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(54) **METHODS AND KITS FOR DETECTING PROSTATE CANCER BIOMARKERS**

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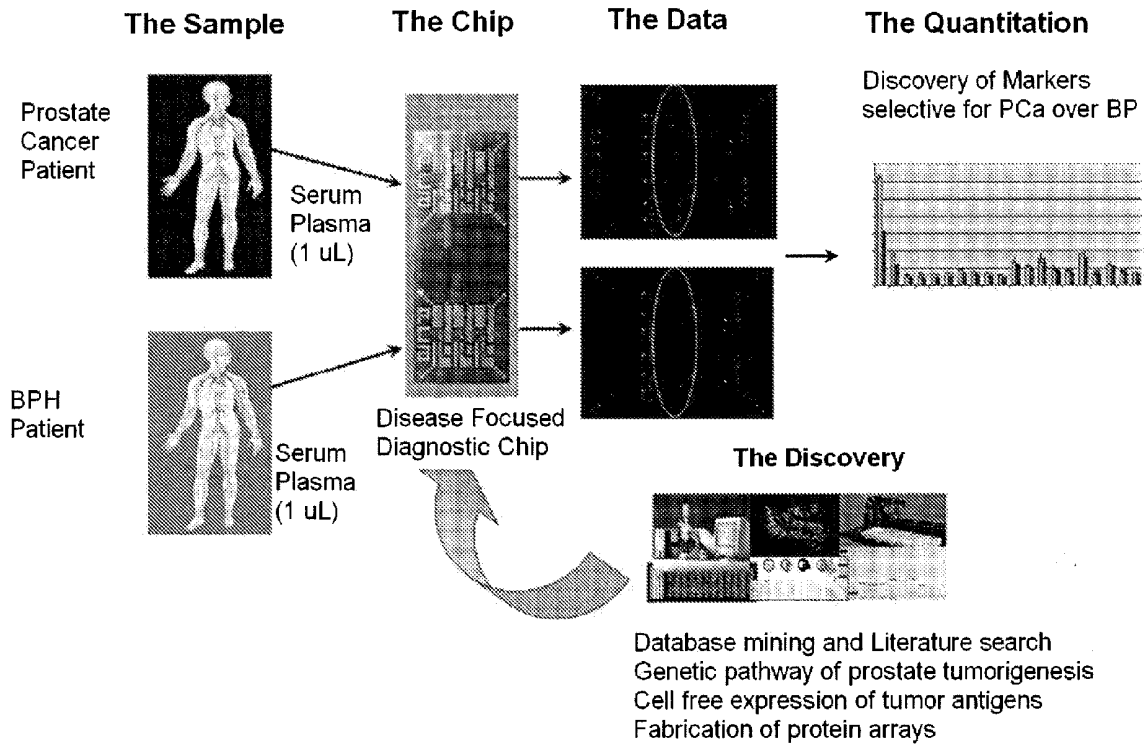
(57) **ABSTRACT**

Provided herein are novel autoantibody biomarkers, and panels for detecting autoantibody biomarkers for prostate cancer, and methods and kits for detecting these biomarkers in the serum of individuals suspected of having prostate cancer.



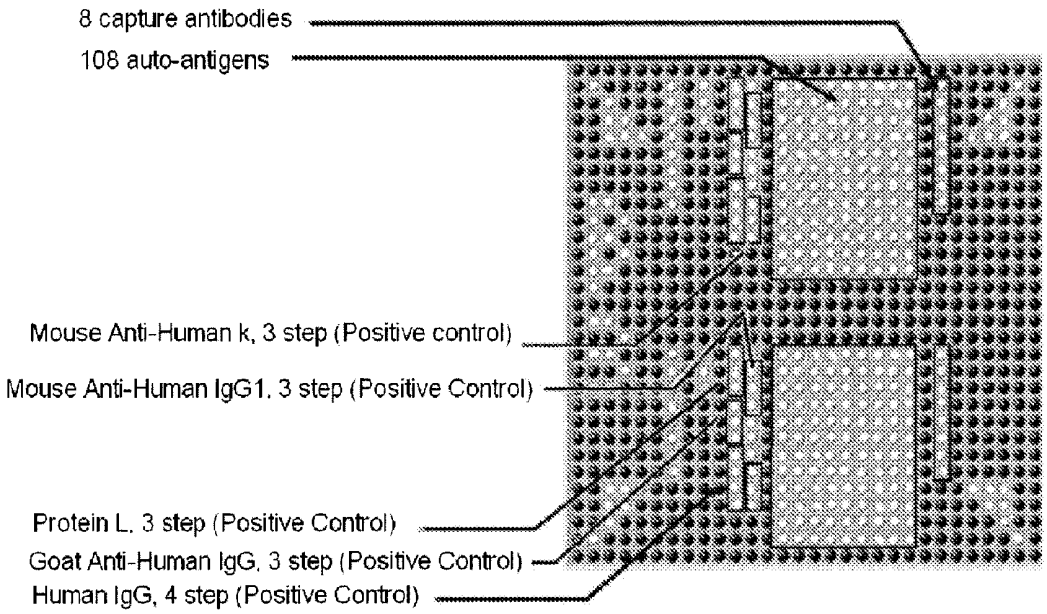

8 capture antibodies
108 auto-antigens
Mouse Anti-Human k, 3 step (Positive control)
Mouse Anti-Human IgG1, 3 step (Positive Control)
Protein L, 3 step (Positive Control)
Goat Anti-Human IgG, 3 step (Positive Control)
Human IgG, 4 step (Positive Control)

FIGURE 1

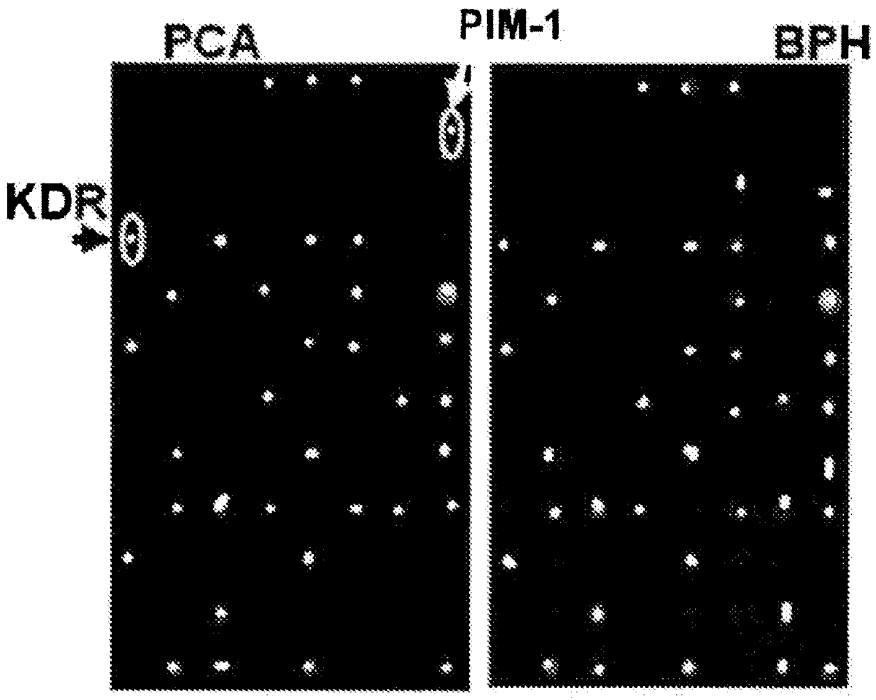


FIGURE 2A

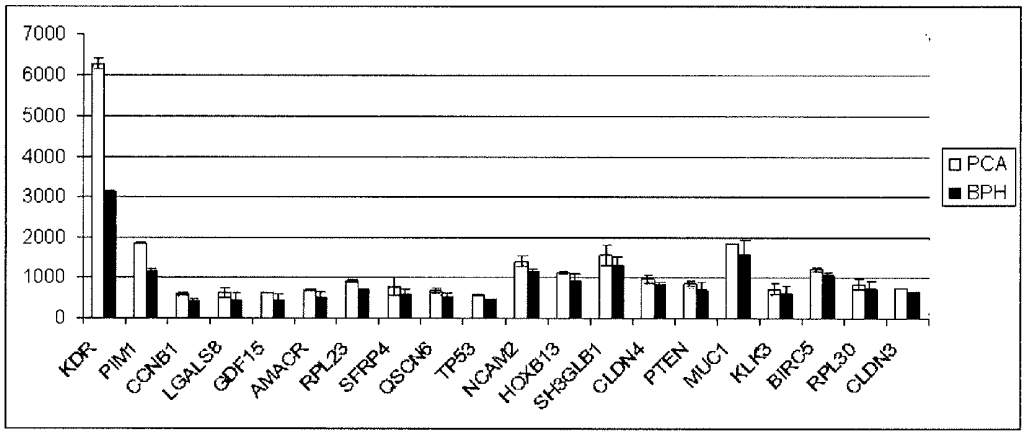

7000
6000
5000
4000
3000
2000
1000
0
PCA
BPH
KDR
PIM1
CCNB1
LGALS8
GDF15
AMACR
RPL23
SFRP4
GSCN6
TP53
NCAM2
HOXB13
SH3GLB1
CLDN4
PTEN
MUC1
KLK3
BIRC5
RPL30
CLDN3

FIGURE 2B

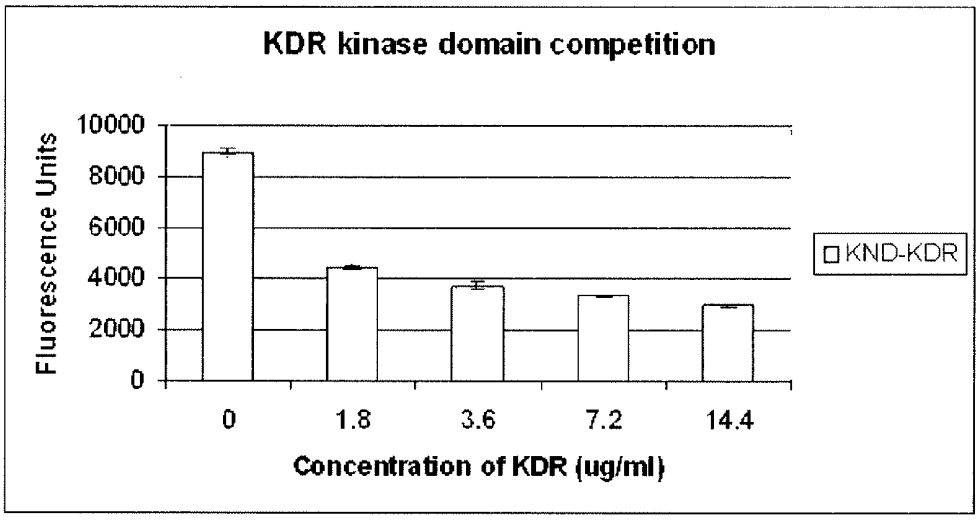


FIGURE 2C

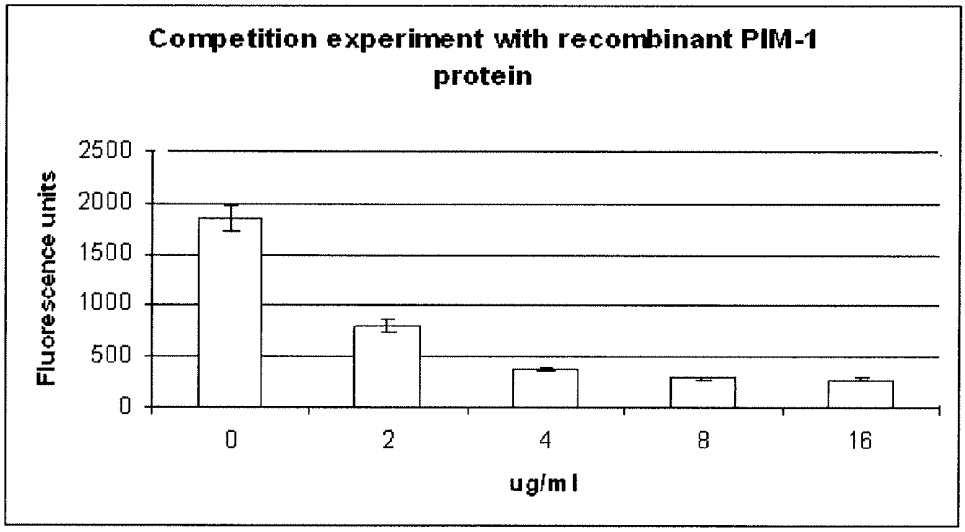
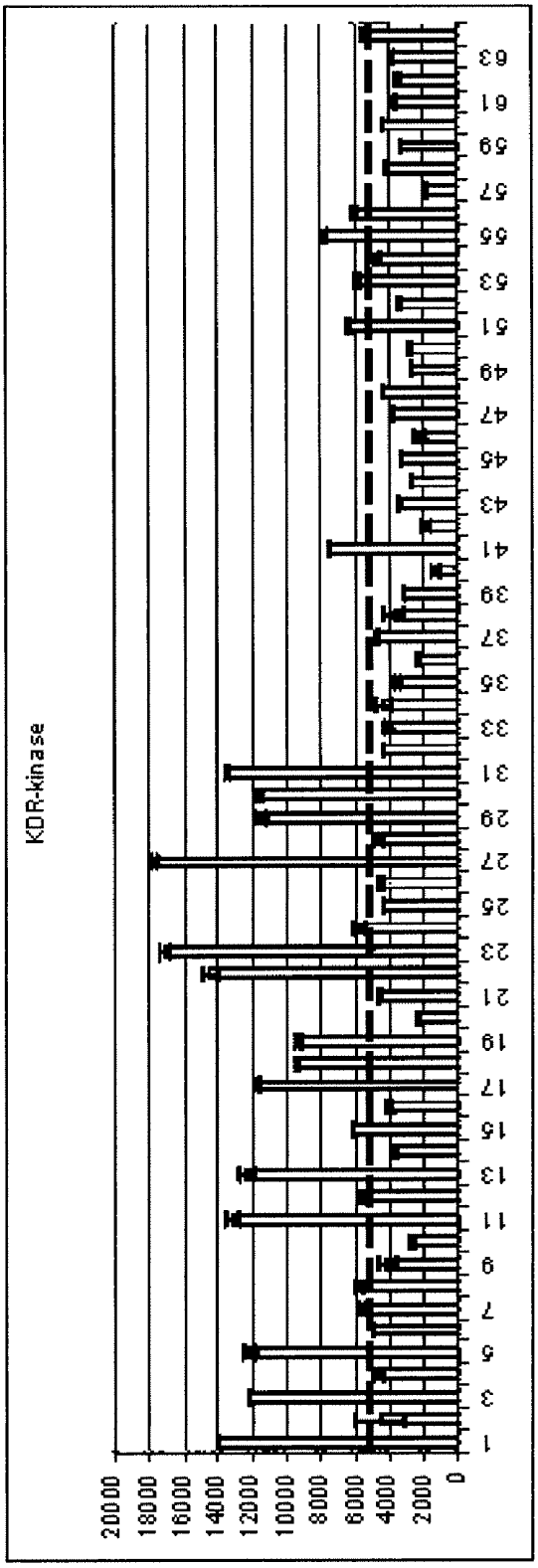


FIGURE 2D


KDR-kinase
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
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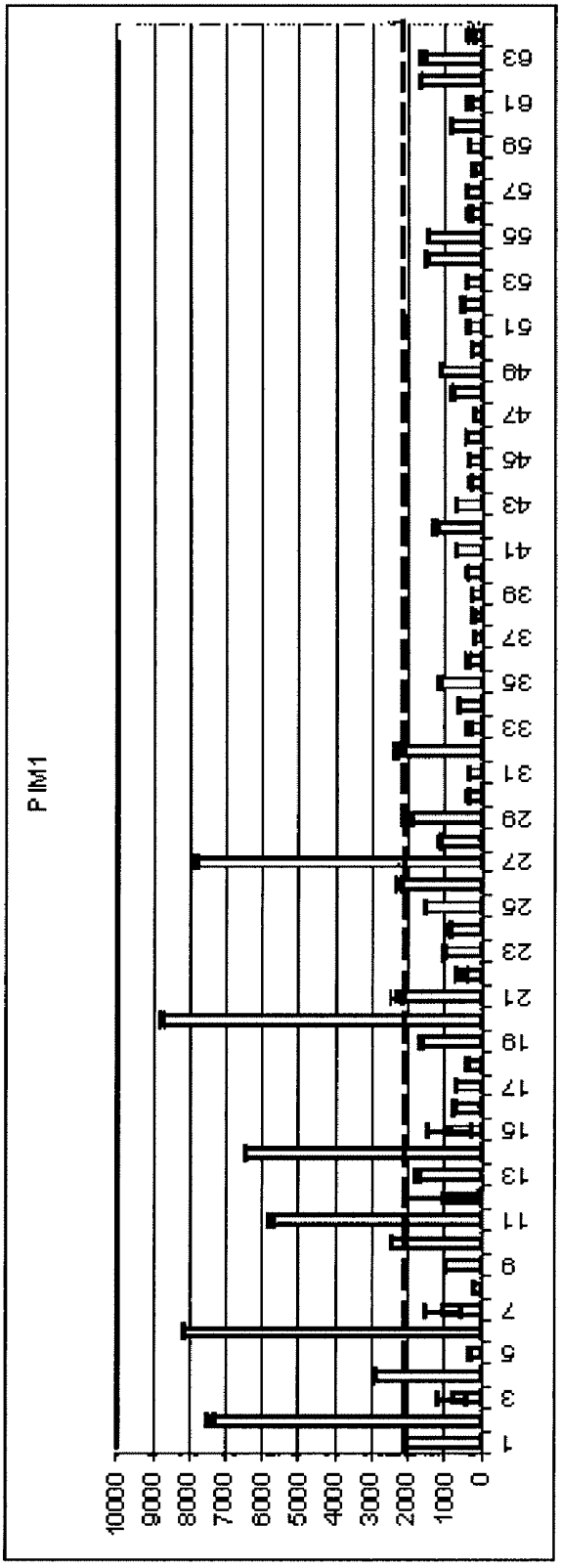
61
63

FIGURE 3A


PIM1
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
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39
41
43
45
47
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51
53
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57
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61
63

FIGURE 3B

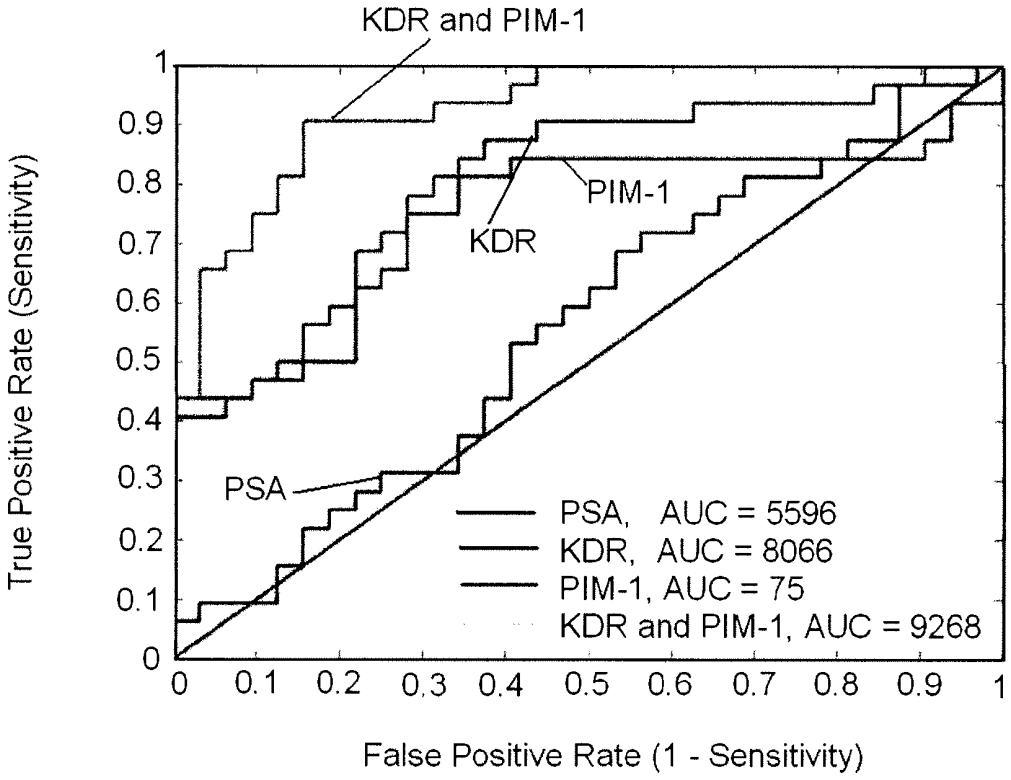
KDR and PIM-1
PIM-1
KDR
PSA
PSA, AUC = 5596
KDR, AUC = 8066
PIM-1, AUC = 75
KDR and PIM-1, AUC = 9268
True Positive Rate (Sensitivity)
False Positive Rate (1 - Sensitivity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

FIGURE 3C

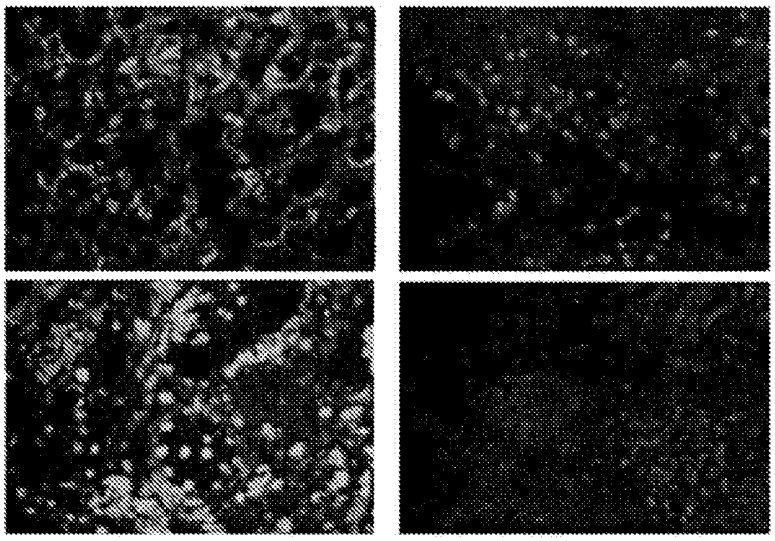

FIGURE 4

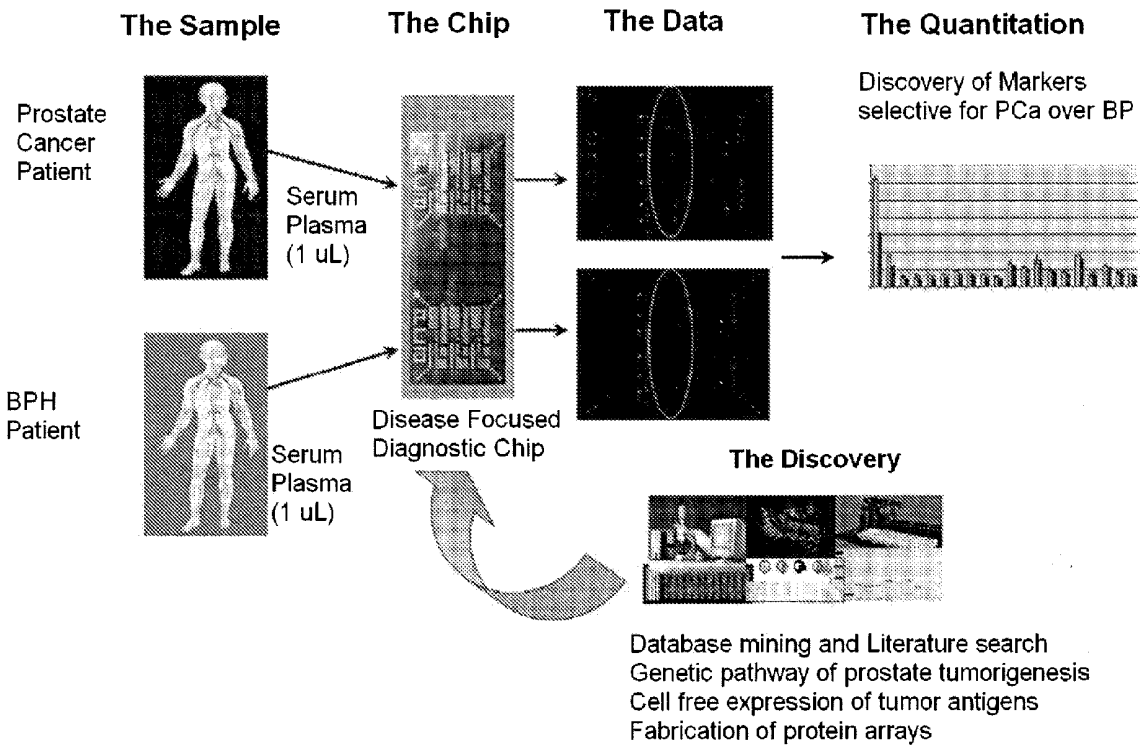
The Sample
The Chip
The Data
The Quantitation
Prostate Cancer Patient
Serum Plasma (1 uL)
BPH Patient
Serum Plasma (1 uL)
Disease Focused Diagnostic Chip
Discovery of Markers selective for PCa over BP
The Discovery
Database mining and Literature search
Genetic pathway of prostate tumorigenesis
Cell free expression of tumor antigens
Fabrication of protein arrays

FIGURE 5

METHODS AND KITS FOR DETECTING PROSTATE CANCER BIOMARKERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 60/865,621, filed Nov. 13, 2006, which is incorporated by reference in its entirety herein to the extent that there is no consistency with the present disclosure.

BACKGROUND

[0002] The invention generally relates to biomarkers associated with prostate cancer, and methods and compositions for the detection, diagnosis, prognosis, and monitoring of the progression of prostate cancer.

[0003] Prostate cancer (also referred to herein as "PCa") is the most prevalent form of cancer and the second most common cause of cancer death in American men (Jemal et al. (2007) "Cancer statistics," CA Cancer J. Clin. 57(1):43-66). When prostate cancer is diagnosed in its early stages, however, the prognosis is very good, with a ten year survival rate of greater than 85%. Current treatment modalities include radiation therapy, surgery, and androgen deprivation therapy. Treatment of prostate cancer can have serious side effects, including impairment of sexual or urinary function, thus the decision to intervene should be made on the most reliable criteria possible.

[0004] Accurate, early diagnosis of prostate cancer has proven challenging however, as the current diagnostic test for prostate cancer relies on detection of prostate-specific antigen (PSA) levels, an indicator that also correlates with benign prostate hypertrophy (BPH), a non-life threatening condition that does not increase cancer risk. BPH is found in about half of men at age 60, and about 90% of men reaching the age of 85. The PSA test is currently widely used in prostate cancer diagnosis. In general, a blood serum level of 4 ng per ml or higher of PSA is considered suggestive of prostate cancer, while a PSA level of 10 ng per ml or higher is considered highly suggestive of prostate cancer. While the PSA test has a fairly good sensitivity (80%), it suffers from a false positive rate that approaches 75%. It is estimated that for PSA values of 4-10 ng/mL, only one true diagnosis of prostate cancer was found in approximately 4 biopsies performed (Catalona et al. (1994) "Comparison of digital rectal examination and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men," J. Urol. 151(5):1283-90). Tests that measure the ratio of free to total (free plus bound) PSA do not have significantly greater specificity or sensitivity than the standard PSA test.

[0005] Recently a urinary test has been developed based on detection of the PCA3 transcript. However, the reliability of the test depends on its being performed in conjunction with an attentive digital rectal exam (DRE), which means the time and effort of a trained clinician are required, as well as the willingness of the patient to undergo DRE.

[0006] Another condition known as prostate intraepithelial neoplasia (PIN) may precede prostate cancer by five to ten years, but requires no treatment or intervention. Currently there are no specific diagnostic tests for PIN, although the ability to detect and monitor the potentially pre-cancerous condition would contribute to early detection and enhanced survival rates for prostate cancer.

[0007] Autoantibody-based approaches using protein microarrays have a number of distinct advantages for the discovery of quality diagnostic biomarkers. Many of the potentially best disease-based biomarkers are not secreted into the blood at levels that are detectable in a robust manner. Such biomarkers will always be "unavailable" for convenient in-vitro blood-based diagnostic tests. However, autoantibodies to these same specific non-secreted biomarkers (if formed), will circulate beyond the boundaries of the diseased tissue and will be stable in whole-blood for extended periods of time. Using a protein array approach one can very quickly explore of a large number of potential protein targets for the presence of autoantibodies to correlate with specific diseases.

[0008] Cancer initiation and progression has been shown to associate with the process of immunoediting (Dunn et al. (2004), "The three Es of cancer immunoediting," Annu Rev Immunol. 22:329-60). In immunoediting, the immune system interacts with cancer and induces a cancer specific immune response, with unique immune signatures characteristic of the various stages of cancer progression. Tumor associated antigens including peptides, proteins and polysaccarides have been utilized in microarray or ELISA experiments to profile cancer and normal sera.

[0009] Prostate cancer progression involves multiple steps including: prostatic intraepithelial neoplasia (PIN), localized carcinoma, invasive carcinoma and metastasis. The genetic and epigenetic events in prostate tumorigenesis include the loss of function of tumor suppressors, cell cycle and apoptosis regulators, proteins in metabolism machinery and stress response, angiogenesis and metastasis related molecules (Abate-Shen et al. (2000) "Molecular genetics of prostate cancer," Genes Dev. 14(19):2410-34; Ciocca et al. (2005) "Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications," Cell Stress Chaperones 10(2):86-103). These proteins could potentially serve as PCa antigens and induce autoantibody response in PCa patients. Furthermore these induced autoantibodies can be used for PCa diagnosis either in a single- or multiple-marker format. While PCa results from the deregulated proliferation of epithelial cells, BPH majorly results from normal epithelial cell proliferation which does not frequently lead to malignancy (Ziada et al. (1999) "Benign prostatic hyperplasia: an overview," Urology 53(3 Suppl 3D):1-6). Immune profiling using serum samples from PCa and BPH patients will help to identify biomarkers with unique autoantibody patterns in PCa, clearly distinguishable from the BPH autoantibody signature (s).

SUMMARY OF THE INVENTION

[0010] The invention relates generally to the detection of autoantibodies related to cancer, and more particularly to methods of diagnosing, prognosing, and monitoring prostate cancer using panels of antigens for the detection of autoantibodies.

[0011] The invention recognizes the need for an accurate test for prostate cancer, and in particular for a minimally invasive test that can detect prostate cancer and, preferably, distinguish prostate cancer from benign prostate hypertrophy (BPH) with high sensitivity and specificity. The invention is based in part on a collection of target antigens and target antibodies for detecting autoantibodies, and on the detection of autoantibody biomarkers for the detection, diagnosis, prognosis, staging, and monitoring of cancer, particularly prostate cancer. The invention provides biomarkers and

biomarker detection panels that include autoantigens, in which the biomarker detection panels have high selectivity and sensitivity for the detection of prostate cancer and for the diagnosis of prostate cancer over BPH. The invention also provides methods of detecting, diagnosing, prognosing, staging, and monitoring prostate cancer by detecting prostate cancer biomarkers in a test sample of an individual.

[0012] One aspect of the invention is a method of detecting an autoantibody in a sample from an individual. The method includes: contacting a sample from the individual with an autoantibody capture molecule of the invention, and detecting binding of an antibody in the sample to the autoantibody capture molecule, thereby detecting an autoantibody in the individual. The autoantibody capture molecule can be a target antigen that recognizes an autoantibody, or can be a target antibody that can bind an autoantigen complexed with an autoantibody. A target antigen can be an entire protein, such as the protein referred to as a target antigen, or a variant or modified form of the designated proteins, or a target antigen can be an epitope-containing fragment of the protein named as a target antigen. An autoantibody capture molecule that is a target antibody is an antibody that can bind an autoantigen that is complexed with an autoantibody. An autoantibody capture molecule can be, for example, any of the autoantibody capture molecules listed in Table 1 or Table 11a, or can be an antibody to any of the target antigens of Table 1 or Table 11a. In some embodiments, the autoantibody capture molecules are a target antigen of Table 2 or an antibody to any of the target antigens provided in Table 2, in which the antibody can specifically bind an autoantibody-autoantigen complex that includes a target antigen of Table 2.

[0013] In one embodiment, a sample from the individual is contacted with two or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or are target antibodies against an antigen of Table 1 or Table 11a. In further embodiments, the autoantibody capture molecules are autoantibody capture molecules of Table 2, Table 3, Table 4, Table 10, or target antibodies to antigens in these tables (which can be described as subsets of Table 1). An autoantibody, which may correlate to the diagnosis of prostate cancer, is detected in the sample when an antibody or antibody-containing complex is detected to have bound to at least two of the autoantibody capture molecules. Preferably, the binding of the autoantibody capture molecules to antibodies or antibody-containing complexes in the test sample distinguishes between prostate cancer and BPH, and preferably distinguishes between Low Grade prostate cancer and High Grade prostate cancer.

[0014] In some embodiments of the present invention, the methods of detecting and diagnosing PCa and the biomarker detection panels exclude autoantibody capture molecules of PSA.

[0015] In a further embodiment, the sample from the individual is contacted with two or more autoantibody capture molecules, in which at least one of the autoantibody capture molecules is selected from the group consisting of KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, CCNB1, AMACR, TP53, MUC1, KLK3, BIRC5, and target antibodies to KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, CCNB1, AMACR, TP53, MUC1, KLK3, and BIRC5. In some preferred embodiments, the sample is contacted with KDR and/or PIM-1. It is understood that "KDR and/or PIM-1" encompasses full length KDR, a variant of KDR recognized by an antibody that recognizes KDR, a fragment of KDR comprising an epitope recognizable by an antibody, and/or full length PIM-1, a variant of PIM-1 recognized by an antibody that recognizes PIM-1, and a fragment of PIM-1 comprising an epitope recognizable by an antibody.

[0016] In certain aspects, a biomarker panel of the present invention includes a first biomarker that includes an epitope of KDR and a second biomarker that includes an epitope of PIM-1. In certain illustrative embodiments, the epitope of the first biomarker and/or the second biomarker is an epitope that is known to be recognized by autoantibodies in the sera of human subjects. In certain embodiments, the first biomarker and/or the second biomarker are at least 5 kDa or at least 10 kDa. In an illustrative embodiment the first biomarker is full-length PIM-1 and the second biomarker is full-length KDR.

[0017] In another aspect of the invention, any of fourteen novel tumor antigens (or variants or fragments thereof), or antibodies to these antigens, that have not been previously reported as inducing an autoantibody response are contacted with a sample to detect prostate cancer and to distinguish prostate cancer from BPH. In one embodiment, a sample from an individual suspected as having prostate cancer is contacted with one or more autoantibody capture molecules are selected from the group consisting of KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, and target antibodies to KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, and PTEN. Preferably, the sample is contacted with one or more autoantibody capture molecules including KDR and/or PIM-1. It is understood that these terms encompasses full length KDR, a variant of KDR recognized by an antibody that recognizes KDR, a fragment of KDR comprising an epitope recognizable by an antibody, full length PIM-1, a variant of PIM-1 recognized by an antibody that recognizes PIM-1, and a fragment of PIM-1 comprising an epitope recognizable by an antibody.

[0018] The assays suitable for use with the present invention includes assays used to detect autoantibodies in fluid samples from individual, as well non-fluid samples, such as a prostate tissue sample, from an individual. The sample used in the assays and detection and diagnosis methods of the invention can be any type of sample, but preferably is a saliva sample or a blood sample, or a fraction thereof, such as plasma or serum. In some embodiments, the sample is blood or a fraction thereof, such as, for example, serum. In other embodiments, the sample is a non-fluid sample such as a tissue sample. In a further embodiment, the tissue sample is a prostate tissue sample. The individual can be an individual that is being screened for cancer, and in some embodiments is a male individual being screened for prostate cancer.

[0019] In some embodiments, the methods are directed to detecting prostate cancer, in which the methods include: determining the immune reactivity of a test sample from the individual against an autoantibody capture molecule, in which the autoantibody capture molecule is one of molecules of Table 4 (or a variant or fragment thereof), or an antibody to any of the target antigens of Table 4, in which the antibody can specifically bind an autoantibody-autoantigen complex, and correlating the immune reactivity of the test sample to the

capture molecule to a diagnosis of prostate cancer. The method can in some embodiments be used to distinguish prostate cancer from BPH.

[0020] In another aspect, the invention provides methods of diagnosing prostate cancer in an individual by contacting a sample from an individual with a biomarker detection panel comprising two or more autoantibody capture molecules of Table 1, or an antibody to any of the target antigens of Table 1; and detecting the pattern of immune reactivity of the test sample to the biomarker detection panel, in which the pattern of immune reactivity of the test sample to the biomarker detection panel is indicative of the presence of prostate cancer. An autoantibody capture molecule can be a target antibody or a target antigen. A target antigen can be an entire protein, such as a protein referred to as a target antigen, or can be a variant, processed, unprocessed, or modified form of the designated protein, or can be or comprise an epitope-containing fragment of the protein designated. An autoantibody capture molecule that is a target antibody is an antibody that can detect an autoantibody in a sample that is complexed to an autoantigen.

[0021] A biomarker detection panel used in the methods of the invention comprises a plurality of autoantibody capture molecules, for example, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 10 or more, 20 or more, 50 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, 2,000 or more, 5,000 or more, or 10,000 or more, of which from 2 to 214 of the autoantibody capture molecules are from Table 1 and/or Table 11a.

