3-8-2005

Method of identifying and treating invasive carcinomas (CIP)

Xinqing Chai
University of Central Florida

Julie Chao
MUSC Foundation for Research Development

Lee Chao
MUSC Foundation for Research Development

Limei Chen
University of Central Florida

Recommended Citation

Chai, Xinqing; Chao, Julie; Chao, Lee; and Chen, Limei, "Method of identifying and treating invasive carcinomas (CIP)" (2005). *UCF Patents*. 355.
https://stars.library.ucf.edu/patents/355

US006864093B1

(12) **United States Patent**
Chai et al.

(10) **Patent No.: US 6,864,093 B1**
(45) **Date of Patent: Mar. 8, 2005**

(54) **METHOD OF IDENTIFYING AND TREATING INVASIVE CARCINOMAS**

(75) Inventors: **Karl X. Chai**, Winter Springs, FL (US); **Li-Mei Chen**, Winter Springs, FL (US); **Lee Chao**, Mt. Pleasant, SC (US); **Julie Chao**, Mt. Pleasant, SC (US)

(73) Assignee: **University of Central Florida**, Orlando, FL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 90 days.

(21) Appl. No.: **10/235,255**

(22) Filed: **Sep. 5, 2002**

Related U.S. Application Data

(63) Continuation of application No. 09/755,811, filed on Jan. 5, 2001, now Pat. No. 6,569,684.

(60) Provisional application No. 60/174,801, filed on Jan. 6, 2000.

(51) **Int. Cl.**[7] **G01N 33/48**; A61K 49/00; C07H 21/02; C07H 21/04

(52) **U.S. Cl.** **436/64**; 424/9.1; 536/23.1; 536/23.5; 536/24.3

(58) **Field of Search** 435/4, 6, 7.1, 7.21, 435/7.23, 40.5, 40.52, 69.1, 325; 436/64; 424/94.1, 94.64; 536/23.1, 23.5, 24.1, 24.3, 24.31

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,686,419 A	11/1997	Powers et al.	514/18
6,043,033 A	3/2000	Bandman et al.	435/6
6,048,970 A	4/2000	Lal et al.	536/23.5
6,075,136 A	6/2000	Tang et al.	536/23.1
6,090,559 A	7/2000	Russell et al.	435/6
6,090,786 A	7/2000	Augustyns et al.	514/19
6,107,049 A	8/2000	Allard et al.	435/7.1

OTHER PUBLICATIONS

Chen et al., International Journal of Cancer, vol. 97 No. 3, ages 323–29 (Jan. 2002).*

Akazaki K. Stemmerman GN. Comparative study of latent carcinoma of the prostate among Japanese in Japan and Hawaii. Journal of the National Cancer Institute. 50(5):1137–44, 1973.

Berteau P. Laribi A. Eschwege P. Lebars I. Dumas F. Benoit G. Lorie S. Prostasin mRNA to detect prostate cells in blood of cancer patients. Clinical and Chemical Laboratory Medicine 37 (SS): S119, 1999.

Bussemakers MJ van Moorselaar RJ. Giroldi LA. Ichikawa T. Isaacs JT. Takeichi M. Debruyne FM. Schalken JA. Decreased expression of E–cadherin in the progression of rat prostatic cancer. Cancer Research. 52(10):2916–22, 1992.

Catalona WJ. Partin AW. Slawin KM. Brawer MK. Flanigan RC. Patel A. Richie JP. deKernion JB. Walsh PC. Scardino PT. Lange PH. Subong EN. Parson RE. Gasior GH. Loveland KG. Southwick PC. Use of the percentage of free prostate–specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trail [see comments]. JAMA. 279(19):1542–7, 1998.

Dong JT. Lamb PW. Rinker–Schaeffer CW. Vukanovic J. Ichikawa T. Isaacs JT. Barrett JC. KA11, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2 [see comments]. Science. 268(2512):884–6, 1995.

Dong JT. Suzuki H. Pin SS. Bova GS. Schalken JA. Isaacs WB. Barrett JC. Isaacs JT. Down–regulation of the KA11 metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Research. 56(19):4387–90, 1996.

Dunn JE. Cancer epidemiology in populations of the United States—with emphasis on Hawaii and California—and Japan. Cancer Research. 35(11 Pt. 2):3240–5, 1975.

Fornaro M. Tallini G. Bofetiado CJ Bosari S. Languino LR. Down–regulation of beta 1C integrin, an inhibitor of cell proliferation, in prostate carcinoma. American Journal of Pathology. 149(3):765–73, 1996.

(List continued on next page.)

Primary Examiner—James Housel
Assistant Examiner—Zachariah Lucas
(74) *Attorney, Agent, or Firm*—Brian S. Steinberger; Joyce P Morlin; Frances L. Olmsted

(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. Imunohistochemistry 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-453, 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 either cell line. The prostasin gene promoter region was found to be hypermethylated at specific sites in invasive cancer cells.

7 Claims, 9 Drawing Sheets

OTHER PUBLICATIONS

Fornaro M. Manzotti M. Tallini G. Slear AE. Bosari S. Ruoslahti E. Languino LR. Beta1C integrin in epithelial cells correlates with a nonproliferative phenotype: forced expression of beta1C inhibits prostate epithelial cell proliferation. American Journal of Pathology. 153(4):1079–87, 1998.

Fornaro M. Tallini G. Zheng DQ. Flanagan WM. Manzotti M. Languino LR. p27(kip1) acts as a downstream effector of and its coexpressed with the beta1C integrin in prostatic adenocarcinoma. Journal of Clinical Investigation. 103(3):321–9, 1999.

Gao AC. Lou W. Dong JT. Isaacs JT. CD44 is a metastasis suppressor gene for prostatic cancer located on human chromosome 11p13. Cancer Research. 57(5):846–9, 1997.

Goyal J, Smith KM, Cowan JM. Wazer DE. Lee SW. Band V. The role for NESI serine protease as a novel tumor suppressor. Cancer Res 58:4782–4786, 1998.

Greene GF. Kitadai Y. Pettaway CA. von Eschenbach AC. Bucana CD. Fidler IJ. 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.

Greenlee RT. Murray T. Bolden S. Wingo PA. Cancer statistics. 1999. Ca: a Cancer Journal for Clinicians 2000: 50:7–33.

Hooper JD. Nicol DL. Dickinson JL. Eyre HJ. Searman AL. Normyle JF. Stuttgen MA. Douglas ML. Loveland KA. Sutherland GR. Antalis TM. Testisin. a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. Cancer Research. 59(13):3199–205, 1999.

Isaacs JT. 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.

Isaacs JT. Bova GS. Prostate Cancer. In *The Genetic Basis of Human Cancer* (Ed. Vogelstein B and Kinzler KW), pp653–60, McGraw–Hill Health Professions Division. 1998.

Knuutila S. Aalto Y. Autio K. Bjorkqvist AM. El–Rifai W. Hemmer S. Huhta T. Kettunen E. Kiuru–Kuhlefeit S. Larramendy ML. Lushnikova T. Monni O. Pere H. Tapper J. Tarkkanen M. Varis A. Wasenius VM. Wolf M. Zhu Y. DNA copy number losses in human neoplasms. [Review] [197 refs] American Journal of Pathology. 155(3):683–94, 1999.

Liu DF. Rabbani SA. Induction of urinary plasminogen activator by retinoic acid results in increased invasiveness of human prostate cancer cells PC–3, Prostate. 27(5):269–76, 1995.

Lou W. Krill D. Dhir R. Becich MJ. Dong JT. Frierson HF Jr. Isaacs WB. Isaacs JT. Gao AC. Methylation of the CD44 metastasis suppressor gene in human prostate cancer. Cancer Research. 59(10):2329–31, 1999.

Lou J. Lubaroff DM. Hendrix MJ. Suppression of prostate cancer invasive potential and matrix metalloproteinase activity by E–cadherin transfection. Cancer Research. 59(15):3552–6, 1999.

Mirchandani D. Zheng J. Miller GJ. Ghosh AK. Shibata DK. Cote RJ. Roy–Burman P. Heterogeneity in intratumor distribution of p53 mutations in human prostate cancer. American Journal of Pathology, 147(1):92–101, 1995.

Nelson PS. Gan L. Ferguson C. Moss P. Gelinas R. Hood L. Wang K. Molecular cloning and characterization of prostase, an androgen–regulated serine protease with prostate–restricted expression. Proceedings of the National Academy of Sciences of the United States of America, 96(6):3114–9, 1999.

Noordzij MA. van Steenbrugge GJ. Verkaik NS. Schroder FH. van der Kwast TH. The prognostic value of CD44 isoforms in prostate cancer patients treated by radical prostatectomy. Clinical Cancer Research. 3(5):805–15, 1997.

Qian J. Bostwick DG. Takahashi S. Borell TJ. Herath JF. Lieber MM. Jenkins RB. Chromosomal anomalies in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. Cancer Research. 55(22):5408–14, 1995.

Saedi MS. Hill TM. Kuus–Reichel K. Kumar A. Payne J. Mikolajczyk SD. Wolfert RL. Rittenhouse HG. 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.

Sakr WA. Macoska JA. Benson P. Grignon DJ. Wolman SR. Pontes JE. Crissman JD. Allelic loss in locally metastatic, multisampled prostate cancer. Cancer Research, 54(12):3273–7, 1994.

Stamey TA. McNeal JE. Yemoto CM. Sigal BM. Johnstone IM. Biological determinants of cancer progression in men with prostate cancer. JAMA 281:1395–1400, 1999.

Tsihlias J. Kapusta LR. DeBoer G. Morava–Protzner I. Zbieranowski I. Bhattacharya N. Catzavelos GC. Klotz LH. Slingerland JM. Loss of cyclin–dependent kinase inhibitor p27Kipl is a novel prognostic factor in localized human prostate adenocarcinoma. Cancer Research. 58(3):542–8, 1998.

Ueda T. Ichikawa T. Tamaru J. Mikata A. Akakura K. Akimoto S. Imai T. Yoshie O. Shiraishi T. Yatani R. Ito H. Shimazaki J. Expression of the KA11 protein in benign prostatic hyperplasia and prostate cancer. American Journal of Pathology, 149(5):1435–40, 1996.

Umbas R. Schalken JA. Aalders TW. Carter BS. Karthaus HF. Schaafsma HE. Debruyne FM. Isaacs WB. Expression of the cellular adhesion molecule E–cadherin is reduced or absent in high–grade prostate cancer. Cancer Research. 52(18):5104–9, 1992.

Umbas R. Isaacs WB. Bringuier PP. Schaafsma HE. Karthaus HF. Oosterhof GO. Debruyne FM. Schalken JA. Decreased E–cadherin expression is associated with poor prognosis in patients with prostate cancer. Cancer Research. 54(14):3929–33, 1994.

Vallet V. Chraibi A. Gaeggeler HP. Horisberger JD. Rossier BC. An epithelial serine protease activates the amiloride–sensitive sodium channel. Nature. 389(6651):607–10, 1997.

Yu JX. Chao L. Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry, 269(29):18843–8, 1994.

Yu JX. 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.
Yu JX. Chao L. Ward DC. Chao J. Structure and chromosomal localization of the human prostasin (PRSS8) gene. Genomics. 32(3):334–40, 1996.
Zaugg K. Bodis S. Is there a role for molecular prognostic factors in the clinical management of ductal carcinoma in situ (DCIS) of the breast?. [Review] [49 refs] Radiotherapy & Oncology. 55(2):95–9, 2000.

