Abstract: The present invention relates to secreted protein biomarkers for prostate cancer, diagnostic and prognostic kits comprising reagents that bind the secreted protein biomarkers, and methods and systems for using those biomarkers to diagnose and prognostic prostate cancer.
METHODS, KITS, AND SYSTEMS FOR DIAGNOSING AND PROGNOSING PROSTATE CANCER USING SECRETED BIOMARKERS

CROSS REFERENCE TO RELATED APPLICATIONS
[001] This application claims the benefit of United States provisional application No. 60/852,640, filed October 19, 2006, the entire disclosure of which is relied upon and incorporated by reference.

GOVERNMENT INTEREST
[002] The invention described herein may be manufactured, licensed, and used for United States governmental purposes without payment of royalties thereon.

TECHNICAL FIELD
[003] The present invention relates to secreted protein biomarkers for prostate cancer, diagnostic and prognostic kits comprising reagents that bind the secreted protein biomarkers, and methods and systems employing those biomarkers to diagnose and prognose prostate cancer.

BACKGROUND
[004] Prostate cancer is the most common malignancy in American men and the third leading cause of cancer mortality. (Jemal et al., (2006) CA Cancer J. Clin. 56:1 06-30.) The serum prostate specific antigen (PSA) test revolutionized the early detection of prostate cancer. (Barry, M. J., (2001) N. Engl. J. Med. 344:1 373-77.) But while clinicians widely use PSA for prostate cancer screening, serum PSA is also frequently elevated in non-malignant conditions and, in practice, only about 25% of men with elevated serum PSA have prostate cancer on tissue biopsy. Thus, many men who undergo a biopsy could have avoided this invasive procedure. Unnecessary biopsies are not only undesirable from the perspective of the individual, they are also costly to the health care system. Accordingly, there exists a need in the art for prostate cancer diagnostic tests and kits that reduce the need for unnecessary biopsies.

[005] In addition, although PSA can lead to early detection of prostate cancer, it does not permit the clinician to differentiate between indolent prostate
cancer and clinically significant disease. In its early stages, prostate cancer is usually sensitive to androgen therapy, but patients almost uniformly relapse as androgen-independent tumor cells emerge. Further, approximately 30-40% of patients treated with radical prostatectomy for localized prostate cancer have microscopic disease that is not organ-confined and a significant portion of these patients relapse. (Singh et al., (2000) Cancer Cell 1:203-09; Henshell et al., (2003) Cancer Res. 63: 4196-203.) Therefore, discovery of novel biomarkers defining prostate cancer onset and progression is helpful in managing the disease and in predicting which patients have a greater risk of developing aggressive prostate cancer. A need in the art thus also exists for prognostic tests and kits.

[006] Based on the assumption that cancer-specific proteins are present in the biofluids of patients, researchers have directly analyzed patient serum (e.g., Horn et al., (2006) Proteomics 6:559-70; Rai et al., (2004) Ann. N.Y. Acad. Sci. 1022:286-94; Ransohoff, D. F., (2005) J. Natl Cancer Inst. 97:31 5-19), plasma (Jacobs, J. M. et al., (2005) J. Proteome Res. 4:1073-85), and urine (Pang et al., (2002) J. Proteome Res. 1:161-69) using state-of-the-art technologies based on mass spectrometry protein identification strategies. These strategies are not well-suited for cancer protein identification in biofluids, however, because the normal protein content of those biofluids masks the cancer-specific proteins. DNA-based approaches have also been used to identify genes that can be used as diagnostic biomarkers of prostate cancer. (See, e.g., Petrovics et al., 2005, Oncogene 23: 3847-3852.) Gene-based approaches for diagnosis of prostate cancer, however, also require prostate biopsy. And, in spite of these many efforts, PSA is still the only clinically useful biomarker for prostate cancer.

[007] Hence, there is a need in the art for diagnostic and prognostic biomarkers of prostate cancer that can be detected or measured without requiring tissue biopsy. In addition, by developing panels of secreted proteins, false positives can be minimized and the specificity of the test increased compared to tests using a single biomarker, such as PSA. Specificity and sensitivity can be further increased when the biomarker panel data is used in concert with a trained artificial neural network. Panels of biomarkers, therefore, permit optimizing
diagnosis and prognosis and tailoring of treatments to the individual patient's tumor.