[0022] An autoantibody capture molecule included in the present invention, in certain embodiments is at least 70%, 75%, 80%, 85%, 90%, 95%, or 100% identical to an at least 25, 50, 75, 100 or the entire amino acid segment of SEQ ID NO:1 or SEQ ID NO:2. The autoantibody capture molecule in certain illustrative embodiments binds to an autoantibody of KDR or PIM-1.

[0023] In preferred embodiments, at least one of the autoantibody capture molecules of the biomarker detection panel is an autoantibody capture molecule of Table 3. The biomarker detection panel used to detect prostate cancer can in some embodiments comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, between 30 and 35, between 35 and 40, between 40 and 45, between 45 and 50, between 50 and 55, between 55 and 60, between 60 and 65, or between 65 and 70 autoantibody capture molecules of Table 3 or antibodies to any of the antigens of Table 3, in which antibodies to the antigens of Table 3 used on the chip are used for detection of autoantibodies via binding of autoantigen-autoantibody complexes of the sample.

[0024] In some embodiments, the biomarker detection panel comprises at least two autoantibody capture molecules of Table 3. In some embodiments, the biomarker detection panel comprising two or more autoantibody capture molecules of Table 1 includes at least one target antigen selected from the group consisting of Table 4. In some preferred embodiments, the biomarker detection panel used in the methods of the invention has an ROC curve with an AUC value (also referred to as ROC/AUC value) for distinguishing prostate cancer from BPH is 0.800 or greater. In some preferred embodiments of the method, the ROC curve with an AUC value of the biomarker detection panel for distinguishing the presence of PCa from BPH is 0.900 or greater.

[0025] In some embodiments the invention includes methods of diagnosing prostate cancer and methods of distinguishing prostate cancer from BPH that include: contacting a sample from an individual with a biomarker detection panel that includes two or more autoantibody capture molecules of Table 3, in which at least one of the autoantibody capture molecules is from Table 10, and detecting the pattern of immune reactivity of the sample to the biomarker detection panel, in which the pattern of immune reactivity of the sample to the biomarker detection panel distinguishes prostate cancer from BPH in the individual. In some exemplary embodiments, the biomarker detection panel comprises at least one 3-marker autoantibody detection set of Table 5, at least one 4-marker autoantibody detection set of Table 6, at least one 5-marker autoantibody detection set of Table 7, at least one 6-marker autoantibody detection set of Table 8, at least one 7-marker autoantibody detection set of Table 9.

[0026] The biomarker detection panel in some embodiments has a specificity of 80% or greater, 85% or greater, 90% or greater, 96% or greater, or 98% or greater, and/or a sensitivity of 80% or greater, 90% or greater, 96% or greater, 98% or greater, or 100%, for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual. The biomarker detection panel in some embodiments has a Bayesian specificity of 78% or greater, 85% or greater, or 90% or greater, for diagnosing prostate cancer, and/or a Bayesian sensitivity of 80% or greater, 90% or greater, or 95% or greater for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual. The biomarker detection panel in some exemplary embodiments has a Bayesian accuracy of 80% or greater, 85% or greater, 85% or greater, 90% or greater, or 96% or greater for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual.

[0027] In preferred embodiments of the methods for diagnosing prostate cancer, the test sample is blood or a fraction thereof, such as serum. In some embodiments, the individual is a male aged 50 or older. In some embodiments, the method is repeated over time for the individual. In some embodiments, the individual is monitored at regular or irregular intervals after cancer treatment by determining immune reactivity of samples of the patient to a biomarker detection panel of the invention. The immune reactivity of a sample tested at a later date can be compared with the immune reactivity of a sample taken at an earlier date.

[0028] The invention provides in yet other aspects biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, or distinguishing prostate cancer from BPH, that include two or more autoantibody capture molecules of Table 1, in which at least one of the antibody capture molecules is of Table 3. A biomarker detection panel of the invention comprises a plurality of autoantibody capture molecules, for example, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 10 or more, 20 or more, 50 or more, 100 or more, 200 or more, 500 or more, or 1,000 or more autoantibody capture molecules. The biomarker detection panel can comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, between 30 and 35, between 35 and 40, between 40 and 45, between 45 and 50, between 50 and 55, between 55 and 60, between 60 and 65, or between 65 and 70 autoantibody capture molecules of Table 3.

[0029] In some preferred embodiments, a biomarker detection panel includes at least one of the autoantibody capture

molecules selected from Table 2. In some preferred embodiments, a biomarker detection panel includes at least one of the autoantibody capture molecules selected from Table 4. The invention provides biomarker detection panels that include KDR, PIM-1, or both KDR and PIM-1.

[0030] In some embodiments a biomarker detection panel can further comprise antibodies such as but not limited to one or more of antibodies to ACCP, BCL2, PSA (total), PSA (free), CXCR4, PTGER2, IL-6, IL-8, PAP, or PSMA. In some preferred embodiments, biomarker detection panel comprises antibodies to ACCP and/or IL-6.

[0031] In some preferred embodiments of biomarker detection panels, at least one of the autoantibody capture molecules is selected from Table 10. In some exemplary embodiments, a biomarker detection panel of the invention comprises one or more autoantibody detection sets of Table 5, Table 6, Table 7, Table 8, or Table 9.

[0032] In some preferred embodiments, the biomarker detection panel is provided bound to one or more solid or semi-solid supports, such as, for example, a gel or matrix, beads, particles, fibers, rods, filaments, or a filter, strip, sheet, membrane, plate (for example, a multiwell plate), dish, chip or array. In some preferred embodiments, at least 50% of the human proteins bound to the solid support are test antigens of the biomarker detection panel. In some preferred embodiments, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the human proteins bound to the solid support are test antigens of the biomarker detection panel. In some preferred embodiments, the biomarker detection panel is provided in or on a protein array.

[0033] The invention also provides kits that include one or more biomarker detection panels as provided herein. The kits can include one or more reagents for detecting binding of an antibody, or an antigen-antibody complex, from a sample. Detection reagents can include one or more antibodies, labels, labeling reagents, or buffers. In some embodiments, the one or more autoantibody capture molecules of a biomarker panel of a kit are provided bound to a solid support. In some embodiments of kits, the kit provides a biomarker detection panel in which the target antigens of the detection panel are bound to a chip or array.

[0034] A kit of the present invention can include 2 or more autoantibody capture molecules of Table 1 or Table 11a associated with different vessels and/or solid supports.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIG. **1** is a depiction of an autoantibody chip of the present invention that was used to identify prostate cancer autoantibody biomarkers. The array on the chip comprises 8 capture antibodies, 108 auto-antigens, mouse anti-human K 3-step (positive control), mouse anti-human IgG1 3-step (positive control), protein L 3-step (positive control), human IgG 4-step (positive control), and 1137 empty spots. The array shown in FIG. **1** is printed in duplicate on each chip, so every spot on the chip is replicated a total of four times.

[0036] FIGS. **2**A-**2**D illustrate an autoantibody profiling experiment with pooled serum samples from 32 PCa and 32 BPH patients. The protein array is made of 96 protein antigens and the signal normalized using a protein L spot printed on the microarray. FIG. **2**A is a representative image to show the difference between PCa and BPH. Among 90 antigens printed on the cellulose slides about half show significant autoantibody signals. The arrows show KDR and PIM-1 spots which capture significantly higher autoantibody signals in pooled PCa serum than in pooled BPH serum. FIG. **2**B shows the top 20 protein antigens showing the highest fold difference between PCa serum and BPH serum. FIG. **2**C shows an autoantigen competition experiment using purified KDR protein at the concentration as shown. At 1.8 ug/ml concentration, half of the KDR signals were eliminated. Similarly, FIG. **2**D shows an autoantigen competition experiment using purified PIM-1 protein at the concentrations as shown. At 2 ug/ml concentration half of the PIM-1 signal was eliminated.

[0037] FIGS. **3**A-**3**C illustrate autoantibody fluorescence signal profiles of 32 PCa patients (numbered 1-32) and 32 BPH patients (numbered 33-64) for both KDR antigen (FIG. **3**A) and PIM-1 antigen (FIG. **3**B). Only the odd patient numbers are labeled in the figures. The signal threshold level determined by ROC analysis is denoted by horizontal dashed line. FIG. **3**C shows a plot of ROC curves of 64 sera data set using 1-plex analysis of KDR (green), PIM-1 (red), PSA (dark blue) and a 2-plex analysis of KDR & PIM-1 (light blue) combination. KDR and PIM-1 2-plex analysis generates a sensitivity and specificity of 90.6% and 84.4% respectively. AUCs for all analyses are shown in the legend. The experiments were done with a low content microarray containing KDR and PIM-1 antigens.

[0038] FIG. **4** shows images of prostate tissue microarray experiments with anti-KDR and anti-PIM-1 antibodies. Red fluorescence shows the autoantibody signals detected by Alexa 647 labeled goat anti-human IgG and the blue fluorescence indicated the counter-staining of nuclei by DAPI. The images show that KDR and PIM-1 are preferentially expressed in PCa tissues. Over expression of KDR and PIM-1 proteins lead to the aberrant humoral response in PCa patients.

[0039] FIG. **5** shows the scheme for the autoantibody profiling experiment described in FIGS. **2**A-**2**D. Samples from individuals having prostate cancer and BPH were collected and contacted with a chip containing possible target antigens selected based on their role in prostate cancer. The resulting binding of target antigens to autoantibodies was quantified and used to identify biomarkers selective for prostate cancer over BPH.

DETAILED DESCRIPTION

[0040] The invention is based on the identification of candidate antigens for the detection of autoantibodies in samples from individuals. Test antigens and test antibodies provided in Table 1 and are human proteins selected based on knowledge of prostate cancer biology. Of the test antigens listed in Table 1, TP53, PTEN, PDLIM1,SPRX, NUCB1, and PSCA are prostate cancer pathway-specific tumor suppressor genes; FOLH1, KDR, PSIP1, EGFR, ERBB2, CCKBR, XLKD1, MMP9, TMPRSS2, AGR2, PRSS8, MUC1, LGALS8, CD164, CXCR4, NRP1, STEAP1, HPN, MET, PTGER3, CLDN3, CLDN4, NCAM2, EDNRB, FLT1, PECAM1, BDKRB2, CD151, QSCN6, ERG, PCNA, EPCAM, and MAD1L1 are cell surface proteins expressed by some cancer cells; HSPA1A, HSPB1, SERPINH1, HSPA5, TRA1, MICB, PSMAB4, UBE2C, STIP1, HSPD1, and UBQLN1 are proteins involved in innate immunity; EIF4G1 ALOX15, PTGS1, RPL23, RPS14, ELAC1, EIF3S3, TOP2A, RPS6KA1, ACPP, KLK3, FASN, RPL30, and ENO1 are proteins involved in cell metabolism; CCNB1, CCND1, CCNA, CDKN2A, CUL4A, BIRC5, MYC, ETS2, BCL2, BCLG, TP53BP2, GDF15, RASSF1, AKT1, MDM2, PIM1,

SH3GLB1, HIMP2, HIMP3, KHDRBS1, PCNA, and CAV3, are cell cycle or apoptosis-related proteins; and E6 and E7 are human papillomavirus antigens. HIP1, BRD2, AZGP1, COVA1, MLH1, TPD52, PSAP, MIB1, HOXB13, RDH11, HMGA2, ZWINT, RCV1, SFRP4, SPRR1B, HMGA2, HIP2, and HEYL were also found to be cancer-associated.

TABLE 1

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
ABV0G41VX-KLK3	KLK3	KLK3, APS, PSA, hK3, KLK2A1	kallikrein 3, (prostate specific antigen) GI: 22208991 NM_145864	In vitro synthesized wheat germ (WG IVT) - Abnova; Tapei City, Taiwan
ACPP	ACPP	ACPP, PAP, ACP3, ACP-3	acid phosphatase, prostate GI: 6382063 NM_001099	WG IVT
AGR2	AGR2	AGR2, AG2, GOB-4, HAG-2, XAG-2	anterior gradient 2 homolog (*Xenopus laevis*) GI: 20070225NM_006408	WG IVT
AKT1	AKT1	AKT1, PKB, RAC, PRKBA, MGC99656, RAC-ALPHA	v-akt murine thymoma viral oncogene homolog 1 GI: 62241010 NM_005163	WG IVT
ALOX15	ALOX15	ALOX15	arachidonate 15-lipoxygenase GI: 40316936 NM_001140	WG IVT
AMACR	AMACR	AMACR, RACE	alpha-methylacyl-CoA racemase NM_014324 GI: 42794624	WG IVT
anti-ACPP	(antibody)	anti-PAP mouse capture mAb		United Biotech
anti-BCL2	(antibody)	mouse anti-bcl-2		Zymed
anti-CXCR4	(antibody)	mouse anti-CXCR4 monoclonal		Zymed
anti-IL6	(antibody)	cap Ab from cytosets assay kit for hIL-6 58.126.09 mu clone 677B 6A2 IgG1		Biosource
anti-IL8	(antibody)	capture Ab from IL8 Cytosets Kit		Biosource
anti-PSA(f)	(antibody)	(Free PSA coat Ab)		Biospacific
anti-PSA(t)	(antibody)	(Total PSA coat Ab)		Medix
anti-PTER2	(antibody)	mouse anti-PTER2 monoclonal		GeneTex
AZGP1	AZGP1	AZGP1, ZAG, ZA2G	alpha-2-glycoprotein 1, zinc GI: 38372939 NM_001185	WG IVT
BCL2	BCL2	BCL2, Bcl-2	B-cell CLL/lymphoma 2 (BCL2), nuclear gene encoding mitochondrial protein GI: 72198188 NM_000633	WG IVT
BCLG	BCL2L14	BCL2L14, BCLG	BCL2-like 14 (apoptosis facilitator) GI: 13540528 NM_030766	WG IVT
BDKRB2	BDKRB2	BDKRB2, B2R, BK2, BK-2, BKR2, BRB2, DKFZp686O088	bradykinin receptor B2 GI: 17352499 NM_000623	WG IVT
BIRC5	BIRC5	BIRC5, API4, EPR-1	baculoviral IAP repeat-containing 5 (survivin) GI: 59859879 NM_001012270	WG IVT

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
BRD2	BRD2	BRD2, NAT, RNF3, FSRG1, RING3, D6S113E, FLJ31942, KIAA9001, DKFZp686N0336	bromodomain containing 2 GI: 12408641 NM_005104	WG IVT
CAV3	CAV3	CAV3, VIP21, LGMD1C, VIP-21, MGC126100, MGC126101, MGC126129	caveolin 1, caveolae protein, 22 kDa GI: 15451855 NM_001753	WG IVT
CCKBR	CCKBR	CCKBR, GASR, CCK-B	cholecystokinin B receptor GI: 33356159 NM_176875	Purified from human serum, (EMD Biosciences, San Diego, CA)
CCNA1	CCNA1	CCNA1	cyclin A1 GI: 16306528 NM_003914	WG IVT
CCNB1	CCNB1	CCNB1, CCNB	cyclin B1 GI: 34304372 NM_031966	WG IVT
CCND1	CCND1	CCND1, BCL1, PRAD1, U21B31, D11S287E	cyclin D1 GI: 77628152 NM_053056	WG IVT
CD151	CD151	CD151, GP27, MER2, RAPH, SFA1, PETA-3, TSPAN24	CD151 molecule (Raph blood group) GI: 87159810 NM_004357	WG IVT
CD164	CD164	CD164, MGC-24, MUC-24, endolyn	CD164 molecule, sialomucin GI: 34222157 NM_006016	WG IVT
CDKN2A	CDKN2A	CDKN2A, ARF, MLM, p14, p16, p19, CMM2, INK4, MTS1, TP16, CDK4I, CDKN2, INK4a, p14ARF, p16INK4, p16INK4a	cyclin-dependent kinase inhibitor 2A GI: 47132606 NM_000077	WG IVT
CLDN3	CLDN3	CLDN3, RVP1, HRVP1, C7orf1, CPE-R2, CPETR2	claudin 3 GI: 21536298 NM_001306	WG IVT
CLDN4	CLDN4	CLDN4, CPER, CPE-R, CPETR, CPETR1, WBSCR8, hCPE-R	Claudin 4 GI: 34335232 NM_001305	WG IVT
COVA1	COVA1	COVA1, APK1, tNOX	cytosolic ovarian carcinoma antigen 1 GI: 32528292 NM_006375	WG IVT
CUL4A	CUL4A	CUL4A	cullin 4A (CUL4A), transcript variant 2 GI: 57165422 NM_003589	WG IVT
CXCR4	CXCR4	CXCR4, FB22, HM89, LAP3, LCR1, NPYR, WHIM, CD184, LESTR, NPY3R, NPYRL, HSY3RR, NPYY3R, D2S201E	chemokine (C—X—C motif) receptor 4 GI: 56790926 NM_001008540	WG IVT
E6	NA		HPV (viral) protein	WG IVT
E7	NA		HPV (viral) protein	WG IVT
EDNRB	EDNRB	EDNRB, ETB, ETRB, HSCR, ABCDS, HSCR2	endothelin receptor type B GI: 4557546 NM_000115	WG IVT

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
EGFR	EGFR	EGFR, ERBB, mENA, ERBB1	epidermal growth factor receptor GI: 41327737 NM_005228	WG IVT
EIF3S3	EIF3S3	EIF3S3, eIF3-p40, MGC102958, eIF3-gamma	eukaryotic translation initiation factor 3, subunit 3 gamma, 40 kDa GI: 83656776 NM_003756	WG IVT
EIF4G1	EIF4G1	EIF4G1, p220, EIF4F, EIF4G, DKFZp686A1451	eukaryotic translation initiation factor 4 gamma, 1 GI: 38201620 NM_182917	WG IVT
ELAC1	ELAC1	ELAC1, D29	elaC homolog 1 (*E. coli*) GI: 50726987 NM_018696	WG IVT
ENO1	ENO1	ENO1, NNE, PPH, MPB1, MBP-1, ENO1L1	enolase 1, (alpha) GI: 16507965 NM_001428	WG IVT
EP-CAM	TACSTD1	TACSTD1, EGP, KSA, M4S1, MK-1, CD326, EGP40, MIC18, TROP1, Ep-CAM, hEGP-2, CO17-1A, GA733-2	tumor-associated calcium signal transducer 1 precursor GI: 49457558 NM_002354	WG IVT
ERBB2	ERBB2	ERBB2, NEU, NGL, HER2, TKR1, HER-2, c-erb B2, HER-2/neu	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) GI: 54792097 NM_001005862	WG IVT
ERG	ERG	ERG, p55, erg-3	v-ets erythroblastosis virus E26 oncogene homolog (avian) GI: 46255021 NM_004449	WG IVT
ETS2	ETS2	ETS2	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) GI: 41327737 NM_005228	WG IVT
FASN	FASN	FASN, FAS, OA-519, MGC14367, MGC15706	fatty acid synthase GI: 41872630 NM_004104	WG IVT
FLT1	FLT1	FLT1, FLT, VEGFR1	fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) GI: 32306519 NM_002019	WG IVT
FOLH1	FOLH1	FOLH1, PSM, FGCP, FOLH, GCP2, PSMA, mGCP, GCPII, NAALAD1, NAALAdase	folate hydrolase (prostate-specific membrane antigen) 1 GI: 4758397 NM_004476	WG IVT
GDF15	GDF15	GDF15, PDF, MIC1, PLAB, MIC-1, NAG-1, PTGFB, GDF-15	growth differentiation factor 15 GI: 4758935 NM_004864	WG IVT
HEYL	HEYL	HEYL, HRT3, MGC12623	hairy/enhancer-of-split related with YRPW motif-like	WG IVT

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
HIP1	HIP1	HIP1, ILWEQ, MGC126506	GI: 105990530 NM_014571 huntingtin interacting protein 1 GI: 38045918 NM_005338	In vitro Synthesized wheat germ (Abnova; Tapei City, Taiwan)
HIP2	HIP2	HIP2, LIG, HYPG, UBE2K	huntingtin interacting protein 2 GI: 21536483 NM_005339	Synthesized in *E. coli* (U of MI)
HMGA2	HMGA2	HMGA2, BABL, LIPO, HMGIC, HMGI-C	high mobility group AT-hook 2 GI: 62912481 NM_003484	WG IVT
HOXB13	HOXB13	HOXB13, PSGD	homeobox B13 GI: 84043952 NM_006361	WG IVT
HPN	HPN	HPN, TMPRSS1	hepsin (transmembrane protease, serine 1) GI: 4504480 NM_002151	WG IVT
HSPA1A	HSPA1A	HSPA1A, HSP72, HSPA1, HSPA1B, HSP70-1	heat shock 70 kDa protein 1A GI: 26787973 NM_005345	WG IVT
HSPA5	HSPA5	HSPA5, BIP, MIF2, GRP78, FLJ26106	heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) GI: 21361242 NM_005347	WG IVT
HSPB1	HSPB1	HSPB1, CMT2F, HSP27, HSP28, Hsp25, HS.76067, DKFZp586P1322	heat shock 27 kDa protein 1 GI: 4996892 NM_001540	WG IVT
HSPD1	HSPD1	HSPD1, CPN60, GROEL, HSP60, HSP65, SPG13, HuCHA60	heat shock 60 kDa protein 1 (chaperonin) GI: 41399283 NM_002156	WG IVT
IMP-2	IGF2BP2	IGF2BP2, p62, IMP2, IMP-2, VICKZ2	insulin-like growth factor 2 mRNA binding protein 2 GI: 64085376 NM_006548	WG IVT
IMP-3	IGF2BP3	IGF2BP3, IMP3, KOC1, IMP-3, VICKZ3, DKFZp686F1078	insulin-like growth factor 2 mRNA binding protein 3 GI: 30795211 NM_006547	WG IVT
KDR	KDR	KDR, FLK1, CD309, VEGFR, VEGFR2	kinase insert domain receptor (a type III receptor tyrosine kinase) GI: 11321596 NM_002253	WG IVT
KHDRBS1	KHDRBS1	KHDRBS1, p62, Sam68	KH domain containing, RNA binding, signal transduction associated 1 GI: 5730026 NM_006559	WG IVT
LGALS8	LGALS8	LGALS8, Gal-8, PCTA1, PCTA-1, Po66-CBP	lectin, galactoside-binding, soluble, 8 GI: 42544184 NM_006499	WG IVT
MAD1L1	MAD1L1	MAD1L1, MAD1, PIG9, HsMAD1, TP53I9, TXBP181	MAD2 mitotic arrest deficient-like 1 GI: 6466452 NM_002358	WG IVT
MDM2	MDM2	MDM2, hdm2, MGC71221	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein GI: 46488903 NM_002392	WG IVT

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
MET	MET	MET, HGFR, RCCP2	met proto-oncogene (hepatocyte growth factor receptor) GI: 42741654 NM_000245	WG IVT
MIB1	MIB1	MIB1, MIB, ZZZ6, DIP-1, ZZANK2, FLJ90676, MGC129659, MGC129660, DKFZp686I0769, DKFZp761M1710	mindbomb homolog 1 (*Drosophila*) GI: 62868229 NM_020774	WG IVT
MICB	MICB	MICB, PERB11.2	MHC class I polypeptide-related sequence B GI: 26787987 NM_005931	WG IVT
MLH1	MLH1	MLH1, FCC2, COCA2, HNPCC, hMLH1, HNPCC2, MGC5172	mutL homolog 1, colon cancer, nonpolyposis type 2 GI: 28559089 NM_000249	WG IVT
MMP9	MMP9	MMP9, GELB, CLG4B, MMP-9	matrix metallopeptidase 9 GI: 74272286 NM_004994	WG IVT
MUC1	MUC1	MUC1, EMA, PEM, PUM, MAM6, PEMT, CD227, H23AG	mucin 1, cell surface associated GI: 65301116 NM_002456	WG IVT
MYC	MYC	MYC, c-Myc	v-myc myelocytomatosis viral oncogene homolog (avian) GI: 71774082 NM_002467	WG IVT
NCAM2	NCAM2	NCAM2, NCAM21, MGC51008	neural cell adhesion molecule 2 GI: 33519480 NM_004540	WG IVT
NRP1	NRP1	NRP1, NRP, CD304, VEGF165R, DKFZp781F1414, DKFZp686A03134	neuropilin 1 GI: 57162075 NM_015022	WG IVT
NUCB1	NUCB1	NUCB1, NUC, FLJ40471, DKFZp686A15286	nucleobindin 1 GI: 39725676 NM_006184	WG IVT
PCNA	PCNA	PCNA, MGC8367	proliferating cell nuclear antigen GI: 33239450 NM_182649	WG IVT
PDLIM1	PDLIM1	PDLIM1, CLIM1, CLP36, ELFIN, CLP-36, hCLIM1	PDZ and LIM domain 1 (elfin) GI: 20127594 NM_020992	WG IVT
PECAM1	PECAM1	PECAM1, CD31, PECAM-1	platelet/endothelial cell adhesion molecule (CD31 antigen) GI: 110347450 NM_000442	WG IVT
PIM1	PIM1	PIM1, PIM	pim-1 oncogene GI: 31543400 NM_002648	WG IVT
PRL	PRL	PRL	Prolactin GI: 40254429 NM_000948	IV synthesized in WG system (Abnova, Taipei City, Taiwan)
PRL	PRL	PRL	Prolactin GI: 40254429 NM_000948	Purified from human serum (Fitgerald Industries

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
				International, Concord, MA)
PRSS8	PRSS8	PRSS8, CAP1, PROSTASIN	*Homo sapiens* protease, serine, 8 (prostasin) GI: 21536453 NM_002773	WG IVT
PSA	KLK3	KLK3, APS, PSA, hK3, KLK2A1	kallikrein 3, (prostate specific antigen) GI: 22208991 NM_145864	WG IVT
PSAP	PSAP	PSAP, GLBA, SAP1, FLJ00245, MGC110993	prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy) GI: 110224477 NM_002778	WG IVT
PSCA	PSCA	PSCA, PRO232	prostate stem cell antigen GI: 83641882 NM_005672	WG IVT
PSIP1	PSIP1	PSIP1, p52, p75, PAIP, DFS70, LEDGF, PSIP2, MGC74712	PC4 and SFRS1 interacting protein 1 GI: 19923652 NM_033222	WG IVT
PSMB4	PSMB4	PSMB4, HN3, HsN3, PROS26	proteasome (prosome, macropain) subunit, beta type, 4 GI: 22538466 NM_002796	WG IVT
PTEN	PTEN	PTEN, BZS, MHAM, TEP1, MMAC1, PTEN1, MGC11227	phosphatase and tensin homolog GI: 110224474 NM_000314	WG IVT
PTGER3	PTGER3	PTGER3, EP3, EP3e, EP3-I, EP3-II, EP3-IV, EP3-III, MGC27302, MGC141828, MGC141829	prostaglandin E receptor 2 GI: 31881629 NM_000956	WG IVT
PTGS1	PTGS1	PTGS1, COX1, COX3, PHS1, PCOX1, PGHS1, PTGHS, PGG/HS, PGHS-1	prostaglandin-endoperoxide synthase 1 GI: 18104966 NM_000962	WG IVT
QSCN6	QSCN6	QSCN6, Q6, QSOX1	quiescin Q6 GI: 52493187 NM_002826	WG IVT
RASSF1	RASSF1	RASSF1, 123F2, RDA32, NORE2A, RASSF1A, REH3P21	Ras association (RaIGDS/AF-6) domain family 1 GI: 25777678 NM_007182	WG IVT
RCV1	RCVRN	RCVRN, RCV1	recoverin GI: 56550117 NM_002903	WG IVT
RDH11	RDH11	RDH11, MDT1, PSDR1, RALR1, SCALD, ARSDR1, CGI-82, HCBP12, FLJ32633	retinol dehydrogenase 11 GI: 20070271 NM_016026	WG IVT
RNF14	RNF14	RNF14, ARA54, HFB30, FLJ26004, HRIHFB2038	ring finger protein 14 GI: 34577094 NM_004290	WG IVT
RPL23	RPL23	RPL23, rpL17, MGC72008, MGC111167, MGC117346	ribosomal protein L23a GI: 78190460 NM_000984	WG IVT
RPL30	RPL30	RPL30	ribosomal protein L30 GI: 15812218 NM_000989	WG IVT

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
RPS14	RPS14	RPS14, EMTB	ribosomal protein S14 (RPS14) GI: 68160914 NM_001025070	WG IVT
RPS6KA1	RPS6KA1	RPS6KA1, RSK, HU-1, RSK1, MAPKAPK1A, S6K-alpha 1	ribosomal protein S6 kinase, 90 kDa, polypeptide 1 GI: 56243479 NM_002953	WG IVT
SERPINH1	SERPINH1	SERPINH1, CBP1, CBP2, gp46, AsTP3, HSP47, PIG14, RA-A47, SERPINH2	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1) GI: 32454740 NM_001235	WG IVT
SFRP4	SFRP4	SFRP4, FRP-4, FRPHE, MGC26498	secreted frizzled-related protein 4 GI: 8400733 NM_003014	WG IVT
SH3GLB1	SH3GLB1	SH3GLB1, Bif-1, CGI-61, KIAA0491, dJ612B15.2	SH3-domain GRB2-like endophilin B1 GI: 108936948 NM_016009	WG IVT
SPRR1B	SPRR1B	SPRR1B, SPRR1, GADD33, CORNIFIN, MGC61901	small proline-rich protein 1B (cornifin) GI: 83582814 NM_003125	WG IVT
STEAP	STEAP1	STEAP1, STEAP, PRSS24, MGC19484	six transmembrane epithelial antigen of the prostate 1 GI: 22027487 NM_012449	WG IVT
STIP1	STIP1	STIP1, HOP, P60, STI1L, IEF-SSP-3521	stress-induced-phosphoprotein 1 GI: 110225356 NM_006819	WG IVT
TMPRSS2	TMPRSS2	TMPRSS2, PRSS10	transmembrane protease, serine 2 GI: 14602458 NM_005656	WG IVT
TOP2A	TOP2A	TOP2A, TOP2, TP2A	topoisomerase (DNA) II alpha 170 kDa GI: 19913405 NM_001067	WG IVT
TP53	TP53	TP53, p53, LFS1, TRP53	tumor protein p53 (Li-Fraumeni syndrome) GI: 8400737 NM_000546	WG IVT
TPD52	TPD52	TPD52, D52, N8L, PC-1, PrLZ, hD52	tumor protein D52 GI: 70608192 NM_005079	WG IVT
TRA1(-SP)	HSP90B1	HSP90B1, ECGP, GP96, TRA1, GRP94	heat shock protein 90 kDa beta (Grp94), member 1 GI: 4507676 NM_003299	WG IVT
UBE2C	UBE2C	UBE2C, UBCH10, dJ447F3.2	ubiquitin-conjugating enzyme E2C GI: 32967292 NM_007019	WG IVT
UBQLN1	UBQLN1	UBQLN1, DA41, DSK2, XDRP1, PLIC-1, FLJ90054	ubiquilin 1 GI: 44955932 NM_013438	WG IVT
XLKD1	XLKD1	XLKD1, HAR, LYVE-1, CRSBP-1	extracellular link domain containing 1 GI: 40549450 NM_006691 NM_016164	WG IVT

TABLE 1-continued

Test Antigens and Test Antibodies

Marker (Autoantibody Capture Molecule)	HUGO designation	Protein/Gene Aliases	Genbank Identifiers	Source
ZWINT	ZWINT	ZWINT, KNTC2AP, HZwint-1, MGC117174	ZW10 interactor GI: 53729319 NM_001005413	WG IVT

[0041] The Examples provided herein demonstrate that test antigens and test antigens from the 108 test antigens and 8 test antibodies of Table 1, when tested using an immunoassay, detected antibodies or antibody-antigen complexes, respectively, in blood samples of prostate cancer patients. Seventy of the test antigens and all eight of the test antibodies of Table 1 detected autoantibodies or antibody-antigen complexes, respectively, in serum from prostate cancer patients tested using the methods for detecting autoantibodies provided herein.

[0042] In addition, ninety-nine target antigens that detected autoantibodies in serum of prostate cancer patients were identified on the PROTOARRAY™ high density protein chip (Invitrogen, Carlsbad, Calif.). The detection of autoantibodies that bind these test antigens (thereby confirmed as autoantigens) in a sample of an individual can be, alone or in combination with the presence or levels of other biomarkers, indicative of cancer. Table 11a provides target antigens identified through the PROTOARRAY™ high density protein chip that demonstrate ability to detect prostate cancer while distinguishing prostate cancer from BPH. Table 11a also indicates whether each antigen has significance for distinguishing High Grade prostate cancer, Low Grade prostate cancer, or both (overall) from BPH. Tables 11b and 11c provide statistical support showing that these antigens are able to distinguish prostate cancer from BPH.