* cited by examiner

Fig. 1

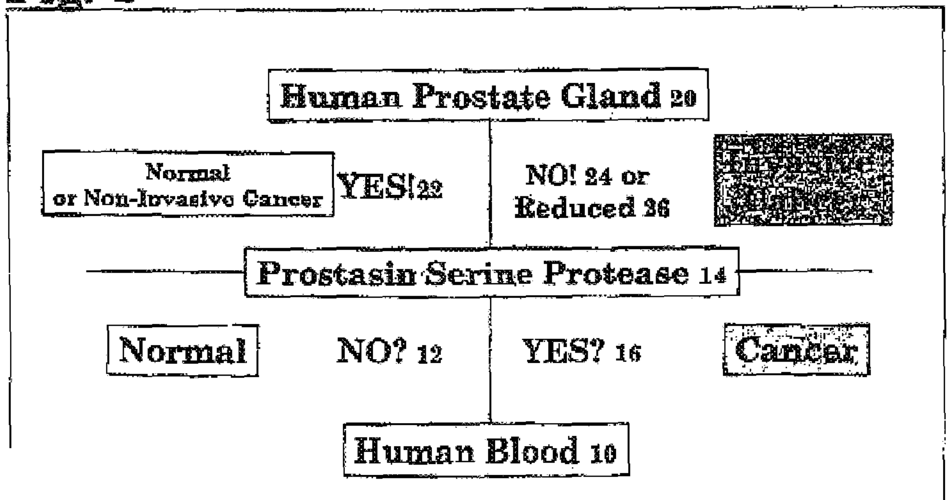

Human Prostate Gland 20
Normal or Non-Invasive Cancer
YES! 22
NO! 24 or Reduced 26
Invasive Cancer
Prostasin Serine Protease 14
Normal
NO? 12
YES? 16
Cancer
Human Blood 10

Fig. 2

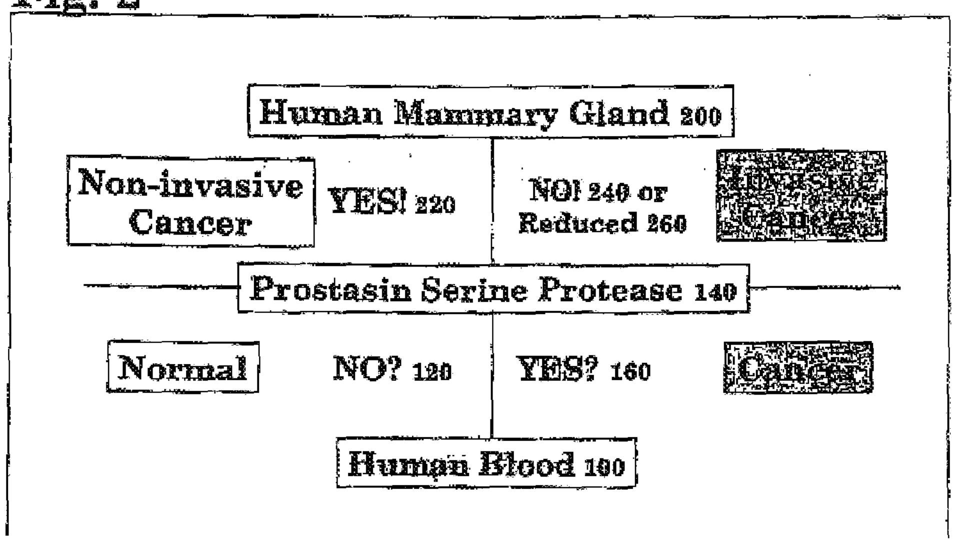

Human Mammary Gland 200
Non-invasive Cancer
YES! 220
NO! 240 or Reduced 260
Invasive Cancer
Prostasin Serine Protease 140
Normal
NO? 120
YES? 160
Cancer
Human Blood 100

Fig. 3

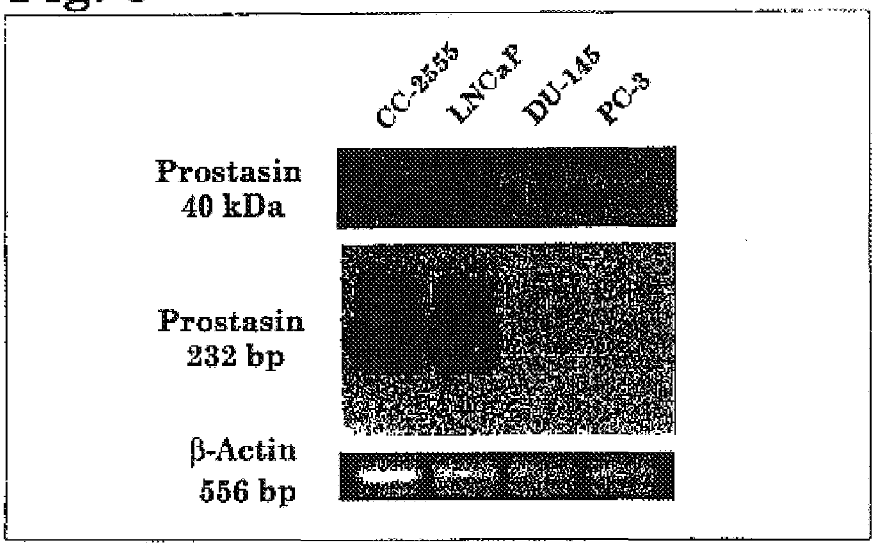

CC-2555
LNCaP
DU-145
PC-3
Prostasin
40 kDa
Prostasin
232 bp
β-Actin
556 bp

Fig. 4A

Fig. 4B

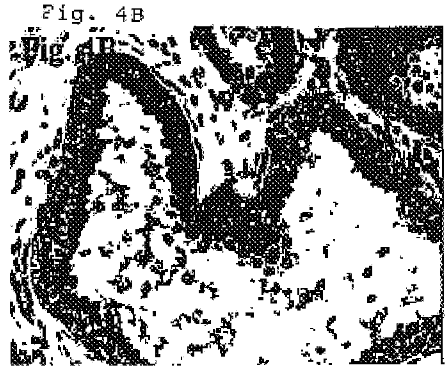


Fig. 4C

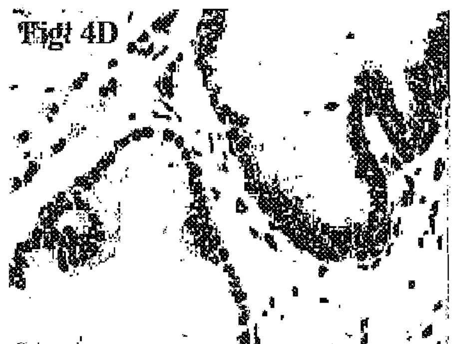


Fig. 4D

Fig. 4E

Fig. 4F

Fig. 4G

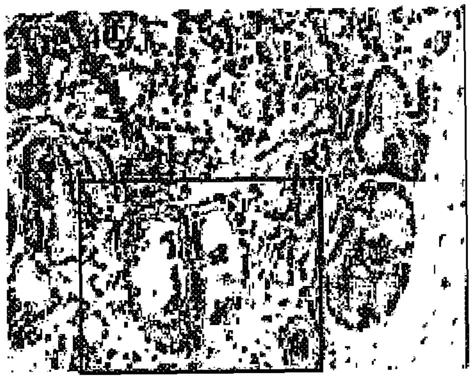


Fig. 4H

Fig. 4I

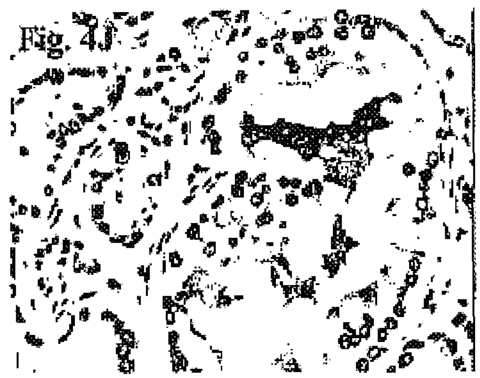


Fig. 4J

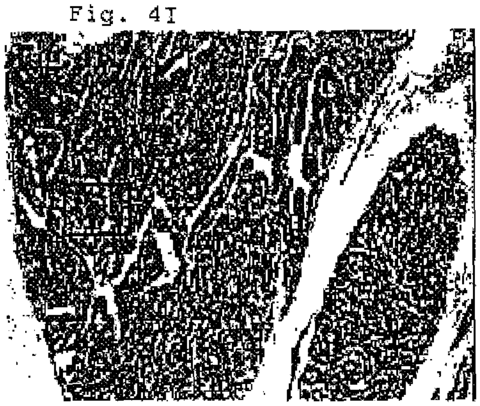


Fig. 4K

Fig. 4L

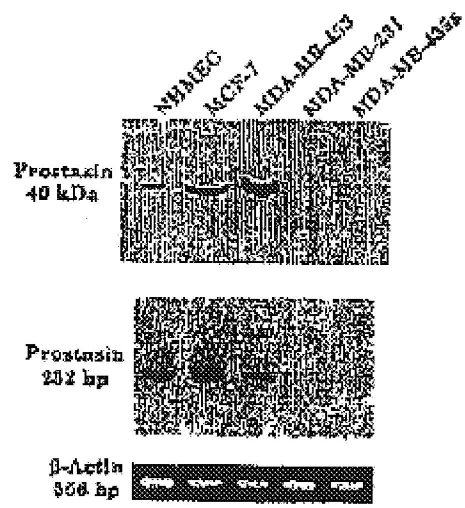

NHMEC
MCF-7
MDA-MB-453
MDA-MB-231
MDA-MB-435s
Prostasin
40 kDa
Prostasin
232 bp
β-Actin
356 bp

Figure 5

Fig. 6

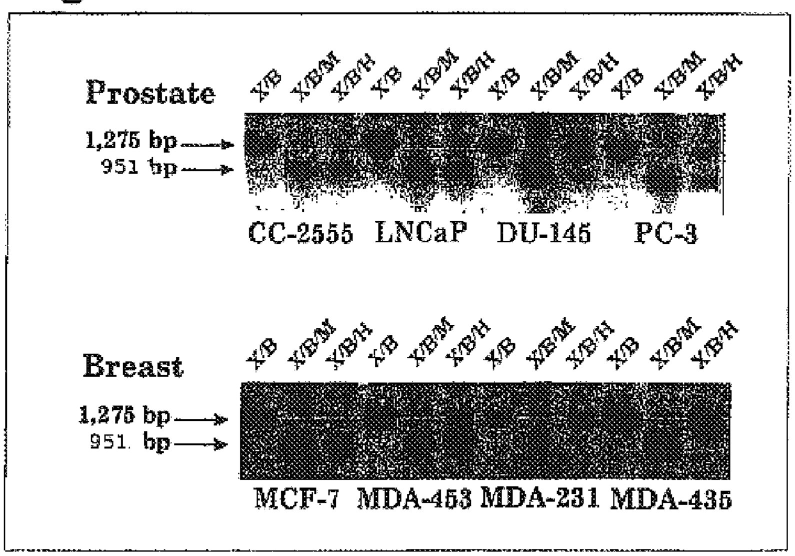

Prostate
X/B
X/B/M
X/B/H
X/B
X/B/M
X/B/H
X/B
X/B/M
X/B/H
X/B
X/B/M
X/B/H
1,275 bp
951 bp
CC-2555
LNCaP
DU-145
PC-3
Breast
X/B
X/B/M
X/B/H
X/B
X/B/M
X/B/H
X/B
X/B/M
X/B/H
X/B
X/B/M
X/B/H
1,275 bp
951 bp
MCF-7
MDA-453
MDA-231
MDA-435