SUMMARY

[008] The present application provides secreted protein biomarkers for prostate cancer, methods and systems for using those biomarkers to diagnose and prognose prostate cancer, and diagnostic and prognostic kits comprising reagents that bind the secreted protein biomarkers. In some embodiments, one of the disclosed biomarkers is measured or detected in a diagnostic assay to determine whether prostate tumor cells are present in a sample. In other embodiments, the biomarkers comprise a panel of two or more different secreted proteins, and these proteins are measured or detected in a diagnostic assay to determine whether prostate tumor cells are present in a sample. A diagnostic kit can be used to detect or measure the biomarkers. In various embodiments, the diagnostic kits can be used to measure the levels of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, or more secreted proteins.

[009] In certain embodiments, the diagnostic assay detects or measures the levels of one or more of the secreted proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5. In some embodiments, the assay further comprises detecting or measuring PSA, %PSA, PSA doubling time, PSA velocity, prostate volume, or a combination of these indicators.

[010] The present application also provides prognostic kits that detect or measure the levels of one or more secreted proteins in a sample. The prognostic kits are used in methods of predicting prostate cancer progression or severity, such as whether the prostate cancer is a moderate risk prostate cancer or a high risk prostate cancer, or to predict whether the prostate cancer is progressing, regressing, or in remission. The prognostic kits can also be used to predict disease-free survival following radical prostatectomy. In some embodiments, one prognostic secreted protein is detected. In other embodiments, the prognostic panel comprises two or more different secreted proteins. Accordingly, assays using the prognostic kits can detect or measure the levels of one or more secreted proteins. For example, a prognostic kit can be used to measure the levels of one,
two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, or more secreted proteins.

[011] In certain embodiments, the prognostic assay detects or measures the levels of one or more of the secreted proteins SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOd, AGR2, SERPINA3, AZGP1, FAM3B, and CD164. In some embodiments, the assay further comprises detecting or measuring PSA, %fPSA, PSA doubling time, PSA velocity, prostate volume or a combination of these indicators.

[012] In some diagnostic embodiments, an increase in the levels of the secreted protein(s) indicates the presence of prostate tumor cells. In other diagnostic embodiments, it is a decrease in expression levels of the secreted protein(s) that indicates the presence of prostate tumor cells. In still other diagnostic embodiments, the expression level of one or more of the secreted protein(s) is increased, while the expression level of one or more other secreted protein(s) is decreased. In yet other diagnostic embodiments, the relevant parameter is whether one or more proteins in the secreted protein panel is detectable or undetectable.

[013] Similarly, in some prognostic embodiments, an increase in the levels of the secreted protein(s) is used to predict a moderate or a high risk prostate cancer. In other prognostic embodiments, a decrease in expression levels of the secreted protein(s) is used to predict a moderate or a high risk prostate cancer. In still other prognostic embodiments, the expression level of one or more of the secreted protein(s) is increased, while the expression level of one or more other secreted protein(s) is decreased. In yet other prognostic embodiments, the relevant parameter is whether a secreted protein is detectable or undetectable.

[014] In diagnostic and prognostic embodiments, the method of diagnosing or prognosing prostate cancer can comprise:

(a) detecting or measuring in a biological sample from a patient the expression of one or more secreted proteins from Table 1 or Table 2; and

(b) comparing, for each secreted protein detected or measured in (a), the results obtained in (a) with the expression of the same protein in a control sample. When the method is a diagnostic method, the altered expression of the
one or more secreted proteins in the patient sample relative to the control indicates
the presence of prostate cancer. When the method is a prognostic method, the
altered expression of the one or more secreted proteins in the patient sample
relative to the control is predictive of disease severity, for example a moderate risk
prostate cancer or a high risk prostate cancer, or is predictive of whether the
prostate cancer is progressing, regressing, or in remission.

[015] Although increases and decreases of at least 10% relative to a
control can be used in the diagnostic and prognostic methods, other values may
also be used. For example, the increase or decrease may be at least 20, 30, 40,
50, 60, 70, 80, 90, 100, 200, 300, 400, or even 500%. The increase or decrease
may also be expressed in terms of statistical significance, where a statistically
significant increase or decrease in expression, such as p< 0.05, p< 0.01 , p< 0.005,
or p< 0.001, indicates the presence of prostate cancer or a higher predisposition
to develop prostate cancer, prostate cancer progression, or disease severity.