TABLE 11a

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH

Gene Symbol	Genbank Accession	UltimateORF ™ Clone ID	Significance Call
ACAD9	BC001817	IOH5174	Sig in HG
B2M	BC032589	IOH21955	Sig in HG
BMX	NM_001721	IOH11645	Sig Overall
BRAF	NP_004324		Sig in LG
BRD3	BC032124	IOH23093	Sig in HG, Sig Overall
C10orf65	BC011916	IOH12850	Sig in HG
C11orf9	BC004938	IOH5465	Sig in HG
C14orf126	NM_080664	IOH9768	Sig in HG
C14orf147	BC021701	IOH23015	Sig in HG
C1orf36	NM_183059	IOH39968	Sig in HG
C4orf15	NM_024511	IOH5198	Sig in HG
C7orf31	BC043269	IOH25865	Sig in HG
C9orf123	BC009510	IOH12049	Sig in HG, Sig Overall
CA14	NM_012113	IOH27401	Sig in HG
CASQ2	BC022288	IOH12278	Sig in HG, Sig Overall

TABLE 11a-continued

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH

Gene Symbol	Genbank Accession	UltimateORF ™ Clone ID	Significance Call
CD58	BC005930	IOH7549	Sig in HG
CDCA8	BC001651	IOH3740	Sig in HG, Sig Overall
CDKN1A	BC001935	IOH5068	Sig in HG
CRABP2	NM_001878	IOH1673	Sig Overall
CYB5-M	NM_030579	IOH5585	Sig in HG
DKFZp434L142	NM_016613	IOH11008	Sig in HG, Sig Overall
DTNBP1	NM_032122	IOH13153	Sig in HG
DYRK1A	NM_001396		Sig in HG
EFS	NM_032459	IOH21413	Sig in HG
FER	NM_005246		Sig in HG
FHIT	NM_002012	IOH21676	Sig Overall
FKBP6	BC036817	IOH22107	Sig in HG
FLJ10052	BC004888	IOH5668	Sig in HG
FLJ13150	BC039014	IOH26125	Sig in HG
FLJ13910	NM_022780	IOH13276	Sig in HG
FLJ30294	BC020898	IOH13022	Sig in HG
FLJ30473	BC032485	IOH21724	Sig in HG
FLJ32884	BC033790	IOH21793	Sig in LG
FLJ44216	BC032390	IOH27534	Sig Overall
FRMD3	BC023560	IOH27849	Sig in LG
FTL	BC016715	IOH27895	Sig Overall
HADHSC	BC000306	IOH3456	Sig Overall
HCK	BC014435	IOH14630	Sig in HG
HEY1	BC001873	IOH4800	Sig in HG, Sig Overall
HLA-DRB2	BC033827	IOH21889	Sig in HG
HNRPK	NM_002140	IOH3670	Sig Overall
HSPA4	BC002526	IOH4058	Sig in HG
IL17RB	BC000980	IOH2952	Sig in HG, Sig Overall
JDP2	NM_130469	IOH28073	Sig in HG, Sig Overall
LARP	BC033856	IOH21797	Sig in HG, Sig Overall
LEPREL1	BC005029	IOH6657	Sig Overall
LIG3	NM_013975	IOH40893	Sig in HG
LOC196394	NM_207337	IOH40127	Sig in HG, Sig Overall
LOC441046	BC025996	IOH10875	Sig in HG
MGC31967	NM_174923	IOH14835	Sig in HG
MGC40168	NM_153709	IOH21517	Sig in HG, Sig Overall
MGC52010	NM_194326	IOH26706	Sig in HG
MGC59937	NM_199001	IOH28105	Sig in HG
MLKL	BC028141	IOH21529	Sig in HG
MPG	BC014991	IOH12177	Sig in HG
MPPE1	BC002877	IOH5717	Sig in HG
MS4A4A	NM_024021	IOH36738	Sig in HG
MTHFD2	BC017054	IOH10366	Sig in HG
MVD	NM_002461	IOH4651	Sig in HG

TABLE 11a-continued

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH

Gene Symbol	Genbank Accession	UltimateORF ™ Clone ID	Significance Call
MYC	BC000141	IOH2954	Sig in HG
MYLC2PL	BC002778	IOH5313	Sig Overall
NAP1L2	BC026325	IOH11158	Sig Overall
PDE4DIP	BC025406	IOH11226	Sig in HG
PDYN	NM_024411	IOH11247	Sig in HG
PPAP2B	BC009196	IOH12943	Sig in HG
PPIA	BC007104	IOH7532	Sig in HG
PRKACB	BC035058	IOH27691	Sig Overall
PSMD11	NM_002815	IOH3459	Sig in LG
PTGS2	NM_000963	IOH11237	Sig in LG
RFX5	NM_000449	IOH10040	Sig in HG
RNF5	NM_006913	IOH3743	Sig in HG, Sig Overall
RPL14	BC005134	IOH5666	Sig Overall
RPS19	NM_001022	IOH4572	Sig Overall
RPS6KA3	NM_004586		Sig in HG
RPS6KC1	NM_012424		Sig Overall
RRAGB	BC034726	IOH25776	Sig in HG
SERPINI2	NM_006217	IOH11838	Sig in HG
SFRS7	BC000997	IOH2939	Sig in HG
SMARCD2	BC018953	IOH13650	Sig in HG
SMN2	NM_017411	IOH10903	Sig in LG

TABLE 11a-continued

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH

Gene Symbol	Genbank Accession	UltimateORF ™ Clone ID	Significance Call
SMR3B	NM_006685	IOH11229	Sig in HG
SNAI2	NM_003068	IOH10082	Sig in HG
SPG21	NM_016630	IOH4511	Sig Overall
SPRR1B	BC056240	IOH29466	Sig Overall
SRP9	BC021995	IOH14627	Sig in HG
TTYH2	BC004233	IOH5231	Sig in HG, Sig Overall
TXNL4A	NM_006701	IOH3749	Sig Overall
TYRO3	NM_006293		Sig in HG
UBOX5	NM_199415	IOH26936	Sig in HG
UCK2	NM_012474	IOH40599	Sig in LG
URG4	BC018426	IOH9673	Sig in HG
UROS	NM_000375	IOH4136	Sig Overall
UXS1	BC009819	IOH12608	Sig Overall
VAPB	NM_004738	IOH4934	Sig in HG
VIPR2	NM_003382	IOH9624	Sig in HG
WDR4	NM_033661	IOH6391	Sig in HG, Sig Overall
ZCCHC4	BC016914	IOH10995	Sig in HG
ZNF581	NM_016535	IOH4783	Sig in HG, Sig Overall

TABLE 11b

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH; Statistics including P-values

Gene Symbol	Low Grade Cancer/Normal Ratio	High Grade Cancer/Normal Ratio	All PCA vs BPH P-Value	HG PCA vs BPH P-Value	LG PCA vs BPH P-Value
ACAD9	1.162470024	0.757246377	0.05988135	0.000969619	0.259609431
B2M	1.219371915	0.956939082	0.023031288	0.000161603	0.250773994
BMX	1.23719588	0.799912988	0.000707711	0.003393665	0.054227197
BRAF	1.06137257	0.756860044	0.03294643	0.127989657	0.000357228
BRD3	1.007407407	1.46509851	0.00022316	0.000161603	0.003611971
C10orf65	1.27312196	0.778762307	0.002055424	0.000969619	0.057791538
C11orf9	1.13630708	1.341577122	0.001913559	0.000969619	0.015440184
C14orf126	1.634055501	0.804034796	0.042772393	0.000969619	0.187306502
C14orf147	1.353750582	0.908549821	0.004574786	0.000969619	0.03989045
C1orf36	0.970859211	0.772121966	0.002055424	0.000969619	0.018059856
C4orf15	1.243298599	0.706382334	0.004574786	0.000969619	0.057791538
C7orf31	1.276783872	0.971358584	0.001301768	0.000161603	0.049122807
C9orf123	1.333791089	0.783155116	0.000317583	0.000969619	0.004437564
CA14	1.197577425	0.759441992	0.058881043	0.000969619	0.204929745
CASQ2	1.300961325	0.853836373	0.000223218	0.000969619	0.015440184
CD58	2.010870829	0.946903077	0.005988135	0.000161603	0.105572755
CDCA8	1.48758564	0.735950982	0.00022316	0.000161603	0.014447884
CDKN1A	1.312358322	0.790184407	0.002863891	0.000969619	0.105572755
CRABP2	1.339278029	0.907247212	0.0003608	0.009049774	0.003929507
CYB5-M	0.993521615	0.477181166	0.01197627	0.000161603	0.187306502
DKFZp434L142	1.208883709	0.907434598	0.000317583	0.000969619	0.004437564
DTNBP1	1.518970007	1.030859029	0.009484313	0.000161603	0.259609431
DYRK1A	1.372657432	1.010271961	0.042772393	0.000161603	0.306501548
EFS	2.247215006	0.48469795	0.005988135	0.000969619	0.014447884
FER	1.324652778	0.955782313	0.01197627	0.000969619	0.156918314
FHIT	1.039032959	0.743245033	0.000707711	0.003393665	0.003215051
FKBP6	1.312099253	0.857584561	0.002055424	0.000969619	0.049122807
FLJ10052	2.431110974	0.702468544	0.009484313	0.000969619	0.03989045
FLJ13150	1.215104062	0.770332481	0.003932941	0.000969619	0.049122807
FLJ13910	1.424118335	0.983937883	0.001913559	0.000969619	0.156918314
FLJ30294	1.357971014	0.973864326	0.009484313	0.000969619	0.057791538
FLJ30473	2.007543999	0.540415322	0.034106334	0.000969619	0.296181631
FLJ32884	1.143609646	0.626325474	0.019690961	0.278280543	0.000357228

TABLE 11b-continued

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH; Statistics including P-values

Gene Symbol	Low Grade Cancer/Normal Ratio	High Grade Cancer/Normal Ratio	All PCA vs BPH P-Value	HG PCA vs BPH P-Value	LG PCA vs BPH P-Value
FLJ44216	1.248492407	0.90009307	0.000707711	0.003393665	0.003215051
FRMD3	1.146829664		0.001301768	0.014705882	0.000722394
FTL	0.88004925	0.761439346	0.000223218	0.003393665	0.003215051
HADHSC	1.551796828	0.880630631	0.000235304	0.00210084	0.010216718
HCK	1.330952381	0.696443342	0.003932941	0.000161603	0.023839009
HEY1	1.397489485	1.102228373	0.0003608	0.000161603	0.024886878
HLA-DRB2	1.215223401	0.629726665	0.002863891	0.000161603	0.003929507
HNRPK	1.303875824	0.773978726	0.000557901	0.052521008	0.014447884
HSPA4	1.580284949	0.992612842	0.001913559	0.000969619	0.015440184
IL17RB	1.282353347	0.93388687	0.00022316	0.000161603	0.009883306
JDP2	1.240702174	0.934734513	0.000849575	0.000969619	0.057791538
LARP	1.020773383	1.600998404	0.000557901	0.000969619	0.014447884
LEPREL1	0.997990926	0.728299735	0.000707711	0.003393665	0.001309836
LIG3	0.810788225	0.790472468	0.018485112	0.000969619	0.049122807
LOC196394	1.150393767	0.717851917	0.000849575	0.000969619	0.018059856
LOC441046	0.871319142	0.697274808	0.001019651	0.000969619	0.023839009
MGC31967	1.702403255	0.739953427	0.023031288	0.000161603	0.147368421
MGC40168	0.749691945	0.882877794	0.000223218	0.000969619	0.003215051
MGC52010	1.089363462	0.766755587	0.001301768	0.000969619	0.049122807
MGC59937	1.433770866	1.171278591	0.076990307	0.000161603	0.296181631
MLKL	1.545161861	0.623150295	0.002863891	0.000161603	0.147368421
MPG	1.621703212	0.93860869	0.005988135	0.000161603	0.014447884
MPPE1	1.137113311	0.679554968	0.001301768	0.000161603	0.018059856
MS4A4A	1.250713572	1.1002849	0.001913559	0.000161603	0.015440184
MTHFD2	1.573891626	0.959472228	0.002863891	0.000161603	0.102167183
MVD	0.982767902	0.761473708	0.004574786	0.000969619	0.003215051
MYC	1.294859551	1.060884219	0.005988135	0.000161603	0.105572755
MYLC2PL	1.28086945	0.770674519	0.0003608	0.020361991	0.023839009
NAP1L2	1.135174419	0.76985755	0.000334293	0.009049774	0.03989045
PDE4DIP	1.222619048	0.832978193	0.058881043	0.000969619	0.137254902
PDYN	1.343532744	0.749279144	0.013765295	0.000969619	0.102167183
PPAP2B	1.487720911	0.725823454	0.001913559	0.000161603	0.147368421
PPIA	0.894068691		0.003932941	0.000969619	0.051083591
PRKACB	1.24862016	0.840965587	0.000223218	0.003393665	0.015440184
PSMD11	0.903461389	0.733500484	0.05988135	0.207983193	0.000770025
PTGS2	1.165972222	0.706594445	0.002863891	0.073529412	0.000722394
RFX5	2.30012442	0.609409763	0.005725735	0.000969619	0.052115583
RNF5	0.918109106	0.739568234	0.000849575	0.000969619	0.004437564
RPL14	1.048172957	0.648122651	0.0003608	0.009857789	0.024886878
RPS19	2.27209198	0.613443063	0.000849575	0.009049774	0.018059856
RPS6KA3	1.295793226	0.899175064	0.01197627	0.000969619	0.147368421
RPS6KC1	1.063906337	0.772026543	0.000235304	0.020361991	0.010216718
RRAGB	1.514367816	0.705171652	0.005988135	0.000161603	0.049122807
SERPINI2	1.332480407	1.035150924	0.002863891	0.000161603	0.156918314
SFRS7	2.065030947	1.51100029	0.113756025	0.000969619	0.187306502
SMARCD2	1.246801706	0.936639229	0.001913559	0.000161603	0.052115583
SMN2	1.059886664	0.612138501	0.003291672	0.009049774	0.000770025
SMR3B	1.298064611	1.065819421	0.023031288	0.000161603	0.187306502
SNAI2	1.306347607	0.959687052	0.002055424	0.000161603	0.057791538
SPG21	1.432676317	1.012578455	0.000317583	0.052521008	0.015440184
SPRR1B	0.853978677	1.527526395	0.000707711	0.074660633	0.003215051
SRP9	1.577757079	0.990385933	0.01197627	0.000161603	0.102167183
TTYH2	1.392587894	1.165611587	0.00022316	0.000161603	0.03989045
TXNL4A	0.744509392	1.281679936	0.000849575	0.009857789	0.004437564
TYRO3	4.096516716		0.023031288	0.000969619	0.049122807
UBOX5	1.472284007	0.935727116	0.009484313	0.000969619	0.153147575
UCK2	0.751419118	0.882890123	0.004574786	0.009857789	0.000770025
URG4	1.232855865	1.917607328	0.002863891	0.000969619	0.014447884
UROS	1.462585682	0.873990628	0.000849575	0.009049774	0.004437564
UXS1	1.209117235	1.030439466	0.000707711	0.003393665	0.137254902
VAPB	1.4075032	0.905413431	0.009484313	0.000161603	0.057791538
VIPR2	1.624015347	0.782106501	0.043280283	0.000969619	0.147368421
WDR4	1.284354133	0.671563203	0.000557901	0.000969619	0.014447884
ZCCHC4	1.492037651	0.840563712	0.002863891	0.000161603	0.049122807
ZNF581	1.29159949	0.856237167	0.000849575	0.000969619	0.003611971

TABLE 11c

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH; Statistics including prevalence

Gene Symbol	BPH Prevalence	All PCa Prevalence	HG PCA Prevalence	LG PCA Prevalence
ACAD9	21.43%	40.91%	85.71%	40.00%
B2M	14.29%	36.36%	85.71%	50.00%
BMX	28.57%	77.27%	85.71%	80.00%
BRAF	71.43%	50.00%	28.57%	30.00%
BRD3	21.43%	63.64%	85.71%	60.00%
C10orf65	21.43%	63.64%	85.71%	50.00%
C11orf9	28.57%	72.73%	85.71%	70.00%
C14orf126	14.29%	31.82%	85.71%	80.00%
C14orf147	21.43%	59.09%	85.71%	70.00%
C1orf36	85.71%	40.91%	28.57%	50.00%
C4orf15	21.43%	59.09%	85.71%	50.00%
C7orf31	14.29%	54.55%	85.71%	40.00%
C9orf123	21.43%	72.73%	85.71%	70.00%
CA14	35.71%	59.09%	85.71%	60.00%
CASQ2	28.57%	81.82%	85.71%	70.00%
CD58	14.29%	45.46%	85.71%	80.00%
CDCA8	14.29%	63.64%	85.71%	50.00%
CDKN1A	14.29%	50.00%	85.71%	80.00%
CRABP2	64.29%	13.64%	28.57%	20.00%
CYB5-M	14.29%	40.91%	85.71%	80.00%
DKFZp434L142	21.43%	72.73%	85.71%	70.00%
DTNBP1	21.43%	54.55%	85.71%	40.00%
DYRK1A	14.29%	31.82%	85.71%	80.00%
EFS	14.29%	45.46%	85.71%	50.00%
FER	14.29%	40.91%	85.71%	70.00%
FHIT	78.57%	36.36%	28.57%	40.00%
FKBP6	85.71%	40.91%	28.57%	70.00%
FLJ10052	21.43%	54.55%	85.71%	70.00%
FLJ13150	42.86%	9.09%	28.57%	70.00%
FLJ13910	28.57%	72.73%	85.71%	70.00%
FLJ30294	21.43%	54.55%	85.71%	50.00%
FLJ30473	85.71%	59.09%	28.57%	70.00%
FLJ32884	28.57%	59.09%	71.43%	90.00%
FLJ44216	28.57%	77.27%	85.71%	80.00%
FRMD3	14.29%	54.55%	57.14%	70.00%
FTL	78.57%	22.73%	28.57%	30.00%
HADHSC	50.00%	95.46%	71.43%	90.00%
HCK	42.86%	9.09%	28.57%	20.00%
HEY1	42.86%	90.91%	85.71%	80.00%
HLA-DRB2	92.86%	54.55%	28.57%	20.00%
HNRPK	92.86%	45.46%	57.14%	60.00%
HSPA4	28.57%	72.73%	85.71%	70.00%
IL17RB	14.29%	63.64%	85.71%	80.00%
JDP2	21.43%	68.18%	85.71%	50.00%
LARP	14.29%	59.09%	85.71%	50.00%
LEPREL1	78.57%	27.27%	28.57%	20.00%
LIG3	85.71%	54.55%	28.57%	70.00%
LOC196394	85.71%	36.36%	28.57%	50.00%
LOC441046	50.00%	9.09%	28.57%	20.00%
MGC31967	14.29%	36.36%	85.71%	80.00%
MGC40168	78.57%	22.73%	28.57%	30.00%
MGC52010	14.29%	54.55%	85.71%	40.00%
MGC59937	14.29%	27.27%	85.71%	70.00%
MLKL	92.86%	54.55%	28.57%	80.00%
MPG	92.86%	59.09%	28.57%	60.00%
MPPE1	14.29%	54.55%	85.71%	60.00%
MS4A4A	78.57%	31.82%	28.57%	40.00%
MTHFD2	14.29%	50.00%	85.71%	90.00%
MVD	85.71%	45.46%	28.57%	30.00%
MYC	14.29%	45.46%	85.71%	80.00%
MYLC2PL	64.29%	13.64%	28.57%	20.00%
NAP1L2	71.43%	22.73%	28.57%	40.00%
PDE4DIP	71.43%	45.46%	28.57%	50.00%
PDYN	71.43%	95.46%	85.71%	90.00%
PPAP2B	28.57%	72.73%	85.71%	80.00%
PPIA	64.29%	95.46%	85.71%	90.00%
PRKACB	78.57%	22.73%	28.57%	40.00%
PSMD11	85.71%	63.64%	28.57%	30.00%
PTGS2	14.29%	50.00%	42.86%	70.00%

TABLE 11c-continued

Target Antigens Screened on PROTOARRAY ™ Protein Array having Significance for distinguishing High Grade or Low Grade prostate cancer from BPH; Statistics including prevalence

Gene Symbol	BPH Prevalence	All PCa Prevalence	HG PCA Prevalence	LG PCA Prevalence
RFX5	57.14%	86.36%	85.71%	60.00%
RNF5	85.71%	36.36%	28.57%	40.00%
RPL14	42.86%	90.91%	71.43%	80.00%
RPS19	21.43%	68.18%	85.71%	60.00%
RPS6KA3	14.29%	40.91%	85.71%	80.00%
RPS6KC1	57.14%	9.09%	28.57%	20.00%
RRAGB	14.29%	45.46%	85.71%	40.00%
SERPINI2	14.29%	50.00%	85.71%	70.00%
SFRS7	50.00%	68.18%	85.71%	30.00%
SMARCD2	28.57%	72.73%	85.71%	60.00%
SMN2	71.43%	27.27%	28.57%	30.00%
SMR3B	14.29%	36.36%	85.71%	80.00%
SNAI2	21.43%	63.64%	85.71%	50.00%
SPG21	21.43%	72.73%	57.14%	70.00%
SPRR1B	78.57%	27.27%	28.57%	30.00%
SRP9	14.29%	40.91%	85.71%	90.00%
TTYH2	14.29%	63.64%	85.71%	70.00%
TXNL4A	85.71%	36.36%	42.86%	40.00%
TYRO3	92.86%	68.18%	28.57%	40.00%
UBOX5	85.71%	50.00%	28.57%	70.00%
UCK2	85.71%	45.46%	57.14%	30.00%
URG4	14.29%	50.00%	85.71%	50.00%
UROS	85.71%	36.36%	28.57%	40.00%
UXS1	28.57%	77.27%	85.71%	50.00%
VAPB	21.43%	54.55%	85.71%	50.00%
VIPR2	57.14%	81.82%	85.71%	80.00%
WDR4	14.29%	59.09%	85.71%	50.00%
ZCCHC4	14.29%	50.00%	85.71%	40.00%
ZNF581	85.71%	36.36%	28.57%	60.00%

[0043] The invention therefore provides test antigens and target antigens that specifically bind autoantibodies present in samples of individuals. The invention also provides test antibodies and target antibodies that specifically bind autoantigens present in samples from individuals, in which the target antibodies can be used to detect autoantibodies bound to the recognized autoantigens. The invention provides methods of using target antigens and target antibodies, collectively referred to herein as autoantibody capture molecules, to detect autoantibodies in samples from individuals, and methods of using autoantibody capture molecules to detect cancer by detecting autoantibodies in samples from individuals. The invention also provides methods of using autoantibody capture molecules to detect prostate cancer by detecting autoantibodies, and biomarker detection panels that comprise autoantibody capture molecules for detecting prostate cancer autoantibody biomarkers. The invention provides methods of detecting, diagnosing, prognosing, staging, and monitoring prostate cancer using the identified target antigens and target antibodies, provides biomarker detection panels for detecting autoantibodies, and also provides kits that include autoantibody capture molecules.

[0044] A target antibody used in any of the methods and compositions provided herein can be any antibody that specifically binds an autoantigen in a sample from an individual, in which the autoantigen can be bound by the capture antibody while bound to an autoantibody from the sample. Target antibodies include, for example, the antibodies listed in Table 1, such as antibodies to ACPP, BCL2, CXCR4, IL-6, IL-8, PSA(F) (free PSA), PSA(T) (total, or free plus bound PSA), or PTGER2, that are able to detect autoantibodies by binding

antigen-antibody complexes, and also include antibodies to any of the proteins of Table 1 or Table 11a, in which the antibodies are able to detect autoantibodies in a sample by specifically binding to an autoantigen-autoantibody complex present in a sample.

[0045] A target antigen in any of the aspects or embodiments of the invention can be an entire protein, such as the protein referred to as a target antigen, including a precursor of the protein, or an unprocessed form, processed form, or post-translationally modified form of the protein, or a form of the protein that is not post-translationally modified, or a form of the protein that is partially, atypically, or abnormally post-translationally modified. A target antigen used in the methods or compositions provided herein can be an isoform of the designated protein (e.g., a splice variant), or an allelic variant, or a target antigen can be an epitope-containing fragment of the protein named as a target antigen.

[0046] The Genbank sequence identifiers and accession numbers provided in the tables that list target antigens do not limit the proteins to being encoded by those specific sequences. In particular, the identified proteins may, at a future date, have updated sequences submitted, or may have isoforms, allelic variants, homologs, etc., that are also included as a target protein (target antigen) identified by the particular Genbank sequence identifiers or accession numbers. Sequence variants of antigens or antigen fragments of proteins in the referenced tables also include peptides and polypeptides having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence homology to a protein or a fragment thereof that is at least four amino acids in length, in which the sequence variant binds an antibody that recognizes the target antigen listed in the table.

[0047] Specifically included in the term "target antigen" is a molecule that comprises an epitope-containing fragment of the protein named as a target antigen (or a sequence variant thereof), such that the molecule is specifically recognized by an antibody that recognizes the target antigen epitope. A molecule that comprises an epitope-containing fragment can be any type of molecule, and can be a polymer, including a synthetic polymer, or a biomolecule such as a polypeptide, nucleic acid, peptide nucleic acid, etc.

[0048] A fragment of a protein that includes an epitope recognized by an antibody can be at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 750, or 1000 amino acids in length, where the amino acids in the fragment correspond to consecutive amino acids in the full length protein sequence. Preferably, the fragment is at least 15 amino acids in length. A fragment that includes an epitope recognized by an antibody can be greater than 1000 amino acids in length. The fragment can also be between 5,10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, or 2000 amino acids and one amino acid less than the entire length of an autoantigen. Typically, such epitopes are characterized in advance such that it is known that autoantibodies for a given antigen recognize the epitope. Methods for epitope mapping are well known in the art.

[0049] An autoantibody capture molecule that is a target antibody is an antibody that can detect an autoantibody in a sample. An autoantibody capture molecule that can detect an autoantibody in a sample detects an autoantibody that is complexed to an autoantigen to which the target antibody specifically binds. Antibodies can be tested for their ability to bind autoantigen-autoantibody complexes by methods disclosed herein, for example, by detecting binding using a directly or indirectly labeled species-specific secondary antibody that recognizes antibodies of the species from which the sample has been provided.

[0050] An autoantibody capture molecule can be, for example, any of the target antigens or target antibodies listed in Table 1 or Table 11a, or can be an antibody to any of the target antigens of Table 1 or Table 11a. For example, Table 2 lists a set of 55 proteins selected from Table 1 which are believed to be particularly useful antigens in detecting prostate cancer. Markers from Table 1 that were detectable in prostate cancer serum and exhibited signals that were at least two times background are provided in Table 3. Table 4 lists a set of 83 proteins selected from Table 1, including proteins from Table 2, which are believed to be particularly useful antigens in detecting prostate cancer. In some embodiments an autoantibody capture molecule is a target antigen of Table 2, or is an antibody to any of the target antigens provided in Table 2, in which the antibody can specifically bind an autoantibody-autoantigen complex that includes a target antigen of Table 2. In some embodiments and antibody capture molecule is a is of Table 3. In some embodiments an autoantibody capture molecule is a target antigen of Table 4, or is an antibody to any of the target antigens provided in Table 4, in which the antibody can specifically bind an autoantibody-autoantigen complex that includes a target antigen of Table 4.

TABLE 2

Proteins of Interest Current Term and Hugo Symbol Term
AGR2
ALOX15
AZGP1
BCLG
BDKRB2
BIRC5
BRD2
CCKBR
CCNA1
CCNB1
CCND1
CD151
CLDN3
CLDN4
COVA1
CUL4A
EIF3S3
EIF4G1
ELAC1
ETS2
FLT1
HEYL
HIP2
HOXB13
HPN
MAD1L1
MICB
MLH1
NCAM2
NRP1
NUCB1
PDLIM1
PIM1
PRSS8
PSAP
PSCA
PSMB4

TABLE 2-continued

Proteins of Interest Current Term and Hugo Symbol Term
PTGER3
PTGS1
QSCN6
RASSF1
RDH11
RNF14
RPL23
RPL30
RPS14
RPS6KA1
SFRP4
SH3GLB1
SPRR1B
STEAP
TOP2A
UBE2C
UBQLN1
ZWINT

TABLE 3

Autoantibody Capture Molecules that Detected Autoantibodies in Sera of PCa Patients

MARKER	Genbank ID	
ACPP	NM_001099	antigen
AMACR	NM_014707	antigen
AZGP1	NM_001185	antigen
BCL2	NM_000633	antigen
BCLG	NM_030766	antigen
BDKRB2	NM_000623	antigen
BIRC5	NM_001012270	antigen
CAV3	NM_001753	antigen
CCNA1	NM_003914	antigen
CCNB1	NM_031966	antigen
CCND1	NM_053056	antigen
CD151	NM_004357	antigen
CD164	NM_006016	antigen
CLDN3	NM_001306	antigen
COVA1	NM_006375	antigen
EGFR	NM_005228	antigen
EIF3S3	NM_003756	antigen
EIF4G1	NM_182917	antigen
ENO1	NM_001428	antigen
ERBB2	NM_001005862	antigen
ERG	NM_004449	antigen
ETS2	NM_005239	antigen
HEYL	NM_014571	antigen
HIP1	NM_005338	antigen
HMGA2	NM_003484	antigen
HSPA1A	NM_005345	antigen
HSPA5	NM_005347	antigen
HSPB1	NM_001540	antigen
HSPD1	NM_002156	antigen
IMP-2	NM_006548	antigen
IMP-3	NM_006559	antigen
KDR	NM_002253	antigen
KHDRBS1	NM_006559	antigen
MAD1L1	NM_002358	antigen
MET	NM_000245	antigen
MICB	NM_005931	antigen
MLH1	NM_000249	antigen
MMP9	NM_004994	antigen
MUC1	NM_002456	antigen
MYC	NM_002467	antigen
NCAM2	NM_004540	antigen
NRP1	NM_015022	antigen
NUCB1	NM_006184	antigen

TABLE 3-continued

Autoantibody Capture Molecules that Detected Autoantibodies in Sera of PCa Patients

MARKER	Genbank ID	
PCNA	NM_182649	antigen
PRL	NM_000948	antigen
PRSS8	NM_002773	antigen
PSA	NM_145864	antigen
PSAP	NM_002778	antigen
PSIP1	NM_033222	antigen
PSMB4	NM_002796	antigen
PTEN	NM_000314	antigen
PTGER3	NM_000956	antigen
QSCN6	NM_002826	antigen
RASSF1	NM_007182	antigen
RCV1	NM_002903	antigen
RDH11	NM_016026	antigen
RNF14	NM_004290	antigen
RPL30	NM_000989	antigen
RPS6KA1	NM_002953	antigen
SH3GLB1	NM_016009	antigen
SPRR1B	NM_003125	antigen
STEAP	NM_012449	antigen
STIP1	NM_006819	antigen
TMPRSS2	NM_005656	antigen
TOP2A	NM_001067	antigen
TP53	NM_000546	antigen
TPD52	NM_005079	antigen
TRA1(-SP)	NM_003299	antigen
XLKD1	NM_016164	antigen
ZWINT	NM_001005413	antigen
a-ACPP		antibody
a-BCL2		antibody
a-CXCR4		antibody
a-II-6-1		antibody
a-II-8-1		antibody
a-PSA (F)		antibody
a-PSA(T)		antibody
a-Pter2		antibody

TABLE 4

Proteins of Interest Current Term
ACPP
AGR2
AKT1
ALOX15
AZGP1
BCL2
BCLG
BDKRB2
BIRC5
BRD2
CAV3
CCKBR
CCNA1
CCNB1
CCND1
CD151
CD164
CDKN2A
CLDN3
CLDN4
COVA1
CUL4A
CXCR4
EDNRB
EGFR
EIF3S3
EIF4G1

TABLE 4-continued

Proteins of Interest Current Term
ELAC1
EP-CAM
ERBB2
ETS2
FASN
FLT1
GDF15
HEYL
HIP2
HMGA2
HOXB13
HPN
HSPA1A
HSPB1
HSPD1
IMP-3
KDR
LGALS8
MAD1L1
MDM2
MICB
MLH1
NCAM2
NRP1
NUCB1
PCNA
PDLIM1
PECAM1
PIM1
PRSS8
PSAP
PSCA
PSMB4
PTEN
PTGER3
PTGS1
QSCN6
RASSF1
RDH11
RNF14
RPL23
RPL30
RPS14
RPS6KA1
SERPINH1
SFRP4
SH3GLB1
SPRR1B
STEAP
STIP1
TOP2A
TRA1
UBE2C
UBQLN1
XLKD1
ZWINT

[0051] In certain embodiments, one or more diagnostic (or prognostic) biomarkers, such as one or more autoantibody biomarkers, are correlated to a condition or disease by the presence or absence of the biomarker(s). In other embodiments, threshold level(s) of a diagnostic or prognostic biomarker(s) can be established, and the level of the biomarker(s) in a sample can be compared to the threshold level(s). Levels can be relative or absolute, and are preferably normalized with respect to one or more controls.

[0052] In the methods provided herein, the test sample can be contacted with an autoantibody capture molecule provided in solution phase, or the autoantibody capture molecule can be provided bound to a solid support. The sample can be diluted or concentrated, or subjected to one or more processing steps prior to contacting with an autoantibody capture molecule. In some preferred embodiments, the sample is a serum sample that is diluted in a binding buffer. The dilution can be any useful dilution for obtaining a detectable binding signal with acceptable background, such as, for example, from no dilution to 1:10,000, 1:1 to 1:1,000, or from 1:2 to 1:500, or from 1:5 to 1:200, or from 1:10 to 1:100. An incubation step is performed under conditions of temperature, ionic strength, and pH that are permissive of antibody binding, and for a sufficient period of time to allow antibody-antigen binding. Antibody-antigen binding conditions and assay parameters are well known in the art.

[0053] Detection can be by an immunological assay, described in further detail in a later section, such as radioimmune assay or ELISA performed in any of a wide variety of formats, or by detecting binding on a solid support or semi-solid support using labeled reagents, which can be signal-generating reagents. Detection of binding of a biomarker to a solid support can be detection on or in a gel, matrix, filter, strip, sheet, strip, membrane, slide, plate (for example, a multiwell plane), well, dish, bead, particle, filament, rod, fiber, chip, or array. In some preferred embodiments, binding of autoantigens present in the sample to autoantibody capture molecules on a protein array is detected. The protein array can have proteins other than autoantibody capture molecules bound to the array, such as, but not limited to, antibodies that are used to detect proteins that are not necessarily bound to autoantibodies, negative or positive control proteins, proteins used for normalization of signal intensity, and proteins (including but not limited to antibodies) whose reactivity or binding status to a test sample is unknown.

[0054] The invention provides autoantibody capture molecules, such as target antigens and target antibodies, for detecting autoantibodies in a sample from an individual, methods for detecting cancer, such as prostate cancer, by detecting autoantibodies in an individual, and biomarker detection panels comprising combinations of the target antigens and/or target antibodies of Table 1 that can be used to detect and diagnose prostate cancer with high sensitivity and specificity. Biomarker detection panels can include sets of autoantibody capture molecules that have high sensitivity and specificity for detecting prostate cancer, including the biomarker detection sets provided in Table 5, Table 6, Table 7, Table 8, and Table 9. Target antigens for detecting autoantibodies present in samples of prostate cancer that were identified using the PROTOARRAY™ human protein microarray (Invitrogen, Carlsbad, Calif.) are also provided (Table 11a). Biomarker detection panels can include one or both of KDR, PIM-1, or variants or fragments thereof (Example 3). Accordingly, methods, compositions, and kits are provided herein for the detection, diagnosis, staging, and monitoring of cancer, such as prostate cancer, in individuals.

DEFINITIONS

[0055] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0056] The term "about" as used herein refers to a value within 10% of the underlying parameter (i.e., plus or minus 10%), and is sometimes a value within 5% of the underlying parameter (i.e., plus or minus 5%), a value sometimes within 2.5% of the underlying parameter (i.e., plus or minus 2.5%),

or a value sometimes within 1% of the underlying parameter (i.e., plus or minus 1%), and sometimes refers to the parameter with no variation. Thus, a distance of "about 20 nucleotides in length" includes a distance of 19 or 21 nucleotides in length (i.e., within a 5% variation) or a distance of 20 nucleotides in length (i.e., no variation) in some embodiments.

[0057] As used herein, the article "a" or "an" can refer to one or more of the elements it precedes (e.g., a protein microarray "a" protein may comprise one protein sequence or multiple proteins).

[0058] The term "or" is not meant to be exclusive to one or the terms it designates. For example, as it is used in a phrase of the structure "A or B" may denote A alone, B alone, or both A and B.

[0059] By "biomarker" is meant a biochemical characteristic that can be used to diagnose, or to measure the progress of a disease or condition, or the effects of treatment of a disease or condition. A biomarker can be, for example, the presence of a nucleic acid, protein, or antibody associated with the presence of cancer or another disease in an individual. The present invention provides biomarkers for prostate cancer that are antibodies present in the sera of subjects diagnosed with prostate cancer. The biomarker antibodies in the present invention are the autoantibodies displaying increased reactivity in individuals with prostate cancer, most likely as a consequence of their increased abundance. The autoantibodies can be detected with autoantigens, human proteins that are specifically bound by the antibodies. Established biomarkers vary widely in the frequency with which they are observed. Importantly, biomarkers need not be expressed in a majority of disease individuals to have clinical value. The receptor tyrosine kinase Her2 is known to be over-expressed in approximately 25% of all breast cancers (J. S. Ross et al., Mol Cell Proteomics 3, 379-98 (April, 2004)), and yet is a clinically important indicator of disease progression as well as specific therapeutic options.

[0060] "Biomolecule" refers to an organic molecule of biological origin, e.g., steroids, fatty acids, amino acids, nucleotides, sugars, peptides, polypeptides (proteins), antibodies, polynucleotides, complex carbohydrates or lipids.

[0061] As used herein, the word "protein" refers to a full-length protein, a portion of a protein, or a peptide. The term protein includes antibodies. Proteins can be produced via fragmentation of larger proteins, or chemically synthesized. Proteins may, for example, be prepared by recombinant over-expression in a species such as, but not limited to, bacteria, yeast, insect cells, and mammalian cells. Proteins to be placed in a protein microarray of the invention, may be, for example, are fusion proteins, for example with at least one affinity tag to aid in purification and/or immobilization. In certain aspects of the invention, at least 2 tags are present on the protein, one of which can be used to aid in purification and the other can be used to aid in immobilization. In certain illustrative aspects, the tag is a His tag, a FLAG tag, a GST tag, or a biotin tag. These examples are non-limiting. Where the tag is a biotin tag, the tag can be associated with a protein in vitro or in vivo using commercially available reagents (Invitrogen, Carlsbad, Calif.). In aspects where the tag is associated with the protein in vitro, a Bioease tag can be used (Invitrogen, Carlsbad, Calif.).

[0062] As used herein, the term "peptide," "oligopeptide," and "polypeptide" are used interchangeably with protein herein and refer to a sequence of contiguous amino acids linked by peptide bonds. As used herein, the term "protein" refers to a polypeptide that can also include post-translational modifications that include the modification of amino acids of the protein and may include the addition of chemical groups or biomolecules that are not amino acid-based. The terms apply to amino acid polymers in which one or more amino acid residue is an analog or mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Polypeptides can be modified, e.g., by the addition of carbohydrate residues to form glycoproteins. The terms "polypeptide," "peptide" and "protein" include glycoproteins, as well as non-glycoproteins.

[0063] The term "antigen" or "test antigen" as used herein refers to proteins or polypeptides to be used as targets for screening test samples obtained from subjects for the presence of autoantibodies. "Autoantigen" is used to denote antigens for which the presence of antibodies in a sample of an individual has been detected. These antigens, test antigens, or autoantigens are contemplated to include any fragments thereof of the so-identified proteins, in particular, immunologically detectable fragments. The terms antigen and test antigen are also meant to include immunologically detectable products of proteolysis of the proteins, as well as processed forms, post-translationally modified forms, such as, for example, "pre" "pro," or "prepro" forms of markers, or the "pre," "pro," or "prepro" fragment removed to form the mature marker, as well as sequence variants, including but not limited to allelic variants and splice variants of the antigens, test antigens, or autoantigens or fragments thereof. The identification or listing of antigens, test antigens, and autoantigens also includes amino acid sequence variants of these, for example, sequence variants that include a fragment, domain, or epitope that shares immune reactivity with the identified antigen, test antigen, and autoantigen protein. The fragment, domain, or epitope can be provided as part of or attached to a larger molecule or compound.

[0064] As used herein, "target antigen" refers to a protein, or to a portion, fragment, variant, isoform, processing product thereof having immunoreactivity of the protein, that is used to determine the presence, absence, or amount of an antibody in a sample from a subject. A "test antigen" is a protein evaluated for use as a target antigen. A test antigen is therefore a candidate target antigen, or a protein used to determine whether a portion of a test population has antibodies reactive against it. Use of the terms "target antigen", "test antigen", "autoantigen", and, simply, "antigen" is meant to include the complete wild type mature protein, or can also denote a precursor, processed form (including, a proteolytically processed or otherwise cleaved form) unprocessed form, post-translationally modified, or chemically modified form of the protein indicated, in which the target antigen, test antigen, or antigen retains or possesses the specific binding characteristics of the referenced protein to one or more autoantibodies of a test sample. The protein can have, for example, one or more modifications not typically found in the protein produced by normal cells, including aberrant processing, cleavage or degradation, oxidation of amino acid residues, atypical glycosylation pattern, etc. The use of the terms "target antigen", "test antigen", "autoantigen", or "antigen" also include splice isoforms or allelic variants of the referenced proteins, or can be sequence variants of the referenced protein, with the proviso that the "target antigen", "test antigen", "autoantigen", or "antigen" retains or possesses the immunological reactivity of the referenced protein to one or more autoantibodies of a test sample. Use of the term "target antigen", "test antigen",

"autoantigen", or simply "antigen" specifically encompasses fragments of a referenced protein ("antigenic fragments") that have the antibody binding specificity of the reference protein. The fragment can be provided as part of or attached to a larger molecule or compound.

[0065] An "autoantibody" is an antibody present in an individual that specifically recognizes a biomolecule present in the individual. Typically an autoantibody specifically binds a protein expressed by the individual, or a modified form thereof present in a sample from the individual. Autoantibodies are generally IgG antibodies that circulate in the blood of an individual, although the invention is not limited to IgG autoantibodies or to autoantibodies present in the blood.