Fig. 7

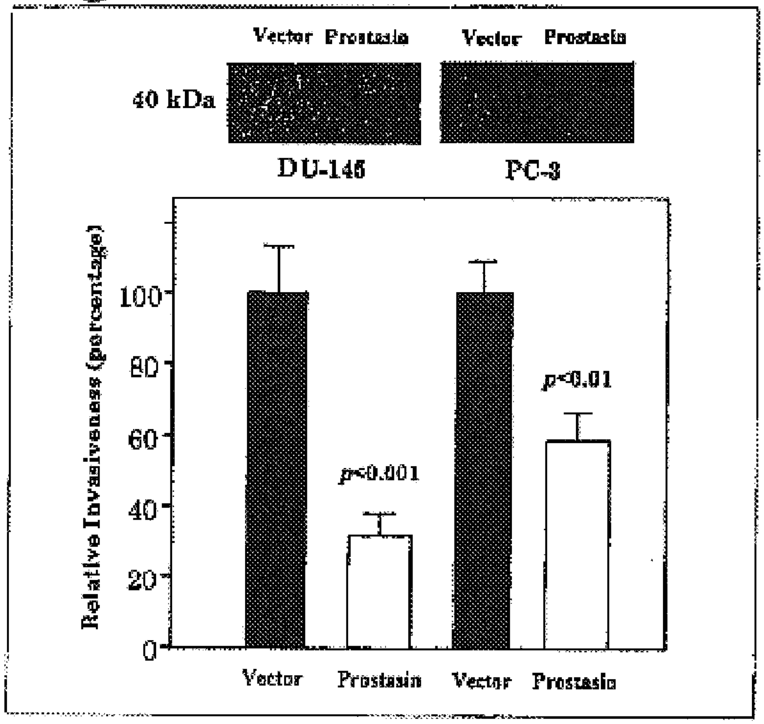
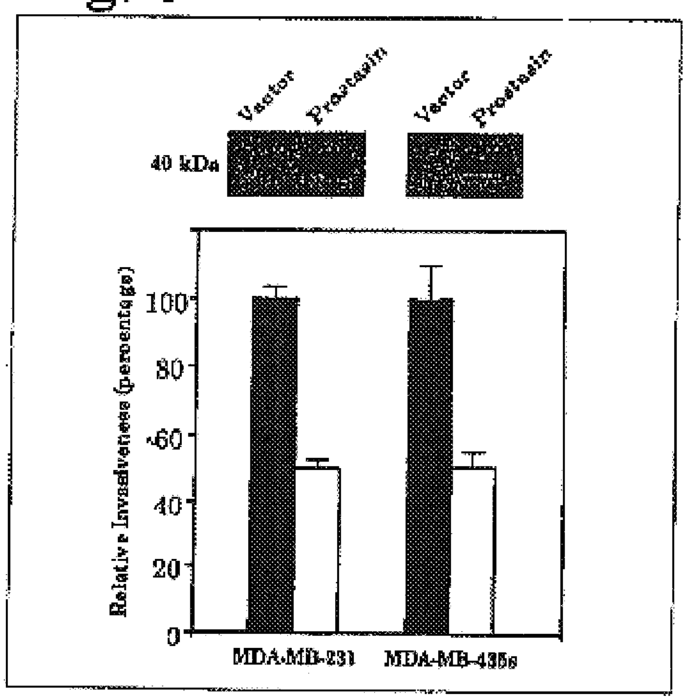

Vector
Prostasin
Vector
Prostasin
40 kDa
DU-145
PC-3
Relative Invasiveness (percentage)
100
80
60
40
20
0
p<0.001
p<0.01
Vector
Prostasin
Vector
Prostasin

Fig. 8


Vector
Prostasin
Vector
Prostasin
40 kDa
Relative Invasiveness (percentage)
100
80
60
40
20
0
MDA-MB-231
MDA-MB-435s

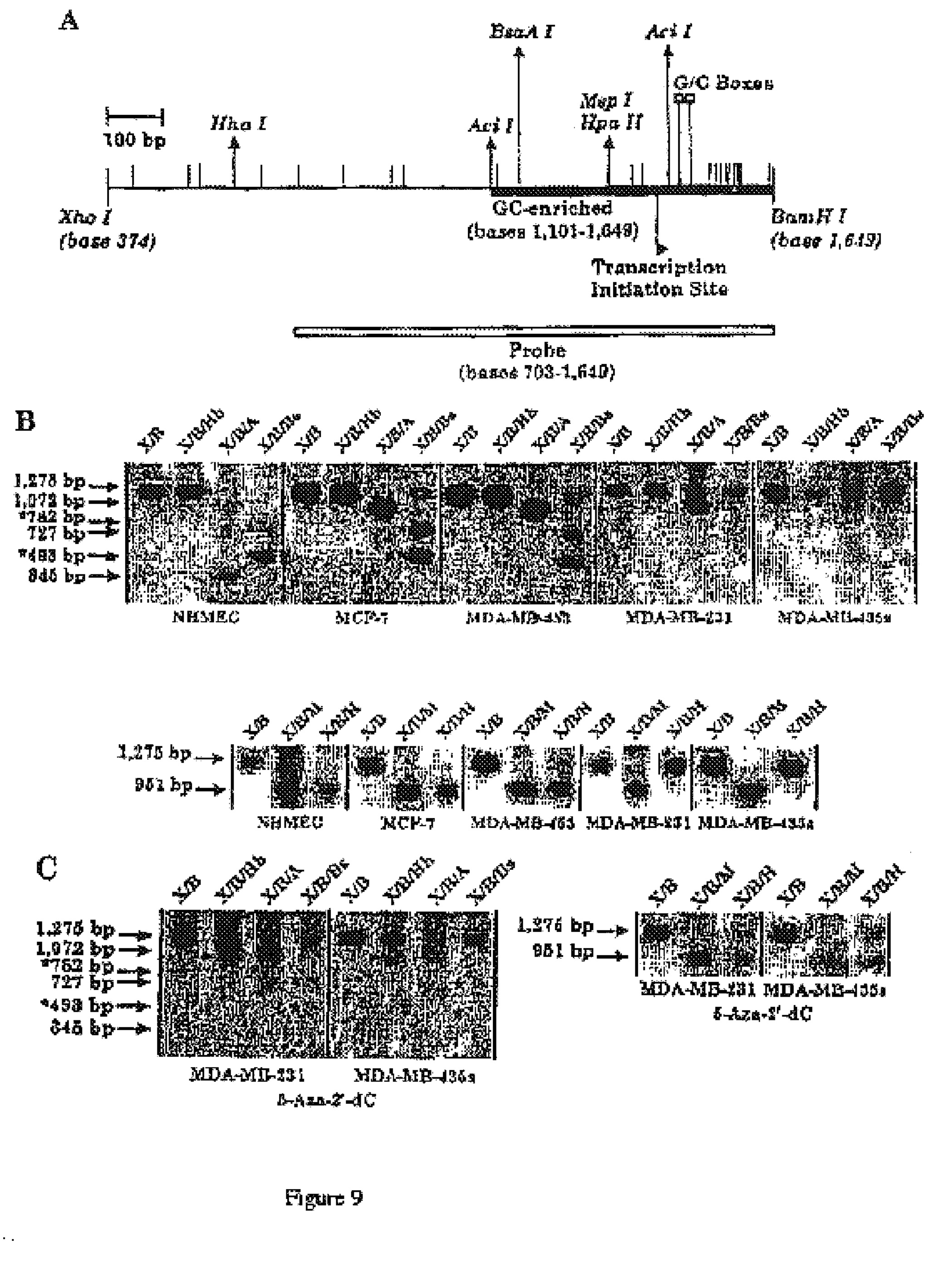

A
100 bp
Hha I
Aci I
BssA I
Msp I
Hpa II
Aci I
G/C Boxes
Xho I
(base 374)
GC-enriched
(bases 1,101-1,649)
BamH I
(base 1,649)
Transcription
Initiation Site
Probe
(bases 703-1,649)
B
1,275 bp
1,072 bp
*752 bp
727 bp
*493 bp
345 bp
NHMEC
MCF-7
MDA-MB-231
MDA-MB-435s
1,275 bp
951 bp
C
5-Aza-2'-dC