[016] The disclosure also provides computer systems comprising artificial
neural network (ANN) models that have been trained with prostate cancer
diagnostic or prognostic data. In one embodiment, the ANN is a diagnostic ANN.
Accordingly, it has been trained with data obtained from detecting or measuring
one or more of the secreted proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2,
F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5. In other
embodiments, the ANN has been trained with data obtained from detecting or
measuring one or more of the secreted proteins SPOCK, PDGFD, CHGA, CAV1,
RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOG, AGR2, SERPINA3, AZGP1,
FAM3B, and CD164. In these cases, the ANN models are used to prognose
prostate cancer. Both diagnostic and prognostic ANN models can also be trained
with additional clinical data, including but not limited to prostate volume, PSA
values, %fPSA, PSA doubling time, PSA velocity, digital rectal exam results,
patient ethnicity, and patient age.

[017] The biological sample tested in either the diagnostic or prognostic
assays can be tissue, including prostate tissue, or a biofluid. Examples of biofluids
include serum, plasma, whole blood, urine (including post digital rectal exam ("post-
DRE") urine), saliva, and prostatic fluid.
The diagnostic and prognostic kits comprise, for each secreted protein to be detected or measured, a reagent that detects or measures the secreted protein. The reagent can be an antibody, including an antibody that is, optionally, detectably labeled and that binds to the secreted protein. In other embodiments, the reagent is a binding protein other than an antibody. Examples of binding proteins include soluble forms of natural receptors for the biomarkers. Binding proteins can also be detectably labeled, if desired. The antibodies and binding proteins can be used in soluble form, or they can be attached to a solid support, such as a bead, chip, or plate. In some embodiments, the kit comprises one or more antibodies and one or more binding proteins. When the secreted protein is an enzyme, it can be detected or measured by detecting or measuring its effect on a substrate. Accordingly, in some embodiments, a kit may comprise a substrate in combination with one or more antibodies, one or more binding proteins, or a combination of antibodies and binding proteins.

The secreted proteins in the biological sample can be detected or measured using techniques known in the art, such as enzyme-linked immunosorbant assays (ELISA), protein arrays, or colorimetric assays.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 compares the expression levels of certain genes selected as diagnostic markers. The data show normalized expression units for each gene in two databases: the CPDR database and the Oncomine database.

Figure 2 compares protein expression levels of selected diagnostic markers in pooled serum of patients with prostate cancer (CaP) and with metastatic disease (Met).

Figure 3 compares protein expression levels of two biomarkers and evaluates the sensitivity of those biomarkers. Expression levels of SPOCK1 (Fig. 3A) and PLA2G7 (Fig. 3B) are presented for a cohort of patients with prostate cancer (CaP) and for controls. In Figure 3C and 3D, a linear classifier algorithm (Support Vector Machine ("SVM")) and an artificial neural network (ANN) are used to evaluate PLA2G7 and PSA sensitivity. The results for each marker and algorithm at 90% and 95% sensitivity cutoffs is shown in Figure 3E.
[023] Figure 4 presents protein expression levels of SPOCK in individual control (CTL) and prostate cancer (CaP) samples in the top panel, while the bottom panel provides a comparison between the two sets of data.

[024] Figure 5 presents protein expression levels of CRISP3 in individual control (CTL) and prostate cancer (CaP) samples in the top panel, while the bottom panel provides a comparison between the two sets of data.

[025] Figure 6 presents protein expression levels of SMOC1 (Fig. 6A) and F5 (Fig. 6B) in urine samples as compared to serum PSA levels (Fig. 6C).

[026] Figure 7 correlates the probability of prostate cancer as a function of the number of up-regulated genes analyzed.

[027] Figure 8 shows that the probability of prostate cancer diagnosis increases as expression data for increasing numbers of genes encoding secreted proteins is combined.

[028] Figure 9 illustrates the training of an artificial neural network (ANN) using gene expression data from TMEFF2, NPY, CRISP3, SMOC2, PLA2G7, SPOCK, and F5. The upper left panel presents the normalization histogram of data distribution for the level of each biomarker, the upper right panel plots various transfer functions for the best classifiers of an ANN model, and the lower panel presents the performance of the ANN tested and validated by the gene expression data.

**DETAILED DESCRIPTION**

I. **Definitions**

[029] The term "altered protein expression," "altered expression" refers both to qualitative differences (i.e., that a protein is detectable versus undetectable) and to quantitative differences (i.e., differences in measured levels of a protein).