[0066] An "autoantibody capture molecule" is a reagent that specifically binds a particular autoantibody, or an autoantigen-autoantibody complex, in a sample from an individual. An autoantibody capture molecule can be, for example, a protein (or target antigen) that can directly bind an autoantibody, or can be an antibody (for example a target antibody) that indirectly binds an autoantibody that is complexed with an autoantigen that can specifically bind to the target antibody.

[0067] The term "target antibody" is herein used to mean an antibody that can bind an antigen-autoantibody complex.

[0068] A "variant" of a polypeptide or protein, as used herein, refers to an amino acid sequence that is altered with respect to the referenced polypeptide or protein by one or more amino acids. In the present invention, a variant of a polypeptide retains the antibody-binding property of the referenced protein. In preferred aspects of the invention, a variant of a polypeptide or protein can be specifically bound by the same population of autoantibodies that are able to bind the referenced protein. Preferably a variant of a polypeptide has at least 60% identity to the referenced protein over a sequence of at least 10 amino acids. More preferably a variant of a polypeptide is at least 70% identical to the referenced protein over a sequence of at least 4 amino acids. Protein variants can be, for example, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to referenced polypeptide over a sequence of at least 4 amino acids. Protein variants of the invention can be, for example, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to referenced polypeptide over a sequence of at least 10 amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). A variant may also have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing immunological reactivity may be found using computer programs well known in the art, for example, DNASTAR software.

[0069] As used herein, a "biomarker detection panel" or "biomarker panel" refers to a collection of biomarkers that are provided together for detection, diagnosis, prognosis, staging, or monitoring of a disease or condition, based on detection values for the set (panel) of biomarkers. The set of biomarkers is physically associated, such as by being packaged together, or by being reversibly or irreversibly bound to a solid support. For example, the biomarker detection panel can be provided, in separate tubes that are sold and/or shipped together, for example as part of a kit, or can be provided on a chip, membrane, strip, filter, or beads, particles, filaments, fibers, or other supports, in or on a gel or matrix, or bound to the wells of a multiwell plate. A biomarker detection panel can in addition or in the alternative be associated by a list, table, or program provided to a user or potential user that provides an internet address that provides computer-based linkage of the biomarker identities and information stored on a web site. A computer-based program can provide links between biomarker identities, information, and/or purchasing functions for a collection of biomarkers that make up a biomarker detection panel, based on the user's entered selections.

[0070] The phrase "differentially present" refers to differences in the quantity of a biomolecule (such as an antibody) present in a sample taken from patients having prostate cancer as compared to a comparable sample taken from patients who do not have prostate cancer (e.g., have benign prostate hyperplasia). A biomolecule is differentially present between the two samples if the amount of the polypeptide in one sample is significantly different from the amount of the polypeptide in the other sample. For example, a polypeptide is differentially present between the two samples if it is present in an amount (e.g., concentration, mass, molar amount, etc.) at least about 150%, at least about 200%, at least about 500% or at least about 1000% greater than it is present in the other sample, or if it is detectable (gives a signal significantly greater than background or a negative control) in one sample and not detectable in the other. Any biomolecules that are differentially present in samples taken from prostate cancer patients as compared to subjects who do not have prostate cancer (e.g., benign prostate hypertrophy patients) can be used as biomarkers.

[0071] A "sample" as used herein can be any type of sample, such as a sample of cells or tissue, or a sample of bodily fluid, preferably from an animal, most preferably a human. The sample can be a tissue sample, such as a swab or smear, or a pathology or biopsy sample of tissue, including tumor tissue. Samples can also be tissue extracts, for example from tissue biopsy or autopsy material. A sample can be a sample of bodily fluids, such as but not limited to blood, plasma, serum, cerebral-spinal-fluid, sputum, semen, urine, lung aspirates, nipple aspirates, tears, or a lavage. Samples can also include, for example, cells or tissue extracts such as homogenates or solubilized tissue obtained from a patient. A preferred sample is a blood or serum sample. By "blood" is meant to include whole blood, plasma, serum, or any derivative of blood. A blood sample may be, for example, serum.

[0072] A "patient" is an individual diagnosed with a disease or being tested for the presence of disease. A patient tested for a disease can have one or more indicators of a disease state, or can be screened for the presence of disease in the absence of any indicators of a disease state. As used herein an individual "suspected" of having a disease can have one or more indicators of a disease state or can be part of a population routinely screened for disease in the absence of any indicators of a disease state.

[0073] By "an individual suspected of having prostate cancer," is meant an individual who has been diagnosed with prostate cancer, or who has at least one indicator of prostate cancer, including but not limited to, a prostate biopsy pathology report that states at least one of the biopsy cores contains carcinoma or adenocarcinoma and gives a Gleason score

value, a PSA level of greater than 4 ng per ml, a PSA serum level of greater than 10 ng per mL, enlarged prostate, or a positive PCA3 test.

[0074] As used herein, the term "array" refers to an arrangement of entities in a pattern on a substrate. Although the pattern is typically a two-dimensional pattern, the pattern may also be a three-dimensional pattern. The individual entities are localized to particular positions, or loci on the array, sometimes referred to as "spots". In a protein array, the entities are proteins. In certain embodiments, the array can be a microarray or a nanoarray. A "nanoarray" is an array in which separate entities are separated by 0.1 nm to 10 μm, for example from 1 nm to 1 μm. A "microarray" is an array in the density of entities on the array is at least 100 distinct loci/cm^2. A high density array has at least 400 distinct loci per cm^2. For example, a high density protein array has at least 400 distinct protein spots per cm^2. In some embodiments, a high density array has at least 1,000 distinct loci per cm^2. For example, a high density protein array has at least 1,000 distinct protein spots per cm^2. On microarrays separate entities can be separated, for example, by more than 1 μm.

[0075] The term "protein array" as used herein refers to a protein array, a protein microarray or a protein nanoarray. A protein array may include, for example, but is not limited to, a "ProtoArray™" human protein high density array (Invitrogen, Carlsbad, Calif., available on the Internet at Invitrogen.com)) The ProtoArray™ high density protein array can be used to screen complex biological mixtures, such as serum, to assay for the presence of autoantibodies directed against human proteins. Alternatively, a custom protein array that includes autoantigens, such as those provided herein, for the detection of autoantibody biomarkers, can be used to assay for the presence of autoantibodies directed against human proteins. In certain disease states including autoimmune diseases and cancer, autoantibodies are expressed at altered levels relative to those observed in healthy individuals.

[0076] The term "protein chip" is used in the present application synonymously with protein array or microarray.

[0077] The phrase "diagnosis" as used herein refers to methods by which the skilled artisan can estimate and/or determine whether or not a patient is suffering from a given disease or condition. The skilled artisan often makes a diagnosis on the basis of one or more diagnostic indicators, i.e., a marker, the presence, absence, or amount of which is indicative of the presence, severity, or absence of the condition, physical features (lumps or hard areas in or on tissue), or histological or biochemical analysis of biopsied or sampled tissue or cells, or a combination of these.

[0078] Similarly, a prognosis is often determined by examining one or more "prognostic indicators", the presence or amount of which in a patient (or a sample obtained from the patient) signal a probability that a given course or outcome will occur. For example, when one or more prognostic indicators reach a sufficiently high level in samples obtained from such patients, the level may signal that the patient is at an increased probability of having a disease or condition in comparison to a similar patient exhibiting a lower marker level. A level or a change in level of a prognostic indicator, which in turn is associated with an increased probability of morbidity or death, is referred to as being "associated with an increased predisposition to an adverse outcome" in a patient. Preferred prognostic markers can predict the onset of prostate cancer in a patient with PIN, or a more advanced stage of prostate cancer in a patient diagnosed with prostate cancer.

[0079] The term "correlating," as used herein in reference to the use of diagnostic and prognostic indicators, refers to comparing the presence or amount of the indicator in a patient to its presence or amount in persons known to suffer from, or known to be at risk of, a given condition; or in persons known to be free of a given condition. As discussed above, a marker level in a patient sample can be compared to a level known to be associated with prostate cancer. The sample's marker level is said to have been correlated with a diagnosis; that is, the skilled artisan can use the marker level to determine whether the patient has prostate cancer, and respond accordingly. Alternatively, the sample's marker level can be compared to a marker level known to be associated with a good outcome (e.g., the absence of prostate cancer, etc.). In preferred embodiments, a profile of marker levels are correlated to a global probability or a particular outcome using ROC curves.

[0080] The phrase "determining the prognosis" as used herein refers to methods by which the skilled artisan can predict the course or outcome of a condition in a patient. The term "prognosis" does not refer to the ability to predict the course or outcome of a condition with 100% accuracy, or even that a given course or outcome is more likely to occur than not. Instead, the skilled artisan will understand that the term "prognosis" refers to an increased probability that a certain course or outcome will occur; that is, that a course or outcome is more likely to occur in a patient exhibiting a given condition, when compared to those individuals not exhibiting the condition. For example, in individuals not exhibiting the condition, the chance of a given outcome may be about 3%. In preferred embodiments, a prognosis is about a 5% chance of a given outcome, about a 7% chance, about a 10% chance, about a 12% chance, about a 15% chance, about a 20% chance, about a 25% chance, about a 30% chance, about a 40% chance, about a 50% chance, about a 60% chance, about a 75% chance, about a 90% chance, and about a 95% chance. The term "about" in this context refers to +/−1%.

[0081] "Diagnosis" means identifying the presence or nature of a pathologic condition. Diagnostic methods differ in their sensitivity and specificity. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

[0082] "Sensitivity" is defined as the percent of diseased individuals (individuals with prostate cancer) in which the biomarker of interest is detected (true positive number/total number of diseased×100). Nondiseased individuals diagnosed by the test as diseased are "false positives".

[0083] "Specificity" is defined as the percent of nondiseased individuals for which the biomarker of interest is not detected (true negative/total number without disease×100). Diseased individuals not detected by the assay are "false negatives." Subjects who are not diseased and who test negative in the assay, are termed "true negatives."

[0084] A "diagnostic amount" of a marker refers to an amount of a marker in a subject's sample that is consistent with a diagnosis of prostate cancer. A diagnostic amount can be either in absolute amount (e.g., X nanogram/ml) or a relative amount (e.g. relative intensity of signals).

[0085] A "test amount" of a marker refers to an amount of a marker present in a sample being tested. A test amount can be either in absolute amount (e.g., X nanogram/ml) or a relative amount (e.g., relative intensity of signals).

[0086] A "control amount" of a marker can be any amount or a range of amount which is to be compared against a test

amount of a marker. For example, a control amount of a marker can be the amount of a marker (e.g., seminal basic protein) in a prostate cancer patient, a BPH patient or a person without prostate cancer or BPH. A control amount can be either in absolute amount (e.g., X nanogram/ml) or a relative amount (e.g., relative intensity of signals).

[0087] "Detect" refers to identifying the presence, absence or relative or absolute amount of the object to be detected.

[0088] "Label" or a "detectable moiety" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electromagnetic, or chemical means. For example, useful labels include radiolabels such as ^{32}P, ^{35}S, or ^{125}I; heavy isotopes such as ^{15}N or ^{13}C or heavy atoms such as selenium or metals; fluorescent dyes; chromophores, electron-dense reagents; enzymes that generate a detectable signal (e.g., alkaline phosphatase or peroxidase, as commonly used in an ELISA); or spin labels. The label or detectable moiety has or generates a measurable signal, such as a radioactive, chromogenic, or fluorescent signal, that can be used to quantify the amount of bound detectable moiety in a sample. The detectable moiety can be incorporated in or attached to a molecule (such as a protein, for example, an antibody) either covalently, or through ionic, van der Waals or hydrogen bonds, e.g., or by incorporation of labeled precursors. The label or detectable moiety may be directly or indirectly detectable. Indirect detection can involve the binding of a second directly or indirectly detectable moiety to the detectable moiety. For example, the detectable moiety can be the ligand of a binding partner, such as biotin, which is a binding partner for streptavidin, which can be linked to a directly detectable label. The binding partner may itself be directly detectable, for example, an antibody may be itself labeled with a fluorescent molecule. The binding partner also may be indirectly detectable, for example, it may be bound by another moiety that comprises a label. Quantitation of the signal is achieved by any appropriate means, e.g., fluorescence detection, spectrophotometric detection (e.g., absorption at a particular wavelength), scintillation counting, mass spectrometry, densitometry, or flow cytometry.

[0089] "Measure" in all of its grammatical forms, refers to detecting, quantifying or qualifying the amount (including molar amount), concentration or mass of a physical entity or chemical composition either in absolute terms in the case of quantifying, or in terms relative to a comparable physical entity or chemical composition.

[0090] "Antibody" refers to a polypeptide ligand substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically recognizes and binds a molecule or a region or domain of a molecule (an epitope). The recognized immunoglobulin genes include the kappa and lambda light chain constant region genes, the alpha, gamma, delta, epsilon and mu heavy chain constant region genes, and the myriad immunoglobulin variable region genes. Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. This includes, e.g., Fab' and $F(ab)'_2$ fragments. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA methodologies. It also includes polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, or single chain antibodies. "Fc" portion of an antibody refers to that portion of an immunoglobulin heavy chain that comprises one or more heavy chain constant region domains, CH1, CH2 and CH3, but does not include the heavy chain variable region.

[0091] An "antibody to a protein" or an "antibody that recognizes a protein" is an antibody that specifically binds the protein.

[0092] "Immunoassay" is an assay in which an antibody specifically binds an antigen. An immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, capture, target, and/or quantify the antigen.

[0093] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at a level that is statistically significantly different from background, and do not substantially bind in a significant amount to other proteins present in the sample. In methods of the invention in which an antibody is used as a capture molecule, the antibody is selected for its specificity for a particular protein, and also, in some embodiments, for its ability to specifically bind an antigen that is complexed with an autoantibody. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). In some embodiments, a specific or selective reaction will be at least twice background signal or noise and more typically more than five times the background signal, and can be, for example, 10 to 100 times background.

[0094] "Immune reactivity" as used herein means the presence or level of binding of an antibody or antibodies in a sample to one or more target antigens. A "pattern of immune reactivity" refers to the profile of binding of antibodies in a sample (autoantibodies) to a plurality of target antigens and/or target antibodies. The profile includes the subset of target antigens and/or target antibodies to which the sample specifically binds, and/or the relative or absolute level(s) of binding to members of the subset of target antigens and/or target antibodies to which binding is detected.

[0095] An "epitope" is a site on an antigen, such as an autoantigen disclosed herein, recognized by an antibody.

Methods

[0096] The invention provides, in one aspect, a method of detecting an autoantibody in a sample from an individual. The method includes: contacting a sample from the individual with an autoantibody capture molecule of the invention, and detecting binding of an antibody in the sample to the autoantibody capture molecule, thereby detecting an autoantibody in the individual.

[0097] The autoantibody capture molecule can be a target antigen that can specifically bind an autoantibody, or can be an antibody that can specifically bind an autoantigen that is complexed with an autoantibody. The autoantibody capture molecule can be any of the target antigens or target antibodies provided in Table 1, or can be an antibody to a protein of Table 1 that can bind an antigen-autoantibody complex; and in some preferred embodiments is a protein of Table 2, or is an anti-

body that recognizes a protein of Table 2, in which the antibody can bind a target antigen of Table 2 that is complexed with an autoantibody in the sample. Target antigens specifically include variants and modified forms of the proteins listed in Table 2, and specifically include epitope-containing fragments of the proteins listed in Table 2. In some exemplary embodiments, the autoantibody capture molecule used in the methods is HEYL, MLH1, BDKRB2, PTGER3, RPL30, ZWINT, BIRC5, TOP2A, AZGP1, CLDN3, MAD1L1, PRSS8, PSAP, PSMAB4, QSCN6, RPS6KA1, SPRR1B, EIF3S3, CCNA1, RNF14, CD151, NCAM2, ETS2, MICB, NUCB1, COVA1, RASSF1, STEAP, NRP1, SH3GLB1, RDH11, BCLG, CCNB1, CCND1, or EIF4G1, or is an antibody to HEYL, MLH1, BDKRB2, PTGER3, RPL30, ZWINT, BIRC5, TOP2A, AZGP1, CLDN3, MAD1L1, PRSS8, PSAP, PSMAB4, QSCN6, RPS6KA1, SPRR1B, EIF3S3, CCNA1, RNF14, CD151, NCAM2, ETS2, MICB, NUCB1, COVA1, RASSF1, STEAP, NRP1, SH3GLB1, RDH11, BCLG, CCNB1, CCND1, or EIF4G1, in which the antibody can bind an autoantigen-autoantibody complex. In some exemplary embodiments, the autoantibody capture molecule is HEYL, BDKRB2, PTGER3, ZWINT, TOP2A, PSAP, RPS6KA1, SPRR1B, CCNA1, RNF14, NCAM2, ETS2, COVA1, RASSF1, SH3GLB1, CCNB1, or EIF4G1, or is an antibody to HEYL, BDKRB2, PTGER3, ZWINT, TOP2A, PSAP, RPS6KA1, SPRR1B, CCNA1, RNF14, NCAM2, ETS2, COVA1, RASSF1, SH3GLB1, CCNB1, or EIF4G1 in which the antibody can bind an autoantigen-autoantibody complex.

[0098] In some exemplary embodiments, the autoantibody capture molecule used in the methods is KDR or PIM-1, or is an antibody to KDR or PIM-1 in which the antibody can bind an autoantigen-autoantibody complex.

[0099] In the methods provided herein, the sample can be any sample of cells or tissue (including extracts thereof), or of any bodily fluid. Since the autoantibodies being screened for circulate in the blood and are fairly stable in blood samples, in certain illustrative embodiments, the test sample is blood or a fraction thereof, such as, for example, serum. The sample can be unprocessed prior to contact with the test antigen, or can be a sample that has undergone one or more processing steps. For example, a blood sample can be processed to remove red blood cells and obtain serum.

[0100] The individual from whom the test sample is taken can be any individual, and in some embodiments is an individual that is being screened for cancer. Autoantibodies detected in a sample from an individual can be indicative of more than one type of cancer. Individuals testing positive for autoantibodies indicative of more than one type of cancer can be further screened to determine whether a given type of cancer is present in the individual. In some embodiments, an individual from whom a sample is taken can be an individual being screened for any of prostate, breast, liver, ovarian, pancreatic, uterine, stomach, bone, brain, colorectal, bladder, or lung cancer, or a leukemia or lymphoma.

[0101] In some embodiments, the individual from whom a sample is taken for contacting with a target antigen of the invention can be a male individual being screened for prostate cancer. In some of these embodiments, the method includes: contacting a sample from the individual with a protein of Table 4, or an antibody to a protein of Table 4, and detecting binding of an antibody in the sample to the protein of Table 4, or binding of an antigen-antibody complex of the sample to the antibody to a protein of Table 4. The method can be used to detect, diagnose, prognose, stage, or monitor prostate cancer or prostate intraepithelial neoplasia (PIN) in an individual. In some embodiments, the method is used to distinguish prostate cancer from BPH. In some exemplary embodiments, the method includes contacting a sample from an individual with one or more of the target antigens: HEYL, MLH1, PTEN, BDKRB2, BCL2, PTGER3, RPL30, ZWINT, ERBB2, BIRC5, TOP2A, ACPP, AZGP1, CLDN3, HSPB1, CAV3, HSPD1, KDR, MAD1L1, PRSS8, PSAP, PSMB4, QSCN6, RPS6KA1, SPRR1B, TRA1, HMGA2, EIF3S3, CCNA1, RNF14, CD151, NCAM2, EGFR, ETS2, HSPA1A, MICB, CD164, NUCB1, COVA1, IMP-3, STIP1, RASSF1, STEAP, NRP1, SH3GLB1, RDH11, XLKD1, BCLG, CCNB1, CCND1, PCNA, or EIF4G1 or with one or more antibodies to HEYL, MLH1, PTEN, BDKRB2, BCL2, PTGER3, RPL30, ZWINT, ERBB2, BIRC5, TOP2A, ACPP, AZGP1, CLDN3, HSPB1, CAV3, HSPD1, KDR, MAD1L1, PRSS8, PSAP, PSMB4, QSCN6, RPS6KA1, SPRR1B, TRA1, HMGA2, EIF3S3, CCNA1, RNF14, CD151, NCAM2, EGFR, ETS2, HSPA1A, MICB, CD164, NUCB1, COVA1, IMP-3, STIP1, RASSF1, STEAP, NRP1, SH3GLB1, RDH11, XLKD1, BCLG, CCNB1, CCND1, PCNA, or EIF4G1; and detecting an autoantibody to one or more of the target antigens or antibodies to target antigens to detect, diagnose, monitor, stage, or prognose prostate cancer or PIN in an individual. In some exemplary embodiments, the method includes contacting a sample from an individual with one or more of the target antigens: HEYL, BDKRB2, PTGER3, ZWINT, TOP2A, PSAP, RPS6KA1, SPRR1B, HMGA2, CCNA1, RNF14, NCAM2, ETS2, CD164, COVA1, RASSF1, SH3GLB1, XLKD1, CCNB1, PCNA, ERBB2, or EIF4G1 or with one or more antibodies to one or more of these target antigens (HEYL, BDKRB2, PTGER3, ZWINT, TOP2A, PSAP, RPS6KA1, SPRR1B, HMGA2, CCNA1, RNF14, NCAM2, ETS2, CD164, COVA1, RASSF1, SH3GLB1, XLKD1, CCNB1, PCNA, ERBB2, or EIF4G1); and detecting an autoantibody to one or more of the target antigens to detect, diagnose, monitor, stage, or prognose prostate cancer or PIN in an individual. In some exemplary embodiments, the method includes contacting a sample from an individual with one or more of the target antigens: HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, RPL30, PSMB4, MICIB, IMP-3, or CCNB1 or with one or more antibodies to one or more of the target antigens (HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, RPL30, PSMB4, MICIB, IMP-3, or CCNB1), and detecting an autoantibody in the sample to one or more of HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, RPL30, PSMB4, MICIB, IMP-3, or CCNB1 to detect, diagnose, monitor, stage, or prognose prostate cancer or PIN in an individual. In some exemplary embodiments, the method includes contacting a sample from an individual with one or more of the target antigens: HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, or CCNB1 or with an antibody to one or more of the target antigens HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, or CCNB1; and detecting an autoantibody in the sample to one or more of HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, or CCNB1 to detect, diagnose, monitor, stage, or prognose prostate cancer or PIN in an individual.

[0102] The individual from which the sample is taken can be, for example, a male individual that can have one or more risk factors for prostate cancer, including but not limited to, being of age 50 or older, being of African heritage, or having a family history of prostate cancer. The individual can have

one or more indicators of prostate cancer. For example, the individual can be an individual with enlarged prostate. The individual can be a male with a PSA level of greater than 4 ng per ml, or greater than 10 ng per ml. The individual can also be a male with a PSA level of less than 4 ng per ml, such as between 0 and 4 ng per ml. The individual can be a male with a ratio of free to total PSA of less than 0.25. The individual can be a male with a level of PCA3 transcript that is suggestive of prostate cancer or BPH. The individual can be an individual previously diagnosed with PIN. The individual can also be an individual who has previously been tested or biopsied for prostate cancer and found to be negative.

[0103] The test sample can be contacted with an autoantibody capture molecule provided in solution phase, or the autoantibody capture molecule can be provided bound to a solid support. The sample can be diluted or concentrated, or subjected to one or more processing steps prior to contacting with an autoantibody capture molecule. After contacting the sample with the autoantibody capture molecule, incubation is performed under conditions of temperature, ionic strength, and pH that are permissive of antibody binding, for a sufficient period of time to allow antibody-antigen binding. Antibody-antigen binding conditions and assay parameters are well known in the art. Detection of autoantibodies can be performed using an immunoassay, which can be in any of various formats that include that detection of proteins by antibodies, including those described in further detail below.

[0104] Detection of binding in certain illustrative embodiments makes use of one or more solid or semi-solid supports to which the autoantibody capture molecule is immobilized and to which the sample from an individual, in preferred embodiments a human subject, is applied. In exemplary embodiments, after incubation of the sample with the immobilized autoantibody capture molecule (and, preferably, subsequent wash steps), or optionally, concurrently with the incubation of the sample and autoantibody capture molecule, an antibody that is reactive against antibodies of the species from which the sample is taken, e.g., anti-human antibodies (for example, an anti-human IgG antibody that is from a species other than human, for example, goat, rabbit, pig, mouse, etc.) can be applied to the solid or semi-solid support with which the sample is incubated. The anti-human IgG antibody is directly or indirectly labeled. In some embodiments, the anti-human IgG antibody is labeled in one or more additional steps after the anti-human IgG antibody is contacted with the immobilized antigen that has been contacted with a sample. After removing nonspecifically bound antibody, signal from the label that is significantly above background level is indicative of binding of a human antibody from the sample to an autoantibody capture molecule on the solid or semi-solid support.

[0105] Detection antibodies, such as for example anti-species-specific antibodies can be used to detect a captured autoantibody bound directly to a target antigen or can be used to detect a captured autoantibody bound indirectly to a target antibody. Autoantibodies bound indirectly to a target antibody are autoantibodies that are captured as autoantibody-autoantigen complexes.

[0106] The invention thus in some aspects provides methods of detecting an autoantibody in a sample from an individual, in which the method includes: contacting a sample from an individual with at least one target antibody, in which the target antibody specifically binds an antigen of Table 2 or Table 4, and detecting binding of an autoantibody-autoantigen complex of the sample to the target antibody to detect an autoantibody in the sample. A target antibody can be any antibody that specifically binds to an antigen of Table 2 or Table 4, and in some exemplary embodiments is an antibody to HEYL, MLH1, BDKRB2, PTGER3, RPL30, ZWINT, BIRC5, TOP2A, AZGP1, CLDN3, MAD1L1, PRSS8, PSAP, PSMAB4, QSCN6, RPS6KA1, SPRR1B, EIF3S3, CCNA1, RNF14, CD151, NCAM2, ETS2, MICB, NUCB1, COVA1, RASSF1, STEAP, NRP1, SH3GLB1, RDH11, BCLG, CCNB1, CCND1, or EIF4G1.

[0107] Detection of binding of the autoantibody to a target antibody is by detecting binding of an autoantigen-autoantibody complex to a target antibody. Detection of binding can be by any means that detects the autoantibody. The detection preferably uses anti-species-specific antibodies. For example, where the sample is from a human, the method can include detecting an autoantibody by detecting binding of an anti-human IgG antibody to the autoantigen-autoantibody complex bound to the target antibody. The anti-species specific IgG antibodies can be directly or indirectly labeled.

[0108] The detection of autoantibodies bound by either target antigens or target antibodies can in some preferred embodiments be performed on a solid or semi-solid support of any type, such as a bead, particle, matrix, gel, filament, fiber, rod, dish, plate, well, sheet, membrane, slide, chip, or array, such as a protein array, which can be a microarray, and can optionally be a high density microarray. Analysis on the high density PROTOARRAY™ human protein microarray (Invitrogen, Carlsbad, Calif.) is shown in Example 2, and markers of the array correlating positively with prostate cancer are provided in Table 11a.

[0109] The detection method can provide a positive/negative binding result, or can give a value that can be a relative or absolute value for the level of the autoantibody biomarker in the sample. For example, detection of binding of the target sample to a test antigen indicates the presence of an autoantibody that specifically binds the test antigen in the sample. Identifying an autoantibody present in a sample from an individual can be used to identify biomarkers of a disease or condition, or to diagnose a disease or condition. In other embodiments, the level or levels of one or more autoantibodies can be detected using the methods of the invention. An increased or decreased level of an autoantibody that is detected in the assay in exemplary embodiments is a signal of at least two, and in some embodiments at least three, standard deviations above or below the signal level when a normal control (for example, serum from an individual who does not have cancer) is assayed. For example, autoantibodies can be detected in prostate cancer samples with an increased signal or a decreased signal when compared with BPH samples. The value of the autoantibody level in some exemplary embodiments can be detected as above or below a threshold value. The autoantibody level value is preferably normalized with respect to one or more controls provided in, alongside (in parallel with, preferably, or in tandem with) the detection assay. The result can provide a diagnosis or prognosis, or can be used as an indicator for conducting further tests or evaluation that may or may not result in a diagnosis or prognosis.

[0110] In certain illustrative embodiments, the methods provided herein for detecting autoantibodies are used to detect, diagnose, prognose, stage, monitor a disease state, pre-disease state, or medical condition. For example, the methods can be used to detect, diagnose, prognose, stage, or monitor cancer or a pre-cancerous state, such as but not lim-

ited to prostate cancer or PIN. In certain illustrative embodiments, the methods provided herein for detecting autoantibodies are used for managing a disease state, pre-disease state, or medical condition, including managing treatment regimes, or contributing to the decision of whether to treat a disease, pre-disease state, or medical condition, for example, pharmaceutically, with radiation therapy, surgically, or a combination of any of these. In some embodiments the methods can be used to test for recurrence of cancer after remission or anti-cancer treatment, such as but not limited to surgery or chemotherapy.

[0111] In some embodiments, the method includes detecting an autoantibody using an autoantibody capturing molecule from Table 2 or Table 4 in a sample from an individual and detecting one or more additional biomarkers for cancer in a sample from the same individual, in which the cancer can be any kind of cancer, including but not limited to: lung, liver, breast, uterine, ovarian, pancreatic, colorectal, stomach, esophageal, head and neck, brain, bone, or prostate cancer, or a lymphoma or leukemia. The additional biomarker for cancer can be of any type, for example, a protein, peptide, antibody, nucleic acid, hormone, growth factor, or metabolic marker, and detection can provide a positive or negative result, or can be detection of a relative or absolute value. The method can be used to detect, diagnose, stage, monitor, or prognose cancer in the individual. In some preferred embodiments, the method distinguishes between prostate cancer and BPH in an individual.

[0112] The detection and diagnostic methods of the invention can be repeated over time for the individual. For example, the individual can be an individual diagnosed with PIN that is being monitored for the presence of prostate cancer. The individual can be an individual diagnosed with prostate cancer in its early stages or with a low or intermediate Gleason score.

[0113] The individual can in some embodiments of the invention be an individual who has been treated or is being treated for prostate cancer, for example, using one or more pharmaceuticals, "nutriceuticals" or dietary regime, chemical anticancer agents, radiation therapy, or surgery. The results of a diagnostic test that determines the immune reactivity of a patient sample to a test antigen can be compared with the results of the same diagnostic test done at an earlier time. Significant differences in immune reactivity over time can contribute to a diagnosis or prognosis of prostate cancer. In these aspects, the methods and compositions provided herein can be used to detect regression, progression, or recurrence of cancer, such as prostate cancer.

[0114] The methods of the invention also include detecting two or more autoantibodies in a sample from an individual that bind to two or more target antigens, in which at least one of the two or more target antigens used to detect autoantibodies is a target antigen of Table 2 or Table 4, or an epitope-containing fragment thereof. In some embodiments, the method includes: contacting a sample from the individual with a plurality of autoantibody capture molecules of the invention, and detecting binding of an antibody in the sample to at least one of the plurality of autoantibody capture molecules, thereby detecting an autoantibody in the individual. In some embodiments, the method includes: contacting a sample from the individual with a plurality of target autoantibody capture molecules of the invention, and detecting binding of at least two autoantibodies in the sample to at least two of the plurality of autoantibody capture molecules, thereby detecting at least two autoantibodies in the individual. In exemplary embodiments, at least one of the plurality of target antigens used to detect autoantibodies in the individual is an autoantibody capture molecule of Table 2 or Table 4. One or more additional autoantibody capture molecules can be from Table 1 or Table 3. In some exemplary embodiments, at least two of the plurality of target antigens used to detect autoantibodies in the individual are target antigens of Table 2 or Table 4.

[0115] In one study described in Example 3, 96 selected proteins, hypothesized to be linked to prostate cancer (PCa) progression, were expressed, purified and then printed onto nitrocellulose slides. Each printed protein spot only contained approximately 0.03 picograms of protein, so cell free expression of approximately one to ten micrograms of protein was sufficient for even an extended protein array based study. Many of these proteins were subsequently revealed to have increased levels of autoantibodies correlating with PCa, and were not present in patients with benign prostatic hyperplasia (BPH).

[0116] This study revealed twenty antigens, including KDR and PIM-1 that are listed in Table 2 and Table 4, that induced significant humoral autoantibody response. Further studies with a low content protein chip showed that autoantibodies against KDR and PIM-1 differentiated prostate cancer from benign prostatic hyperplasia with 90.6% sensitivity and 84.4% specificity in thirty-two prostate cancer and thirty-two benign prostatic hyperplasia patients. Protein array signals were specific, and could be competed away by spiking pure antigen into the sera in a dose dependent manner. Additionally fluorescence immunohistochemistry of prostate cancer tissue arrays showed that KDR and PIM-1 were differentially expressed in prostate cancer tissues with reduced expression in benign prostatic hyperplasia tissues, suggesting over-expression of KDR and PIM-1 tumor antigens lead to the aberrant humoral response.

[0117] The use of a single autoantibody marker, KDR or PIM-1 alone, gave specificities of 62.5% and 65.6% respectively, while the combination of these two markers gave significant higher specificity of 84.4%. KDR autoantibody was present in ~62% of the prostate cancer patient population, a significantly higher percentage than reported for some other tumor antigens. Since autoimmunity involves a polyclonal antibody response, using a truncated KDR may have exposed more epitopes for autoantibody capture, thereby yielding a higher frequency of positive responses in the cancer patient population.

[0118] The methods of the invention include detecting autoantibodies in a sample from an individual that bind to KDR and PIM-1, or an epitope-containing fragment thereof. In some embodiments, the method includes: contacting a sample from the individual with a plurality of autoantibody capture molecules of the invention, and detecting binding of an antibody that in the sample to at least one of KDR or PIM-1, or a fragment or variant thereof, thereby detecting an autoantibody to KDR or PIM-1 in the individual. In some embodiments, the method includes: contacting a sample from the individual with a plurality of target autoantibody capture molecules of the invention, and detecting binding of at least two autoantibodies in the sample to KDR or PIM-1, or a fragment or variant thereof, thereby detecting an autoantibody to KDR or PIM-1 in the individual. In some embodiments, the method is a method for diagnosing prostate cancer

in an individual. In some embodiments, the method is a method for distinguishing prostate cancer from BHP in an individual

[0119] Another aspect of the invention is a method of diagnosing prostate cancer in an individual by contacting a sample from an individual with at least one autoantibody capture molecule that is either an antigen of Table 11a or an antibody that specifically binds an antigen of Table 11a, in which the antigen of Table 11a is complexed with an autoantibody, and detecting binding of the autoantibody capture molecule with at least one autoantibody of the sample, in which binding of the autoantibody capture molecule to an autoantibody of the sample is indicative of prostate cancer. In some embodiments, the method includes contacting a sample from the individual with two or more autoantibody capture molecules that are either antigens or antibodies that specifically bind, directly or indirectly, autoantibodies to the antigens of Table 11a that are present in a sample, and detecting binding of the sample to at least one of the one or more antigens or one or more antibodies to an autoantibody, in which binding of the sample to an antigen or Table 11a or an antibody to an antigen of Table 11a is indicative of prostate cancer. The method can include detecting binding of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, between 10 and 15, between 15 and 20, between 20 and 25, between 25 and 30, between 30 and 35, between 35 and 40, between 40 and 45, between 45 and 50, between 50 and 60, between 60 and 70, between 70 and 80, between 80 and 90, or between 90 and 98 autoantibodies to antigens of Table 11a or to antibodies to the antigens listed in Table 11a. Antigens of Table 11a include variants and modified forms of the proteins listed in Table 11a, and include epitope-containing fragments of the proteins of Table 11a.

[0120] The methods can be used to diagnose, prognose, monitor, or stage prostate cancer or PIN. As demonstrated in Example 2 (discussed below), the methods can be used to distinguish High Grade and Low Grade prostate cancer. The methods can be used to distinguish prostate cancer from BPH.

[0121] In another aspect of the invention, the invention provides a method for detecting, diagnosing, staging, prognosing, and/or monitoring prostate cancer or PIN, that includes determining the pattern of immune reactivity of a sample from an individual to a biomarker detection panel that includes two or more autoantibody capture molecules of Table 1, in which one or more of the autoantibody capture molecules is from Table 3.

[0122] Autoantibody capture molecules include target antigens (including variants and modified forms thereof, and including epitope-containing fragments thereof), as well as target antibodies that can detect autoantibodies in a sample from an individual, such as autoantibodies present in a sample as autoantibody-autoantigen complexes. In these methods, the target antibodies can be those designated as antibodies in the tables provided herein, or can be antibodies to any of the designated target antigens (for example, antibodies to any of the target antigens of Table 1 or Table 3), in which the target antibodies specifically bind an antigen, in which the antigen is complexed with an autoantibody. The method includes: contacting a test sample from an individual with a biomarker detection panel that comprises two or more autoantibody capture molecules of Table 1, in which at least one of the autoantibody capture molecules is from Table 3; and determining the pattern of immune reactivity to the biomarker detection panel to diagnose, stage, prognose, or monitor prostate cancer. In preferred embodiments, the sample is a blood sample or a sample derived from a blood sample, such as serum.

[0123] The invention therefore includes in some aspects methods of diagnosing prostate cancer by contacting a sample of an individual with a biomarker detection panel that includes two or more autoantibody capture molecules of Table 1, in which at least one of the autoantibody capture molecules is a target antigen and at least one of the autoantibody capture molecules is a target antibody, and detecting at least one autoantibody bound to the target antigen and at least one autoantibody bound to the target antibody. In some preferred embodiments, at least one of the two auto or more autoantibody capture molecules is an autoantibody capture molecule of Table 3. In these methods, an autoantibody of a sample that is bound to a target antibody of a biomarker detection panel as an antigen-autoantibody complex and an autoantibody directly bound to a target antigen can both be detected using directly or indirectly labeled anti-species-specific antibodies, such as, for example, anti-species-specific IgG antibodies, such as anti-human IgG antibodies. The autoantibody capture molecules of the panel can optionally be bound to a common solid support, such as a protein array, such that in exemplary embodiments the addition of anti-species specific antibodies for detection of autoantibodies bound to both target antigen(s) and target antibody(ies) can be performed in a single step.