Figure 9

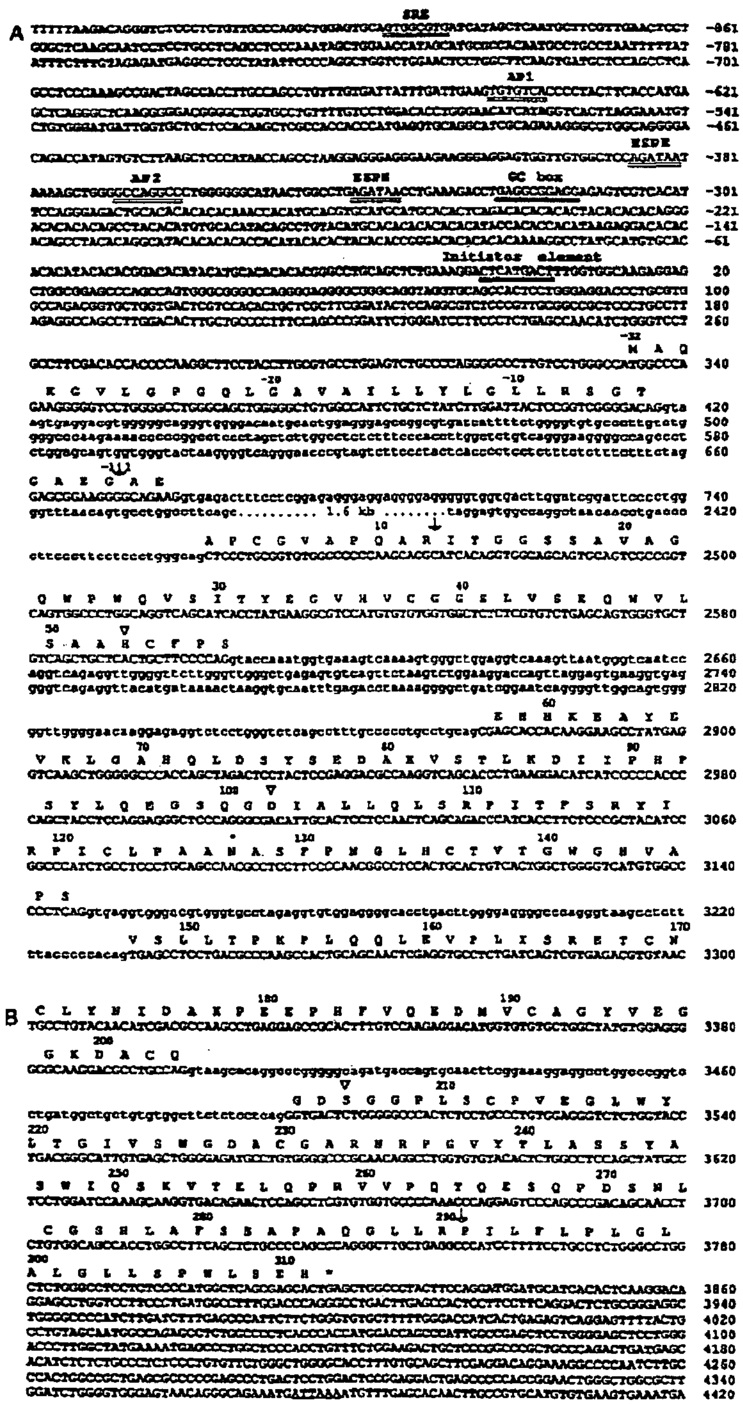


Fig. 10

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 is a Continuation-In-Part (CIP) of application Ser. No. 09/755,811 filed Jan. 5, 2001 now U.S. Pat. No. 6,569,684 and claims the priority to U.S. Provisional application Ser. No. 60/174,801 filed Jan. 6, 2000 and was supported in part by 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 F. Bolden S, Wingo P A. 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. Resnick M. Door R. et al.), pp1–9, Plenum Press, 1988; Carter H B Piantadosi S. Issacs J T. Clinical evidence for implications of the multistep development of prostate cancer. Journal of Urology. 143(4):742–6, 1990; Scardino P T. Weaver R. Hudson M A. Early detection of prostate cancer. [Review] [102 refs] Human Pathology. 23(3):211–22, 1999). Prostate cancers are diagnosed clinically by an estimate of size and location using the TNM staging system (Denis L J. Staging and prognosis of prostate cancer. European Urology. 24 Suppl 2:13–8, 1993), and by pathological staging based on an examination of the histology of the removed prostate via either biopsy or prostatectomy using a system by D. F. Gleason, (Gleason D F. Classification of prostatic carcinomas. Cancer Chemotherapy Reports—Part 1. 50(3):125–8, 1966). About 50% of prostate cancer cases receiving treatment are diagnosed clinically as advanced, or, non-organ-confined (Scardino P T. Weaver R. Hudson M A. Early detection of prostate cancer. [Review] [102 refs] Human Pathology. 23(3): 211–22, 1992), for which no effective treatment exists (Yagoda A. Petrylak D. Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer. [Review] [63 refs] Cancer. 71(3 Suppl): 1098–109, 1993 and Petrylak, 1993). Of the remaining 50% cases, ⅓ (~50,000) are diagnosed as organ-confined but micrometastasis may be present. The final group of patients (~100,000) have truly organ-confined prostate cancer and can be cured by radical prostatectomy (Sgrignoli A R Walsh P C. Steinberg G D. Steiner M S. Epstein J I. Prognostic factors in men with stage D 1 prostate cancer: identification of patients less likely to have prolonged survival after radical prostatectomy [see comments]. Journal of Urology. 152(4):1077–81, 1994; Zincke H. Oesterling J E. Blute M L. Bergstralh E J. Myers R P. Barrett D M. long term (15 years) results after radical prostatectomy for clinically localized (stageT2c or lower) prostate cancer [see comments]. Journal of Urology.152(5 Pt 2): 1850–7, 1994) or left untreated (watchful waiting) 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. Cytotixic chemotherapy for 1098-109, 1993). Consequently, it will be ideal, both medically and economically, if one could precisely predict 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—Part1. 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. Mellinger 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 (Knuutila S. Aalto Y. Bjorkqvist A M. EL-Rifai W. Hemmer

S. Huhta T. Kettunen E. Kiuru-Kuhlefelt S. Larramendy ML. Lushnikova T. Monni O. Pere H. Tapper J. Tarkkanen M. Varis A. Wasenius V M. Wolf M. Zhu Y. DNA copy number losses in human neoplasms. [Review] [197 refs] American Journal of Pathology. 155(3):683–94, 1999); and, DNA repair genes (Knuutila S. Aslto Y. Bjorkqvit A M. EL-Rifai W. Hemmer S. Huhta T. Kettunen E. Kiuru-Kuhlefelt S. Larramedy M L. Lushnikova T. Monni O. Pere H. Tapper J. Tarkkanen M. Varis A. Wasenius V M. Wolf M. Zhu Y. DNA copy number losses in human neoplasms. [Review] [197 refs] American Journal of Pathology. 155(3): 683–94, 1999). The histological prostate cancers for which the prediction of clinical aggressiveness is difficult (those with the intermediate Gleason scores 5–7) probably have not gone through the necessary "multi-step" transformation to acquire the potentials to behave aggressively (as would the high-grade cancers). This notion was supported by studies comparing the course of prostate cancer development among men in Japan and in the U.S., and Japanese men who migrated to the U.S. (Carter H B. Piantadosi S. Isaacs J T. Clinical evidence for and implications of the multistep development of prostate cancer. Journal of Urology, 1990; Haenszel W. Kurihara M. Studies of Japanese Migrants. 1. Mortality form cancer and other diseases among Japanese in the United States. Journal of the National Cancer Institute.40 (1):43–68, 1968; Akazaki K. Stemmerman G N. Compararative study of latent carcinoma of the prostate among Japanese in Japan and Hawaii. Journal of the National Cancer Institute. 50(5):1137–44, 1973; Dunn J E. Cancer epidemiology in populations of the United States—with emphasis on Hawaii and California—and Japan. Cancer Research. 35(11 Pt.2):3240–5, 1975). The findings were that first- and second-generation Japanese men who migrated to the U.S. have a higher prostate cancer incident rate than native Japanese men. The emigrant Japanese men's prostate cancer incident rate is similar to that of men in the U.S. Investigating the changes of expression in these three classes of cancer-relevant genes during the course of prostate cancer development will lead to a better understanding of the processes by which prostate cancers acquire their aggressive potentials. The discovery of molecules whose changes can be correlated to the staging of prostate cancer will provide new tools to improve our ability to better predict the aggressive behaviors of prostate cancer. This approach is now commonly referred to as "molecular staging".

Down-regulated genes such as tumor suppressors, invasion suppressors or metastasis suppressors may be used as prostate cancer markers. Examples of these genes that can potentially serve as prostate cancer markers for "molecular staging" are: KAII (Dong J T. Suzuki H. Pin S S. Bova G S., Schalken JA, Issacs W B, Barrett JC. Issace I T. Down-regulation of the KAI I metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Research. 56(19): 4387–90, 1996); (Ueda T, Ichikawa T., Tamaru j, Mikata A, Akakura K, Akimoto S, Imai T. Yoshie O. Shiraishi T. Yatani R. Ito H. Shimazaki J., Expression fof the **KA11** protein in benign prostatic hyperplasia and prostate cancer. American Journal of Pathology 149(5): 1435–40, 1996); E-cadherin (Umbas R. Isaacs WB BringuierP P. Schaafsma H E. Karthaus H F. Oosterhof G O. Debruyne F M. Schalken J A. Decreased E-Cadherin expression is associated with poor prognosis is patients with prostate cancer. Cancer Research. 52(18):5104–9, 1992, Umbas R. Isaacs W B. Bringuier P P. Schaafsma H E. Karthaus H F. Oosterhof G O. Debruyne F M. Schalken J A. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. Cancer Research. 54(14): 3929–33, 1994); β_{1C} integrin (Formaro M. Tallini G. Bofetiado C J. Bosari S. Languino L R. Down-regulation of β1C integrin, an inhibitor of cell proliferation, in prostate carcinoma. American Journal of Pathology. 149(3):765–73,1996 Formaro M. Manzotti M. Tallini G. Stear A E. Bosari S. Ruoslahti E. Languino L R. β1C integrin in epithelial cells correlates with a nonproliferative phenotype: forced expression of β1C inhibits prostate epithelial cell proliferation. American Journal of Pathology. 153(4):1079–87, 1998; p27 (kip1) (Tsihlias J. Kapusta LR. DeBoer G. Morava-Protzner I. Zbieranowski I. Bhattacharya N. Catzavelos G C. Klotz L H. Slingerland J M. Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. Cancer Research. 58(3):542–8, 1998); and CD44 (Lou W. Krill D. Dhir R. Becich M J. Dong J T. Frierson H F Jr. Isaacs W B. Isaacs J T. Gao A C. Methylation of the CD44 metastasis suppressor gene in human prostate cancer. Cancer Research. 59(10):2329–31, 1999). By using the method of immunohistochemistry, these genes were found to be down-regulated in prostate cancer. KAI's down regulation is a potential predictor of metastasis (Dong, et al., 1996: Ueda et al., 1996). While E-cadherin'cadherin's down regulation is strongly correlated to higher Gleason grades. Functional studies of these genes have given clues to their role in prostate cancer or normal prostate biology. (Debruyne F M. Isaacs W B. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. Cancer Research. 52(18):5104–9, 1992; Umbas R. Isaacs W B. Bringuier P P. Schaafsma H E. Karthaus H F. Oosterhof G O. Debruyne F M. Schalken J A. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. Cancer Research. 54(14):3929–33, 1994; metastasis suppressor gene during the progression of human prostatic cancer infrequently involves gene mutation or allelic loss. Cancer Research. 56(19):4387–90; 1996 Ueda T. Ichikawa T. Tamaru J. Mikata A. Akakura K. Akimoto S. Imai T. Yoshie O. Shiraishi T. Yatani R. Ito H. Shimazaki J. Expression of the KAI1 protein in benign prostatic hyperplasia and prostate cancer. American Journal of Pathology. 149(5): 1435–40, 1996) while E-cadherin's down-regulation is strongly correlated to higher Gleason grades (Umbas R. Schalken J A. Aalders T W. Carter B S. Karthaus H F. Schaafsma H E. Debruyne F M. Isaacs W B. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. Cancer Research. 52(18):5104–9,1992; Umbas R. Isaacs W B. Bringuier P P. Schaafsma H E. Karthaus H F. Oosterhof G O. Debruyne F M. Schalken J A. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. Cancer Research. 54(14):3929–33, 1994) Functional studies of these genes have given clues to their role in prostate cancer or normal prostate biology. Human KAI suppress metastasis of rat prostate cancer cells upon gene transfer (Dong J T. Lamb P W. Rinker-Schaeffer C W. Vukanovic J. Ichikawa T. Isaacs J T. Barrett J C. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2 [see comments]. Science. 268(5212):884–6,1995). β_{1C} and p27(kip1) are involved in signaling pathway that inhibits cell proliferation (Formaro M. Tallini G. Zheng D Q. Flanagan W M. Manzotti M. Languino L R. p27(kipl) acts as a downstream effector of and is coexpressed with the beta1C integrin in prostatic adenocarcinoma. Journal of Clinical Investigation. 103(3):321–9, 1999). E-cadherin expression is progressively lost during the transformation of rat prostate cancer from non-invasive to invasive