[030] "NPY" is neuropeptide Y (tyrosine). It is encoded, for example, by NCBI Entrez GenelD: 4852. An example of the amino acid sequence of preproNPY is published in the NCBI database under the accession number NP_000896, and is:
Cleavage of the 28 amino acid signal sequence results in the 69 amino acid pro-NPY polypeptide (residues 29-97 of SEQ ID NO: 1). This sequence undergoes additional cleavage following Arg$^{67}$ of SEQ ID NO: 1. Mature NPY is then formed by removal of the Gly$^{65}$-Lys$^{66}$-Arg$^{67}$ carboxy terminus and amidation of Tyr$^{64}$. (Minth et al., (1984) PROC. NATL. ACAD. SCI. USA 81:4577-81.) Thus, the peptide defined by amino acids 29-64 of SEQ ID NO: 1 is an example of a mature NPY polypeptide. NPY is also known as PYY4. Binding of NPY by its receptor on prostate cancer cells lines alters proliferation of those cell lines. (Ruscica et al., (2006) ENDOCRINOL. 147: 1466-73.) In addition, Rasiah et al. detected altered NPY expression using immunohistochemistry of tissue microarrays prepared from prostate samples, showed that there was an increase in the proportion of cells expressing NPY in prostate cancer compared to benign epithelium, and found that aberrant expression of NPY is associated with recurrence. (Rasiah et al. (2006) CANCER EPIDEMIOL. BIOMARKERS PREV. 15: 711-16.)

"SPOCK" is sparc/osteonectin, cwcv and kazal-like domains proteoglycan 1. It is encoded, for example, by NCBI Entrez GenelID NOS: 6695 (SPOCK1), 9806 (SPOCK2), and 50859 (SPOCK3). An example of the amino acid sequence of SPOCK1 is published in the NCBI database under the accession number NP_004589, and is:

```
MLGNKRLGLS GLTLALSLLV CLGALAEAYP KPDNPGEDA
PAEDMARYYS ALRHYINLIT RQRYGKRSSP ETLISDLLMR
ESTENVPRTR LEDPAMW
```
Amino acids 1-21 of SEQ ID NO: 2 correspond to a signal peptide. The mature form of SPOCK1 corresponds to amino acids 22-439 of SEQ ID NO: 2.

[033] An example of the amino acid sequence of SPOCK2 is published in the NCBI database under the accession number NP_055582, and is:

```
MRAPGCGLRV LPLLLLAAAA LAEADAKGLK EGETPGNFME
DEQWLSSISQ YSGKIKHWRN FRDEVEDDYI KSWEDNQQGD
EALDTRKPC QKVCSRHKV CIAQGYQRAM CISRKKLEHR
IQQPTVKLH GNDSCICKPS MAQLASVCGS DGGTYSSVCK
LEQQACLSSK VALRCEGPHC PCPTEQAATS TADGKPETCT
GQDLADLDR LRDWQOLLHE NSKQNGSASS VAGPASGLDK
SLGASCNDKI GMSTKLDTS ADLFLDQTEL AAINLKYEV
CIRPPFNSCD TYKDGTVSTA EWCFCFWREK PPCPLAELERI
QIQAIAKJKPP GIPICSCDED GYYRKKMDQ SSGDCWCVDQ
LGLELTGTRT HGSPDCDDIV GFSGDFGSGV GWEDDEEEEK
EEAGEAESEE EGEAGEADDG GYIW (SEQ ID NO: 3).
```

The mature form of SPOCK2 corresponds to amino acids 1-424 of SEQ ID NO: 3.

[034] An example of the amino acid sequence of SPOCK3 is published in the NCBI database under the accession number NP_001035249, and is:

```
MLKVSAVLCV CAAACWSQSL AAAAAVAAAG GRSDGNFLD
DOKWLTTISQ YDKEVGQWNK FRDDYFRTW SPGKPFDQAL
DPADPCLKLM KCSRHKVCIA QDQTAVCIS HRRLTHRMKE
AGVQHVRQWRG PILSTCKQCP WYPSPVCGS DGHYTFQCK
LEYQACVLKQ QISVKCEGHC PCPSDKPTST SRNVKACSD
LEFREVANRL RDWFKALHES GSQNMKTCL LRPERSRFDT
SILPICDSL GMFNNRLTIN YDLDLDQSEL RSIYLDRKNEQ
CTKAFFNCSD TYKDSLSNN EWCYCFQRQO DPPCQTELSN
IQKROGVKKE LQGUYIPLCDE DGGYKPTQCH GSVGQGCVD
RYGNEVMGSR GINGVADCAID FEISGDFASG DFHEWTDDED
```
The mature form of SPOCK3 corresponds to amino acids 1-433 of SEQ ID NO: 4.