[0124] In some embodiments, the biomarker detection panel used in the methods of the invention includes 3, 4, 5, 6, 7, or 8 autoantibody capture molecules of Table 1. In some embodiments, the biomarker detection panel used in the methods of the invention includes 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more autoantibody capture molecules of Table 1. In some embodiments, the test sample is contacted with a biomarker detection panel comprising 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 autoantibody capture molecules of Table 1. A biomarker detection panel can comprise between 30 and 35 autoantibody capture molecules of Table 1, between 35 and 40 autoantibody capture molecules of Table 1, between 40 and 45 autoantibody capture molecules of Table 1, between 45 and 50 autoantibody capture molecules of Table 1, between 50 and 55 autoantibody capture molecules of Table 1, between 55 and 60 autoantibody capture molecules of Table 1, between 60 and 65 autoantibody capture molecules of Table 1, between 65 and 70 autoantibody capture molecules of Table 1, between 70 and 75 autoantibody capture molecules of Table 1, between 75 and 80 autoantibody capture molecules of Table 1, between 80 and 85 autoantibody capture molecules of Table 1, between 85 and 90 autoantibody capture molecules of Table 1, between 90 and 95 autoantibody capture molecules of Table 1, between 95 and 100 autoantibody capture molecules of Table 1, between 100 and 110 autoantibody capture molecules of Table 1, or between 110 and 116 autoantibody capture molecules of Table 1. In all of these embodiments, one or more of the autoantibody capture molecules of Table 1 present in the biomarker detection panel can be an autoantibody capture molecule of Table 3.

[0125] In some embodiments, the biomarker detection panel used in the methods comprises two or more autoantibody capture molecules of Table 3. A biomarker detection panel can comprise, as nonlimiting examples, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24,

25, 26, 27, 28, 29, 30, 30-35, 40-45, 45-50, 50-55, 55-60, 60-65, or 65-70 autoantibody capture molecules of Table 3.

[0126] In some preferred embodiments, one or more autoantibody capture molecules of a biomarker panel used in the methods is a protein of Table 2. In some embodiments, one or more autoantibody capture molecules is a protein of Table 4. In some embodiments, prostate cancer is diagnosed using a biomarker panel that comprises one or more of: HEYL, MLH1, PTEN, BDKRB2, BCL2, PTGER3, RPL30, ZWINT, ERBB2, BIRC5, TOP2A, ACPP, AZGP1, CLDN3, HSPB1, CAV3, HSPD1, KDR, MAD1L1, PRSS8, PSAP, PSMB4, QSCN6, RPS6KA1, SPRR1B, TRA1, HMGA2, EIF3S3, CCNA1, RNF14, CD151, NCAM2, EGFR, ETS2, HSPA1A, MICB, CD164, NUCB1, COVA1, IMP-3, STIP1, RASSF1, STEAP, NRP1, SH3GLB1, RDH11, XLKD1, BCLG, CCNB1, CCND1, PCNA, and EIF4G1, or an antibody to one or more of: HEYL, MLH1, PTEN, BDKRB2, BCL2, PTGER3, RPL30, ZWINT, ERBB2, BIRC5, TOP2A, ACPP, AZGP1, CLDN3, HSPB1, CAV3, HSPD1, KDR, MAD1L1, PRSS8, PSAP, PSMB4, QSCN6, RPS6KA1, SPRR1B, TRA1, HMGA2, EIF3S3, CCNA1, RNF14, CD151, NCAM2, EGFR, ETS2, HSPA1A, MICB, CD164, NUCB1, COVA1, IMP-3, STIP1, RASSF1,STEAP, NRP1, SH3GLB1, RDH11, XLKD1, BCLG, CCNB1, CCND1, PCNA, and EIF4G1. In some embodiments, prostate cancer is diagnosed using a biomarker panel that comprises on or more of: HEYL, BDKRB2, PTGER3, ZWINT, TOP2A, PSAP, RPS6KA1, SPRR1B, HMGA2, CCNA1, RNF14, NCAM2, ETS2, CD164, COVA1, RASSF1, SH3GLB1, XLKD1, CCNB1, PCNA, ERBB2, and EIF4G1, or an antibody to one or more of HEYL, BDKRB2, PTGER3, ZWINT, TOP2A, PSAP, RPS6KA1, SPRR1B, HMGA2, CCNA1, RNF14, NCAM2, ETS2, CD164, COVA1, RASSF1, SH3GLB1, XLKD1, CCNB1, PCNA, ERBB2, and EIF4G1. In some exemplary embodiments, prostate cancer is diagnosed using a biomarker panel that comprises on or more of: HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, RPL30, PSMB4, MICIB, IMP-3, or CCNB1; or comprises an antibody to one or more of HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, RPL30, PSMB4, MICIB, IMP-3, and CCNB1. In some exemplary embodiments, prostate cancer is diagnosed using a biomarker panel that comprises one or more of: HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, and CCNB1; or comprises one or more of HEYL, PTGER3, ZWINT, SPRR1B, SH3GLB1, and CCNB1.

[0127] In some embodiments, the biomarker detection panel used in the methods of the invention has an ROC/AUC value of 0.800 or greater, or 0.850 or greater. In some embodiments, the biomarker detection panel has an ROC/AUC value of 0.950 or greater.

[0128] In some embodiments, the biomarker detection panel used in the methods that comprises two or more autoantibody capture molecules of Table 1 comprises one or more autoantibody capture molecules of Table 10. In some exemplary embodiments, a biomarker detection panel used in the methods of the invention comprises two or more autoantibody capture molecules of Table 3, in which at least one of the two or more autoantibody capture molecules of Table 3 is an autoantibody capture molecule of Table 10.

[0129] In some embodiments, the invention includes methods for detecting, diagnosing, staging, prognosing, and/or monitoring prostate cancer or PIN, or a method for differentiating BPH from prostate cancer in an individual, that includes determining the immune reactivity of a test sample from the individual against a biomarker detection panel that includes sets of autoantibody capture molecules ("autoantibody detection sets") that when used together have a high specificity and sensitivity for diagnosing prostate cancer and for distinguishing prostate cancer from BPH. The autoantibody detection sets can in some embodiments comprise one or more autoantibody capture molecules of Table 10.

[0130] In exemplary embodiments, the invention includes methods for detecting, diagnosing, staging, prognosing, and/or monitoring prostate cancer, or a method for differentiating BPH from prostate cancer in an individual, that includes determining the immune reactivity of a test sample from the individual against a biomarker detection panel that comprises a 3-entity autoantibody detection set of Table 5, a 4-marker autoantibody detection set of Table 6, a 5-marker autoantibody detection set of Table 7, a 6-marker autoantibody detection set of Table 8, or a 7-marker autoantibody detection set of Table 9, and correlating the immune reactivity of the test sample to the biomarker detection panel with a diagnosis, stage, or prognosis of prostate cancer. In one exemplary embodiment, a biomarker detection panel comprises a 3-marker detection set, including HEYL, RNF14 and PCNA. In one exemplary 4-biomarker embodiment, the biomarker detection panel comprises: an anti-IL-6 antibody, TRA1-SP, XLKD1, and PCNA. In one illustrative 5-biomarker embodiment, the biomarker detection panel comprises: SPRR1B, CCNA1, ERG, CCNB1, PSIP1. In one illustrative 6-biomarker embodiment, the biomarker detection panel comprises: ERBB2, CCNA1, KHDRBS1, RASSF1, NRP1, PCNA. In one illustrative 7-biomarker embodiment, the biomarker detection panel comprises: HEYL, BDKRB2, PSAP, MAD1L1, CCNA1, ERG, PCNA.

[0131] The biomarker detection panels can optionally include additional autoantibody capture molecules such as but not limited to autoantibody capture molecules from Table 11a, Table 1, Table 3, or Table 10.

[0132] An autoantibody capture molecule present in a biomarker detection panel can be a protein referred to as a target antigen (for example, a target antigen listed in Table 11a, Table 1, Table 3, or Table 10), including variants or modified forms thereof, or fragments thereof, that detect autoantibodies. An autoantibody capture molecule can also be an antibody that can bind an autoantigen that is complexed with one or more autoantibodies. A biomarker detection panel can comprise one or more target antigens and one or more target antibodies.

[0133] In addition to autoantibody capture molecules, a biomarker detection panel used to detect prostate cancer can optionally include one or more antibodies that capture antigens that are not necessarily complexed with autoantibodies. Binding of one or more test antigens of the biomarker detection panel to autoantibodies in the test sample and binding of one or more antibodies of the biomarker detection panel to antigens in the test sample can be detected and analyzed in determining the presence of prostate cancer in the individual, or distinguishing between the BPH and prostate cancer in a subject. An antibody present on a biomarker detection panel of the invention can be any antibody, including but not limited to an antibody that specifically binds any of the autoantigens of Table 1. Such antibodies can be used to detect antigens in a sample in the same assays used to detect autoantibodies using the autoantibody capture molecules. For example, in some embodiments of the methods directly or indirectly labeled secondary antibodies can be added to the panel prior to detection, in which the antibodies recognize the antigen captured by an antibody of the panel.

[0134] In certain embodiments, the pattern of immune reactivity is determined by quantitating the amount of binding of the one or more autoantibodies to target antigens. The quantitation can be absolute or relative. The quantitation can

include normalizing the detection values with respect to one or more controls that are preferably but optionally provided in the same detection assay. In some embodiments, the method includes detecting a level of binding of antibodies of a sample to two or more autoantibody capture molecules, wherein the level of binding is above a threshold or cutoff value.

[0135] As will be understood, one or more test antigens may have poor diagnostic or prognostic value when considered alone, but when used as part of a panel that includes other reagents for biomarker detection (such as but not limited to other test antigens), such test antigens can contribute to making a particular diagnosis or prognosis. In preferred embodiments, particular threshold values for one or more test antigens in a biomarker detection panel are not relied upon to determine if a profile of marker levels obtained from a subject are indicative of a particular diagnosis or prognosis. Rather, the present invention may utilize an evaluation of the entire marker profile of a biomarker detection panel by plotting ROC curves for the sensitivity of a particular biomarker detection panel versus 1-(specificity) for the panel at various threshold cutoffs. The analyses performed herein determined threshold levels using logistic regression analysis, but similar results has been obtained using K-nearest neighbor analysis (statistical analyses are known in the art, and are described in detail in references such as, for example, Hastings, Tibshirani, and Friedman (2003) *Elements of Statistical Learning*, Springer). In these methods, a profile of biomarker measurements from a sample of an individual is considered together to provide an overall probability (expressed either as a numeric score or as a percentage risk) that an individual has prostate cancer, for example. In such embodiments, an increase in a certain subset of biomarkers (such as a subset of biomarkers that includes one or more autoantibodies) may be sufficient to indicate a particular diagnosis (or prognosis) in one patient, while an increase in a different subset of biomarkers (such as a subset of biomarkers that includes one or more autoantibodies) may be sufficient to indicate the same or a different diagnosis (or prognosis) in another patient. Weighting factors may also be applied to one or more biomarkers being detected. As one example, when a biomarker is of particularly high utility in identifying a particular diagnosis or prognosis, it may be weighted so that at a given level it alone is sufficient to indicate a positive diagnosis. In another example, a weighting factor may provide that no given level of a particular marker is sufficient to signal a positive result, but only signals a result when another marker also contributes to the analysis.

[0136] Increasing the specificity of a diagnostic test also decreases its sensitivity to some degree. In exemplary embodiments of the invention, the biomarker detection panel provides a test for the presence of prostate cancer in an individual that has at least as high a sensitivity as the currently used PSA test (that is greater than 80%), and a lower false positive rate than the currently used PSA test (about 75% false positives). The biomarker detection panel used in the methods of the invention in some exemplary embodiments has a specificity of 80% or greater, 85% or greater, 88% or greater, 90% or greater, 92% or greater, 94% or greater, 96% or greater, 98% or greater, or 100%, for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual. The biomarker detection panel in exemplary embodiments has a sensitivity of 80% or greater, 82% or greater, 84% or greater, 86% or greater, 88% or greater, 90% or greater, 92% or greater, 94% or greater, 96% or greater, 98% or greater, or 100%, for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual. The biomarker detection panel in exemplary embodiments has a Bayesian specificity of 78% or greater, 80% or greater, 85% or greater, 88% or greater, 90% or greater, or 92% or greater for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual. The biomarker detection panel in exemplary embodiments has a Bayesian sensitivity of 80% or greater, 82% or greater, 84% or greater, 85% or greater, 90% or greater, or 95% or greater for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual. The biomarker detection panel in exemplary embodiments has a Bayesian specificity of 78% or greater, 80% or greater, 85% or greater, 88% or greater, 90% or greater, or 92% or greater. The biomarker detection panel in exemplary embodiments has a Bayesian accuracy of 80% or greater, 81% or greater, 84% or greater, 85% or greater, 85% or greater, 87% or greater, 90% or greater, 93% or greater, or 96% or greater for diagnosing prostate cancer, or for discriminating prostate cancer from BPH in an individual.

[0137] Determining the immune reactivity of the sample can be performed by detection of binding of antibodies of the sample to the autoantibody capture molecules of the biomarker detection panel, and can be done in separate assays, in which each autoantibody capture molecule of the panel is contacted independently by the sample, or in a single assay, in which multiple autoantibody capture molecules are contacted with the sample in a single assay. In the latter case, different autoantibody capture molecules are preferably spatially separated, such as by binding to separate solid support surfaces or by binding of individual autoantibodies to specific locations on a single solid support, so that binding to individual autoantibody capture molecules can be independently assessed. Assays for detecting binding, including immunoassays, are described herein.

[0138] The test sample can be contacted with a autoantibody capture molecule provided in solution phase, or autoantibody capture molecules can be provided bound to a solid support. Detection of autoantibodies can be performed using an immunoassay, which can be in various formats as described in detail below. Detection of binding in certain illustrative embodiments makes use of one or more solid supports to which the autoantibody capture molecule is immobilized and to which the sample from an individual, in this case a human subject, is applied. The detection can be performed on any solid or semi-solid support, such as a gel, matrix, bead, particle, filament, fiber, rod, dish, plate, well, sheet, filter, strip, membrane, slide, chip, or array, such as a protein array, which can be a microarray, and can optionally be a high density microarray.

[0139] After incubation of the sample with the immobilized autoantibody capture molecules, or optionally, concurrently with the incubation of the sample, an antibody that is reactive against human antibodies (for example, an anti-human IgG antibody that is from a species other than human, for example, goat, rabbit, pig, mouse, etc.) is applied to the solid support with which the sample is incubated. The anti-human IgG antibody is directly or indirectly labeled. In some embodiments, the anti-human IgG antibody is labeled in one or more additional steps after the anti-human IgG antibody is contacted with the immobilized autoantibody capture molecule that has been contacted with a sample. After removing non-specifically bound antibody, signal from the label that is significantly above background level is indicative of binding of a human antibody from the sample to an autoantibody capture molecule on the solid support.

[0140] The methods of diagnosing prostate cancer by contacting a sample from an individual with a biomarker detection panel can be repeated over time, for example, to monitor a pre-cancerous or pre-malignant state, or to monitor regression, progression, or recurrence of prostate cancer after or

during a treatment or treatment regime (such as, for example, surgery, radiation therapy, chemotherapy, etc.). The results of a diagnostic test that determines the immune reactivity of a patient sample to a test antigen can be compared with the results of the same diagnostic test done at an earlier time. Significant differences in immune reactivity over time can contribute to a diagnosis or prognosis of prostate cancer.

[0141] In some embodiments, the individual has had at least one examination or diagnostic test that has indicated the presence of BPH or prostate cancer. In some embodiments, the individual is a male of age 50 or older. In some embodiments, the individual is a male with enlarged prostate. The individual can be a male previously diagnosed with BPH or PIN. The individual can have one or more risk factors for prostate cancer such as, for example, being age 55 or older, being African-American, or having a family history of prostate cancer. The individual can have one or more positive indicators of prostate cancer, such as, for example, a PSA level of greater than 4 ng per ml, a PSA level of greater than 10 ng per ml, a significant rise in PSA level over time, a free PSA to total PSA ratio of 0.25 or less, prostate abnormalities detected in a DRE, or a positive PCA3 urine test. The provided values are examples only, and test values used for diagnosis may differ. In some embodiments, for example, the individual can have PSA levels of less than 4 ng per ml.

[0142] In some embodiments, the method further includes testing for one or more additional indicators of prostate cancer, for example, PSA level or the PCA3 transcript level. The testing can be performed at the same time as determining the immune reactivity of the sample to a target antigen, or can be performed earlier or later than the test to detect autoantibodies to target antigens. Such additional indicators can contribute to the diagnosis.

[0143] A particular immune reactivity pattern can be correlated with a diagnosis of prostate cancer using statistical analysis based on comparison of immune reactivity of samples from prostate cancer patients and individuals not exhibiting prostate cancer using the same biomarker sets, as illustrated in the Examples below. Algorithms can be applied to the analysis of the binding patterns of test samples that can preferably be provided in computer readable format and integrated with signal detection devices to correlate binding patterns of samples with diagnoses of prostate cancer.

[0144] As will be understood, for any particular biomarker, a distribution of biomarker levels for subjects with and without a disease will likely overlap. Under such conditions, a test does not absolutely distinguish normal from disease with 100% accuracy, and the area of overlap indicates where the test cannot distinguish normal from disease. A threshold is selected, above which (or below which, depending on how a biomarker changes with the disease) the test is considered to be abnormal and below which the test is considered to be normal. Receiver Operating Characteristic curves, or "ROC" curves, are typically generated by plotting the value of a variable versus its relative frequency in "normal" and "disease" populations. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification of a condition. ROC curves can also be generated using relative, or ranked, results. Methods of generating ROC curves and their use are well known in the art. See, e.g., Hanley et al., Radiology 143: 29-36 (1982).

Immunoassays

[0145] Immunoassays can be employed in any of the foregoing embodiments. Virtually any immunoassay technique known in the art can be used to detect antibodies that bind an antigen according to methods and kits of the present invention. Such immunoassay methods include, without limitation, radioimmunoassays, immunohistochemistry assays, competitive-binding assays, Western Blot analyses, ELISA assays, sandwich assays, test strip-based assays, assays using immunoprecipitation, assays combining antibody binding with two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass spectrometry or protein interaction profiling, all known to those of ordinary skill in the art. These methods may be carried out in an automated manner, as is known in the art. Such immunoassay methods may also be used to detect the binding of antibodies in a sample to a target antigen.

[0146] In one example of an ELISA method, the method includes incubating a sample with a target protein (such as an autoantibody capture molecule) and incubating the reaction product formed (which includes the captured autoantibody of the sample) with a binding partner, such as a secondary antibody (for example, an anti-species specific antibody), that binds to the reaction product by binding to an antibody from the sample that associated with the target protein to form the reaction product. In some cases these may comprise two separate steps, in others, the two steps may be simultaneous, or performed in the same incubation step. Examples of methods of detection of the binding of the target protein to an antibody include the use of an anti-human IgG (or other isotype specific) antibody or protein A. This detection antibody may be fluorescently labeled, or directly or indirectly linked to, for example, an alkaline phosphatase or a peroxidase, such as horseradish peroxidase. The CARD technique can optionally be employed to enhance the signal generated by a substrate converted by a peroxidase enzyme (Bobrow et al. (1989) J. Immunol. Methods 125: 279-285; Bhattacharya et al. (1999) J. Immunol. Methods 227: 31-39).

[0147] Using microarrays for immunoassays allows the simultaneous analysis of multiple proteins. For example, target antigens or antibodies that recognize biomarkers that may be present in a sample are immobilized on microarrays. Then, the biomarker antibodies or proteins, if present in the sample, are captured on the cognate spots on the array by incubation of the sample with the microarray under conditions favoring specific antigen-antibody interactions. The binding of protein or antibody in the sample can then be determined using secondary antibodies or other binding labels, proteins, or analytes. Comparison of proteins or antibodies found in two or more different samples can be performed using any means known in the art. For example, a first sample can be analyzed in one array and a second sample analyzed in a second array that is a replica of the first array.

[0148] The term "sandwich assay" refers to an immunoassay where the molecule to be detected is sandwiched between two binding reagents, which are typically antibodies. The first binding reagent/antibody is attached to a surface and the second binding reagent/antibody comprises a detectable moiety or label. In exemplary embodiments of the present invention, the first binding reagent is an autoantibody capture molecule, which can be an antigen or antibody, and the second

binding reagent is anti-species specific antibody which can be directly or indirectly labeled, either when applied to the capture molecules to which sample has been added, or can be directly or indirectly labeled in a subsequent step. Examples of labels include, for example and without limitation: fluorophores, chromophores, enzymes that generate a detectable signal, or epitopes for binding a second binding reagent (for example, when the second binding reagent/antibody is a mouse antibody, which is detected by a fluorescently-labeled anti-mouse antibody), for example an antigen or a member of a binding pair, such as biotin. The surface may be a planar surface, such as in the case of a typical grid-type array (for example, but without limitation, 96-well plates and planar microarrays), as described herein, or a non-planar surface, as with coated bead array technologies, where each "species" of bead is labeled with, for example, a fluorochrome (such as the Luminex technology described herein and in U.S. Pat. Nos. 6,599,331, 6,592,822 and 6,268,222), or quantum dot technology (for example, as described in U.S. Pat. No. 6,306,610).

[0149] A variety of different solid and semi-solid phase substrates can be used to detect a protein or antibody in a sample, or to quantitate or determine the concentration of a protein or antibody in a sample. The choice of substrate can be readily made by those of ordinary skill in the art, based on convenience, cost, skill, or other considerations. Useful substrates include without limitation: gels, matrices, beads, particles, bottles, surfaces, substrates, fibers, wires, framed structures, tubes, filaments, plates, sheets, filters, strips, and wells. These substrates can be made from: polystyrene, polypropylene, polycarbonate, glass, silica, silicon, plastic, metal, alloy, ceramics, cellulose, cellulose derivatives, nylon, coated surfaces, acrylamide or its derivatives and polymers thereof, agarose, or latex, or combinations thereof. This list is illustrative rather than exhaustive.

[0150] After contacting the sample with the biomarker detection panel, the panel is incubated under conditions of temperature, ionic strength, and pH compatible with antibody-antigen binding and for a time sufficient to allow antigen-antibody binding to occur. In preferred embodiments, after one or more washing steps, binding reagents (in exemplary embodiments species-specific antibodies) for detection are applied to the biomarker detection panel and also incubated with the biomarker detection panel under conditions of temperature, ionic strength, and pH compatible with binding and for a time sufficient to allow binding to occur.

[0151] Other methods of protein detection and measurement described in the art can be used as well. For example, a single antibody can be coupled to beads or to a well in a microwell plate, and quantitated by immunoassay. In this assay format, a single protein can be detected in each assay. The assays can be repeated with antibodies to many analytes to arrive at essentially the same results as can be achieved using the methods of this invention. Bead assays can be multiplexed by employing a plurality of beads, each of which is uniquely labeled in some manner. For example each type of bead can contain a pre-selected amount of a fluorophore. Types of beads can be distinguished by determining the amount of fluorescence (and/or wavelength) emitted by a bead. Such fluorescently labeled beads are commercially available from Luminex Corporation (Austin, Tex.; see the worldwide web address of luminexcorp.com). The Luminex assay is very similar to a typical sandwich ELISA assay, but utilizes Luminex microspheres conjugated to antibodies or proteins (Vignali, J. Immunol. Methods 243:243-255 (2000)).

[0152] The methodology and steps of various antibody assays are known to those of ordinary skill in the art. Additional information may be found, for example, in Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Chap. 14 (1988); Bolton and Hunter, "Radioimmunoassay and Related Methods," in Handbook of Experimental Immunology (D. M. Weir, ed.), Blackwell Scientific Publications, 1996; and Current Protocols in Immunology, (John E. Coligan, et al., eds) (1993).

[0153] The antibodies used to perform the foregoing assays can include polyclonal antibodies, monoclonal antibodies and fragments thereof as described supra. Monoclonal antibodies can be prepared according to established methods (see, e.g., Kohler and Milstein (1975) Nature 256:495; and Harlow and Lane (1988) Antibodies: A Laboratory Manual (C.H.S.P., N.Y.)).

[0154] An antibody can be a complete immunoglobulin or an antibody fragment. Antibody fragments used herein, typically are those that retain their ability to bind an antigen. Antibodies subtypes include IgG, IgM, IgA, IgE, or an isotype thereof (e.g., IgG1, IgG2a, IgG2b or IgG3). Antibody preparations can by polyclonal or monoclonal, and can be chimeric, humanized or bispecific versions of such antibodies. Antibody fragments include but are not limited to Fab, Fab', $F(ab)'_2$, Dab, Fv and single-chain Fv (ScFv) fragments. Bifunctional antibodies sometimes are constructed by engineering two different binding specificities into a single antibody chain and sometimes are constructed by joining two Fab' regions together, where each Fab' region is from a different antibody (e.g., U.S. Pat. No. 6,342,221). Antibody fragments often comprise engineered regions such as CDR-grafted or humanized fragments. Antibodies sometimes are derivitized with a functional molecule, such as a detectable label (e.g., dye, fluorophore, radioisotope, light scattering agent (e.g., silver, gold)) or binding agent (e.g., biotin, streptavidin), for example.

[0155] Detection can use any means compatible with the label and format employed. For example, for a scanner can be used to detect the signal, such as a fluorescent signal, from arrays, filters, plate, or bead assays. A plate reader can also be used for ELISAs that use chromogenic reagents. Detection can also use scintillation counters or autoradiography/densitometry where the signal is generated by a radioisotope label.

[0156] Automated systems for performing immunoassays, such as those utilized in the methods herein, are widely known and used in medical diagnostics. For example, random-mode or batch analyzer immunoassay systems can be used, as are known in the art. These can utilize magnetic particles or non-magnetic particles or microparticles and can utilize a fluorescence or chemiluminescence readout, for example. As non-limiting examples, the automated system can be the Beckman ACCESS paramagnetic-particle, chemiluminescent immunoassay, the Bayer ACS:180 chemiluminescent immunoassay or the Abbott AxSYM microparticle enzyme immunoassay. Such automated systems can be designed to perform methods provided herein for an individual antigen or for multiple antigens without multiple user interventions.

Biomarker Detection Panels

[0157] The invention also provides biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, in which the biomarker detection panels comprise two or more autoantibody capture molecules selected from Table 1 or Table 11a, in which at least 50% of the

proteins of the test panel are autoantibody capture molecules of Table 1 or Table 11a. The set of autoantibody capture molecules in a biomarker detection panel are associated, either electronically, for example by linking the identities and/or information about the purchase or use of particular autoantibody capture molecules that are members of a biomarker detection panel, or preferably, physically.

[0158] For example, each biomarker of a biomarker detection panel can be provided in isolated form, in separate tubes or vials or bound to separate solid supports such as strips or beads that are sold and/or shipped together, for example as part of a kit. The autoantibody capture molecules of a biomarker panel can also be mixed together in the same solution. The autoantibody capture molecules of a biomarker panel can also be physically associated by being bound to one or more solid supports in the form of beads, one or more matrices (e.g., gels or resins), or one or more dishes, wells, plates, slides, sheets, membranes, strips, filters, fibers, chips, or arrays.

[0159] In certain embodiments, isolated autoantibody capture molecules are formed into a detection panel by attaching them to the same solid support. In some preferred embodiments, the proteins of the biomarker detection panel are provided on a protein array in which 50% or more of the proteins on the array are autoantibody capture molecules of the biomarker detection panel. A protein array that comprises a biomarker panel can in some exemplary embodiments be a high-density array.

[0160] The invention also provides biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, in which the biomarker detection panels comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more target antigens selected from Table 1 or Table 11a, or in certain preferred embodiments, Table 3, in which at least 50%, 55%, 60%, 65%, 70%, or 75% of the proteins of the test panel are proteins of Table 1, Table 11a, or Table 3, respectively. In some preferred embodiments, the proteins of the biomarker detection panel are provided on one or more solid supports, in which at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the proteins on the one or more solid supports to which the proteins of the panel are bound are of Table 1, Table 11a, or Table 3. In some preferred embodiments, the proteins of the biomarker detection panel are provided on a protein array in which at least 55%, 60%, 65%, 70%, or 75%, 80%, 85%, 90%, 95% or 100% of the proteins on the array are autoantibody capture molecules of the biomarker detection panel.

[0161] The invention provides biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, in which the biomarker detection panels comprise KDR or PIM-1, a variant thereof, or a fragment thereof. The invention provides biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, in which the biomarker detection panels comprise KDR and PIM-1, variants thereof, or fragments thereof. The invention provides biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, in which the biomarker detection panels comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more target antigens selected from Table 1, that comprise one or both of KDR or PIM-1, fragments thereof, or variants thereof. In some preferred embodiments, the proteins of the biomarker detection panel that include one or both of KDR or PIM-1 are provided on one or more solid supports, in which at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the proteins on the one or more solid supports to which the proteins of the panel are bound are of Table 1. In some preferred embodiments, the proteins of the biomarker detection panel are provided on a protein array in which at least 55%, 60%, 65%, 70%, or 75%, 80%, 85%, 90%, 95% or 100% of the proteins on the array are autoantibody capture molecules of the biomarker detection panel.

[0162] In some embodiments, the biomarker detection panel used in the methods of the invention includes 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 proteins of Table 1, Table 11a, or Table 3. In some embodiments, the biomarker detection panel used in the methods of the invention includes 13, 14, 15, 16, 17, 18, 19, 20, or more proteins of Table 1, Table 11a, or Table 3. In some embodiments, the test sample is contacted with a biomarker detection panel comprising 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 proteins of Table 1, Table 11a, or Table 3. A biomarker detection panel can comprise between 30 and 35 autoantibody capture molecules; between 35 and 40 autoantibody capture molecules; between 40 and 45 autoantibody capture molecules; between 45 and 50 autoantibody capture molecules; between 50 and 55 autoantibody capture molecules; between 55 and 60 autoantibody capture molecules; between 60 and 65 autoantibody capture molecules; between 65 and 70 autoantibody capture molecules; between 70 and 75 autoantibody capture molecules; between 75 and 80 autoantibody capture molecules; between 80 and 85 autoantibody capture molecules; between 85 and 90 autoantibody capture molecules; between 90 and 95 autoantibody capture molecules; between 95 and 100 autoantibody capture molecules, between 100 and 105 autoantibody capture molecules, or between 105 and 110 autoantibody capture molecules of Table 1, Table 11a, or Table 3.

[0163] One embodiment of the invention provides a biomarker panel comprising three autoantibody capture molecules from Table 1 or Table 11a. Table 5a provides ten autoantibody detection sets (labeled 5-1 through 5-10) having three autoantibody capture molecules. Table 5b shows the specificity and sensitivity of these biomarker panels, including their Bayesian accuracy. Both tables use a known positive control, such as mouse anti human IgG1, to normalize the data. In one embodiment, the biomarker detection panel comprising three autoantibody capture molecules is a panel provided in Table 5a.

TABLE 5a

Three Marker Biomarker Detection Panels, ranked by Bayesian Accuracy, Identities of Markers

Normalization Method	Panel	Marker 1	Marker 2	Marker 3
Mouse Anti Human IgG1	5-1	TOP2A	COVA1	RASSF1
Goat Anti-Human IgG	5-2	HEYL	RNF14	PCNA
Mouse Anti Human Kappa 6.3	5-3	IMP-2	XLKD1	PCNA
Mouse Anti Human Kappa 6.3	5-1	TOP2A	COVA1	RASSF1
Protein L 1.6	5-4	a-ACPP	RPS6KA1	EIF4G1
Mouse Anti Human Kappa 6.3	5-5	HEYL	CCNA1	PCNA
Protein L 1.6	5-6	a-II-6	COVA1	RASSF1
Protein L 1.6	5-7	SPRR1B	XLKD1	CCNB1
Mouse Anti Human Kappa 6.3	5-8	SPRR1B	CCNA1	RASSF1
Protein L 1.6	5-9	a-II-6	AZGP1(-SP)	COVA1
Mouse Anti Human IgG1	5-10	ZWINT	COVA1	RASSF1

TABLE 5b

Three Marker Biomarker Detection Panels, ranked by Bayesian Accurac Statistics

Normalization Method	Panel	AUC	Specificity	Sensitivity	Bayesian Specificity	Bayesian Sensitivity	Bayesian Accuracy
Mouse Anti Human IgG1	5-1	0.88158	91.67%	89.47%	85.71%	85.71%	87.88%
Goat Anti-Human IgG	5-2	0.91228	83.33%	89.47%	78.57%	85.71%	84.85%
Mouse Anti Human Kappa 6.3	5-3	0.89474	83.33%	89.47%	78.57%	85.71%	84.85%
Mouse Anti Human Kappa 6.3	5-1	0.87719	83.33%	89.47%	78.57%	85.71%	84.85%
Protein L 1.6	5-4	0.87281	83.33%	89.47%	78.57%	85.71%	84.85%
Mouse Anti Human Kappa 6.3	5-5	0.86842	83.33%	89.47%	78.57%	85.71%	84.85%
Protein L 1.6	5-6	0.86404	91.67%	84.21%	85.71%	80.95%	84.85%
Protein L 1.6	5-7	0.86842	83.33%	84.21%	78.57%	80.95%	81.82%
Mouse Anti Human Kappa 6.3	5-8	0.85088	83.33%	84.21%	78.57%	80.95%	81.82%
Protein L 1.6	5-9	0.81579	83.33%	84.21%	78.57%	80.95%	81.82%
Mouse Anti Human IgG1	5-10	0.80702	83.33%	84.21%	78.57%	80.95%	81.82%

[0164] One embodiment of the invention provides a biomarker panel comprising four autoantibody capture molecules from Table 1 or Table 11a. Table 6a provides twenty-one autoantibody detection sets (labeled 6-1 through 6-21) having four autoantibody detection molecules. Table 6b shows the specificity and sensitivity of these biomarker panels, including their Bayesian accuracy. The tables use a known positive control, such as mouse anti human IgG1, to normalize the data. In one embodiment of the invention, the biomarker detection panel comprising four autoantibody detection molecules is a panel provided in Table 6a.

TABLE 6a

Four Marker Biomarker Detection Panels, ranked by Bayesian Accuracy, Identities of Markers

Panel	Normalization Method	Marker 1	Marker 2	Marker 3	Marker 4
6-1	Protein L 1.6	a-II-6	TRA1(-SP)	XLKD1	PCNA
6-2	Mouse Anti Human Kappa 6.3	ZWINT	ACPP	CCNA1	RASSF1
6-3	Mouse Anti Human Kappa 6.3	a-ACPP	CCNA1	CD164	RASSF1
6-4	Mouse Anti Human IgG1	NCAM2	KHDRBS1	UBE2C	RASSF1
6-5	Protein L 1.6	a-II-6	a-PSA (F)	RPS6KA1	EIF4G1
6-6	Mouse Anti Human IgG1	SPRR1B	RASSF1	XLKD1	CCND1
6-7	Mouse Anti Human IgG1	TOP2A	RNF14	CD164	RASSF1
6-8	Protein L 1.6	a-II-8	CCNA1	CD164	RASSF1
6-9	Protein L 1.6	a-II-6	a-PSA(T)	RPS6KA1	EIF4G1
6-10	Goat Anti-Human IgG	PTGER3	HMGA2	EGFR	COVA1
6-11	Mouse Anti Human IgG1	PTGER3	SPRR1B	NCAM2	RASSF1
6-12	Mouse Anti Human IgG1	SPRR1B	STIP1	RASSF1	H3GLB1
6-13	Protein L 1.6	a-II-6	RPS6KA1	CD151	EIF4G1
6-14	Mouse Anti Human Kappa 6.3	a-II-6	NRP1	XLKD1	PCNA
6-15	Mouse Anti Human Kappa 6.3	a-II-8	ACPP	CCNA1	RASSF1
6-16	Human IgG 1.6	PTGER3	MAD1L1	SPRR1B	CCNA1
6-17	Protein L 1.6	RCV1	H3GLB1	CCNB1	PCNA
6-18	Goat Anti-Human IgG	MLH1	RPS6KA1	SPRR1B	RASSF1
6-19	Protein L 1.6	PTGER3	PSMB4	CCNA1	COVA1
6-20	Protein L 1.6	ZWINT	H3GLB1	CCNB1	EIF4G1
6-21	Protein L 1.6	TRA1(-SP)	H3GLB1	CCNB1	EIF4G1

TABLE 6b

Four Marker Biomarker Detection Panels, ranked by Bayesian Accurac Statistics

Panel	AUC	Specificity	Sensi-tivity	Bayesian Specificity	Bayesian Sensitivity	Bayesian Accuracy
6-1	0.89035	91.67%	94.74%	85.71%	90.48%	90.91%
6-2	0.86842	91.67%	94.74%	85.71%	90.48%	90.91%
6-3	0.89474	83.33%	94.74%	78.57%	90.48%	87.88%
6-4	0.85965	83.33%	94.74%	78.57%	90.48%	87.88%
6-5	0.89474	83.33%	89.47%	78.57%	85.71%	84.85%
6-6	0.89035	83.33%	89.47%	78.57%	85.71%	84.85%
6-7	0.88596	91.67%	84.21%	85.71%	80.95%	84.85%
6-8	0.88158	83.33%	89.47%	78.57%	85.71%	84.85%
6-9	0.87281	83.33%	89.47%	78.57%	85.71%	84.85%
6-10	0.86404	83.33%	89.47%	78.57%	85.71%	84.85%
6-11	0.85965	83.33%	89.47%	78.57%	85.71%	84.85%
6-12	0.85526	83.33%	89.47%	78.57%	85.71%	84.85%
6-13	0.85088	91.67%	84.21%	85.71%	80.95%	84.85%
6-14	0.8114	91.67%	84.21%	85.71%	80.95%	84.85%
6-15	0.7807	83.33%	89.47%	78.57%	85.71%	84.85%
6-16	0.88596	83.33%	84.21%	78.57%	80.95%	81.82%
6-17	0.82018	83.33%	84.21%	78.57%	80.95%	81.82%
6-18	0.81579	83.33%	84.21%	78.57%	80.95%	81.82%
6-19	0.81579	83.33%	84.21%	78.57%	80.95%	81.82%
6-20	0.80702	83.33%	84.21%	78.57%	80.95%	81.82%
6-21	0.79386	83.33%	84.21%	78.57%	80.95%	81.82%

[0165] One embodiment of the invention provides a biomarker panel comprising five autoantibody capture molecules from Table 1 or Table 11a. Table 7a provides seven autoantibody detection sets (labeled 7-1 through 7-7) having five autoantibody detection molecules. Table 7b shows the specificity and sensitivity of these biomarker panels, including their Bayesian accuracy. The tables use a known positive control, such as mouse anti human IgG1, to normalize the data. In one embodiment of the invention, the biomarker detection panel comprising five autoantibody detection molecules is a panel provided in Table 7a.