(Bussemakers M J. van Moorselaar R J. Giroldi L A. Ichikawa T. Isaacs J T. Takeichi M. Debruyne F M. Schalken J A. Decreased expression of E-cadherin in the progression of rat prostatic cancer. Cancer Research. 52(10):2916–22, 1992), and it has also been shown to be an invasion suppressor (Lou W. Krill D. Dhir R. Becich M J. Dong J T. Frierson H F Jr. Isaacs W B. Isaacs J T. Gao A C. Methylation of the CD44 metastasis suppressor gene in human prostate cancer. Cancer Research. 59(10):2329–31, 1999). CD44 loss of expression is associated with high metastatic ability and transfection of CD44 suppresses metastasis without affecting tumorigenecity of rat prostate cancer cells (Gao A C. Lou W. Dong J T. Isaacs J T. CD44 is a metastasis suppressor gene for prostatic cancer located on human chromosome 11p13. Cancer Research. 57(5):846–9, 1997). CD44 down-regulation is a prognostic marker for prostate cancer (Noordzij M A. van Steenbrugge G J. Verkaik N S. Schroder F H. van der Kwast T H. The prognostic value of CD44 isoforms in prostate cancer patients treated by radical prostatectomy. Clinical Cancer Research. 3(5):805–15, 1997). Human prostate cancer histology is heterogeneous, or "multi-focal" in nature (Isaacs JT. Bova GS. Prostate Cancer. In The Genetic Basis of Human Cancer (Eds. Vogelstein B and Kinzler KW), pp653–60, McGraw-Hill Health Professions Division, 1998 and Bova, 1998), with an average of five lesions in a patient (Bastacky S I. Wojno K J. Walsh PC. Carmichael M J. Epstein J I. Pathological features of hereditary prostate cancer. Journal of Urology. 153(3 Pt 2):987–92, 1995). Between the tumor regions of a prostate and within a single tumor region, the genetic causes to the cancer are heterogeneous and independent as well (Sakr W A. Macoska J A. Benson P. Grignon D J. Wolman S R. Pontes J E. Crissman J D. Allelic loss in locally metastatic, multisampled prostate cancer. Cancer Research. 54(12):3273–7, 1994; Qian J. Bostwick D G. Takahashi S. Borell T J. Herath J F. Lieber M M. Jenkins R B. Chromosomal anomalies in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence in situ hybridization. Cancer Research. 55(22): 5408–14, 1995; Mirchandani D. Zheng 1. Miller G J. Ghosh A K. Shibata D K. Cote R J. Roy-Burman P. Heterogeneity in intratumor distribution of p53 mutations in human prostate cancer. American Journal of Pathology. 147(1):92–101, 1995). In the end, the best predictor of prostate cancer's potential to gain invasiveness may be a consideration of a number of genes whose expression levels change along the course of cancer development, as demonstrated in principle by Greene et al. (Greene G F. Kitadai Y. Pettaway C A. von Eschenbach A C. Buucana 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). Hence, expanding the repertory of such genes, including both the onco-genes and the suppressor genes, will enhance the accuracy and dependability of this approach. 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. Petrylak D. Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer. [Review] [63 refs] Cancer. 71(3 Suppl): 1098–109, 1993). Breast cancer is the most diagnosed cancer in women and the second leading cancer related cause of death in women (Greenlee R T, Murray T, Bolden S, Wingo P A. Cancer statistics, 1999. Ca: a Cancer Journal for Clinicians 2000;50:7–33). Breast ductal carcinoma in situ (hereinafter indicated as DCIS) incidence has increased dramatically since 1983 as a result of implementing screening programs (Ernster V L. Barclay J. Increases in ductal carcinoma in situ (DCIS) of the breast in relation to mammography: a dilemma. [Review] [34 refs] Journal of the National Cancer Institute. Monographs.

(22): 151–6, 1997). DCIS is described as a malignant growth of epithelial cells within the ducts and lobules of the breast, and is believed to be the precursor of all invasive breast carcinoma. DCIS itself is non-life-threatening; however, current treatment options for DCIS include masectomy, lumpectomy, radiotherapy or tamoxifen (Ernster V L. Barclay J. Increases in ductal carcinoma in situ (DCIS) of the breast in relation to mammography: a dilemma. [Review] [34 refs] Journal of the National Cancer Institute. Monographs. (22): 151–6, 1997; Hwang E S. Esserman L J. Management of ductal carcinoma in situ. [Review] [91 refs] Surgical Clinics of North America. 79(5):1007–30, viii, 1999). These treatment options for DCIS are at best controversial due primarily to a lack of precision in diagnosis and prognosis of whether the detected DCIS will progress to invasive breast cancer and whether recurrence is likely after treatment, usually with a high percentage being invasive breast cancer (Zaugg K. Bodis S. Is there a role for molecular prognostic factors in the clinical management of ductal carcinoma in situ (DCIS) of the breast?. [Review] [49 refs] Radiotherapy & Oncology. 55(2):95–9, 2000). At present, histological grading (nuclear grading and whether come do-type necrosis is present) and the size of the DCIS are used to provide assessments of risk of DCIS to progress into invasive breast cancer (Zaugg K. Bodis S. Is there a role for molecular prognostic factors in the clinical management of ductal carcinoma in situ (DCIS) of the breast?. [Review] [49 refs] Radiotherapy & Oncology. 55(2):95–9, 2000; Shoker BS. Sloane JP. DCIS grading schemes and clinical implications. [Review] [40 refs] Histopathology. 35(5):393–400, 19; van de Vijver M J. Ductal carcinoma in situ of the breast: histological classification and genetic alterations. [Review] 169 refs] Recent Results in Cancer Research. 152:123–34, 1998). These parameters are far from being adequate for making the most accurate choice of treatment, resulting in a choice either overly excessive or conservative, in either case, the patient will suffer unnecessarily. Molecular markers can help improve our ability to better diagnose DCIS and stratify treatment options, especially the molecular markers that, themselves, play a role in the progression of DCIS to invasive breast cancer (Zaugg K. Bodis S. Is there a role for molecular prognostic factors in the clinical management of ductal carcinoma in situ (DCIS) of the breast?. [Review] [49 refs] Radiotherapy & Oncology. 55(2):95–9, 2000; Silverstein M J. Masetti R. Hypothesis and practice: are there several types of treatment for ductal carcinoma in situ of the breast?. [Review] [53 refs] Recent Results in Cancer Research. 152:105–22, 1998). The tumorigenesis process is a multi-step transformation in which molecular events escalate to the final stage of invasive phenotype (Silverstein MJ. Masetti R. Hypothesis and practice: are there several types of treatment for ductal carcinoma in situ of the breast?. [Review] [53 refs] Recent Results in Cancer Research. 152:105–22, 1998). The conventional paradigm of protease involvement in the development and progression of cancer has been the assignment of a usually negative role to the proteases, such as promoting tumor invasion (Mignatti P. Rifkin D B. Biology and biochemistry of proteinases in tumor invasion. [Review] [306 refs] Physiological Reviews. 73(1):161–95, 1993). In turn, the conventional paradigm of protease inhibitors in relation to cancer is usually regard of a beneficial effect for the presence of these molecules (Kennedy AR. Chemopreventive agents: protease inhibitors. Pharmacol Therapeut 78:167–209, 1998). Recently, however, the picture of a new paradigm is beginning to emerge for several serine proteases in breast, prostate, and testicular cancers. A "normal epithelial cell specific-**1**" (NES**1**) serine protease was found to be down-regulated in breast and prostate cancers, and it functions as a tumor suppressor (Goyal J, Smith K M, Cowan J M, Wazer D E, Lee S W, Band V. The role for NES**1** serine protease as a novel tumor suppressor. Cancer Res 58:4782–4786, 1998). A prostate-specific serine protease, prostase (Nelson P S. Gan L. Ferguson C. Moss P. Gelinas R. Hood L. Wang K. Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proceedings of the National Academy of Sciences of the United States of America. 96(6):3114–9, 1999), was shown to be expressed in normal prostate but not in prostate cancer cell lines DU-145 and PC-3. The expression of a testis-specific serine protease, testisin, was shown to be lost in testicular cancer through either a loss of gene (Hooper J D. Nicol D L. Dickinson J L. Eyre H J. Scarman A L. Normyle J F. Stuttgen M A. Douglas M L. Loveland K A. Sutherland G R. Antalis T M. Testisin, a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. Cancer Research. 59(13):3199–205, 1999) or methylation in the promoter (Boucaut K, Douglas M, Clements J, Antalis T. The serine proteinase testisin may act as a tumor and/or growth suppressor in the testis and may be regulated by DNA methylation. Cancer Genetics and Tumor Suppressor Genes, Cold Spring Harbor Laboratory, 2000). Further, transfection of human testicular cancer cells with a testisin cDNA reduced the tumor growth of xenografts of these cells in nude mice, suggesting a tumor suppressor function for testisin (Boucaut K, Douglas M, Clements J, Antalis T. The serine proteinase testisin may act as a tumor and/or growth suppressor in the testis and may be regulated by DNA methylation. Cancer Genetics and Tumor Suppressor Genes, Cold Spring Harbor Laboratory, 2000). The testisin serine protease is potentially membrane-bound as suggested by its structure and confirmed by immunohistochemistry gene (Hooper J D. Nicol D L. Dickinson J L. Eyre H J. Scarman A L. Normyle J F. Stungen M A. Douglas M L. Loveland K A. Sutherland GR. Antalis TM. Testisin, a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. Cancer Research. 59(13): 3199–205, 1999.) Prostasin serine protease is an acidic protein (pI 4.5–4.8) of approximately 40 kDa in molecular mess (Yu JX. Chao L. Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994). It is predominantly made in the prostate gland (~146 ng/mg protein), with lesser amounts (2–6 ng/mg protein) also found in the bronchi, colon, kidney, liver, lung, pancreas, and the salivary glands (Yu JX. Chao L. Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994). Prostasin is secreted in the prostatic fluid, and can be detected in the semen (~9 μg/ml). Prostasin expression is localized to the epithelial cells of human prostate gland by in situ hybridization histochemistry using an antibody or an anti-sense RNA probe (Yu J X. Chao L. Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994; (Yu JX. 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). Molecular cloning of a full-length human prostasin cDNA revealed that its predicted amino acid residue sequence contains a carboxyl-terminal hydrophobic region that can potentially anchor the protein on the membrane (Yu JXChao L. Chao J. Molecular cloning, tissue-specific expression, and cellular localization of human prostasin mRNA. Journal of Biological Chemistry. 270(22):13483–9, 1995). At the amino acid level, prostasin is similar to plasma kallikrein, coagulation factor XI, hepsin, plasminogen, acrosin, prostase, and, in particular, testisin (sharing 44% sequence identity) [Nelson PS. Gan L. Ferguson C. Moss P. Gelinas R. Hood L. Wang K. Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. Proceedings of the National Academy of Sciences of the United States of America 96(6):3114–9, 1999; Hooper J D. Nicol D L. Dickinson J L. Eyre H J. Scarman A L. Normyle J F. Stuttgen M A. Douglas M L. Loveland K A. Sutherland G R. Antalis T M. Testisin, a new human serine proteinase expressed by premeiotic testicular germ cells and lost in testicular germ cell tumors. Cancer Research. 59(13):3199–205, 1999; Yu JX. Chao L. Chao J. Molecular cloning, tissue-specific expression, and cellular localization of human prostasin mRNA. Journal of Biological sodium channel-activating protease (CAP**1**) was shown to be highly homologous to human prostasin as well (sharing 53% sequence identity at the amino acid level) (Vallet V. Chraibi A. Gaeggeler H P. Horisberger J D. Rossier B C. An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature. 389(6651):607–10, 1997). Prostasin is encoded by a single-copied gene, which is located on human chromosome 16pl 1.2 (Yu JX. Chao L. Ward D C. Chao J. Structure and chromosomal localization of the human prostasin (PRSS8) gene. Genomics. 32(3):334–40,1996). The secreted prostasin cleaves synthetic substrates in vitro preferentially at the carboxyl-terminal side of Arg residue, and is thus considered a trypsin-like serine protease (Yu JX. Chao L. Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29): 18843–8, 1994). The physiological function of prostasin, however, has remained unknown. By comparing gene expression of normal tissues, pre-invasive cancer, and invasive cancer, it would be highly advantageous to discover molecular markers that display a differential expression pattern between the pre-invasive and the invasive phenotypes and use these markers for a more precise diagnosis and prognosis of prostate and/or breast cancers. With the enhanced precision in diagnosis and prognosis, treatment options for patients with DCIS could then be stratified. Those with low risks of developing invasive breast cancer will have a higher confidence in choosing breast-conserving options, while those at high risks will ponder more aggressive options with the necessary follow-up treatments. Similarly, those males with low risks of developing invasive prostate cancer will have a higher confidence in choosing prostate-conserving options, while those at high risks will ponder more aggressive options with the necessary follow-up treatments.

SUMMARY OF THE INVENTION

The first objective of the present invention is to reduce deficiencies in the prior art with specific regard to differential 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 gene promoter DNA methylation levels,

as well as a method of treating invasive human carcinomas comprising the steps of: incorporating human prostasin nucleic acid into a selected gene delivery vector nucleic acid to form a recombinant nucleic acid; preferably wherein the nucleic acid includes a gene or cDNA delivering the recombinant nucleic acid into a human carcinoma; and reducing invasiveness of the human carcinoma.