[035] There are two isoforms of SPOCK3. The amino acid of the second isoform is given in NP_058646.2 and is:

```
MLKVSALCV CAAWCSQSL AAAAAVAAG GRSDGGNFDL
DKQWLTIISQ YDKEVQGWK FRDEVEDDDYF RTWSPGKPD
QLDTPAKDC LKMKCSRHKV CIAQDSQTAV CISHRRLHR
MKEAGVDHRQ WRGPILSTCK QCPWYPSPV CGSDGHTY
QCKLEYQACV LGKQISVKCE GHCPCSSDKP TTSRNVKRA
CSDLEFREVA NLRDWFKAL HESGDSNKT KTLRPFERSR
FTSILPICK DSLGWMFNRL DTNYDLLLDQ SELRSIYLDK
NEQCTKAFN SCDTYKDSLI SNNEWCYCFQ RQQDPPCQTE
LSNIQKRQGV KKLQGQYIPL CDEDGYKPT QCHGSVGCW
CVDYRGNEVM GSRINGVADCF AIDFEISGDF ASGDFHETD
DEDDEDDIMN DEDEIIEDDDE DEGDGDGGD DHDVY
```

(SEQ ID NO: 5).

The mature form of isoform2 of SPOCK3 corresponds to amino acids 1-436 of SEQ ID NO: 5.

[036] Unless otherwise indicated, "SPOCK" refers to SPOCK1, SPOCK2, and SPOCK3. SPOCK is also known as testican, SPARC, and osteonectin. It has been associated with prostate cancer metastasis to the bone. (Thomas et al., (2000) CLIN. CANCER RES. 6; 1140-49; de et al., (2003) J. BIOL. CHEM. 278(40): 39044-50.) Antibody-based assays have also shown that it is present at elevated levels in the serum of melanoma patients. (Ikuta et al., (2005) CLIN. CANCER RES. 11: 8079-88.)

[037] "CRISP3" is cysteine-rich secretory protein 3. It is encoded, for example, by NCBI Entrez GeneID: 10321. An example of the amino acid sequence is published in the NCBI database under the accession number NP_006052 and is:

```
MTLPVLFL VAGLPSFPA NEDKPAFTA LTTQTQVQR
EIVNKHNELR RAVSPPARNM LKMENKEAA ANAQKWANQC
NYRHSNPKDR MTSLKCGENL YMMSSSSWS QAIQSWFDEY
NDFDFGVGPK TPNAWGHYT QWWYSSYLV GCGNAYCPNQ
```
KVLKYYYVCQ YCPAGNWANR LYVPYEQGAP CASCPDNCDD
GLCTNGCKYE DLYSNCKSLK LTLTCKHQLV RDSCKASCNC SNSIY
(SEQ ID NO: 6).

In SEQ ID NO: 6, amino acids 1-20 correspond to the signal peptide and amino acids 21-245 correspond to the mature protein. CRISP3 is also known as SGP28. Its mRNA is overexpressed in prostate cancer. (Kosari et al., (2002) CANCER EPIDEMIOL. BIOMARKERS & PREV. 11: 141 9-26.) Both glycosylated and non-glycosylated forms of CRISP3 exist, and ELISA and immunoblotting have been used to detect CRISP3 in human plasma and seminal plasma. (Udby et al., (2002) J. IMMUNOL. METH. 263: 43-55.)

[038] "PLA2G7" is phospholipase A2, group VII. It is encoded, for example, by NCBI Entrez GeneID: 7941. An example of the amino acid sequence is published in the NCBI database under the accession number NP_005075, and is:

MVPPKLHVLF CLCGLAWY PDFWQYINPV AHMKSSAWVN KIQVLMAAAS FGQTKIPRGN GPYSVGCSDL MFDHTNKGTF LRLYYPQDNN DRLDTLWIPN KEYFWGLSKF LGTHWLMGNI LRLFGLSSH PANWNSPLRP GEKYPLWFS HGLGAFRTLY SAIGIDLASH GFIYAAVEHR DRSSATYYF KDQSAEIGD KSWLYLRTLK QEEETHIRNE QVRQRAKECS QALSLILDID HGKPVKNAIY FKFDMEQLKD SIDREKAVPS GHSGGATVI QTLSEDOQRF CGIALDAWMF PLGDEVYRSI PQPLFINSE YFPQYPANIIE MKKCYSPOKE RKMITHRGVS HQNCFADTFKA TGKIIHGMLK LGKDSDSNA IALSNKASLA FLQKHLGHLH KDFOQWDCMIE GDDELIPGT NINNTNHIM LQNSSGIEKY N
(SEQ ID NO: 7).

