells are expressed as being 100% invasive (solid bar), the open bar represents the relative invasiveness of the human prostasin cDNA-transfected cells. The data were analyzed by a Student t-test using the Stat view software (Abacus Concepts, Inc., Berkeley, Calif.).

Stable, polyclonal, episomal transfectants were obtained and the expression of human prostasin protein was confirmed by western blot analysis (FIG. 8, upper panel). In vitro Matrigel chemoinvasion assays, the invasiveness of either cell lines expressing human prostasin was reduced by 50% as compared to the vector-transfected controls (FIG. 8, lower panel).

Taken together, the foregoing evidences linking prostasin level reduction or prostasin absence to the invasiveness of prostate and breast cancer cell lines, or linking prostasin expression to reduced invasiveness. The evidence qualifies prostasin as an invasion suppressor, which thus is a marker for diagnosis of invasiveness of prostate and breast cancers, or as a therapeutic agent to treat invasive prostate and breast cancers.

Pathological grading by the Gleason system is performed after either surgery or biopsy, both highly invasive procedures, while blood tests such as that for the PSA prostate cancer marker can offer the hope of accurate diagnosis and prognosis without the harm of an invasive procedure. In practice, however, single markers suffer from an intrinsic limitation that the “positive” identifications are not always confirmed for the “diagnosed” disease. Biopsy is still required for a true positive identification of prostate cancer even in the case of the application of the PSA prostate cancer marker (Catalona W J. Partin A W. Slawin K M. Brewer M K. Flanagan R C. Patel A. Richie J P. deKernion J B. Walsh P C. Scardino P T. Lange P H. Subong E N. Parson R E. Gasior G H. Loveland K G. Southwick P C. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial [see comments]. JAMA. 270(19):1542–7, 1998). As stated above, it has been demonstrated in principle that many markers used in a multivariate approach may provide a highly accurate diagnosis (Greene G F. Kitai Y. Pettiaway C A. von Eschenbach A C. Bucana C D. Fidler I J. Correlation of metastasis-related gene expression with metastatic potential in human prostate carcinoma cells implanted in nude mice using an in situ messenger RNA hybridization technique. American Journal of Pathology. 150(5):1571–82, 1997). From the standpoint of prostate cancer genetics, the multivariate approach is well supported by our current understanding. A serine protease structurally and genetically related to the PSA, the hK2 (human glandular kallikrein 2) has shown some promise of joining the list of markers applicable for prostate cancer diagnosis (Saedi M S. Hill T M. Kuus-Reichel K. Kumar A. Payne J. Mikolajczyk S D. Wollert R L. Rittenhouse H G. The precursor form of the human kallikrein 2, a kallikrein homologous to prostate-specific antigen, is present in human sera and is increased in prostate cancer and benign prostatic hyperplasia. Clinical Chemistry. 44(10):2115–9, 1998). Structurally and in prostate gland biology, prostasin shares many common characteristics with both PSA and hK2, as being a secreted serine protease made in large abundance in prostate epithelial cells (Yu J X. Chao L. Chao J. Prostasin is a novel human serine protease from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994; Yu J X. Chao L. Chao J. Molecular cloning, tissue-specific expression, and cellular localization of human prostasin mRNA. Journal of Biological Chemistry. 270(22):13483–9, 1995). While high-grade prostate cancer cells produce less PSA protein than normal prostate cells or low-grade prostate cancer cells (Hakalahli L. Vilhko P. Henttu P. Autio-Harmainen H. Soini Y. Vilhko R. Evaluation of PAP and PSA gene expression in prostate hyperplasia and prostatic carcinoma using northern-blot analyses, in situ hybridization and immunohistochemical stainings with monoclonal and biopspecific antibodies. International Journal of Cancer. 55(4):590–7, 1; Sakai H. Yogi Y. Minami Y. Yushita Y. Kanetake H. Saito Y. Prostate specific antigen and prostatic acid phosphatase immunoreactivity as prognostic; Sakai H. Yogi Y. Minami Y. Yushita Y. Kanetake H. Saito Y. Prostate specific antigen and prostatic acid phosphatase immunoreactivity as prognostic), the serum PSA levels in prostate cancer patients increase due to tissue damage caused by invasive cancer (Rittenhouse H G. Finlay J A. Mikolajczyk S D. Partin A W. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. [Review] [457 refs] Critical Reviews in Clinical Laboratory Sciences. 35(4): 275–368, 1998). By comparison, we also believe prostasin is in the forensic field of prostate cancer patients. As a result, a blood test for the circulating prostasin to indicate the presence and/or the stage of prostate cancer would be highly useful. (as illustrated in FIG. 1). In principal, the feasibility of a blood test based on prostasin detection to indicate
cancer has been demonstrated by Berteau et al. (1999). These authors (Berteau P, Laribi Eschwege P, Lebars I, Dumas F, Benoit G, Lorinc S) demonstrated highly promising potential of using prostasin as a marker to detect circulating prostate epithelial cells, a sign of prostate tissue damage caused by invasive prostate cancer leading to the dissemination of prostate epithelial cells into the circulation. It is expected that a blood test for circulating prostasin to indicate the presence and/or the stage of breast cancer would be highly useful (as illustrated in FIG. 2).