TABLE 7a

Five marker Biomarker Detection Panels, ranked by Bayesian Accuracy, Identities of Markers

Marker	Panel	AUC	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5
Protein L 1.6	7-1	0.91667	SPRR1B	CCNA1	ERG	CCNB1	PSIP1
Mouse Anti Human IgG1	7-2	0.90351	HEYL	CCNA1	ERG	KHDRBS1	PCNA
Mouse Anti Human IgG1	7-3	0.85965	HEYL	ERBB2	CCNA1	KHDRBS1	PCNA
Human IgG 1.6	7-4	0.82018	HEYL	RNF14	CCNB1	PCNA	EIF4G1
Human IgG 1.6	7-5	0.74561	HEYL	CCNA1	MMP9	BCLG	PCNA
Mouse Anti Biotin 25	7-6	0.74561	HEYL	BDKRB2	RNF14	HSPA5	PCNA
Human IgG 1.6	7-7	0.76316	HEYL	ERBB2	RNF14	CCNB1	PCNA

TABLE 7b

Five marker Biomarker Detection Panels, ranked by Bayesian Accuracy, Statistics

Panel	AUC	Speci-ficity	Sensi-tivity	Bayesian Specificity	Bayesian Sensitivity	Bayesian Accuracy
7-1	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
7-2	0.90351	83.33%	100.00%	78.57%	95.24%	90.91%
7-3	0.85965	83.33%	94.74%	78.57%	90.48%	87.88%
7-4	0.82018	91.67%	89.47%	85.71%	85.71%	87.88%
7-5	0.74561	83.33%	89.47%	78.57%	85.71%	84.85%
7-6	0.74561	83.33%	89.47%	78.57%	85.71%	84.85%
7-7	0.76316	83.33%	84.21%	78.57%	80.95%	81.82%

[0166] One embodiment of the invention provides a biomarker panel comprising six autoantibody capture molecules from Table 1 or Table 11a. Table 8a provides eighteen autoantibody detection sets (labeled 8-1 through 8-18) having six autoantibody detection molecules. Table 8b shows the specificity and sensitivity of these biomarker panels, including their Bayesian accuracy. The tables use a known positive control, such as mouse anti human IgG1, to normalize the data. In one embodiment of the invention, the biomarker detection panel comprising six autoantibody detection molecules is a panel provided in Table 8a.

TABLE 8a

Six Marker Biomarker Detection Sets, ranked by Bayesian Accuracy, Identities of Markers

Marker Set	Method	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5	Marker 6
8-1	Mouse Anti Human Kappa 6.3	ERBB2	CCNA1	KHDRBS1	RASSF1	NRP1	PCNA
8-2	Mouse Anti Biotin 25	MAD1L1	SPRR1B	HMGA2	ETS2	IMP-2	CCNB1
8-3	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ETS2	PCNA
8-4	Human IgG 1.6	HEYL	RPL30	ERBB2	CCNA1	MMP9	PCNA
8-5	Mouse Anti Human IgG1	HEYL	CCNA1	ERG	IMP-3	KHDRBS1	PCNA
8-6	Human IgG .4	CCNA1	RNF14	ERG	MICB	CCNB1	EIF4G1
8-7	Mouse Anti Biotin 25	HEYL	BDKRB2	CCNA1	ERG	RASSF1	PCNA
8-8	Mouse Anti Biotin 25	HEYL	RNF14	ERG	AMACR	PCNA	EIF4G1
8-9	Human IgG 1.6	HEYL	PRL	BIRC5	CCNA1	CD164	PCNA
8-10	Mouse Anti Biotin 25	HSPB1	SPRR1B	HMGA2	ERG	IMP-2	CCNB1
8-11	Human IgG .4	a-ACPP	PSAP	CCNA1	ERG	MICB	EIF4G1
8-12	Mouse Anti Human Kappa 6.3	a-II-8	E7	BDKRB2	CCNA1	RASSF1	EIF4G1
8-13	Mouse Anti Biotin 25	HEYL	RNF14	STEAP	BCLG	CCNB1	PCNA
8-14	Human IgG 1.6	HEYL	CCNA1	ERG	MMP9	CCND1	PCNA
8-15	Mouse Anti Biotin 25	HEYL	BDKRB2	CCNA1	ETS2	RASSF1	PCNA
8-16	Human IgG 1.6	HEYL	BDKRB2	PTGER3	ENO1	CCNA1	PCNA
8-17	Mouse Anti Biotin 25	HEYL	CLDN3	RNF14	BCLG	CCNB1	PCNA
8-18	Mouse Anti Biotin 25	MYC	PSAP	NCAM2	ETS2	CCNB1	EIF4G1

TABLE 8b

Six Marker Biomarker Detection Sets, ranked by Bayesian Accuracy, Statistics

Marker Set	AUC	Specificity	Sensitivity	Bayesian Specificity	Bayesian Sensitivity	Bayesian Accuracy
8-1	0.99123	100.00%	94.74%	92.86%	90.48%	93.94%
8-2	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
8-3	0.94298	100.00%	89.47%	92.86%	85.71%	90.91%
8-4	0.9386	100.00%	89.47%	92.86%	85.71%	90.91%
8-5	0.91228	83.33%	100.00%	78.57%	95.24%	90.91%
8-6	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
8-7	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
8-8	0.89035	91.67%	89.47%	85.71%	85.71%	87.88%
8-9	0.86404	91.67%	89.47%	85.71%	85.71%	87.88%
8-10	0.84211	100.00%	84.21%	92.86%	80.95%	87.88%
8-11	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
8-12	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
8-13	0.77193	91.67%	84.21%	85.71%	80.95%	84.85%
8-14	0.74561	83.33%	89.47%	78.57%	85.71%	84.85%
8-15	0.74561	83.33%	89.47%	78.57%	85.71%	84.85%
8-16	0.70175	83.33%	84.21%	78.57%	80.95%	81.82%
8-17	0.70175	83.33%	84.21%	78.57%	80.95%	81.82%
8-18	0.70175	83.33%	84.21%	78.57%	80.95%	81.82%

[0167] One embodiment of the invention provides a biomarker panel comprising seven autoantibody capture molecules from Table 1 or Table 11a. Table 9a provides eighty-one autoantibody detection sets (labeled 9-1 through 9-81) having seven autoantibody detection molecules. Table 9b shows the specificity and sensitivity of these biomarker panels, including their Bayesian accuracy. The tables use a known positive control, such as human IgG 1.6, to normalize the data. In one embodiment of the invention, the biomarker detection panel comprising seven autoantibody detection molecules is a panel provided in Table 9a.

TABLE 9a

Seven-marker Biomarker Detection Sets, ranked by Bayesian Accuracy, Identities of Markers

Panel	Method	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5	Marker 6	Marker 7
9-1	Human IgG 1.6	HEYL	BDKRB2	MAD1L1	PSAP	CCNA1	ERG	PCNA
9-2	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	IMP-3	PCNA
9-3	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	PIM1	PCNA
9-4	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ETS2	RDH11	PCNA
9-5	Human IgG 1.6	HEYL	BDKRB2	PRSS8	PSAP	CCNA1	ERG	PCNA
9-6	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	KHDRBS1	PCNA
9-7	Human IgG .4	a-ACPP	MYC	PSAP	CCNA1	ETS2	MICB	EIF4G1
9-8	Human IgG 1.6	HEYL	BDKRB2	HSPB1	PSAP	CCNA1	ERG	PCNA
9-9	Human IgG 1.6	HEYL	BDKRB2	PSAP	PSMB4	CCNA1	ERG	PCNA
9-10	Human IgG 1.6	HEYL	ACPP	CCNA1	ERG	TPD52	PSA	PCNA
9-11	Human IgG 1.6	a-PSA(T)	HEYL	BDKRB2	PSAP	CCNA1	ERG	PCNA
9-12	Human IgG 1.6	HEYL	BDKRB2	FLT1	PSAP	CCNA1	ERG	PCNA
9-13	Human IgG 1.6	HEYL	BDKRB2	MUC1	PSAP	CCNA1	ERG	PCNA
9-14	Human IgG 1.6	HEYL	BDKRB2	PSAP	SPRR1B	CCNA1	ERG	PCNA
9-15	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	MMP9	PCNA
9-16	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	TPD52	PCNA
9-17	Human IgG 1.6	HEYL	BDKRB2	CCNA1	ERG	MMP9	CCKBR	PCNA
9-18	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ETS2	KHDRBS1	PCNA
9-19	Human IgG .4	a-ACPP	PSAP	QSCN6	CCNA1	ETS2	MICB	EIF4G1
9-20	Human IgG 1.6	HEYL	BDKRB2	HSPD1	PSAP	CCNA1	ERG	PCNA
9-21	Human IgG .4	a-ACPP	BIRC5	CCNA1	RNF14	MICB	CCNB1	EIF4G1
9-22	Human IgG .4	a-ACPP	PSAP	CCNA1	ETS2	MICB	RDH11	EIF4G1
9-23	Human IgG .4	BIRC5	CCNA1	RNF14	MICB	ELAC1	CCNB1	EIF4G1
9-24	Human IgG 1.6	HEYL	BDKRB2	CAV3	PSAP	CCNA1	ERG	PCNA
9-25	Human IgG 1.6	HEYL	TP53	BDKRB2	PSAP	CCNA1	ERG	PCNA
9-26	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	LGALS8	PCNA
9-27	Human IgG 1.6	HEYL	BDKRB2	HMGA2	CCNA1	ERG	MMP9	PCNA
9-28	Human IgG .4	a-ACPP	PSAP	CCNA1	ERG	MICB	UBE2C	EIF4G1
9-29	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	ELAC1	PCNA
9-30	Human IgG .4	RCV1	CCNA1	RNF14	ERG	MICB	CCNB1	EIF4G1
9-31	Human IgG .4	BDKRB2	CCNA1	RNF14	ERG	MICB	CCNB1	EIF4G1
9-32	Human IgG .4	BDKRB2	CCNA1	RNF14	ETS2	MICB	CCNB1	EIF4G1
9-33	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	RASSF1	PCNA
9-34	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	PSA	PCNA
9-35	Human IgG .4	EIF3S3	CCNA1	RNF14	ERG	MICB	CCNB1	EIF4G1
9-36	Human IgG 1.6	HEYL	BDKRB2	PSAP	RCV1	CCNA1	ERG	PCNA
9-37	Human IgG 1.6	HEYL	BDKRB2	CCNA1	ERG	MMP9	HOXB13	PCNA
9-38	Human IgG 1.6	HEYL	BDKRB2	CCNA1	MMP9	ETS2	HOXB13	PCNA
9-39	Human IgG 1.6	HEYL	ACPP	FLT1	CCNA1	ERG	MMP9	PCNA
9-40	Human IgG 1.6	HEYL	MET	BDKRB2	CCNA1	MMP9	H3GLB1	PCNA
9-41	Human IgG 1.6	HEYL	BDKRB2	BCL2	PSAP	CCNA1	ERG	PCNA
9-42	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	HOXB13	PCNA
9-43	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	HIP1	PCNA
9-44	Human IgG .4	a-ACPP	PTGER3	PSAP	CCNA1	ETS2	MICB	EIF4G1
9-45	Human IgG 1.6	a-BCL2	HEYL	BDKRB2	CCNA1	ERG	MMP9	PCNA
9-46	Human IgG 1.6	a-IL-6	HEYL	BDKRB2	PSAP	CCNA1	ERG	PCNA
9-47	Human IgG 1.6	HEYL	BDKRB2	PTGS1	PSAP	CCNA1	ERG	PCNA
9-48	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	UBE2C	PCNA
9-49	Human IgG .4	a-ACPP	PSAP	QSCN6	CCNA1	ERG	MICB	EIF4G1
9-50	Human IgG .4	MAD1L1	CCNA1	NRP1	CCNB1	CCKBR	PCNA	EIF4G1
9-51	Human IgG 1.6	HEYL	BDKRB2	CXCR4	PSAP	CCNA1	ERG	PCNA
9-52	Human IgG 1.6	HEYL	BDKRB2	KDR	PSAP	CCNA1	ERG	PCNA
9-53	Human IgG 1.6	HEYL	BDKRB2	PSAP	TRA1(-SP)	CCNA1	ERG	PCNA
9-54	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	ALOX15	PCNA
9-55	Human IgG .4	a-IL-8	CCNA1	RNF14	ERG	MICB	CCNB1	EIF4G1
9-56	Human IgG .4	CCNA1	RNF14	ETS2	MICB	PDLIM1	CCNB1	EIF4G1

TABLE 9a-continued

		Seven-marker Biomarker Detection Sets, ranked by Bayesian Accuracy, Identities of Markers						
Panel	Method	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5	Marker 6	Marker 7
9-57	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	BCLG	PCNA
9-58	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	CCKBR	PCNA
9-59	Human IgG 1.6	HEYL	ACPP	CCNA1	ERG	MMP9	KHDRBS1	PCNA
9-60	Human IgG .4	a-ACPP	PTEN	PSAP	CCNA1	ERG	MICB	EIF4G1
9-61	Human IgG .4	a-ACPP	PSAP	CCNA1	ERG	NCAM2	MICB	EIF4G1
9-62	Human IgG 1.6	HEYL	BDKRB2	BCL2	CCNA1	ERG	MMP9	PCNA
9-63	Human IgG 1.6	HEYL	BDKRB2	PSAP	CUL4A	CCNA1	ERG	PCNA
9-64	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	BRD2	PCNA
9-65	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	CD164	PCNA
9-66	Human IgG 1.6	HEYL	BDKRB2	CCNA1	ERG	GDF15(-SP)	HOXB13	PCNA
9-67	Human IgG 1.6	HEYL	MET	BDKRB2	CCNA1	MMP9	TMPRSS2	PCNA
9-68	Human IgG 1.6	HEYL	BDKRB2	BIRC5	PSAP	CCNA1	ERG	PCNA
9-69	Human IgG .4	a-Pter2	PTGER3	HSPB1	HSPD1	CCNA1	MICB	EIF4G1
9-70	Human IgG .4	a-ACPP	BIRC5	PSAP	CCNA1	GDF15(-SP)	MICB	EIF4G1
9-71	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	STEAP	PCNA
9-72	Human IgG .4	a-ACPP	PTGER3	PSAP	CCNA1	ERG	MICB	EIF4G1
9-73	Human IgG .4	a-ACPP	PSAP	CCNA1	ERG	MICB	RDH11	EIF4G1
9-74	Human IgG .4	a-ACPP	PSAP	CCNA1	GDF15(-SP)	ETS2	MICB	EIF4G1
9-75	Human IgG .4	PRL	HSPB1	EIF3S3	CCNA1	ERG	MICB	EIF4G1
9-76	Human IgG 1.6	a-CXCR4	HEYL	ACPP	CCNA1	ERG	PSA	PCNA
9-77	Human IgG 1.6	HEYL	BDKRB2	PSAP	CCNA1	ERG	EGFR	PCNA
9-78	Human IgG 1.6	HEYL	BDKRB2	RPL30	PSAP	CCNA1	ERG	PCNA
9-79	Human IgG .4	CCNA1	RNF14	ERG	MICB	PDLIM1	CCNB1	EIF4G1
9-80	Human IgG .4	CCNA1	RNF14	ERG	MICB	MIB1	CCNB1	EIF4G1
9-81	Human IgG 1.6	HEYL	BDKRB2	PSAP	SFRP4	CCNA1	ERG	PCNA

TABLE 9b

	Seven-marker Biomarker Detection Sets, ranked by Bayesian Accuracy, Statistics					
Panel	AUC	Specificity	Sensitivity	Bayesian Specificity	Bayesian Sensitivity	Bayesian Accuracy
9-1	1	100.00%	100.00%	92.86%	95.24%	96.97%
9-2	1	100.00%	100.00%	92.86%	95.24%	96.97%
9-3	1	100.00%	100.00%	92.86%	95.24%	96.97%
9-4	1	100.00%	100.00%	92.86%	95.24%	96.97%
9-5	0.99561	91.67%	100.00%	85.71%	95.24%	93.94%
9-6	0.99123	91.67%	100.00%	85.71%	95.24%	93.94%
9-7	0.94737	100.00%	94.74%	92.86%	90.48%	93.94%
9-8	0.94737	100.00%	94.74%	92.86%	90.48%	93.94%
9-9	0.94737	100.00%	94.74%	92.86%	90.48%	93.94%
9-10	0.94737	100.00%	94.74%	92.86%	90.48%	93.94%
9-11	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-12	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-13	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-14	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-15	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-16	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-17	0.91667	91.67%	100.00%	85.71%	95.24%	93.94%
9-18	0.98246	100.00%	89.47%	92.86%	85.71%	90.91%
9-19	0.94298	100.00%	89.47%	92.86%	85.71%	90.91%
9-20	0.94298	100.00%	89.47%	92.86%	85.71%	90.91%
9-21	0.9386	91.67%	94.74%	85.71%	90.48%	90.91%
9-22	0.9386	100.00%	89.47%	92.86%	85.71%	90.91%
9-23	0.9386	91.67%	94.74%	85.71%	90.48%	90.91%
9-24	0.9386	100.00%	89.47%	92.86%	85.71%	90.91%
9-25	0.93421	100.00%	89.47%	92.86%	85.71%	90.91%
9-26	0.91228	83.33%	100.00%	78.57%	95.24%	90.91%
9-27	0.91228	83.33%	100.00%	78.57%	95.24%	90.91%
9-28	0.90789	91.67%	94.74%	85.71%	90.48%	90.91%
9-29	0.90789	91.67%	94.74%	85.71%	90.48%	90.91%
9-30	0.89474	100.00%	89.47%	92.86%	85.71%	90.91%
9-31	0.86842	91.67%	94.74%	85.71%	90.48%	90.91%

TABLE 9b-continued

Seven-marker Biomarker Detection Sets, ranked by Bayesian Accuracy, Statistics

Panel	AUC	Specificity	Sensitivity	Bayesian Specificity	Bayesian Sensitivity	Bayesian Accuracy
9-32	0.86842	91.67%	94.74%	85.71%	90.48%	90.91%
9-33	0.86842	91.67%	94.74%	85.71%	90.48%	90.91%
9-34	0.86842	91.67%	94.74%	85.71%	90.48%	90.91%
9-35	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
9-36	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
9-37	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
9-38	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
9-39	0.83333	83.33%	100.00%	78.57%	95.24%	90.91%
9-40	0.92982	100.00%	84.21%	92.86%	80.95%	87.88%
9-41	0.92544	100.00%	84.21%	92.86%	80.95%	87.88%
9-42	0.92105	100.00%	84.21%	92.86%	80.95%	87.88%
9-43	0.89912	91.67%	89.47%	85.71%	85.71%	87.88%
9-44	0.86404	91.67%	89.47%	85.71%	85.71%	87.88%
9-45	0.86404	91.67%	89.47%	85.71%	85.71%	87.88%
9-46	0.86404	91.67%	89.47%	85.71%	85.71%	87.88%
9-47	0.86404	91.67%	89.47%	85.71%	85.71%	87.88%
9-48	0.86404	91.67%	89.47%	85.71%	85.71%	87.88%
9-49	0.85965	91.67%	89.47%	85.71%	85.71%	87.88%
9-50	0.85965	91.67%	89.47%	85.71%	85.71%	87.88%
9-51	0.85965	91.67%	89.47%	85.71%	85.71%	87.88%
9-52	0.85965	91.67%	89.47%	85.71%	85.71%	87.88%
9-53	0.85965	91.67%	89.47%	85.71%	85.71%	87.88%
9-54	0.82456	83.33%	94.74%	78.57%	90.48%	87.88%
9-55	0.82018	91.67%	89.47%	85.71%	85.71%	87.88%
9-56	0.82018	91.67%	89.47%	85.71%	85.71%	87.88%
9-57	0.82018	91.67%	89.47%	85.71%	85.71%	87.88%
9-58	0.82018	91.67%	89.47%	85.71%	85.71%	87.88%
9-59	0.82018	91.67%	89.47%	85.71%	85.71%	87.88%
9-60	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-61	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-62	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-63	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-64	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-65	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-66	0.78947	83.33%	94.74%	78.57%	90.48%	87.88%
9-67	0.92105	83.33%	89.47%	78.57%	85.71%	84.85%
9-68	0.85088	91.67%	84.21%	85.71%	80.95%	84.85%
9-69	0.81579	83.33%	89.47%	78.57%	85.71%	84.85%
9-70	0.80702	91.67%	84.21%	85.71%	80.95%	84.85%
9-71	0.80702	91.67%	84.21%	85.71%	80.95%	84.85%
9-72	0.78509	83.33%	89.47%	78.57%	85.71%	84.85%
9-73	0.78509	83.33%	89.47%	78.57%	85.71%	84.85%
9-74	0.78509	83.33%	89.47%	78.57%	85.71%	84.85%
9-75	0.78509	83.33%	89.47%	78.57%	85.71%	84.85%
9-76	0.78509	83.33%	89.47%	78.57%	85.71%	84.85%
9-77	0.78509	83.33%	89.47%	78.57%	85.71%	84.85%
9-78	0.77632	83.33%	89.47%	78.57%	85.71%	84.85%
9-79	0.77193	91.67%	84.21%	85.71%	80.95%	84.85%
9-80	0.74561	83.33%	89.47%	78.57%	85.71%	84.85%
9-81	0.74561	83.33%	89.47%	78.57%	85.71%	84.85%

[0168] Individual markers (autoantibody capture molecules) that were present in at least 10% of two or more of the statistically significant classifiers of Tables 5, 6, 7, 8, and 9 are provided in Table 10. The number of normalization techniques (out of seven) in which the marker (autoantibody capture molecule) was present in 10% or more of the identified statistically significant classifiers is provided. Normalization was done with various positive controls at various concentrations. Additionally, the percentage of normalization techniques in which the (autoantibody capture molecule) was present in 10% or more of the identified statistically significant classifiers is provided.

TABLE 10

Target Antigens Having 10% or Above of Statistical Significant Classifiers for Prostate Cancer versus BPH

Markers	Number of Normalization Techniques Marker was in 10% or Above of Classifiers	Percentage of Normalization Techniques
CCNA1	6	85.71%
PCNA	6	85.71%
HEYL	5	71.43%
SPRR1B	5	71.43%
RASSF1	5	71.43%
RNF14	4	57.14%
ERG	4	57.14%
COVA1	4	57.14%
EIF4G1	4	57.14%
BDKRB2	3	42.86%
PTGER3	3	42.86%
PSAP	3	42.86%
XLKD1	3	42.86%
CCNB1	3	42.86%
a-ACPP	2	28.57%
a-II-6	2	28.57%
ZWINT	2	28.57%
ERBB2	2	28.57%
TOP2A	2	28.57%
RPS6KA1	2	28.57%
HMGA2	2	28.57%
NCAM2	2	28.57%
ETS2	2	28.57%
CD164	2	28.57%
IMP-2	2	28.57%
KHDRBS1	2	28.57%
H3GLB1	2	28.57%

[0169] Biomarker detection panels of the invention specifically include but are not limited to any biomarker detection panels disclosed in this application. In some preferred embodiments, the biomarker panel includes at least one autoantibody capture molecule of Table 2. In some preferred embodiments, at least one of the proteins of the biomarker panel is selected from Table 10. In some preferred embodiments, a biomarker panel includes at least one biomarker detection set of Table 5, Table 6, Table 7, Table 8, or Table 9.

[0170] Biomarker detection panels of the invention can include proteins or protein fragments that are not antibodies as well as proteins that are antibodies, such as but not limited to antibodies to ACPP, BCL2, CXR4, IL-6, IL-8, PSA(free), PSA(total), or PTGER2. In some embodiments, a biomarker detection panel can includes an antibody to any of the target antigens of Table 1 or Table 11a. A biomarker detection panel of the invention can also include one or more antibodies that are not listed in Table 1 and are not antibodies to target antigens of Table 1 or Table 11a. As nonlimiting examples, antibodies to PAP or PSMA can also be part of a biomarker detection panel of the invention.

[0171] Also included in the invention is a composition that comprises a biomarker detection panel for diagnosing, prognosing, monitoring, or staging prostate cancer that comprises two or more autoantibody capture molecules selected from Table 1 or Table 11a, in which at least one of the two or more autoantibody capture molecules is bound to an autoantibody from a sample of an individual. The invention includes a biomarker detection panel for diagnosing, prognosing, monitoring, or staging prostate cancer that comprises 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more autoantibody capture molecules selected from Table 1 or Table 11a, in which at least one of the two or more autoantibody capture molecules is bound to an autoantibody from a sample of an individual.

[0172] The invention provides biomarker detection panels for diagnosing, prognosing, monitoring, or staging prostate cancer, in which the biomarker detection panels comprise KDR and PIM-1, variants thereof, or fragments thereof, in which at least one of the two or more autoantibody capture molecules is bound to an autoantibody from a sample of an individual. The invention includes a biomarker detection panel for diagnosing, prognosing, monitoring, or staging prostate cancer that comprises 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more autoantibody capture molecules, in which at least one of the autoantibody capture molecules is KDR or PIM-1, and in which at least one of the two or more autoantibody capture molecules is bound to an autoantibody from a sample of an individual. In some embodiments, at least one of the KDR or PIM-1 autoantibody capture molecules is bound to an autoantibody from a sample of an individual. In some embodiments, both of the KDR or PIM-1 autoantibody capture molecules are bound to an autoantibody from a sample of an individual.

[0173] Also included in the invention is a composition that comprises a biomarker detection panel for diagnosing, prognosing, monitoring, or staging prostate cancer that comprises two or more autoantibody capture molecules selected from Table 3, in which at least one of the autoantibody capture molecules of Table 3 of the array is bound to an autoantibody from a sample of an individual. Also included in the invention is a composition that comprises a biomarker detection panel for diagnosing, prognosing, monitoring, or staging prostate cancer that comprises two or more autoantibody capture molecules selected from Table 3, in which the array includes at least one autoantibody capture molecule of Table 10, and at least one of the autoantibody capture molecules of Table 3 of the array is bound to an autoantibody from a sample of an individual. The arrays having bound antibody from a sample can be arrays in which at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, of 95% of the protein bound to the arrays are proteins of Table 3.

Methods for Identifying Autoantigen/Autoantibody Biomarker Detection Panels

[0174] The present disclosure identifies a population of autoantibodies and panels thereof, associated with prostate cancer based on knowledge of biological pathways altered by cancer progression (including immunological pathways), analogy with other cancers, and literature searching to compile a list of proteins that were overexpressed, inappropriately expressed, or differentially modified or degraded in prostate cancer cells when compared with normal prostate cells. A

similar method can be used with other cancers, such as, for example, breast cancer, lung cancer, colorectal cancer, brain cancer, stomach cancer, bladder cancer, pancreatic cancer, ovarian cancer, liver cancer, leukemia, etc. Accordingly, provided herein is a method for identifying one or more autoantigens or one or more panels of autoantigens that are differentially present in a sample from an individual having a target cancer from an individual not having the target cancer, that includes identifying proteins that are overexpressed, inappropriately expressed, or differentially modified or degraded in a target cancer compared with normal cells or cells of a benign condition, performing an immunoassay that compares the pattern of immune reactivity of a sample from an individual having the target cancer to a sample for an individual that does not have the cancer, against a biomarker detection panel that includes one or more, two or more, three or more, four or more five or more, 10 or more, 20 or more 25 or more, 100 or more, 200 or more 250 or more 500 or more, or 1000 or more of the identified proteins, wherein antibodies against the identified proteins that are differentially present in the sample from the individual having the target cancer compared to the sample from the individual not having the target cancer, identifies the identified protein as an autoantigen for the target cancer. In certain aspects, the method further comprises repeating the immunoassay for a group of samples from different individuals having the target cancer and repeating the immunoassay for a group of samples from different individuals not having the target cancer.

Method for Synthesizing Protein Antigens

[0175] The methods, kits, and systems provided herein include autoantigens, which typically are protein antigens. To obtain protein antigens to be used in the methods provided herein, known methods can be used for making and isolating viral, prokaryotic or eukaryotic proteins in a readily scalable format, amenable to high-throughput analysis. For example, methods include synthesizing and purifying proteins in an array format compatible with automation technologies.

[0176] In some exemplary embodiments, proteins are expressed from gene constructs using in vitro synthesis systems or cell culture systems. Any expression construct having an inducible promoter to drive protein synthesis can be used in accordance with the methods of the invention. The expression construct may be, for example, tailored to the cell type to be used for transformation. Compatibility between expression constructs and host cells are known in the art, and use of variants thereof are also encompassed by the invention.

[0177] Therefore, in one embodiment, protein microarrays for the invention a method for making and isolating eukaryotic proteins comprising the steps of growing a cell transformed with a vector having a heterologous sequence operatively linked to a regulatory sequence, contacting the regulatory sequence with an inducer that enhances expression of a protein encoded by the heterologous sequence, lysing the cell, contacting the protein with a binding agent such that a complex between the protein and binding agent is formed, isolating the complex from cellular debris, and isolating the protein from the complex, wherein each step is conducted in a 96-well format. For example, bacterial, yeast, mammalian, or insect cells can be used for the production of proteins.

[0178] In a particular embodiment, eukaryotic proteins can be made and purified in a 96-array format (i.e., each site on the solid support where processing occurs is one of 96 sites), e.g., in a 96-well microtiter plate. In another embodiment, the solid support does not bind proteins (e.g., a non-protein-binding microtiter plate).

[0179] In certain embodiments, proteins are synthesized by in vitro translation according to methods commonly known in the art. For example, a wheat germ expression (WGE) system can be used to synthesize proteins used as autoantibody capture molecules. A variety of commercial WGE systems are available, the majority of this work has been performed using Cell Free Sciences WGE system (Yokahama Japan). Alternatively, proteins used as autoantibody capture molecules can be synthesized in other in vitro synthesis systems or in cell culture. As nonlimiting examples, *E. coli* in vitro translation systems or reticulocyte lysate in vitro translation systems can be used for synthesis of autoantibody capture molecules. Proteins used as autoantibody capture molecules can also be isolated from organisms, for example, from sera.

[0180] In some exemplary embodiments, proteins are synthesized in vitro or in culture systems as GST-fusion constructs, and are purified from cell culture or a cell-free expression system using GST-beads or columns. Invitrogen's Ultimate™ ORF clone collection is an ideal platform to generate a large number of antigens in a facile manner.

[0181] In a particular embodiment, the fusion proteins have GST tags and are affinity purified by contacting the proteins with glutathione beads. In further embodiment, the glutathione beads, with fusion proteins attached, can be washed in a 96-well box without using a filter plate to ease handling of the samples and prevent cross contamination of the samples.

[0182] In addition, fusion proteins can be eluted from the binding compound (e.g., glutathione bead) with elution buffer to provide a desired protein concentration. In a specific embodiment, fusion proteins are eluted from the glutathione beads with elution buffer to provide a desired protein concentration.

[0183] For purified proteins that will eventually be spotted onto microscope slides, the glutathione beads are separated from the purified proteins. In one example, all of the glutathione beads are removed to avoid blocking of the microarrays pins used to spot the purified proteins onto a solid support. In one embodiment, the glutathione beads are separated from the purified proteins using a filter plate, for example, comprising a non-protein-binding solid support. Filtration of the eluate containing the purified proteins should result in greater than 90% recovery of the proteins.

[0184] The elution buffer may, for example, comprise a liquid of high viscosity such as, for example, 15% to 50% glycerol, for example, about 40% glycerol. The glycerol solution stabilizes the proteins in solution, and prevents dehydration of the protein solution during the printing step using a microarrayer.

[0185] Purified proteins may, for example, be stored in a medium that stabilizes the proteins and prevents desiccation of the sample. For example, purified proteins can be stored in a liquid of high viscosity such as, for example, 15% to 50% glycerol, for example, in about 40% glycerol. In one example, samples may be aliquoted containing the purified proteins, so as to avoid loss of protein activity caused by freeze/thaw cycles.

[0186] The skilled artisan can appreciate that the purification protocol can be adjusted to control the level of protein purity desired. In some instances, isolation of molecules that associate with the protein of interest is desired. For example,

dimers, trimers, or higher order homotypic or heterotypic complexes comprising an overproduced protein of interest can be isolated using the purification methods provided herein, or modifications thereof. Furthermore, associated molecules can be individually isolated and identified using methods known in the art (e.g., mass spectroscopy).

[0187] The protein antigens once produced, can be used in the biomarker panels, methods and kits provided herein as part of a "positionally addressable" array. The array includes a plurality of target antigens, with each target antigen being at a different position on a solid support. The array can include, for example, 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,24, 25, 26, 27, 28, 29, 30, 40, 50, 100, 200, 300, 400, or 500 different proteins. The array can include 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or all the proteins of Table 1. In one aspect, the majority of proteins on an array includes proteins identified as autoantigens that can have diagnostic value for a particular disease or medical condition when provided together autoantigen biomarker detection panel.

[0188] In one aspect, the protein array is a bead-based array. In another aspect, the protein array is a planar array. Methods for making protein arrays, such as by contact printing, are well known. In some embodiments, the detection is performed on a protein array, which can be a microarray, and can optionally be a microarray that includes proteins at a concentration of at least 100/cm^2 or 1000/cm^2, or greater than 400/cm^2.

[0189] In this embodiment, amino-terminal tagged GST proteins were utilized. Proteins were placed into 386-well printing "masterplates".

Kits

[0190] In certain embodiments of the invention, kits are provided. Thus, in some embodiments, a kit is provided that comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74, 75-79, 80-84, 85-89, 90-94, 95-100, 100-105, or 106-108 of the autoantibody capture molecules provided in Table 1. A kit of the invention can include any of the biomarker detection panels disclosed herein, including, but not limited to, a biomarker panel comprising two or more autoantibody capture molecules of Table 1, a biomarker panel comprising two or more biomarker capture molecules of Table 1, in which one or more of the capture molecules is a protein of Table 3, a biomarker panel comprising two or more biomarker capture molecules of Table 3, a biomarker panel comprising two or more autoantibody capture molecules of Table 1, in which one or more of the capture molecules is an autoantibody capture molecule of Table 10, and a biomarker panel comprising a 3-marker biomarker detection set of Table 5, a 4-marker biomarker detection set of Table 6, a 5-marker biomarker detection set of Table 7, a 6-marker biomarker detection set of Table 8, or a 7-marker biomarker detection set of Table 9.

[0191] In some preferred embodiments, a kit of the invention can include an autoantibody capture molecule that binds an autoantibody to KDR (for example, KDR, or a variant or fragment thereof) or an autoantibody capture molecule that binds an autoantibody to PIM-1 (for example, PIM-1, or an antibody or fragment thereof). A kit of the invention can include an autoantibody capture molecule that binds an autoantibody to KDR (for example, KDR, or a variant or fragment thereof) and an autoantibody capture molecule that binds an autoantibody to PIM-1 (for example, PIM-1, or an antibody or fragment thereof). The kit can further include other autoantibody capture molecules, such as but not limited to those provided in Table 1. A kit can include biomarker detection panel that includes KDR, PIM-1, or both KDR and PIM-1. The detection panel can include any number of autoantibody capture molecules, for example, from one to 10, from 10-20, from 20-30, from 30-40, from 40-50, from 50-100, or more than 100.

[0192] A kit can include one or more positive controls, one or more negative controls, and/or one or more normalization controls.

[0193] The proteins of the kit may, for example, be immobilized on a solid support or surface. The proteins may, for example, be immobilized in an array. The protein microarray may use bead technology, such as the Luminex technology (Luminex Corp., Austin, Tex.). The test protein array may or may not be a high-density protein microarray that includes at least 100 proteins/cm^2. The kit can provide a biomarker detection panel of proteins as described herein immobilized on an array. At least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the proteins immobilized on the array can be proteins of the biomarker test pane. The array can include immobilized on the array one or more positive control proteins, one or more negative controls, and/or one or more normalization controls.

[0194] A kit may further comprise detection reagents and/or one or more reporter reagents to detect binding of human antibody to the proteins of the biomarker detection panel, such as, for example, an a species-specific antibody that binds to human antibodies, such as, for example, anti-human IgG antibody. The species specific antibody can be linked to a detectable label.