Further objects and advantages of this invention will be apparent from the following detailed description of a presently preferred embodiment, which is illustrated schematically in the accompanying drawings.

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.

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

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

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

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

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

FIG. **4**F shows immunohistochemical detection of prostasin protein in benign human prostate tissue surrounded by prostate carcinomas.

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

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

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

FIG. **4**J is an enlarged view of the boxed region of FIG. **41**.

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

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

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

FIG. **6** shows promoter hypermethylation of the human prostasin gene in human prostate and 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.

FIG. **9***a* Is a schematic illustration of the promoter-exon region of the prostasin gene.

FIG. **9***b* shows a genomic Southern blot analysis of DNA from NHMEC and human breast cancer cell lines.

FIG. **9***c* shows a genomic Southern blot analysis of MDA-MB-231 and MDA-MB-435s cells following demethylation.

FIG. **10** shows DNA sequence and the deduced amino acid sequence of the PRSS8 (prostasin) gene.

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 normal 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. 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 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 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 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 **10** 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, 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, CA). 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. **4*a*-4*l***, which provides immunohistochemical detection of prostasin protein in tissues. Paraffin-embedded human prostate sections were stained for prostasin protein expression evaluation using a specific antibody as described (Yu JX. Chao L. Chao J. Prostasin is a novel human serine proteinase 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 JX. Chao L. Chao J. Prostasin is a novel human serine proteinase 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-4L**. 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. **4**I and **4**J). 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. **4**K and **4**L, 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 KW), 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 independently 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-MB453, 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-MB453 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, 16p11.2, 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 promoter hypermethylation of the human prostasin gene 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 a 0.8% agarose gel and transferred to an Immobilon-N membrane for hybridization with a nick-translated prostasin promoter probe (bases 703-1,649 of the prostasin gene sequence U33446). The probe detects a promoter fragment of 1,275 bp, which is cut by the methylation-insensitive enzyme Msp I to yield a 951-bp fragment for all DNA samples. The methylation-sensitive isoschizomer Hpa II yields the 951-bp fragment in the CC-2555 (normal prostate epithelial cells), 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-MB453 DNA, both the 951-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 951-bp but rather gave the 1,275-bp fragment. This homogeneous methylation pattern indicates that the Msp I/Hpa II site, at location-96 (relative to the transcription initiation site) of the prostasin promoter, is methylated (hypermethylated) in these DNA samples.

Signal intensity variation may be attributed to aneuploidy. (In addition to the Hpa II site at base number 1326/−96 shown in FIG. **6**, example 1 and 2 are provided, more extensively detailing the other prostasin gene promoter methylated sites and experimental methods for determining them. Such sites have been located at bp 615/−807, 1,102/−320, 1,156/−266, 1,326/−96 and 1,445/+24. Nomenclature used in describing the nucleic acid sequences of the prostasin gene are described by Yu, et al in Structure and Chromosomal localization of the human prostasis (PRSS8) gene. Genomics 1996:32 pp. 334–40); page 336 of the Yu, et al reference is incorporated as FIG. **10** in the description of the present invention.

An examination of the prostasin gene promoter region for DNA methylation differences among human prostate and breast cancer cell lines has been made (see FIG. **6**). We found that cells that express prostasin, normal prostate epithelial, LNCaP, and MCF-7, are unmethylated in the prostasin promoter while MDA-MB-453 showed heterogeneous prostasin promoter methylation. For cells that do not express prostasin, DU-145 and PC-3 showed heterogeneous prostasin promoter methylation while MDA-MB-231 and MDA-MB-435 showed homogeneous hypermethylation in the promoter region of the prostasin gene.

Two human prostate cancer cell lines that do not express prostasin, the highly invasive DU-145 and PC-3, show heterogeneous methylation in the promoter region of the prostasin gene. The result suggests that at least one of the 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.

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 immunohistochemistry 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 prostasin protein expression and in vitro invasive properties of the DU-145 and the PC-3 transfectants. DU-145 or PC-3 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 described in (Liu DF. Rabbani SA. Induction of urinary plasmiinogen activator by retinoic 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 DU-145 or PC-3 cells, 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 StatView software (Abacus Concepts, Inc., Berkeley, Calif.).

It can be seen that Polyclonal 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/Vector or PC-3/Vector, 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 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-145Nector cells (or, the reduction of invasiveness was at 68%). The invasiveness of PC-31Pro cells was determined to be at 58% of that of PC-3Nector cells (or, the reduction of invasiveness was at 42%). We performed in vitro cell proliferation assays on DU-145/Pro vs. DU-145/Vector cells, and on PC-3/Pro vs. PC-3/Vector 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-435 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 DF. Rabbani SA. Induction of urinary plasminogen activator by retinoic 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 cells, 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 StatView 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 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 protasin 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. Brawer M K. Flanigan R C. Patel A. Richie J P. deKemion 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. 279(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. Kitadai Y. Pettaway Calif. 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. Wolfert 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 JX. Chao L. Chao J. Prostasin is a novel human serine proteinase from seminal fluid. Purification, tissue distribution, and localization in prostate gland. Journal of Biological Chemistry. 269(29):18843–8, 1994; Yu JX. 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 (Hakalahti L. Vihko P. Henttu P. Autio-Harmainen H. Soini Y. Vihko R. Evaluation of PAP and PSA gene expression in prostatic hyperplasia and prostatic carcinoma using northern-blot analyses, in situ hybridization and immunohistochemical stainings with monoclonal and bispecific 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 circulation 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. Loric S. Prostasin mRNA to detect prostate cells in blood of cancer patients. Clinical and Chemical Laboratory Medicine 37 (SS): SI 19, 1999), demonstrated a 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 MRA 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-435s were found 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: 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-MB453 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 either cell line.

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

The following examples are provided for the purpose of illustration and not limitation:

Example 1

Prostasin Promoter DNA Methylation

Cell Culture Maintenance

All cell culture media, sera and supplements were purchased from Life Technologies (Gaithersburg, Md.) except for those otherwise noted

A normal human mammary epithelial cell primary culture (catalog number CC-255) was obtained from Clonetics (San Diego, Calif.) and maintained in the mammary epithelial basal medium (supplemented with bovine pituitary extract, recombinant human epidermal growth factor, bovine insulin and hydrocortisone) according to the suppliers protocols. The Culture was kept at 37° C. with 5% CO_2 and used for experiments at the 9^{th} overall passage.

Human breast carcinoma cell lines MCF-7, MDA-MB__453, MDA-MB-231 and MDA_MB-435 were obtained from the American Type Culture Collection (ATCC, Manassas, Va.). The MCF-7 cells were maintained in DMEM supplemented with 10% FBS and kept at 37 for experiments at the 9^{th} overall passage.

Human breast carcinoma cell lines MCF-7, MDA-MB__453, MDA-MB-231 and MDA_MB-435 were obtained from the American Type Culture Collection (ATCC, Manassas, Va.). The MCF-7 cells were maintained in DMEM supplemented with 10% FBS and kept at 37° C. with 5% CO_2. The MDA-MB-231 and MDA-MB-453 cells were maintained in Lebovitz-15 (L-15) medium supplemented with 10% FBS and kept at 37° C. without CO_2. The MDA-MB-435 cells were maintained in L-15 medium supplemented with 15% FBS and 10 μg/ml bovine insulin and kept at 37° C. without CO_2.

Transfection Of Cell Lines With Plasmid DNA And Selection Of Stable Transfectants

Construction of a plasmid containing a full-length human prostasin cDNA under the control of a Rous sarcoma virus (RSV) promoter and transfection of cells were carried out as described in Chen, L M, Hodge, G B, Guarda, L A et al. Down-Regulation of Prostasin Serine Protease, A potential Invasion Suppressor in Prostate Cancer, Prostrate 2001, 48:93–103. Briefly, 1,000,000 MDA-MB-231 or MDA-MB-435s cells were resuspended in 0.3 ml of the culture medium and mixed with 50 μg of plasmid DNA dissolved in 0.1 ml of sterile distilled cuvette and pulsed at 200 volts, 1,600 μF, 72 ohms and 500 V/capacitance setting on a BTX-600 Eleciro-cell manipulator (Genetronics, San Diego, Calif.). Selection of transfectants was carried out in the presence of 800 μg/ml G418 (final concentration) until colonies appeared. (5–7 days) Colonies (≈200 in number) were then dispersed via trypsinization and maintained in G418 containing culture medium without colony-isolation. Cells transfected with the human prostasin cDNA construct were assayed in Western blot analysis for expression of the prostasin protein, using vector-transfected cells as controls.

RNA Preparation And Analysis By RT-PCR(Southern Blot

Cells grown to 80% confluence (in 60 nm tissue culture dish) were lysed directly with 1 ml of the Trizol Reagent (life Technologies) and RNA was isolated according to the manufacturer's protocol. The human prostasin-specific RT-PCR/Southern blot analysis was performed as described in Yu, J X, and Chao,J Molecular Cloning, tissue-specific expression and cellular localization of human prostasin mRNA. J Biol Chem 1995; 270:12483-4. One microgram of total RNA was used in the RT-PCR with 2 human prostasin gene-specific oligonucleotide primers, and a Southern blot of the resolved RT-PCR samples was probed with a third prostasin gene-specific oligonucleotide, detecting an amplified 232 bp fragment. A co-amplification of the β-actin mRNA for control of RNA quality and quantity was performed as described in Chen Hodge and Guarda, et al.

Western Blot Analysis

Cells grown to 80–100% confluence were washed 3 times in 1×PBS (pH 7.4) and then lysed in RIPA buffer (1×PBS, pH 7.4, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). The total lysate were centrifuged at 14,000 rpm for 30 minutes at 4° C. to remove the pellet. Protein concentration was determined using a DC (detergent-compatible) protein assay kit (Bio-Rad, Hercules, Calif.). The samples were then subjected to SDS-PAGE followed by Western blot analysis using a prostasin-specific antibody. Briefly, cell lysate were resolved in 10% gels under reducing conditions before electrotransfer to nitrocellulose (NC) membranes (Fisher Scientific, Pittsburgh, Pa.). Upon complete protein transfer, the NC membranes were blocked in BLOTTO (5% nonfat milk made with Tris-buffered saline/0.1% Tween-20, pH 7.6), incubated with the prostasin-specific antibody diluted at a ratio of 1:2000 in BLOTTO, followed by an incubation with the secondary antibody, goat antirabbit IgG conjugated to HRP (horseradish peroxidase; used at 1:10,000) (Sigma-Aldrich, St. Louis Mo.). The bound secondary antibody was detected using enhanced chemi-luminesence (ECL) reagents (Pierce, Rockford, Ill.) according to the suppliers recommendations. All procedures were performed at room temperature, and the membranes were washed between steps.

Matrigel Chemoinvasion Assay

The Matrigel chemoinvasion assay was carried out essentially as described in Chen, L. M., Hodge, G B, Guardia, LA et al. Down-Regulation of prostasin serine protease, a potential invasion suppressor in prostate cancer. Prostate 2001:48:93–103, incorporated herein. Basement membrane Matrigel stock (10 mg/ml; Collaborative Biochemical, Bedford, Mass.) was thawed overnight on ice in a refrigerator (0–4° C.). Transwell invasion chambers (Coster, Cambridge, Mass.) with 8 μm pore polycarbonate filters (growth area 0.33 cm^2) were coated with Matrigel (50 μg/filter, a 1:3 dilution of the stock with chilled serum-free medium was used for coating). The gels were solidified by incubating the coated filters at 37° C. for 60 min in a moist chamber before use. The lower chambers of the Transwell plates were filled with 0.6 ml serum-free medium (1-15), supplemented with 25 μg/ml fibronectin (Sigma-Aldrich). Fifty thousand cells in 0.1 ml serum-free OPTI-MEM I medium were placed onto each Matrigel-coated filter. The filter cartridge was then inserted into the lower chamber and the assays were performed at 37° C. for 24 hours (MDA-MB-435s transfectants) or 72 hour (MDA-MB-231 transfectants). After removing the medium, the filters were washed 3 times in 1×PBS, fixed at room temperature for 20 min in 4% paraformaldehyde made with 0.1 M sodium phosphate buffer (pH 7.4) and then washed 3 times with the sodium phosphate buffer. The filters were then stained with 1% toluidine blue (LabChem, Pittsburgh, Pa.) for 2 min. The cells on the Matrigel surface were removed with a Q-tip and all cells on the underside of the filters were counted under a light microscope after mounting the filter on a glass slide. All invasion assays were done in triplicate for at least 3 times.