In summary of the invention, it has been taught herein that: protein prostasin as well as its mRNA levels and its gene promoter DNA methylation levels can be used to determine the invasiveness level of human carcinomas; and, provide a method of treating invasive human carcinomas by delivery thereto of a recombinant nucleic acid formed by a human prostasin nucleic acid incorporated into a selected gene delivery vector. Those teachings are repeated for emphasis in the following:

1. Immunohistochemistry studies of human prostate cancer specimens revealed a down-regulation of prostasin in high-grade tumors.

2. Using RT-PCR and western blot analyses, prostasin protein and mRNA expression were found in a non-invasive human breast cancer cell line, MCF-7, while invasive human breast cancer cell lines MDA-MB-231 and MDA-MB-453s were found not to express either the prostasin protein or the mRNA. A non-invasive human breast cancer cell line, MDA-MB-453s, was shown to express prostasin mRNA but not prostasin protein: and,

3. Examination of the prostasin gene promoter in the human prostate and breast cancer cell lines by Southern blot analysis revealed heterogeneous methylation of the promoter in DU-145, PC-3 and MDA-MB-453 cells, and homogeneous methylation of the promoter in MDA-MB-231 and MDA-MB-435s cells. The prostasin gene promoter in normal human prostate epithelial cells, the LNCaP and the MCF-7 cells was shown to be unmethylated. Transfection of DU-145 and PC-3 cells with a full-length human prostasin cDNA restored prostasin expression and reduced the in vitro invasiveness by 68% and 42%, respectively. Transfection of MDA-MB-231 and MDA-MB-435s cells with a full-length human prostasin cDNA restored prostasin expression and reduced the in vitro invasiveness by 50% for each cell line.

The preferred methods of separating sampled human carcinoma tissue from neighboring normal tissues is by laser capture micro-dissection.

While the invention has been described, disclosed, illustrated and shown in various terms of certain embodiments or modifications which it has presumed in practice, the scope of the invention is not intended to be, nor should it be deemed to be, limited thereby and such other modifications or embodiments as may be suggested by the teachings herein are particularly reserved especially as they fall within the breadth and scope of the claims here appended. We claim:

1. A method of determining human prostate carcinomas non-invasiveness using prostasin gene promoter DNA methylation levels, comprising the steps of:
   - sampling a human prostate tissue;
   - determining prostasin gene promoter DNA methylation levels in the human prostate tissue; and
   - determining the human prostate cancer is not invasive based on the lack of DNA methylation of the prostasin gene promoter.

2. The method of claim 1, wherein the step of determining gene promoter DNA methylation levels includes:
   - applying prostasin-promoter-specific DNA probes in a southern blot hybridization analysis to determine the prostasin gene promoter DNA methylation levels in the sampled human prostate carcinoma tissue.

3. The method of claim 1, wherein the sampling comprises:
   - separating the human carcinoma tissue from the neighboring normal tissue by laser capture micro-dissection.

4. A method of determining human breast carcinomas non-invasiveness using prostasin gene promoter DNA methylation levels, comprising the steps of:
   - sampling a human breast tissue;
   - determining prostasin gene promoter DNA methylation levels in the human carcinoma tissue; and
   - determining the human prostate cancer is not invasive based on the lack of DNA methylation of the prostasin gene promoter.

5. The method of claim 4, wherein the step of determining gene promoter DNA methylation levels includes:
   - applying prostasin-promoter-specific DNA probes in a southern blot hybridization analysis to determine the prostasin gene promoter DNA methylation levels in the sampled human breast carcinoma tissue.

6. The method of claim 4, wherein the sampling comprises:
   - separating the human carcinoma tissue from the neighboring normal tissue by laser capture micro-dissection.