[0195] A kit may further comprise reagents useful for various immune reactivity assays, such as ELISA, or other immunoassay techniques known to those of skill in the art. The assays in which the kit reagents can be used may be competitive assays, sandwich assays, test strip assays, and the label may be selected from the group of well-known labels used for radioimmunoassay, fluorescent or chemiluminescence immunoassay.

[0196] A kit can include reagents described herein in any combination. For example, in one aspect, the kit includes a biomarker detection panel as provided herein immobilized on a solid support and anti-human antibodies for detection in solution or for detection on a solid support. The detection antibodies can comprise labels.

[0197] The kit can also include a program in computer readable form to analyze results of methods performed using the kits to practice the methods provided herein.

[0198] The kits of the present invention may also comprise one or more of the components in any number of separate containers, packets, tubes, vials, microtiter plates and the like, or the components may be combined in various combinations in such containers.

[0199] The kits of the present invention may also comprise instructions for performing one or more methods described herein and/or a description of one or more compositions or reagents described herein. Instructions and/or descriptions may be in printed form and may be included in a kit insert. A

kit also may include a written description of an Internet location that provides such instructions or descriptions.

EXAMPLES

[0200] The examples set forth below illustrate but do not limit the invention.

Example 1

Test Protein Array for Diagnostic Autoantigens

[0201] Biomarker detection panels were sought having at least as high a sensitivity as the standard PSA test (80%), with higher specificity.

Experimental Design

[0202] A protein array was fabricated by spotting proteins on a planar nitrocellulose substrate. The overall design of the array is depicted in FIG. **1**, which shows half of an array used to test for the diagnostic utility of the 108 antigens and 8 antibodies listed in Table 1. The antigens and antibodies were selected based on biological experiments, knowledge of biological pathways altered by cancer progression (including immunological pathways), analogy with other cancers, and literature searching to compile a list of proteins that were overexpressed, inappropriately expressed, or differentially modified or degraded in prostate cancer cells when compared with normal prostate cells.

[0203] Table 1 provides in the first column ("Marker") the term used throughout this application for the autoantibody capture molecule. The second column provides the term for the protein used by the Human Genome Organization (HUGO). Other names used for the protein in the scientific literature are provided in column 3, and column 4 provides the Genbank gene identifier and reference sequence ID. The table also provides in column 5 the method of synthesis and/or source of the protein.

[0204] The proteins were synthesized in vitro (103 out of the 108 using wheat germ extract, in most cases using Cell Free Sciences WGE system (Yokahama Japan)), or, in some cases, were obtained commercially as proteins synthesized in *E. coli* or isolated from human serum. A variety of clones available from Invitrogen Corp. (Carlsbad, Calif., Invitrogen.com), including the extensive human ORF collection, were used as templates. The cell-free expressed proteins were purified using purification tags (e.g., GST-fusion vectors using GST columns) and then quality-control verified (for correct molecular weight and purity) using high throughput electrophoresis (e.g., Agilent bioanalysers). Antibodies were obtained commercially.

[0205] The chip array (as shown in FIG. **1**) also included as controls: 1137 empty spots (no protein), 35 Alexa 488 fiducials, 16 Alexa 555 fiducials, 24 Alexa 647 fiducials, glutathione S-transferase (GST) at 8 concentrations, bovine serum albumin (BSA) at 8 concentrations, mouse anti-human Kappa antibody at three concentrations (a positive control), mouse anti-human IgG1 antibody at three concentrations (a positive control), goat anti-mouse biotin at three concentrations, mouse anti-biotin at three concentrations, Protein L (an immunoglobulin binding protein from the bacterium *Peptostreptococcus magnus*) at three concentrations, goat anti-human IgG at three concentrations, and human IgG at three concentrations. Positive controls were spotted at from 5 to 8 different concentrations.

[0206] The qc-validated protein antigens were then printed onto slides with various surfaces, including but not limited to nitrocellulose, amine group or epoxy group modified surfaces. Nitrocellulose has the largest binding capacity of these surfaces, so it was used for the final test chip Ver.3. The antigens were printed in a manner so that each is represented using multiple, independent spots, located in geographically distinct spots multiple millimeters away from each other (FIG. **1**). Multiple positive and negative control proteins that were specifically selected and designed for autoantigen profiling experiments, were spotted on the test chip Ver.3 such that they were interspersed around and within the antigen spots. Examples of positive and negative control spots are provided in FIG. **1** (fluorophore fiducials, GST protein, mouse anti-human antibodies, protein L, mouse anti-biotin, BSA protein, and human IgG) along with the tested antigens and antibodies.

[0207] The example chip design format shown in FIG. **1** comprises 8 capture antibodies, 108 auto-antigens, mouse anti-human K 3 step (positive control), mouse anti-human IgG1 3 step (positive control), goat anti-mouse biotin 3 step (biotinylation assay control), mouse anti-biotin 3 step (biotinylation assay control), protein L 3 step (positive control), human IgG 4 step (positive control), and 1137 empty spots. The array is printed in duplicate on each chip, so every spot on the chip is replicated a total of four times. The chip also utilizes a print buffer, BSA 2 step gradient (negative control), GST 8 step gradient (on-chip concentration quantification), 35 Alexa 488 Fiducials, 16 Alexa 555 Fiducials, 24 Alexa 647 Fiducials.

[0208] Protein concentrations for printing were approximately 150 micrograms per mL (ranging from 30 to 250 ug/mL). Approximately 10 uL protein aliquots in 50 mM Tris, 10 mM glutathione pH 8 were diluted 1:1 with this same buffer or Whatman printing buffer (Protein Arraying Buffer, 2× concentration, product number 10 485 331). Master plates were maintained at either 15° C. or at ~4° C. during the printing process. Proteins were printed onto Whatman nitrocellulose slides (FAST slides, 1 pad, 11 um thick nitrocellulose, product number 10 484 182). Approximately 250 pL of protein solution was spotted, or multiples of between three to six times 250 pL spotted sequentially using a Scienion non-contact piezo-based printer (sciFLEXARRAYER S5 Piezo Dispenser). After spotting, slides were placed into a low humidity environment (10% relative humidity at room temperature inside a low humidity chamber) allowed to dry for at least 12 hours. Proteins were printed in batches of 24 slides. A single master plate could generate from 24 to ~200 slides. Every 24^{th} slide was quality control imaged using Alexa Fluor anti-GST antibody. Briefly, auto-antigen array slides were blocked with 1% BSA in PBS, 0.1% Tween-20 (PBST) followed by development with 240 ng/mL rabbit anti GST Alexa Fluor 647 in PBST/0.3% BSA. The intensities of the spots were quantitated using a GenePix 4000B micro-array scanner.

[0209] The Ver.3 test chips were blocked with blocking buffer (1×PBS, 1% BSA, 0.1% Tween-20) for 1 hr at 4 degrees. The blocking was done in glass staining dish with gentle agitation. The serum was diluted 1:150 in probing buffer (1×PBS, 5 mM $Mgcl_2$, 0.5 mM DTT, 0.05% Triton X-100, 5% glycerol, 1% BSA). 100 ul of diluted serum was then applied to the lifterslip (Erie Scientific) to probe the protein array chip with autoantibodies. The lifterslip was applied to cover the chip from one end to the other so that no

bubbles were trapped between the lifeterslip and the chip. The serum probing was done at 4 degrees for one and half hours in a moisture chamber (Evergreen, cat #240-9020-Z10). After serum probing, the chips were washed three times with the probing buffer (10 min/wash) in a pap jar (1 slide per pap jar in 25 ml volume, Evergreen cat #222-5450-G8S). The washed chips were then incubated with secondary antibody goat anti-human IgG labeled with Alexa Fluo 647 (Invitrogen, cat #A21445) for one and half hours at dilution 1:2000 in the probing buffer. The chips were then washed three times (10 min/wash, in pap jar with 25 ml volume) with probing buffer and spin dried. The chips were scanned with an Axon scanner (PMT 600, 33% power).

[0210] The antigen content and experimental design methodologies described above were carried out using serum samples of 19 biopsy-verified prostate-cancer (PCa) patient serum samples and 12 benign hypertrophy (BPH) patient serum samples. One of the main complicating factors in the diagnosis of prostate cancer using PSA as a biomarker, is PSA's inability to discriminate between bone fide' prostate cancer and a non-life threatening condition termed benign prostatic hypertrophy (BPH). Hence, to generate a biomedically relevant autoantibody signature for PCa, the autoantibody detection chips were screened with serum from biopsy-verified PCa and biopsy-verified BPH patients. The serum was in all cases collected before digital rectal examination and before prostate biopsy was performed, such that the results obtained were not dependent on or influenced by having a DRE or biopsy procedure.

[0211] Data analysis was performed on locally background subtracted data, and normalization was performed by calculating the median positive controls signal (either Protein L 1.6 ng/ml, Goat Anti-Human IgG, Human IgG 0.4 ng/ml, Human IgG 1.6 ng/ml, Mouse Anti Biotin 25 ng/ml, Mouse Anti-Human Kappa 6.3, or Mouse Anti-Human IgG1) and dividing it into the median of the antigen/antibody signal to give the normalized signal. Using this normalized signal for each marker, a logistic regression classifier, using a leave-one-out approach, was used to predicted the left out observation. This was done for each normalized signal. Once all of the normalized signals were predicted by the leave-one-out logistic regression, a Receiver Operator Characteristic (ROC) Curve was calculated, and the associated Area Under the Curve (AUC) was calculated. Marker sets were then ranked based on AUC's from largest to smallest. For each marker set, using the ROC, we looked for the optimal point on the curve that maximizes both Sensitivity and Specificity.

[0212] The markers of Table 3 exhibited signals that were at least two times background. Tables 5-9 provide marker sets (autoantibody detection sets) that had greater than 80% Sensitivity and greater than 80% Specificity when analyzed by the above methods. The tables provide the positive control ("Method") that was used to normalize the data on which the classifier was built. Tables 5 through 9 also provide designations for each classifier, or marker set (5-1, 5-2, 6-1, 6-2, etc.) and subsequent columns in Tables 5 through 9 provide the markers of each autoantibody detection set. In Tables 5b, 6b, 7b, 8b, and 9b, statistical values are provided for each marker set. "AUC" is the area under the curve of the associated leave-one-out Receiver Operator Characteristic ("ROC") curve for the classifier. "Specificity" is the frequentist estimate of the specificity of the classifier based on testing and analysis of 19 prostate cancer serum samples and 12 BPH serum samples, i.e., the percentage of negatives (BPH patients) correctly identified. "Sensitivity" is the frequentist estimate of the sensitivity of the classifier based on testing and analysis of 19 prostate cancer serum samples and 12 BPH serum samples, i.e., the percentage of positives (prostate cancer patients) correctly identified. "Bayesian Specificity" is the Bayesian estimate of the specificity of the classifier based on testing and analysis of 19 prostate cancer serum samples and 12 BPH serum samples, i.e., the sum of the number of correctly identified negatives (BPH patients) and one, divided by the sum of the number of BPH samples and two. "Bayesian Sensitivity" is the Bayesian estimate of the sensitivity of the classifier based on testing and analysis of 19 prostate cancer serum samples and 12 BPH serum samples, i.e., the sum of the number of correctly identified positives (prostate cancer patients) and one, divided by the sum of the number of prostate cancer samples and two. "Bayesian Accuracy" is the Bayesian estimate of the accuracy of the classifier based on testing and analysis of 19 prostate cancer serum samples and 12 BPH serum samples, i.e., the sum of the number of correctly identified samples and one, divided by the sum of the number of samples and two.

[0213] Three-marker autoantibody detection sets having greater than 80% Bayesian Accuracy are provided in Table 5. Four-marker autoantibody detection sets having greater than 80% Bayesian Accuracy are provided in Table 6. Five-marker autoantibody detection sets having greater than 80% Bayesian Accuracy are provided in Table 7. Six-marker autoantibody detection sets having greater than 80% Bayesian Accuracy are provided in Table 8. Seven-marker autoantibody detection sets having greater than 80% Bayesian Accuracy are provided in Table 9. Four of the seven-marker autoantibody detection sets exhibited 100% Specificity and 100% Sensitivity for distinguishing prostate cancer from BPH. These autoantibody detection sets were: Set 7-1: HEYL, BDKRB2, MAD1L1, PSAP, CCNA1, ERG, and PCNA; Set 7-2: HEYL, BDKRB2, PSAP, CCNA1, ERG, IMP-3, and PCNA; Set 7-3: HEYL, BDKRB2, PSAP, CCNA1, ERG, PIM1, and PCNA; and Set 7-4: HEYL, BDKRB2, PSAP, CCNA1, EDS, RDH11, and PCNA. Each of these biomarker detection sets had a calculated ROC/AUC of 1.

[0214] Individual markers (autoantibody capture molecules) that were present in at least 10% of two or more of the statistically significant classifiers of Tables 5, 6, 7, 8, and 9 are provided in Table 10. In column 2 of Table 10, the number of normalization techniques (out of seven) in which the marker (autoantibody capture molecule) was present in 10% or more of the identified statistically significant classifiers is provided. In column 3, the percentage of normalization techniques in which the (autoantibody capture molecule) was present in 10% or more of the identified statistically significant classifiers is provided.

Example 2

Identification of Autoantigens Present in Prostate Cancer Sera on ProtoArrav™ Human Protein Array

[0215] The human Protoarray™ high content protein microarray from Invitrogen (Carlsbad, Calif.) was screened with sera using the methods provided in Example 1. A combination of single-patient sample and pooled-patient samples were utilized with these arrays. A total of 32 patient samples were screened (16 prostate cancer and 16 BPH) as well as series of pooled-patient samples representing high, medium, and low PSA values. All of this data was analyzed together to generate a list of approximately 98 candidate prostate cancer biomarkers (Table 11a).

[0216] The high density Protoarray™ microarray data was normalized using a Quantile Normalization method for all chips used. After normalization, the diagnostic value of individual markers was estimated by calculating all possible

order M-statistics and their associated p-values. The order with the lowest p-value was selected for each marker, the prevalence of the marker was calculated using a standard Bayesian estimate of prevalence. Markers with p-value less then 0.002 were determined as significance. For the pooled data analysis the ratio of Low Grade PCa versus BPH and High Grade PCa versus BPH was calculated, any marker that showed at least a 20% increase or decrease in signal was considered significant. Only markers that were determined to be significant in both the normal ProtoArray Analysis and the pooled experiment are provided in Table 11a.

[0217] Table 11a provides the terms used for the markers that were found to have significance for the detection of prostate cancer, high grade (HG) prostate cancer (PCA), or low grade (LG) prostate cancer over BPH. The panel also provides the Genbank identifier and/or reference sequence ID, the Invitrogen ORF designation, and the "Significance Call" of whether the marker had significance for distinguishing prostate cancer overall, high grade prostate cancer, or low grade prostate cancer from BPH. Table 11b provides in one column the "Low Grade Cancer/Normal Ratio", which is the ratio of Low Grade Pool signal to BPH pooled signal; in another column the "High Grade Cancer/Normal Ratio", which is the ratio of High Grade Pool signal to BPH pooled signal; in another column the "All PCA vs BPH P-Value", which is the p-value of the difference between all PCa versus BPH in the individual analysis; in another column the "HG PCA vs BPH P-Value", which is the p-value of the difference between High Grade PCa versus BPH in the individual analysis; and in a final column of the panel the "LG PCA vs BPH P-Value", which is the p-value of the difference between Low Grade PCa vs BPH in the individual analysis (p-values are based on M-Statistics). Table 11c, one column provides the BPH Prevalence, or the estimated Bayesian prevalence of the marker in all BPH samples based on the signals of the individual protoarray experiments; in another column is provided the "All PCA Prevalence" which is the estimated Bayesian prevalence of the marker in all prostate cancer samples based on the signals of the individual protoarray experiments; in another column is provided the "HG PCA Prevalence" which is the estimated Bayesian prevalence of the marker in all High Grade prostate cancer samples based on the signals of the individual protoarray experiments; and a final column of the third panel provides the "LG PCA Prevalence", which is the estimated Bayesian prevalence of the marker in all Low Grade prostate cancer samples based on the signals of the individual protoarray experiments.

Example 3

Autoantibodies Differentiating Prostate Cancer from Benign Prostatic Hyperplasia in Patients

[0218] Autoantibody profiling using a protein microarray chip containing 96 proteins thought to be associated with prostate cancer development was conducted using sera from 32 patients with prostate cancer and 32 patients with benign prostatic hyperplasia. The goal was to find biomarkers that are stable in blood, easily measured using approximately 1 μL of serum (or plasma), and that can differentiate true prostate cancer from the closely-related benign prostatic hyperplasia, the major weakness in the current, clinically used, PSA-based prostate cancer diagnostic test.

[0219] The scheme for testing chips with human sera from individuals with prostate cancer and BPH is provided in FIG. **5**. Serum samples from individuals having prostate cancer and BPH were collected and contacted with a chip containing the possible target antigens. The resulting binding of target antigens to autoantibodies was quantified and used to identify the biomarkers selective for prostate cancer over BPH. The top 20 biomarkers that had significant difference between pooled PCa sera and pooled BPH are shown in FIG. **2**B.

[0220] The selected antigens were expressed using a cell-free expression system, purified and arrayed on microslides. The autoantibody profiling results with the pooled PCA and BPH samples showed that twenty of these protein antigens detected significant autoantibody signals which differentiate PCa from BPH. Among these twenty protein antigens, p53, CCNB1, survivin, and mucin1 are common tumor associated antigens shared by various cancer types such as breast cancer, colon cancer, prostate cancer, lung cancer and melanoma, but which have not been previously utilized as part of a prostate cancer diagnostic assay. Fourteen of these antigens are completely novel tumor antigens which have not been reported before.

[0221] In particular, two novel tumor antigens, KDR and PIM-1 which contribute to PCa development and progression, were shown to have significant sensitivity and specificity with regard to PCa diagnosis. KDR is a type III receptor tyrosine kinase which is involved in the angiogenesis and proliferation of PCa (Huss et al. (2001) "Angiogenesis and prostate cancer: identification of a molecular progression switch," Cancer Res. 61(6):2736-43; Jackson et al. (2002) "A potential autocrine role for vascular endothelial growth factor in prostate cancer," Cancer Res. 62(3):854-9; and Soker et al. (2001) "Vascular endothelial growth factor-mediated autocrine stimulation of prostate tumor cells coincides with progression to a malignant phenotype," Am J. Pathol. 159(2): 651-9). PIM-1 is a serine/threonine kinase and its over expression in the prostate leads to the genomic instability which contributes to the tumor progression (Valdman et al. (2004) "Pim-1 expression in prostatic intraepithelial neoplasia and human prostatecancer," Prostate. 60(4):367-71; Bhattacharya et al. (2002) "Pim-1 associates with protein complexes necessary for mitosis," Chromosoma. 111(2):80-95; Roh et al. (2003) "Overexpression of the oncogenic kinase Pim-1 leads to genomic instability," Cancer Res. 63(23):8079-84; and Cibull et al. (2006) "Overexpression of Pim-1 during progression of prostatic adenocarcinoma," J. Clin. Pathol. 59(3):285-8).

[0222] Autoantibodies against KDR and PIM-1 were shown to be present in ~62% PCA patients and ~30% BPH patients. Furthermore the pairing of KDR and PIM-1 confers a sensitivity of 90.6% and specificity of 84.4% in diagnosing PCa over an equal number of BPH samples. The tissue microarray experiments indicated that KDR and PIM-1 antigens are over expressed in 70% and 30% PCa patients respectively suggesting that over expression of these tumor antigens may account for the aberrant autoantibody induction.

[0223] Interestingly, in PCa, KDR and PIM-1 autoantibodies were very effective in diagnosing with small size tumors (tumors with 1-2 positive cores as pathologically diagnosed) where a PSA assay had limited diagnostic value.

[0224] It is worth noting that this study was performed using very small amounts of cell-free-extract in-vitro synthesized proteins (~10 μg). All that is required to make these antigens is the requisite open-reading-frame genetic constructs combined with a cell-free expression system; both materials are widely commercially available. Autoantibodies discovered in this manner are of very high diagnostic quality because they stably exist in sera. Only 1 μL of sera is required for the assay and the diagnostic-assay development step itself is eliminated. Moreover, generic detection reagents (e.g.,

fluorescently labeled Goat anti-human antibodies) can be used for detection on the protein chip.

Materials and Methods

[0225] Patients and Sera. Sixty four biopsy-proven serum samples from thirty two untreated PCA patients and thirty two untreated BPH patients were collected by BIOCHEMED Corporation (1483 Tobias Blvd., Charles, S.C. 29407) before the patients had DRE and biopsies. All samples were tested for PSA level by Beckman Access (Fullerton, Calif.). A complete medical history was provided for each identified patient with biopsy information on a patient's Gleason scores, number of positive scores for all 8 needle biopsy cores, as well as the percentage of cancer cells in a single core. All samples were collected from patients in the South Carolina area with informed consent forms and the studies were approved by the institutional review boards. Patient information is listed in Table 12.

TABLE 12

Clinical data for prostate cancer and benign prostatic hyperplasia patients

Variables	Prostate Cancer Set	Benign Prostatic Hyperplasia Set
No of patients	32	32
Mean age ± SD	65.17 ± 7.79	61.72 ± 9.24
PSA level		
<4 ng/ml	37.5%	50%
4-10 ng/ml	50%	37.5%
>10 ng/ml	12.5%	12.5%

TABLE 12-continued

Clinical data for prostate cancer and benign prostatic hyperplasia patients

Variables	Prostate Cancer Set	Benign Prostatic Hyperplasia Set
Gleason Score		
<6	72%	NA
>7	28%	NA

[0226] Expression of tumor antigens and fabrication of antigen microarray. The 96 recombinant GST tagged proteins were obtained using an in-vitro wheat germ cell-free expression system (Abnova or Invitrogen) and are shown in Table 13. Cell-free expressed proteins were eluted from GST-columns (Invitrogen, cat# 13-6741) with Tris-Glutathione buffer (pH 8.0). The proteins were expressed in either full-length or truncated forms. Each protein was quality controlled (for correct mass-weight and impurities) using an Agilent Bioanalyzer (Santa Clara, Calif.) and then arrayed as quadruplicate on one-pad nitrocellulose slide (Whatman, cat# 10484182) at concentrations ranging from about 0.1 to about 0.25 μg/ml using a sciFLEXARRAYER S5 Piezo Dispenser (Scienion AG, Berlin, Germany). A low content chip containing KDR and PIM-1 was made on a 16-pad nitrocellulose slide (Whatman, cat# 10485323) in which each protein was printed in duplicate, and each chip contained 12 total arrays. Mouse anti-human IgG1 (Invitrogen, cat# 05-3300) was printed as four identical spots of 6 μg/ml on each array and was used for the normalization of microarray signals. Select microarray slides from each printing run were probed with anti-GST antibodies to measure the final amount of printed protein on each spot on the array.

TABLE 13

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
ABL1	ABL1 (full-length) Invitrogen	JTK7, c-ABL, p150, v-abl	v-abl Abelson murine leukemia viral oncogene homolog 1 NM_007313	Oncogene	Apoptosis
ACPP	ACPP (310-418) Abnova	ACPP, PAP, ACP3, ACP-3	Acid phosphatase, prostate NM_001099	Catalyze phosphate monoester into alcohol and phosphate.	Cellular metabolism
AGR2	AGR2 (full-length) Abnova	AGR2, AG2, GOB-4, HAG-2, XAG-2	Anterior gradient homolog 2 (*Xenopus laevis*) NM_006408	Cell differentiation	Metastasis
AKT1	AKT1 (full-length) Invitrogen	AKT1, PKB, RAC, PRKBA, MGC99656, RAC-ALPHA	v-akt murine thymoma viral oncogene homolog 1 NM_005163	Oncogene	Apoptosis
ALOX15	ALOX15 (full-length) Abnova	ALOX15	Arachidonate 15-lipoxygenase NM_1140	Converts arachidonic acid to 15S-hydro-peroxyeicosate traenoic acid	Cellular metabolism
AMACR	AMACR (full-length) Abnova	AMACR, RACE	Alpha-methylacyl-CoA racemase NM_014707	Racemization of 2-methyl-branched fatty acid CoA esters	Cellular metabolism

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
BCL2	BCL2 (140-239) Abnova	BCL2, Bcl-2	B-cell CLL/lymphoma 2 NM_000633	Suppresses apoptosis	Apoptosis
BCL2L14	BCL2L14 (full-length) Abnova	BCL2L14, BCLG	BCL2-like 14 NM_030766	Apoptosis facilitator	Apoptosis
BDKRB2	BDKRB2 (1-61) Abnova	BDKRB2, B2R, BK2, BK-2, BKR2, BRB2, DKFZp686O088	Bradykinin receptor B2 NM_000623	Receptor for bradykinin	Cell surface protein
BIRC5	BIRC5 (full-length) Abnova	BIRC5, API4, EPR-1	Baculoviral IAP repeat-containing 5 (survivin) NM_001012270	Anti-apoptotic	Apoptosis
CAV3	CAV3 (1-84) Abnova	CAV3, VIP21, LGMD1C, VIP-21, MGC126100, MGC126101, MGC126129	Caveolin 3 NM_001753	Scaffolding protein within caveolar membranes	Tumor suppressor
CCKBR	CCKBR (215-327) Abnova	CCKBR, GASR, CCK-B	Cholecystokinin B Receptor NM_176875	Receptor for gastrin and cholecystokinin	Cell surface protein
CCNA1	CCNA1 (full-length) Abnova	CCNA1	Cyclin A1 NM_003914	Cell cycle regulation	Cell cycle
CCNB1	CCNB1 (1-91) Abnova	CCNB1, CCNB	Cyclin B1 NM_031966	Cell cycle regulation	Cell cycle
CCND1	CCND1 (full-length) Abnova	CCND1, BCL1, PRAD1, U21B31, D11S287E	Cyclin D1 NM_053056	Cell cycle regulation	Cell cycle
CD151	CD151 (full-length) Abnova	CD151, GP27, MER2, RAPH, SFA1, PETA-3, TSPAN24	CD151 molecule (Raph blood group) NM_004357	Essential for the proper assembly of the glomerular and tubular basement membranes in kidney	Metastasis
CD164	CD164 (1-115) Abnova	CD164, MGC-24, MUC-24, Endolyn	CD164 molecule, sialomucin NM_006016	Mucin-like protein	Metastasis
CDKN2A	CDKN2A (full-length) Abnova	CDKN2A, ARF, MLM, p14, p16, p19, CMM2, INK4, MTS1, TP16, CDK4I, CDKN2, INK4a, p14ARF, p16INK4, p16INK4a	Cyclin-dependent kinase inhibitor 2A NM_000077	Tumor suppressor	Tumor suppressor
CHEK1	CHK1 (full-length) Invitrogen	CHK1	CHK1 checkpoint homolog (*S. pombe*) NM_001274	Cell cycle regulation	Cell cycle
CLDN3	CLDN3 (full-length) Abnova	CLDN3, RVP1, HRVP1, C7orf1, CPE-R2, CPETR2	Claudin 3 NM_001306	Cell adhesion	Cell surface protein
CLDN4	CLDN4 (full-	CLDN4, CPER,	Claudin 4 NM_001305	Cell adhesion	Cell surface protein

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
	length) Abnova	CPE-R, CPETR, CPETR1, WBSCR8, hCPE-R			
CUL4A	CUL4A (1-100) Abnova	CUL4A	Cullin 4A NM_003589	DNA repair	Cell cycle
CXCR4	CXCR4 (1-47) Abnova	CXCR4, FB22, HM89, LAP3, LCR1, NPYR, WHIM, CD184, LESTR, NPY3R, NPYRL, HSY3RR, NPYY3R, D2S201E	Chemokine (C—X—C motif) receptor 4 NM_001008540	Tumor metastasis	Metastasis
EDNRB	EDNRB (27-101) Abnova	EDNRB, ETB, ETRB, HSCR, ABCDS, HSCR2	Endothelin receptor type B NM_000115	Non-specific receptor for endothelin 1, 2, and 3	Cell surface protein
EGFR	EGFR (26-126) Abnova	EGFR, ERBB, mENA, ERBB1	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) NM_005228	Growth factor receptor	Cell surface protein
EIF3S3	EIF3S3 (full-length) Abnova	EIF3S3, eIF3-p40, MGC102958, eIF3-gamma	Eukaryotic translation initiation factor 3, subunit 3 gamma, 40 kDa NM_003756	Protein translation	Cellular metabolism
ELAC1	ELAC1 (282-363) Abnova	ELAC1, D29	elaC homolog 1 (*E. coli*) NM_018696	Involved in tRNA maturation	Cellular metabolism
ENO1	ENO1 (full-length) Abnova	ENO1, NNE, PPH, MPB1, MBP-1, ENO1L1	Enolase 1, (alpha) NM_001428	Plays a part in various processes such as growth control, hypoxia tolerance and allergic responses	Cell growth
ENOX2	COVA1 (full-length) Abnova	COVA1, APK1, tNOX	Ecto-NOX disulfide-thiol exchanger 2 NM_006375	Cell growth, tumor antigen	Cell surface protein
ERBB2	ERBB2 (676-1255) Invitrogen	ERBB2, NEU, NGL, HER2, TKR1, HER-2, c-erb B2, HER-2/neu	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) NM_001005862	Tyrosine kinase-type cell surface receptor HER2, Oncogene	Cell surface protein
ERG	ERG (full-length) Abnova	ERG, p55, erg-3	v-ets erythroblastosis virus E26 oncogene homolog (avian) NM_004449	Oncogene	Cell growth and differentiation
ETS2	ETS2 (1-101) Abnova	ETS2	v-ets erythroblastosis	Oncogene	Cell growth and differentiation

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
			virus E26 oncogene homolog 2 (avian) NM_005228		
EZH2	EZH2 (full-length) Abnova	EZH1, ENX-1	Enhancer of zeste homolog 2 (*Drosophila*) NM_004456	Involved in the regulation of gene transcription and chromatin structure.	Metastasis
FASN	FASN (full-length) Abnova	FASN, FAS, OA-519, MGC14367, MGC15706	Fatty acid synthase NM_004104	Fatty acid metabolism	Cellular metabolism
FLT1	FLT1 (aa781-1338) Invitrogen	FLT1, FLT, VEGFR1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) NM_002019	Involved in angiogenesis	Angiogenesis
FOLH1	FOLH1 (547-657) Abnova	FOLH1, PSM, FGCP, FOLH, GCP2, PSMA, mGCP, GCPII, NAALAD1, NAALAdase	Folate hydrolase (prostate-specific membrane antigen) 1 NM_004476	Has both folate hydrolase and N-acetylated-alpha-linked-acidic dipeptidase (NAALADase) activity.	Cell surface protein Cellular metabolism
GDF15	GDF15 (full-length) Abnova	GDF15, PDF, MIC1, PLAB, MIC-1, NAG-1, PTGFB, GDF-15	Growth differentiation factor 15 NM_004864	Cell growth factor	Cell growth
HEYL	HEYL (full-length) Abnova	HEYL, HRT3, MGC12623	Hairy/enhancer-of-split related with YRPW motif-like NM_014571	Transcriptional repressor	Angiogenesis
HIPK3	HIPK3 (163-562) Invitrogen	PKY, DYRK6, YAK1	Homeodomain interacting protein kinase 3 NM_001048200	Regulate apoptosis by promoting FADD phosphorylation	Apoptosis
HMGA2	HMGA2 (1-93) Abnova	HMGA2, BABL, LIPO, HMGIC, HMGI-C	High mobility group AT-hook 2 NM_003484	Transcription regulation	Cell growth and differentiation
HOXB13	HOXB13 (full-length) Abnova	HOXB13, PSGD	Homeobox B13 NM_006361	Transcription factor involved in cell differentiation	Cell differentiation
HPN	HPN (full-length) Abnova	HPN, TMPRSS1	Hepsin (transmembrane protease, serine 1) NM_002151	Cell growth	Cell growth
HSPA5	HSPA5 (full-length) Abnova	HSPA5, BIP, MIF2, GRP78, FLJ26106	Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) NM_005347	Stress response protein	Stress response
HSPD1	HSPD1 (full-length) Abnova	HSPD1, CPN60, GROEL, HSP60, HSP65, SPG13, HuCHA60	Heat shock 60 kDa protein 1 (chaperonin) NM_002156	Stress response protein	Stress response

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
KDR	KDR (789-1356) Invitrogen	KDR, FLK1, CD309, VEGFR, VEGFR2	Kinase insert domain receptor (a type III receptor tyrosine kinase) NM_002253	Angiogenesis	Angiogenesis
KLK3	PSA (full-length) Abnova	KLK3, APS, PSA, hK3, KLK2A1	Kallikrein 3, (prostate specific antigen) NM_145864	Protease	Angiogenesis
LGALS8	LGALS8 (full-length) Abnova	LGALS8, Gal-8, PCTA1, PCTA-1, Po66-CBP	Lectin, galactoside-binding, soluble, 8 (galectin 8) NM_006499	Cell adhesion	Cell surface protein
MAD1L1	MAD1L1 (619-718) Abnova	MAD1L1, MAD1, PIG9, HsMAD1, TP53I9, TXBP181	MAD2 mitotic arrest deficient-like 1 NM_002358	Cell division	Cell cycle
MDM2	MDM2 (101-201) Abnova	MDM2, hdm2, MGC71221	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein NM_002392	Cell cycle regulation	Cell cycle
MET	MET (26-125) Abnova	MET, HGFR, RCCP2	Met proto-oncogene (hepatocyte growth factor receptor) NM_000245	Oncogene	Apoptosis
MIB1	MIB1 (909-1007) Abnova	MIB1, MIB, ZZZ6, DIP-1, ZZANK2, FLJ90676, MGC129659, MGC129660, DKFZp686I0769	Mindbomb homolog 1 (*Drosophila*) NM_020774	E3 ubiquitin-protein ligase that mediates ubiquitination of Delta receptors, which act as ligands of Notch proteins	Cell cycle
MICB	MICB (full-length) Abnova	MICB, PERB11.2	MHC class I polypeptide-related sequence B NM_005931	Ligand for NK cells	Innate Immunity
MLH1	MLH1 (full-length) Abnova	MLH1, FCC2, COCA2, HNPCC, hMLH1, HNPCC2, MGC5172	mutL homolog 1, colon cancer, nonpolyposis type 2 NM_000249	DNA mismatch repair	Cell cycle
MMP9	MMP9 (full-length) Abnova	MMP9, GELB, CLG4B, MMP-9	Matrix metallopeptidase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase) NM_004994	Tumor metastasis	Metastasis
MUC1	MUC1 (315-420) Abnova	MUC1, EMA, PEM, PUM, MAM6, PEMT, CD227, H23DG	Mucin 1, cell surface associated NM_002456	Cell adhesion and tumor metastasis	Metastasis
MYC	MYC (330-440) Abnova	MYC, c-Myc	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian) NM_002467	Oncogene	Cell cycle

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
NCAM2	NCAM2 (598-696) Abnova	NCAM2, NCAM21, MGC51008	Neural cell adhesion molecule 2 NM_004540	Cell adhesion	Cell surface Protein
NKX3-1	NKX3-1 (100-210) Abnova	NKX3-1, BAPX2	NK3 transcription factor related, locus 1 (*Drosophila*) NM_006167	Tumor suppressor specific to prostate cancer	Tumor suppressor
NRP1	NRP1 (full-length) Abnova	NRP1, NRP, CD304, VEGF165R,	Neuropilin 1 NM_015022	Tumor angiogenesis	Angiogenesis
NUCB1	NUCB1 (full-length) Abnova	NUCB1, NUC, FLJ40471,	Nucleobindin 1 NM_006184	Major calcium-binding protein of the Golgi	Cellular Metabolism
PCNA	PCNA (full-length) Abnova	PCNA, MGC8367	Proliferating cell nuclear antigen NM_182649	DNA replication	Cell cycle
PDLIM1	PDLIM1 (123-233) Abnova	PDLIM1, CLIM1, CLP36, ELFIN, CLP-36, hCLIM1	PDZ and LIM domain 1 (elfin) NM_020992	Cytoskeletal protein that may act as an adapter that brings other proteins (like kinases) to the cytoskeleton	Cell growth and Differentiation
PECAM1	PECAM1 (full-length) Abnova	PECAM1, CD31, PECAM-1	Platelet/endothelial cell adhesion molecule (CD31 antigen) NM_000442	Cell adhesion	Cell surface protein
PIM-1	PIM-1(full-length) Abnova	PIM-1, PIM	pim-1 oncogene NM_02648	Oncogene	Cell cycle
PRSS8	PRSS8 (full-length) Abnova	PRSS8, CAP1, PROSTASIN	Homo sapiens protease, serine, 8 (prostasin) NM_002773	Possesses a trypsin-like cleavage specificity.	Cell growth and Differentiation
PSAP	PSAP (full-length) Abnova	PSAP, GLBA, SAP1, FLJ00245, MGC110993	Prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy) NM_002778	Lipid metabolism	Cellular Metabolism
PSCA	PSCA (23-96) Abnova	PSCA, PRO232	Prostate stem cell antigen NM_005672	Unknown	Cell surface Protein
PSMB4	PSMB4 (full-length) Abnova	PSMB4, HN3, HsN3, PROS26	Proteasome (prosome, macropain) subunit, beta type, 4 NM_002796	Involved in proteolytic activity	Cellular Metabolism
PTEN	PTEN (221-320) Abnova	PTEN, BZS, MHAM, TEP1 MMAC1, PTEN1, MGC11227	Phosphatase and tensin homolog (mutated in multiple advanced cancers 1) NM_000314	Tumor suppressor	Tumor Suppressor
PTGER3	PTGER3 (1-90) Abnova	PTGER3, EP3, EP3e, EP3-I, EP3-II, EP3-IV, EP3-III, MGC27302, MGC141828, MGC141829	Prostaglandin E receptor 2 NM_000956	GPCR receptor involved in tumor metastasis	Metastasis