Genomic Southern Blot Analysis Of Prostasin Promoter Methylation

High molecular weight genomic DNA was isolated from various cell lines as described in Chai, K X, Ward, D C, Chao, J, et al Molecular Clonings Sequence Analysis and Chromosomal Localization of the Human Protease Inhibitor 4 (kallistatin) gene (P14) Genomics 1994; 23:370–8. Genomic DNA frm each cell line was digested with the following restriction enzyme combinations:Xho I/BamH I (X/B), Xho I/BamH I/Hha (X/B/Hh), Xho I/BamH I/Aci I (X/B/A), Xho I/Bam H I.Bsa I (X/B/Bs),Xho I/Bam H I/Msp I(X/B/M) or Xho I/Bam H I/Hpa II(X/B/H) using 10 μg of DNA per digestion combination. The X/B cutting sites flank the CpG sies be to be investigated for differential methylation by the methylation-sensitive restriction endonucleases. The digests were resolved in a 0.8% agarose gel and analyzed by Southern blot hybridization as described in Chai, K X, Ward, D C, Chao, J et al. A nick translated prostasin promoter probe (bases 703-1,649) of the prostasin gene sequence, Genbank accession number U33446) was used for the hybridization. The base numbering of the prostasin gene sequence was described in Yu et al.

Demethylation of Prostasin Gene Promoter and Reactivation of Prostasin Expression

For demethylation of prostasin gene promoter, MDA-MB-231 and MDA-MB-435s cells were seeded in 100 mm dishes at an initial density of 50% confluence and cultured in the presence of 500 nM 5-aza-2'-deoxycytidine (5-aza-2'dC; Sigma-Aldrich) for 8 days with renewal of medium and 5-aza-2'-dC performed at 2 day intervals. Genomic DNA was then isolated for Southern blot analysis as described. For reactivation of prostasin expression, MDA-MB-231 and MDA-MB-435s cells were seeded in 60 mm dishes at 80% confluence and cultured in the presence of 500 nM 5-aza-2'-dC for 24 hr, and were then treated for an additional 24 hr with either 1 μM trichostatin A (TSA; Sigma Aldrich) or an equal volume of 95% ethanol used to dissolve TSA. For measuring time-dependence of prostatin gene reactivation, cells were treated as described above but were harvested at 6 or 12 hr after the addition of TSA. RNA was isolated for prostasin-specifric RT-PCR/Southern blot analysis as described. The MCF-7 and the MDA-MB-453 cell ines were subjected tot the same 5-aza-2'-dC/TSA treatment and prostasin RT-PCR Southern blot analysis procedures.

Results

Prostasin Expression In Breast Cancer Lines

Expression of prostasin protein and mRNA in *Normal Human Mammary Epithelial Cells* (*NHMEC*) and human breast carcinoma cell lines was examined by Western blot analysis using a prostasin-specific antibody or RT-PCR/Southern blot analysis as described. For this experiment **4** breast carcinoma cell lines based on their differences in invasiveness and metastatic potential were chosen. The MCF-7 cell line is poorly invasive to noninvasive, tumorigenic but nonmetastatic. The MDA-MB-453 cells are poorly tumorigenic and nonmetastatic. The MDA-MB-231 and MDA-MB-435 cells are both highly invasive and highly metastatic.

As was shown in FIG. **5** (middle panel) a 232 bp amplified prostasin message was detected in total RNA of NHMEC, MCF-7 and MDA-MB-453 but not MDA-MB-231 or MDA-MB-435s. A co-amplification of β-actin message is shown in the bottom panel of FIG. **5** to demonstrate the quality and quality of total RNA used in each RT-PCR. In addition to the expected 232 bp prostasin amplification product, a faint bank of retarded mobility was also detected in the Southern blot analysis. This retarded band does not represent a new prostasin message, but rather is a single-stranded form of the expected prostasin PCR product. In a separate experiment, we were able to remove this single-stranded form by treating the PCR products with SI nuclease prior to electrophoresis (data not shown)

Re-expression of recombinant human prostasin in invasive breast cancer cells reduced in vitro invasiveness

Following the electroporation and drug selection ≈200 colonies formed for each transfected cell type. All colonies for each transfected cell type were kept in a mixed culture as polyclonal transfectants for the ensuing experiments. Polyclonal MDA-MB-231 and MDA-MB-435s cells transfected with the human prostasin cDNA were confimred to express the recombinant human prostasin protein, as shown by Western blot analysis of the cell lysate (FIG. **8**, top panel) The vector transfected cells were used as negative control in the Western blot analysis.

In the in vitro Matrigel chemoinvasion assays shown in FIG. **8**, bottom panel) transfected MDA-MB-231 or MDA-MB-345s cells expressing the recombinant human prostasin protein showed a significantly reduced level of invasiveness, at 50% of that of their vector transfected controls, respectively.

Prostasin Gene Promotor Is Hypermethylated In Invasive Breast Cancer Lines

Examination of the prostasin gene locus by genomic Southern blot-RFLP (restriction fragment length polymorphism) analysis did not reveal any evidence of gene loss or gross rearrangement in the cell lines that did not show prostasin expression, namely, MDA-MB435 (data not shown) Consideration of the possibility of prostasin gene-silencing by epigenetic mechanisms was also considered. Polysynthetic methylation of cytosine residues in the 5'-CpG islands is often involved in long-term silencing of certain genes during mammalian development and in the progression of cancers. An examination of the prostasin promoter and exon I region sequence (GenBank accession number U33446) identified 28 CpG dinucleotides in a segment defined by an Xho I site (vse number 374 of U33446 or −1,048 relative to the transcription initiation site and a BamH I site (base number 1,649 of U33446 or +228 relative to the transcription initiation site) (FIG. **9***a**) A GC enriched domain containing 2 consensus G/C boxes with the sequence GGGCGG was identified as encompassing the transcription initiation site. This GC enriched domain extends from base number 1,101 to the BamH I site (1,649) as a length of 549 bp and has a GC content of 58.6%. The 549 bp GC-enriched domain contains 19 CpG dinucleotides, but failed to qualify as a true CpG island by the standards of Gardiner-Garden, M, and Frommer, M. CpG islands in vertebrate genomes. J Mol Biol 1987:196:261–82.

In all breast cancer cell lines and the NHMEC, XhoI/BamH I/Hha digestion yielded a 1,275 bp prostasin promoter band seen in the Xho I/BamH I/I digestion (**FIG. **9***b* upper panel) indicating that the −807 Hha I site-CpG is homogenously methylated.

In the NHMEC digestion with Xho I/Bam H I/Aci I yielded a 1,072 bp, a 727 bp and a 345 bp band, but no 1,275 bp band, indicating that the −320 Aci I site-CpG is heterogeneously methylated but the +24 Aci I site CpG is homogenously unmethylated. In MCF-7, MDA-MB-453 and MDA-MB-231, digestion with Xho I/BamH I/Aci I yielded only the 1,072 bp band, indicating that the −320 Aci site CpG is homogenously methylated, but the +24 Aci site CpG is homogenously unmethylated. In MDA-MB-435s, digestion with Xho I/BamH/Aci I yielded only the 1,275 bp band, indicating that both the −320 and the +24 Aci I site CpG's are homogenously methylated.

In NHMEC and MCF-7 cells the 1,275 bp Xho I/BamH I band can be digested by the methylation-insensitive Msp I as well as by the methylation sensitive Hpa II yielding an 951 bp band, indicating that the CpG at the −96 M/H restriction site is homogenously unmethylated. In MDA-MB-453, the 1,275 bp band can be fully digested by Msp I but only partially by Hpa II indicating heterogenous methylation of the −96 M/H site CpG. In MDA-MB-231 and MDA-MB-435s, the 1,275 bp band was undigested by Hpa II while having been completely digested by Msp I, indicating that the −96 M/H site CpG is homogenously methylated.

Overall, in the prostasin-expressing cells the NHMEC is the least methylated in this region and the methylation is restricted to beyond −266 relative to the transcription initiation site. The prostasin gene promoter-exon I region in MCF-7 and MDA-MB-453 cells is methylated to a higher extent than that in the NHMEC. In the MCF-7, methylation is restricted to beyond −966, while in the MDA-MB-453, methylation is observed at the −96 position and beyond. In the cells that do not express prostasin, the promoter-exon I region is the most heavily methylated. While in MDA-MB-231 the methylation is still restricted to the 5'flanking region, all CpG sites examined, including 1 of exon 1 (+24 Aci I site CpG) were found to be homogenously methylated in the MDA-MB-435s cells. The results showed that the prostasin gene promoter-exon 1 region CpG methylation patterns correlate with the absence of prostasin expression in the human breast cells examined.

Prostasin Expression Was Reactivated By Demethylation And Histone Dacetylase Inhibition In The Invasive And Metastatic Breast Cancer Cell Lines

Methylated promoter DNA may contribute to repression of gene expression by interfering with the binding of transcription factors, or by interacting with various methyl-CpG binding proteins. Such as the McCP 1 complex, MeCP2 and the MBD's depending on cell type, sequence of the methylated regulatory region and binding of other transcription factors. Histone deacetylases are recruited by the methyl-CpG binding proteins to co-repress gene expression via chromatin restructuring. Histone deacetylases act synergistically with DNA methylation in the co-repression, with DNA methylation being the dominant force for stable maintenance of gene silencing in cancer. Treatment of cancer cells with the demethylation agent 5-aza-2'-deoxycytidine (5-aza-2'dC) may restore a minimal expression for genes silenced by promoter methylation and the combined treatment of 5-aza-2'-dC with the histone deacetylase inhibitor, trichostatin A (TSA) may further stimulate the restored expression. Investigation of demethylation and/or histone deacetylase inhibition could reactive prostasin gene expression in the MDA-MB-231 and MDAA-MB-435s cells proceeded as follows:

After 8 days of treatment with the DNA methyltransferase inhibitor 5-aza-2'-dC, a significant level of demethylation was observed in the prostasin promoter-exon 1 region in the MDA-MB-435s cells, as genomic DNA of this region in these cells was partially digested by Hha I at −807, Aci I at −320, BsaA I at −266 and Hpa H at −96 (***FIG. **9***c*). The +24 Aci I was also partially digested in MDA-Mb-435s following demethylation (FIG. **9***c*) Examination of total RNA of these cells by RT-PCR/Southern blot analysis following the 8-day 5-aza-2'-dC treatment, however, failed to detect prostasin mRNA expression (data not shown).

Investigation of the combined effect of treating the cells with 5-aza-2'-dC and TSA, an inhibitor of histone deacetylase followed. In RT-PCR/Southern blot analysis, a timedependant reactivated expression of prostasin mRNA was observed in both the MDA-MB-231 and the MDA-MB-435s cells after a treatment with 5-aza-2'-dC for 24 hr followed by 95% ethanol (solvent for TSA) did not result in prostasin mRNA re-expression. A co-amplification of the β-actin message confirmed the quality and quantity of the total RNA used in each RT-PCR. When MCF-7 and MDA-MB-453 cells were subjected to the same 5-aza-2'-dC/TSA treatment and prostasin RT-PCR/Southern blot analysis procedures, no increase of prostasin mRNA expression was observed for either cell line.

FIG. **9***a* The solid horizontal line represents the promoter-exon I region of the human promstasin gene (Genbank U33446) The Xho I site is located at Base 1,649 of the U33446 sequence respectively. The methylation sensitive restriction sites used for differential methylation analysis, Hha (615/−807), Aci I (1,102/−320 and 1,445/+24), BsaA I (1,156/−266) and Msp l/Hpa II (1,326/−96) are indicated by arrows. Four additional Aci I sites and a second dMsp Ihpa Ii site are present between the +24 Aci I site and the Bam H I sies but not shown in the figure since they do not affect the results of the genomic DNA Southern blot analysis. The transcription initiation site is indicated by the triangle on an extended vertical bar. The vertical bars map the location of the 28 CpG dinucleotides identified in this region. A 549 bp GC-enriched domain is indicated by the filled rectangular box. The extended vertical bas with open square boxes indicate the locations of the consensus G/Cboxes. The location of the probe used in the genomic DNA Southern blot analysis is shown by the open rectangular box.

FIG. **9***b*. Panels of genomic DNA Southern lot analysis results are as indicated for each cell type. Restriction endonucleases used in each digestion mixture are indentified as follows: X, Xho I, B, Bam H I, Hh, Hha I, A, Aci I, Bs, BsaA I, M, Msp I, H, Hpa II. Hha I, Aci I, BsaA I and Hpa II only cut unmethylated DNA while Msp I, an isoschizomer of Hpa Ii, cuts unmethylated or methylated DNA. Genomic DNA (10 μg) from each cell type was cut with X/B or with XIB/M to serve as controls for the detection of differential methylation. In the upper panel, prostasin promoter DNA will yield a 1,275 bp X/B fragment. When Hha I was added to the X/B digestion mixture a fragment of 1,037 bp was expected if the −807 Hha I site CpG was not methylated. When Aci I was added to the X/B digestion mixture, 3 smaller bands might be expected depending on CpG methylation states at −320 and +24 Aci I sites. They are 1,072 bp(from Xho I to +24 Aci I) 727 bp (from Xho I to −320 Aci I) and 345 bp (from −320 Aci to +24 Aci I) in length, respectively. When BsaA I was added to the X/B digestion mixture, 2 smaller bands might be expected depending on CpG methylation state at the −266 Bsa A I. They are 782 bp (from Xho I to BsaA I) and 493 bp (from BsaA I to BamH I) in length, respectively (indicated by asterisks in the figure). In the lower panel, the upper arrow points to the X/B fragment, while the lower arrow points to the fragment that is generated by Msp I, regardless of DNAmethylation, or by Hpa LI, only when DNA is unmethylated.

FIG. **9***c* Cells treated with 5-aza-2'dC for 8 days were harvested for genomic DNA isolation and southern blot analysis as described. The arrowhead indicates the 1,037 bp Hha I-BamH I fragement when the −807 ha I site was cut by enzyme following DNA demethylation.

Example 2

The same methylated sites investigated in example I above in breast cancer cell lines, were investigated in human prostate cancer cell lines and human prostate epithelial cells. All methods were the same, except as follows:

Cell Culture Maintenance

A normal human prostate epithelial cell (PrEC) primary culture (PrEC primary culture (Catalog Number CC-2555) was obtained from Clonetics (San Diego, Calif.) and maintained in the supplied medium (prostrate epithelial cell basal medium, containing bovine pituitary extract, hydrocortisone, human epidermal growth factor, epinephrine, transferring, insulin, retinoic acid, triiodothyronine, gentamicin, and amphotericin). The culture was kept at 37° C. with 5% CO_2 and used for experiments at the overall passage.

Human prostate cancer cell lines LNCAP, DU-145 and PC-3 were obtained from the American Type Culture Collection (ATCC, Manassas, Va.). The PC-3 cells were maintained in F-12K medium supplemented with 10% fetal bovine serum (FBS) while the LNCaP and the DU-145 cells were maintained in RPMI-1640 medium supplemented with 10% FBS and 1 mM sodim pyruvate, and all were kept at 37° C. with 5% CO_2.

Demethylation Of Prostasin Gene Promoter And Reactivation Of Prostasin Expression

Du-145 and PC-3 cells were seeded in 60-mm dishes at 80% confluence and cultured in the presence of 500 nM 5-aza-2'-dC for 24 hours and were then treated for an additional 24 hours with either 1 μM trichostatin A (TSA, Sigma-Aldrich Co.) or an equal volume of 95% ethanol used to dissolve TSA. TNA was isolated for prostasin-specific RT-PCR/Southern blot analysis as described in example 1.

As in example 1, the prostate cells were subjected to Southern blot analysis. In all three prostate cancer cell lines and the PrEC, XhoI/BamH I/Hha I digestion yielded a 1,275 bp prostasin promoter band seen in the XhoI/BamH I/Hha I digestion (FIG. **10**, upper panel), indicating that the −807 Hha site-CpG is homogeneously methylated.

In the PrEC, digestion with Xho I/Bam H I/Aci I yielded a 1,072 bp, a 727 bp and a 345 bp band, but no 1,275 bp band indicating that the −320 Aci I site CpG is heterogeneously methylated but the +24 Aci site CpG is homogeneously unmethylated. In LNCaP and Du-145, digestion with Xho I/BamH I/Aci I ielded only the 1,072 bp band indicating that the −320 Aci I site-CpG is homogenously methylated but the +24 Aci I site CpG is homogeneously unmethylated. In PC-3, digestion with Xho I/BamH I/Aci I yielded the 1,072 bp band and to a lesser extent the 1,275 band, indicating that the −320 Aci I site-CpG is homogensously methylated, while the +24 Aci I site pG is heterogeneously methylated.

In the PrEC and LNCaP digestion with Xho I/BamH I/BsaA I yielded a 782 bp and a 493 bp band, indicating that the −266 BsaA I site CpG is homogeneously unmethylated. In DU-145, digestion with Xho l/BamH I/BasA I yielded the 782 bp and the 493 bp bands, but also the 1,275 bp band, indicating that the −266 BsaA I site-CpG is heterogeneously methylated. In PC-3, digestion with Xho/BamH I/BsaA I yielded only the 1,275 bp band, indicating that the −266 BsaA I site-CpG is homogeneously methylated.

In the PrEC and LNCaP cells, the 1,275 bp Xho I-BamH I band can be digested by the methylation-insensitive Msp I as well as by the methylation-sensitive Hpa II, yielding a 951 bp band (FIG. **10**, lower panel) indicating that the CpG at the −96 M/H restriction site is homogeneously unmethylated. In DU-145 and PC-3, the 1,275 bp band can be fully digested by Msp I but only partially by Hpa II (FIG. **10**, lower panel) indicating heterogeneous methylation of the −96 M/H site Cpg.

The PrEC express both the prostasin mRNA and protein and is the least methylated in the prostasin promoter-exon 1 region (methylation is only observed at beyond −266 relative to the transcription initiation site. The prostasin gene promoter-exon 1 region in LNCaP cells is methylated to a higher extent than that in the PrEC. In LNCaP, which also express both the prostasin mRNA and protein, methyltion is only observed at beyond −96. In DU-145, which does not express either the prostasin protein or mRNA, the promoter-exon 1 region is the most heavily methylated. While in DU-145, the methylation is limited to the 5 flanking region, all CpG sites examined, including one of exon 1 (+24 Aci I site-CpG) show heterogeneous to homogeneous methylation in the PC-3 cells. The prostasin gene promoter-exon region CpG methylation patterns correlate with the absence of prostasin expression in the human prostate cells.

Table I details the methylation state of the prostate cells. As shown with the breast cancer cells the methylation state of the prostasin promoter region may be examined for a potential diagnostic application to indicate prostate cancer invasiveness. For example, the prostasin gene promoter methylation state at the −96M/H CpG site, for example, is seen methylated in invasive cell types.

TABLE 1

CpG methylation in the prostasin gene promoter-exon1 region in human prostate cells.

Cell Type	Pro-stasin Expres-sion	Hha 1 (−807)	Aci 1 (−320)	BsaA 1 (−266)	Msp1/Hpa 1 1 (−96)	Ac1 1 (+24)
PrEC	Yes	Methyl	Hetero-methyl	Un-Methyl	Un-Methyl	Un-Methyl
LNCaP	Yes	Methyl	Methyl	Un-Methyl	Un-Methyl	Un-Methyl
DU-145	No	Methyl	Methyl	Hetero-Methyl	Hetero-Methyl	Un-Methyl
PC-3	No	Methyl	Methyl	Methyl	Hetero-Methyl	Hetero-Methyl

Table Legend:
Methyl = homogeneously methylated
Hetero-Methyl = heterogeneously methylated
Un-Methyl = homogeneously unmethylated

In investigating whether methylation in the promoter is causal to the lack of prostasin gene expression in the DU-145 and PC-3 cells, as in the breast cancer cells, treatment of these cell lines for 8 days with the DNA methyltransferase inhibitor 5-aza-2'-dC resulted in significant level of demethylation but no prostasin mRNA expression was detected in an RT-PCR/Southern blot analysis of the DNA demethylated cells. Treatment of the cells with the combination of 5-aza-2'-dC and trichostatin A (TSA) an inhibitor or histone deacetylase however restored prostasin mRNA expression in DU-145 and PC-3 cells (data not shown) When investigating whether the prostasin protein was expressed in the 5-aza-2'dC/TSA treated cells by western blot analyis, the result was negative. It appears that although promoter DNA methylation is causal to absence of prostasin expression in the invasive prostate cancer cells, the reactivated expression represents only the basal expression, similar to what was observed in the breast cancer cells.

Examples I and II demonstrate that the methylation patterns of the same specific sites in both breast cancer and prostate cancer provide markers for invasiveness.

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 for determining whether human prostate or breast carcinomas are non invasive, comprising sampling human breast or prostate carcinomas and determining whether the prostasin gene promoter DNA is methylated at bp 1,156/−266, wherein the absence of methylation indicates a non-invasive carcinoma.

2. The method, as in claim **1** wherein the sampling of the carcinoma tissue includes the step of separating the human carcinoma tissue from neighboring normal tissue using a laser capture micro-dissection.

3. The method, as in claim **1**, wherein the step of determining gene promoter DNA methylation patterns includes applying prostasin-promoter-specific oligonucleotide primers in a PCR to determine the prostasin gene promoter DNA methylation patterns in the sampled tissue.

4. A method for determining which cancer patients do not require chemotherapeutic treatment, comprising taking a biopsy of a prostate or breast tumor, and determining the non-invasiveness of the carcinoma tissue based on the absence of methylation of the prostasin gene promoter DNA at bp 1,156/−266.

5. A method for determining whether human prostate carcinomas are non invasive, comprising sampling human prostate carcinomas and determining whether the prostasin gene promoter DNA is methylated at bp 1,156/−266, wherein the absence of methylation indicates a non-invastive caarcinoma and the presence of methylation indicates invasive carcinoma.

6. The method of claim **5** wherein the sampling of the carcinoma tissue includes the step of separating the human carcinoma tissue from neighboring normal tissue using a laser capture micro-dissection.

7. The method of claim **5**, wherein the step of determining gene promoter DNA methylation patterns includes applying prostasin-promoter-specific oligonucleotide primers in a PCR to determine the prostasin gene promoter DNA methylation patterns in the sampled tissue.

* * * * *