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
PTGS1	PTGS1(26-125) Abnova	PTGS1, COX1, COX3, PHS1, PCOX1, PGHS1, PTGHS, PGG/HS, PGHS-1	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) NM_000962	Cell proliferation	Cellular Metabolism
QSOX1	QSCN6 (81-181) Abnova	QSCN6, Q6, QSOX1	Quiescin Q6 NM_002826	Cell cycle regulation	Cell cycle
RASSF1	RASSF1 (241-341) Abnova	RASSF1, 123F2, RDA32, NORE2A, RASSF1A, REH3P21	Ras association (RalGDS/AF-6) domain family 1 NM_007182	Potential tumor suppressor	Tumor Suppressor
RCVRN	RCV1 (101-200) Abnova	RCVRN, RCV1	Recoverin NM_002903	Involved in the inhibition of the phosphorylation of rhodopsin	Cellular Metabolism
RDH11	RDH11 (full-length) Abnova	RDH11, MDT1, PSDR1, RALR1, SCALD, ARSDR1, CGI-82, HCBP12, FLJ32633	Retinol dehydrogenase 11 (all-trans/9-cis/11-cis) NM_016026	NADPH-dependent retinal reductase	Cellular Metabolism
RNF14	RNF14 (217-317) Abnova	RNF14, ARA54, HFB30, FLJ26004, HRIHFB2038	Ring finger protein 14 NM_004290	E3 ubiquitin-protein ligase	Cellular Metabolism
ROCK2	ROCK2 (1-552) Invitrogen	ROCK2	Rho-associated coiled-coil containing protein kinase 2 NM_004850	Protein Kinase involved in regulating the assembly of the actin cytoskeleton	Cell growth
RPL23	RPL23 (full-length) Abnova	RPL23, rpL17, MGC72008, MGC111167, MGC117346	Ribosomal protein L23 NM_000984	Protein translation	Cellular metabolism
RPL30	RPL30 (full-length) Abnova	RPL30	Ribosomal protein L30 NM_000989	Protein translation	Cellular Metabolism
RPS14	RPS14 (full-length) Abnova	RPS14, EMTB	Ribosomal protein S14 (RPS14) NM_001025070	Protein translation	Cellular Metabolism
RPS6KA1	RPS6KA1 (full-length) Invitrogen	RPS6KA1, RSK, HU-1, RSK1, MAPKAPK1A, S6K-alpha 1	Ribosomal protein S6 kinase, 90 kDa, polypeptide 1 NM_002953	Mediating the growth-factor and stress induced activation of the transcription factor CREB.	Stress Response
RPS6KA3	RPS6KA3 (full-length) Invitrogen	RSK, RSK2, HU-3	Ribosomal protein S6 kinase, 90 kDa, polypeptide 3 NM_004586	Mediating the growth-factor and stress induced activation of the transcription factor CREB.	Stress Response

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
SERPINH1	SERPINH1 (full-length) Abnova	SERPINH1, CBP1, CBP2, gp46, AsTP3, HSP47, PIG14, RA-A47, SERPINH2	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1) NM_001235	Involved as a chaperone in the biosynthetic pathway of collagen	Stress Response
SFRP4	SFRP4 (211-313) Abnova	SFRP4, FRP-4, FRPHE, MGC26498	Secreted frizzled-related protein 4 NM_003014	Cell growth and differentiation	Cell growth and Differentiation
SH3GLB1	SH3GLB1 (full-length) Abnova	SH3GLB1, Bif-1, CGI-61, KIAA0491, dJ612B15.2	SH3-domain GRB2-like endophilin B1 NM_016009	Apoptosis	Apoptosis
SPRR1B	SPRR1B (full-length) Abnova	SPRR1B, SPRR1, GADD33, CORNIFIN, MGC61901	Small proline-rich protein 1B (cornifin) NM_003125	Unknown	Cell Differentiation
STEAP1	STEAP (full-length) Abnova	STEAP1 STEAP, PRSS24, MGC19484	Six transmembrane epithelial antigen of the prostate 1 NM_012449	Metalloreductase	Cell surface Protein
STIP1	STIP1 (full-length) Abnova	STIP1, HOP, P60, STI1L, IEF-SSP-3521	Stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein) NM_006819	Stress response	Stress Response
TACSTD1	EP-CAM (full-length) Abnova	TACSTD1, EGP, KSA, M4S1, MK-1, CD326, EGP40, MIC18, TROP1, Ep-CAM, hEGP-2, CO17-1A, GA733-2	Tumor-associated calcium signal transducer 1 precursor NM_002354	Cell adhesion	Cell surface Protein
TMPRSS2	TMPRSS2 (full-length) Abnova	TMPRSS2, PRSS10	Transmembrane protease, serine 2 NM_005656	Cell adhesion	Angiogenesis
TOP2A	TOP2A (1435-1532) Abnova	TOP2A, TOP2, TP2A	Topoisomerase (DNA) II alpha 170 kDa NM_001067	DNA replication	Cell cycle
TP53	TP53 (94-202) Abnova	TP53, p53, LFS1, TRP53	Tumor protein p53 (Li-Fraumeni syndrome) NM_000546	Tumor suppressor	Tumor Suppressor
TPD52	TPD52 (100-185) Abnova	TPD52, D52, N8L, PC-1, PrLZ, hD52	Tumor protein D52 NM_005079	Oncogene	Cell cycle
UBE2C	UBE2C (full-length) Abnova	UBE2C, UBCH10, dJ447F3.2	Ubiquitin-conjugating enzyme E2C NM_007019	Cell cycle regulation. Required for the destruction of mitotic cyclins	Cell cycle
XLKD1	XLKD1 (full-length) Abnova	XLKD1, HAR, LYVE-1, CRSBP-1	Extracellular link domain containing 1 NM_016164	Involved in lymphogenesis	Angiogenesis
ZWINT	ZWINT (full-	ZWINT, KNTC2AP,	ZW10 interactor NM_001005413	Cell division	Cell Cycle

TABLE 13-continued

Function and source of protein antigens used for autoantibody profiling chip

HUGO Designation	Protein source	Protein/Gene Aliases	GenBank Identifiers	Function	Biomarker Grouping
	length) Abnova	HZwint-1, MGC117174			

Note:
Numbers in the parenthesis of column 2 indicate the amino acid number for the partial recombinant protein.

[0227] Immune profiling of prostate cancer and benign prostatic hyperplasia sera. The protein array chips were blocked with blocking buffer (1×PBS, 1% BSA, 0.1% Tween-20) for 1 hr at 4° C. The blocking was done in a glass staining dish with gentle agitation. For the pooled serum experiment, both PCa and BPH serum pool was made by pooling the sera from the corresponding 32 patients. Then a 16 μl sample from each pool was diluted, 1:150, in probing buffer (1×PBS, 5 mM MgCL2, 0.5 mM DTT, 0.05% Triton-X-100, 5% glycerol, 1% BSA) and used for probing of each array. For individual patient screening, the serum for each patient was diluted, 1:150, in probing buffer and 100 μl of diluted serum was then applied to a low content, protein array. Serum probing was done at 4° C. for 1.5 hours in a moisture chamber (Evergreen, cat#240-9020-Z1 0). After serum probing, the chips were washed three times with the probing buffer (10 min/wash) in a pap jar (1 slide per pap jar in 25 ml volume, Evergreen, cat#222-5450-G8S). The washed chips were then incubated with a goat anti-human IgG secondary antibody labeled with Alexa 647 (Invitrogen, cat#A21445) for 1.5 hours at a 1:2000 dilution in the probing buffer. The chips were washed three times (10 min/wash, in pap jar with 25 ml volume) with probing buffer and then spin dried. The chips were scanned with Axon Genepix 4000B scanner (PMT 600, 33% power). Competition assay was performed in the same way except that each sample was incubated with purified antigens for 30 minutes at 4° C. before they were loaded on the protein array.

[0228] Tissue microarray. Prostate cancer tissue array analysis was performed using MaxArray human PCa slides (Invitrogen, cat# 73-5063) with standard protocol. Briefly the tissue microarray slides were twice treated with xylene (Sigma) for 5 minutes. The slides were then treated in absolute ethanol twice for 5 minutes each time, 95% ethanol once for 5 minutes, 80% ethanol once for 5 minutes and finally briefly rinsed with H2O. The rehydrated slides were further treated with Digest-All 4 (Proteinase K) (Invitrogen, cat# 00-3011) for 40 minutes at room temperature in a humid chamber. After blocking with 1% BSA for 1 hour, the slides were probed with a 1:500 dilution of anti-KDR/anti-PIM-1 antibodies to detect KDR or PIM-1 antigens. The KDR antibody was from (Invitrogen) and PIM-1 antibody was from Abcam (ab15002) which was generated against N-terminal peptide amino acid 22-37 (ATKLAPGKEKEPLESQT) of human PIM-1. Both antibodies were labeled with Alexa 647 (Invitrogen). The slides were observed with fluorescence Nikon Eclipse TE 200 (40×) microscope. Images were processed using Image-Pro software (Media Cybernetics). In this experiment, at least 1000 cells from each patient were analyzed and only samples with negative and strong signals were counted.

[0229] Data analysis. Data analysis was done by both normalizing (by positive controls on the assay) and not normalizing the data with no statistically difference in the results, so all analysis presented was done with background subtracted unnormalized data.

[0230] For both single and the duplex autoantibody assays a logistic regression classifier was fit to the data. Since KDR and PIM-1 are spotted on the array twice, the classifying percentages were average for the pair, and the combined logistic regression (all 4 combinations) were average, results are shown in Table 14.

TABLE 14

Comparison of KDR and PIM-1 predictability for prostate cancer

Patient	PSA Measurement	Status	KDR Prediction	PIM-1 Prediction	KDR & PIM-1 Prediction
103	5.25	PCa	99.04%	82.08%	99.98%
104	3.25	PCa	39.80%	100.00%	100.00%
105	174.84	PCa	97.57%	40.15%	99.01%
106	0.41	PCa	40.04%	95.53%	97.06%
107	4.37	PCa	97.41%	21.55%	96.75%
109	6.95	PCa	45.07%	100.00%	100.00%
110	26.63	PCa	53.28%	49.65%	51.96%
111	8.52	PCa	56.47%	19.54%	15.05%
112	5.77	PCa	34.07%	45.51%	21.13%
115	5.95	PCa	17.97%	90.87%	69.02%
116	4.18	PCa	95.16%	99.94%	100.00%
119	3.71	PCa	59.47%	49.84%	61.30%
120	5.01	PCa	97.00%	75.20%	99.82%
123	1.83	PCa	27.73%	99.98%	100.00%
126	1.35	PCa	60.77%	44.01%	55.07%
128	3.66	PCa	32.21%	37.90%	12.66%
130	8.84	PCa	96.66%	36.84%	98.15%
131	5.05	PCa	89.02%	22.95%	77.04%
135	3.21	PCa	89.23%	70.28%	98.46%
136	2.15	PCa	14.74%	100.00%	100.00%
137	3.96	PCa	39.88%	88.57%	88.79%
140	57.22	PCa	98.65%	30.75%	99.11%
141	4.84	PCa	99.81%	49.14%	99.98%
142	6.8	PCa	55.58%	43.45%	46.73%
143	7.53	PCa	36.34%	69.34%	55.62%
144	11.84	PCa	38.61%	87.09%	86.21%
150	5.13	PCa	99.87%	100.00%	100.00%
156	1.38	PCa	40.72%	53.11%	38.94%
169	9	PCa	95.47%	80.57%	99.79%
170	1.38	PCa	95.42%	23.37%	93.11%
176	2.82	PCa	98.34%	22.98%	98.34%
177	5.1	PCa	35.94%	85.78%	82.09%
101	5.96	BPH	35.65%	25.53%	7.91%
102	30.08	BPH	38.50%	36.80%	17.37%
108	1.93	BPH	28.70%	55.65%	23.69%
113	5.75	BPH	16.08%	26.66%	1.99%
114	4.98	BPH	43.09%	22.55%	9.73%
117	0.9	BPH	30.61%	23.42%	5.02%
118	8.87	BPH	24.78%	23.52%	3.38%
121	21.03	BPH	11.37%	27.28%	1.20%
122	7.01	BPH	81.64%	38.70%	78.94%
124	3.58	BPH	13.87%	60.15%	9.73%
125	3.97	BPH	27.87%	38.68%	10.53%
127	1.42	BPH	21.53%	25.27%	2.97%
129	5.96	BPH	26.39%	25.48%	4.37%
132	1.55	BPH	17.07%	29.28%	2.63%
133	2.45	BPH	31.52%	22.84%	5.10%

TABLE 14-continued

Comparison of KDR and PIM-1 predictability for prostate cancer

Patient	PSA Measurement	Status	KDR Prediction	PIM-1 Prediction	KDR & PIM-1 Prediction
134	3.66	BPH	40.06%	42.92%	24.32%
138	3.15	BPH	20.72%	55.83%	14.45%
139	2.47	BPH	23.33%	24.00%	3.13%
145	2.79	BPH	59.84%	26.24%	27.18%
146	0.22	BPH	27.14%	31.67%	6.83%
147	0.61	BPH	60.61%	28.60%	30.38%
148	14.37	BPH	45.56%	74.01%	72.37%
149	6.82	BPH	85.73%	69.76%	98.24%
152	4.56	BPH	60.88%	26.41%	27.48%
153	0.87	BPH	14.31%	28.53%	1.88%
154	3.27	BPH	35.98%	23.28%	6.87%
155	3.24	BPH	26.97%	25.68%	4.63%
157	4.34	BPH	32.81%	43.54%	18.00%
158	6.64	BPH	30.05%	25.42%	5.57%
160	19.43	BPH	29.03%	78.32%	54.98%
161	5.1	BPH	32.25%	76.89%	57.97%
162	7.62	BPH	55.15%	24.52%	19.39%

[0231] Receiver operator characteristic (ROC) curves were calculated using the average logistic regression for the KDR, PIM-1 and combination of markers. The ROC curve for the PSA assay was calculated using the observed PSA concentrations. The area under the curve (AUC) was calculated for each ROC curve. For each marker or combined markers the optimal point on the ROC curve that maximizes both sensitivity and specificity using the ROC by looking for the maximum of the sum of the sensitivity and specificity, if multiple points on the ROC curve give the same sum of sensitivity and specificity, then the maximum of the squared sum of the tied points was used.

Results

[0232] This Experiment identified multiple prostate cancer tumor associated antigens, and can be extended to any disease that elicits a humoral response. Two novel tumor antigens KDR and PIM-1 were found to diagnose prostate cancer with higher specificity and sensitivity than current clinical PSA test.

[0233] Both pooled and individual serum samples were examined in this study. Pooled sera from 32 PCa and pooled sera from 32 BPH were first examined using the high content protein array chip containing all 96 proteins (shown in FIG. **2**A, with the position of KDR and PIM-1 indicated). About half of the 96 protein antigens showed detectable autoantibody signals (measured as a minimal fluorescence signal plus three times standard deviation above background level signal). The top 20 biomarkers that had significant difference between pooled PCa sera and pooled BPH are shown in FIG. **2**B. Among these proteins, cyclin B1 (CCNB1), alpha-methylacyl-CoA racemase (AMACR) and prostate specific antigen or PSA (KLK3), survivin (BIRC5) have been reported previously to induce autoantibodies in PCa patients. Tumor suppressors P53 (TP53) and mucin1 (MUC1) are two actively studied tumor antigens for various tumors (Casiano et al. (2006) "Tumor-associated antigen arrays for the serological diagnosis of cancer," Mol Cell Proteomics. 5(10):1745-59; Megliorino et al. (2005) "Autoimmune response to anti-apoptotic protein survivin and its association with antibodies to p53 and c-myc in cancer detection," Cancer Detect. Prev. 29(3):241-8; and Hirasawa et al. (2000) "Natural autoantibody to MUC1 is a prognostic indicator for non-small cell lung cancer," Am J Respir Crit Care Med. 161:589-94).

[0234] Among the top 20 markers, KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN are novel tumor antigens that have not been previously reported as inducing an autoantibody response. These markers are involved in various stages of PCa progression, including cell growth and apoptosis (KDR, PIM-1, GDF15, SFRP4, HOXB13, QSCN6), metabolism (RPL23 and RPL30), metastasis and angiogenesis (KDR, PTEN, CLDN3, CLDN4, NCAM2, LGALS8).

[0235] KDR and PIM-1 protein spots produced a 2-fold and 1.6-fold higher autoantibody response (respectively) in PCa than in BPH (shown in FIGS. **2**A and **2**B). On the protein microarray, full-length PIM-1 and a truncated KDR protein (derived from its intracellular domain) were utilized. Both proteins were previously reported to be involved in the development of PCa.

[0236] To further characterize and validate the autoantibodies against KDR and PIM-1, a purified protein competition assay was used, as shown in FIG. **2**C (KDR) and FIG. **2**D (PIM-1). At spiked concentration of 1.8 ug/mL purified KDR added to sera, the KDR autoantibody signal on the protein chip was reduced by 50%. The PIM-1 autoantibody protein chip signal was also reduced by half when purified PIM-1 protein was added to sera at a concentration of 2 μg/mL. The autoantibody signals against other proteins on the same chip were not affected (data not shown). These results suggested that KDR and PIM-1 autoantibody signals are specific to the respective tumor antigens.

[0237] Autoantibodies against KDR and PIM-1 differentiate prostate cancer from benign prostatic hyperplasia. To further investigate KDR and PIM-1 autoantibodies for their combined diagnostic value, a low content chip was made which contains full-length PIM-1 and the intracellular domain of KDR protein printed on 16-pad nitrocellulose slides. In this manner all 64 individual (non-pooled) sera samples can rapidly and reproducibly be measured using a relatively small number of protein array chips (6 individual sera samples per slide, performed in duplicate). The lower-content-protein chip was cross-validated against the full 96-element protein chip with the same serum resulting in CV between the two chips of 8-10% (data not shown). The fluorescence signals for both KDR and PIM-1 are plotted against each other for each patient in FIGS. **3**A and **3**B. Although KDR gave stronger autoantibody signal than PIM-1, both KDR and PIM-1 showed significantly higher autoantibody signals in PCa than BPH sera. These results are consistent with the immune profiling data using pooled serum samples.

[0238] Diagnostic quality of an assay is characterized by using the receiver operating characteristic curve (ROC). The ROC curve for each individual biomarker, the combined biomarkers (PIM1 and KDR) using logistic regression and PSA from 32 PCa versus 32 BPH individually sample sera data set is shown in FIG. **3**C. The area under the curve (AUC) for KDR and PIM-1 was 0.8066 and 0.75 respectively, whereas the combined PIM-1 and KDR 2-plex assay gives an AUC of 0.9268, compared to the PSA test with AUC of 0.5596. All combinations of 2-plex and 3-plex assays that included PSA as an additional biomarker were looked at using the ROC (data not shown). Adding PSA did not add any additional diagnostic value in any combination of other markers. As a 1-plex assay, the PSA test yielded 71.9% sensitivity and 43.7% specificity, values similar to previous reports, showing it is not very effective in differentiating PCA from BPH (Etzioni et al. (2002) "Overdiagnosis due to prostate-

specific antigen screening: lessons from U.S. prostate cancer incidence trends," J Natl Cancer Inst. 94(13):981-90).

[0239] All PCa and BPH serum samples were biopsy-based classified. Each patient's sera obtained came with a pathology report including: Gleason score, number of positive scores for all 8 needle biopsy cores and a percentage of carcinoma in any single core. Most PCa patients in this study had Gleason scores of 6 (23 out of 32), representing intermediate grade tumors. Detailed examination of the pathology reports from each patient revealed that KDR and PIM-1 gave good differential power in patients with a very low numbers of cancer-positive cores. Patients with either one or two positive cores the KDR and PIM-1 autoantibody assay detects 90% of the cancer cases while PSA detects only 50% of the cancer cases (Table 15). All 11 patients with just a single positive cancerous core (out of the 8 cores biopsied per patient) were correctly categorized using KDR-PIM-1 2-plex assay. Patients with three or more positive cores, both PSA and 2-plex autoantibody assay showed similar sensitivity in diagnosing cancer 83.3% and 91.7% respectively. Positive core numbers correlate with tumor volume (Lewis et al. (2002) "Carcinoma extent in prostate needle biopsy tissue in the prediction of whole gland tumor volume in a screening population," Am J Clin Pathol. 118(3): 442-450), suggesting that KDR and PIM-1 autoantibodies may be valuable in diagnosing PCa when a tumor may be non-palpable, i.e. not detectable by DRE.

TABLE 15

Correlation of pathology, PSA level and KDR/PIM-1 autoantibody test

Number of positive cores	Number in PCa	Mean of largest percentage of carcinoma in	Gleason score			PSA level 4+	Positive using combined KDR
(out of 8)	patients	single core	6	7	8+	ng/ml	and PIM-1 Test
1	11 (34%)	16%	10/11	1/11	0	5/11	11/11
2	9 (28%)	35%	7/9	2/9	0	5/9	7/9
3 and above	12 (38%)	43%	6/12	2/12	4/12	10/12	11/12

[0240] KDR and PIM-1 are over-expressed in prostate cancer tissues and over-expression of KDR and PIM-1 correlates with autoantibody frequencies in the patient population. Several mechanisms have been proposed for autoantibody induction in cancer patients, including the gene mutations, over expression, abnormal post-translational modifications, misfolding and aberrant cellular and subcellular localization (Casiano et al. (2006) "Tumor-associated antigen arrays for the serological diagnosis of cancer," Mol Cell Proteomics. 5(10): 1745-59; and Tan, EM. (2001) "Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis," J Clin Invest. 108(10):1411-5). Final protein expression level of PIM-1 and KDR were measured to correlate with the autoantibody induction in PCa tissues. An antibody against KDR and PIM-1 respectively were examined for expression patterns of these two proteins in PCa tissues using fluorescence immunohistochemistry.

[0241] An antibody against the intracellular domain of KDR showed that KDR was localized in the cytoplasm of cancer cells (FIG. **4**). Immunostaining with a PIM-1 antibody showed that PIM-1 is localized in both cytoplasm and nucleus (FIG. **4**). As shown in Table 16, the KDR-antigen signal was detected in ~70% of PCa and ~21% BPH patients on tissue microarrays, indicating KDR is preferentially expressed in PCa tissues. This correlates with the high KDR autoantibody signals in PCa patients (~62%) versus low KDR autoantibody signals in BPH patients (~20%). The same trend also observed for PIM-1. PIM-1 expression was detected in ~30% PCa patients tissue arrays, while almost no BPH patients showed detectable PIM-1 signal in accordance with frequencies of PIM-1 autoantibody in PCa patients (~37%) and BPH patients (~0%). In this study only specimens with negative and strong fluorescence signals were used and these results are consistent with previous reports where PIM-1 was detected in 38% cancer cases but not detected in normal controls using the strongest intensity grade (score 3). These results suggest that over expression of KDR and PIM-1 protein, may lead to stronger autoantibody induction in PCa patients.

TABLE 16

KDR and PIM-1 detection in PCa patients and BPH patients

	KDR positive patients by tissue arrays (n = 20)	KDR autoantibody positive patients on assay (n = 32)	PIM-1 positive patients by tissue arrays (n = 20)	PIM-1 autoantibody positive patients on assay (n = 32)
PCa	70%	62%	30%	37%
BPH	21%	20%	2.6%	0%

The percentage of patients with higher autoantibody signals in PCa than in BPH (data not shown) suggests that autoimmune response is more pronounced in PCa than in BPH.

[0242] Having now fully described the present invention in some detail by way of illustration and examples for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

[0243] One of ordinary skill in the art will appreciate that starting materials, reagents, purification methods, materials, substrates, device elements, analytical methods, assay methods, mixtures and combinations of components other than those specifically exemplified can be employed in the practice of the invention without resort to undue experimentation. All art-known functional equivalents, of any such materials and methods are intended to be included in this invention. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention

claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0244] As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. In each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms.

[0245] When a group of materials, compositions, components or compounds is disclosed herein, it is understood that all individual members of those groups and all subgroups thereof are disclosed separately. When a Markush group or other grouping is used herein, all individual members of the group and all combinations and subcombinations possible of the group are intended to be individually included in the disclosure. Every formulation or combination of components described or exemplified herein can be used to practice the invention, unless otherwise stated. Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges and subranges, as well as all individual values included in the ranges given are intended to be included in the disclosure. In the disclosure and the claims, "and/or" means additionally or alternatively. Moreover, any use of a term in the singular also encompasses plural forms.

[0246] All references cited herein are hereby incorporated by reference in their entirety to the extent that there is no inconsistency with the disclosure of this specification. Some references provided herein are incorporated by reference to provide details concerning sources of starting materials, additional starting materials, additional reagents, additional methods of synthesis, additional methods of analysis, additional biological materials, additional nucleic acids, chemically modified nucleic acids, additional cells, and additional uses of the invention. All headings used herein are for convenience only. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. References cited herein are incorporated by reference herein in their entirety to indicate the state of the art as of their publication or filing date and it is intended that this information can be employed herein, if needed, to exclude specific embodiments that are in the prior art. For example, when composition of matter are claimed, it should be understood that compounds known and available in the art prior to Applicant's invention, including compounds for which an enabling disclosure is provided in the references cited herein, are not intended to be included in the composition of matter claims herein

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Sequence for PIM-1 (Accession number NP_002639)
DEFINITION: pim-1 oncogene [Homo sapiens].
VERSION     NP_002639.1 GI:4505811
DBSOURCE    REFSEQ: accession NM_002648.2

                                                                (SEQ ID NO: 1)
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 61 nlpvaikhve kdrisdwgel pngtrvpmev vllkkvssgf sgvirlldwf erpdsfvlil

121 erpepvqdlf dfitergalq eelarsffwq vleavrhchn cgvlhrdikd enilidlnrg

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241 pfehdeeiir gqvffrqrvs secqhlirwc lalrpsdrpt feeiqnhpwm qdvllpqeta

301 eihlhslspg psk

Sequence for KDR (Accession number NP_002244)
DEFINITION: kinase insert domain receptor
            (a type III receptor tyrosine kinase) [Homo sapiens].
VERSION     NP_002244.1 GI:11321597
DBSOURCE    REFSEQ: accession NM_002253.1

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 121 yrspfiasvs dqhgvvyite nknktvvipc lgsisnlnvs lcarypekrf vpdgnriswd

 181 skkgftipsy misyagmvfc eakindesyq simyivvvvg yriydvvlsp shgielsvge

 241 klvlnctart elnvgidfnw eypsskhqhk klvnrdlktq sgsemkkfls tltidgvtrs

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 481 pceewrsved fqggnkievn knqfaliegk nktvstlviq aanvsalykc eavnkvgrge

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SEQUENCE LISTING

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        35                  40                  45

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    50                  55                  60

Ala Ile Lys His Val Glu Lys Asp Arg Ile Ser Asp Trp Gly Glu Leu
65                  70                  75                  80

Pro Asn Gly Thr Arg Val Pro Met Glu Val Val Leu Leu Lys Lys Val
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Ser Ser Gly Phe Ser Gly Val Ile Arg Leu Leu Asp Trp Phe Glu Arg
            100                 105                 110

Pro Asp Ser Phe Val Leu Ile Leu Glu Arg Pro Glu Pro Val Gln Asp
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Leu Phe Asp Phe Ile Thr Glu Arg Gly Ala Leu Gln Glu Glu Leu Ala
    130                 135                 140
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Leu Asn Arg Gly Glu Leu Lys Leu Ile Asp Phe Gly Ser Gly Ala Leu
            180                 185                 190

Leu Lys Asp Thr Val Tyr Thr Asp Phe Asp Gly Thr Arg Val Tyr Ser
        195                 200                 205

Pro Pro Glu Trp Ile Arg Tyr His Arg Tyr His Gly Arg Ser Ala Ala
    210                 215                 220

Val Trp Ser Leu Gly Ile Leu Leu Tyr Asp Met Val Cys Gly Asp Ile
225                 230                 235                 240

Pro Phe Glu His Asp Glu Glu Ile Ile Arg Gly Gln Val Phe Phe Arg
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Gln Arg Val Ser Ser Glu Cys Gln His Leu Ile Arg Trp Cys Leu Ala
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Leu Arg Pro Ser Asp Arg Pro Thr Phe Glu Glu Ile Gln Asn His Pro
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        35                  40                  45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
    50                  55                  60

Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65                  70                  75                  80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
                85                  90                  95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
            100                 105                 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
        115                 120                 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
    130                 135                 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
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Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225                 230                 235                 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
                245                 250                 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
            260                 265                 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
        275                 280                 285

Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
    290                 295                 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305                 310                 315                 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
                325                 330                 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
            340                 345                 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
        355                 360                 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
    370                 375                 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385                 390                 395                 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
                405                 410                 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
            420                 425                 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
        435                 440                 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
    450                 455                 460

Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
465                 470                 475                 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
                485                 490                 495

Ile Glu Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
            500                 505                 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
        515                 520                 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
    530                 535                 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
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Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
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625                 630                 635                 640

Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
                645                 650                 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
            660                 665                 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
        675                 680                 685

Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
    690                 695                 700

Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705                 710                 715                 720

Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Thr
                725                 730                 735

Cys Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe
            740                 745                 750

Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu
        755                 760                 765

Val Gly Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile
    770                 775                 780

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785                 790                 795                 800

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                805                 810                 815

Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp
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        835                 840                 845

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    850                 855                 860

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865                 870                 875                 880

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Lys Asn  Val Val Lys Ile Cys  Asp Phe Gly Leu Ala  Arg Asp Ile
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Tyr Lys  Asp Pro Asp Tyr Val  Arg Lys Gly Asp Ala  Arg Leu Pro
    1055                1060                1065

Leu Lys  Trp Met Ala Pro Glu  Thr Ile Phe Asp Arg  Val Tyr Thr
    1070                1075                1080

Ile Gln  Ser Asp Val Trp Ser  Phe Gly Val Leu Leu  Trp Glu Ile
    1085                1090                1095

Phe Ser  Leu Gly Ala Ser Pro  Tyr Pro Gly Val Lys  Ile Asp Glu
    1100                1105                1110

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    1115                1120                1125

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    1175                1180                1185

Asp Ser  Gly Leu Ser Leu Pro  Thr Ser Pro Val Ser  Cys Met Glu
    1190                1195                1200

Glu Glu  Glu Val Cys Asp Pro  Lys Phe His Tyr Asp  Asn Thr Ala
    1205                1210                1215

Gly Ile  Ser Gln Tyr Leu Gln  Asn Ser Lys Arg Lys  Ser Arg Pro
    1220                1225                1230

Val Ser  Val Lys Thr Phe Glu  Asp Ile Pro Leu Glu  Glu Pro Glu
    1235                1240                1245

Val Lys  Val Ile Pro Asp Asp  Asn Gln Thr Asp Ser  Gly Met Val
    1250                1255                1260

Leu Ala  Ser Glu Glu Leu Lys  Thr Leu Glu Asp Arg  Thr Lys Leu
    1265                1270                1275

Ser Pro  Ser Phe Gly Gly Met  Val Pro Ser Lys Ser  Arg Glu Ser
    1280                1285                1290

Val Ala  Ser Glu Gly Ser Asn  Gln Thr Ser Gly Tyr  Gln Ser Gly
    1295                1300                1305

Tyr His  Ser Asp Asp Thr Asp  Thr Thr Val Tyr Ser  Ser Glu Glu
    1310                1315                1320

Ala Glu  Leu Leu Lys Leu Ile  Glu Ile Gly Val Gln  Thr Gly Ser
    1325                1330                1335

Thr Ala  Gln Ile Leu Gln Pro  Asp Ser Gly Thr Thr  Leu Ser Ser
    1340                1345                1350

Pro Pro  Val
    1355
```

1. A method of detecting an autoantibody in an individual suspected of having prostate cancer, comprising:
a) contacting a sample from the individual with two or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or target antibodies against an antigen of Table 1 or Table 11a; and
(b) detecting binding of an antibody or antibody-containing complex in the sample to the autoantibody capture molecules, thereby detecting the autoantibody in the individual.

2. The method of claim **1** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 2 or target antibodies against an antigen of Table 2.

3. The method of claim **1** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 3 or target antibodies against an antigen of Table 3.

4. The method of claim **1** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 4 or target antibodies against an antigen of Table 4.

5. The method of claim **1** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 10 or target antibodies against an antigen of Table 10.

6. The method of claim **1** wherein the autoantibody capture molecules are selected from the group consisting of KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, CCNB1, AMACR, TP53, MUC1, KLK3, BIRC5, and target antibodies to KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, CCNB1, AMACR, TP53, MUC1, KLK3, and BIRC5.

7. The method of claim **1** wherein the autoantibody capture molecules comprise KDR or PIM-1.

8. The method of claim **1** comprising contacting a sample from the individual with an autoantibody detection set of Table 5, Table 6, Table 7, Table 8, or Table 9.

9. The method of claim **1** comprising contacting a sample from the individual with ten or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or target antibodies against an antigen of Table 1 or Table 11a.

10. The method of claim **1** comprising contacting a sample from the individual with fifty or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or target antibodies against an antigen of Table 1 or Table 11a.

11. The method of claim **1** wherein the detecting is performed by immunoassay, wherein the autoantibody capture molecules are immobilized on a solid support.

12. The method of claim **1** wherein the test sample is blood or a fraction thereof.

13. The method of claim **1** further comprising correlating the binding of the autoantibody capture molecules to antibodies or antibody-containing complexes in the test sample with a diagnosis of prostate cancer.

14. The method of claim **1** wherein the binding of the autoantibody capture molecules to antibodies or antibody-containing complexes in the test sample distinguishes between prostate cancer and BPH in the individual.

15. The method of claim wherein the binding of the autoantibody capture molecules to antibodies or antibody-containing complexes in the test sample distinguishes between High Grade and Low Grade prostate cancer.

16. A method of detecting an autoantibody in an individual suspected of having prostate cancer, comprising:
a) contacting a sample from the individual with one or more autoantibody capture molecules, wherein the autoantibody capture molecules are selected from the group consisting of KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, and target antibodies to KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, and PTEN; and
(b) detecting binding of an antibody or antibody-containing complex in the sample to the one or more autoantibody capture molecules, thereby detecting the autoantibody in the individual.

17. The method of claim **16** wherein the one or more autoantibody capture molecules comprise KDR or PIM-1.

18. A biomarker detection panel for diagnosing, prognosing, monitoring, detecting or staging prostate cancer comprising two or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or target antibodies against an antigen of Table 1 or Table 11a.

19. The detection panel of claim **18** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 2 or target antibodies against an antigen of Table 2.

20. The detection panel of claim **18** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 3 or target antibodies against an antigen of Table 3.

21. The detection panel of claim **18** wherein the autoantibody capture molecules are autoantibody capture molecules of Table 10 or target antibodies against an antigen of Table 10.

22. The detection panel of claim **18** wherein the autoantibody capture molecules are selected from the group consisting of KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, CCNB1, AMACR, TP53, MUC1, KLK3, BIRC5, and target antibodies to KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, CCNB1, AMACR, TP53, MUC1, KLK3, and BIRC5.

23. The detection panel of claim **18** wherein the autoantibody capture molecules comprise KDR or PIM-1.

24. The detection panel of claim **18** wherein the autoantibody capture molecules are immobilized on a solid support.

25. The detection panel of claim **18** comprising at least one biomarker detection set of Table 5, Table 6, Table 7, Table 8, or Table 9.

26. The detection panel of claim **18** comprising ten or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or target antibodies against an antigen of Table 1 or Table 11a.

27. The detection panel of claim **18** comprising fifty or more autoantibody capture molecules, wherein the autoantibody capture molecules are autoantibody capture molecules of Table 1 or Table 11a, or target antibodies against an antigen of Table 1 or Table 11a.

28. The detection panel of claim **18** wherein the biomarker detection panel has an ROC/AUC value for distinguishing the presence of prostate cancer from BPH of 0.780 or greater.

29. A biomarker detection panel for diagnosing, prognosing, monitoring, detecting or staging prostate cancer comprising an autoantibody capture molecule selected from the group consisting of KDR, PIM-1, LGALS8, GDF15, RPL23,

RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, PTEN, fragments thereof comprising an epitope recognizable by an antibody, and target antibodies to KDR, PIM-1, LGALS8, GDF15, RPL23, RPL30, SFRP4, QSCN6, NCAM2, HOXB13, SH3GLB1, CLDN3, CLDN4, and PTEN.

30. The biomarker detection panel of claim **29** comprising KDR, a fragment of KDR comprising an epitope recognizable by an antibody, PIM-1, or a fragment of PIM-1 comprising an epitope recognizable by an antibody.

31. The biomarker detection panel of claim **29** wherein the panel comprises a first biomarker comprising an autoantibody-binding epitope of KDR and a second biomarker comprising an autoantibody-binding epitope of PIM-1.

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专利名称(译)	用于检测前列腺癌生物标志物的方法和试剂盒		
公开(公告)号	US20080254481A1	公开(公告)日	2008-10-16
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[标]申请(专利权)人(译)	茵维特罗根公司		
申请(专利权)人(译)	Invitrogen公司		
当前申请(专利权)人(译)	Invitrogen公司		
[标]发明人	LOVE BRADLEY ROGERS JEFFREY BEECHEM JOSEPH WANG LILIN		
发明人	LOVE, BRADLEY ROGERS, JEFFREY BEECHEM, JOSEPH WANG, LILIN		
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摘要(译)

本文提供了新的自身抗体生物标志物，和用于检测前列腺癌的自身抗体生物标志物的组，以及用于检测怀疑患有前列腺癌的个体的血清中的这些生物标志物的方法和试剂盒。