PLA2G7 is also known as platelet-activating factor acetylhydrolase. Monoclonal antibodies to PLA2G7 have been produced (e.g., Rodrigo et al. (2001) BIOCHEM. J. 354: 1-7), but the protein can also be detected in biofluids such as seminal plasma by measuring enzymatic digestion of a substrate (e.g., Zhu et al., (2006) FERTILITY & STERILITY 85: 391-94).
"TMEFF2" is a transmembrane protein with EGF-like and two follistatin-like domains. It is encoded, for example, by NCBI Entrez Gene ID: 23671. An example of the amino acid sequence is published in the NCBI database under the accession number NP_057276, and is:

MVLWESPRQC SSWLCEGFC WLLLPVMLL IVARPVKLAA
FPTSLSDCQT PTGWNCSCGYD DRENDFLCLD TNTCKFDGEC
LRIGDVTTCV CQFKCNNDYV PVCGSGESY QNECYLRQAA
CKQQSEILW SEGSCATDAG SGSGDVHEG SGETSKEH
TCDICQFGAE CDEDAEDVWC VCNIDCSQTN FNPLCASDGK
SYDNACQIKE ASCQKQEKIE VMSLGRCDN TTTTTKSEDG
HYARTDYEAN ANKLEESARE HHIPCEHYN GFCMHGKEH
SINMQEPSCR CDAGYTGQHC EKKDYSVLYV VPGPVRFQYV
LIAAVIGTIQ IAIVCWVLC ITRKPRSRN IHRQKQNTGH
YSSDNTRAS TRLI (SEQ ID NO: 8).

TMEFF2 is also known as tomoregulin. It is an androgen-regulated transmembrane protein whose expression decreases as tumor cells acquire androgen-independence. (Gery et al. (2002) ONCOGENE 21:4739-46; Chen et al., (2006) CANCER LETTERS 244:274-88.) TMEFF2 has been used as a target in immunotherapy of prostate cancer with monoclonal antibodies conjugated to radiolabels or a cytotoxic agent. (Zhao et al., (2005) CANCER RES. 65; 2846-53; Afar et al., (2004) MOL. CANCER THER. 3: 921-32.)

A truncated isoform of TMEFF2 is secreted by prostate cancer cells. (Quayle et al., (2006) GENOMICS 87: 633-37.) The amino acid sequence of a secreted form of TMEFF2, TMEFF2-S, is:

MVLWESPRQC SSWLCEGFC WLLLPVMLL IVARPVKLAA
FPTSLSDCQT PTGWNCSCGYD DRENDFLCLD TNTCKFDGEC
LRIGDVTTCV CQFKCNNDYV PVCGSGESY QNECYLRQAA
CKQQSEILW SEGSCATDAG SGSGDVHEG SGETSKEH
ILLCIFTYVC SISDI (SEQ ID NO: 9).

"F5" is coagulation factor V. It is encoded, for example, by NCBI Entrez Gene ID: 2153. An example of the amino acid sequence is published in the NCBI database under the accession number NP_000121, and is:
MFPGCPRLWV  IWLGTSWVG  WGSQGTEAAQ  LRQFYVAAQG
ISWSYRPEPT  NSSLNLSVTS  FKKIVYREYE  PYFKKEKPQS
TISGLLGPHTL  YAEVGDIKVV  HFKNKADKPL  SIHPQGIRYS
KLSEGASYLDD  HTFPAEKMDD  AVAPGREYT  EWSISEDSP
THDDPPCLTLH  IYYSHENLIE  DFNSGLIGPL  LICKGTLTE
GGTQKTFDKQV  IVLLFAVFDE  SKWSQSQSSL  MYTVNGYVNG
TMPDITVCAH  DHISSHLLGM  SSGPELFSIH  FNGQVLEQNH
HKVASAITLVS  ATSTTANMTV  GPEGKIWISS  LTPKHLQAGM
QAYIDIKNCNP  KKTRNLKKIT  REQRRHMKRW  EYFIAAEV
WDYAPVIPAN  MDKKYRSQHL  DNFSNQIGKH  YKKVMYTQYE
DESFTKHTVN  FNMKEDGIIG  PIIRAQVRDT  LKIVFKNMAS
RPYSIYPHGV  TFSPYEDENV  SSFTSGRNNT  MIRAVQPGET
YTYKWNILEF  DEPTENDAQO  LTRPYYSDVD  IMRDIAISLI
GLLLICKSRH  LRRDGIQRAA  DIEQQAVFAV  FDENKSWYLE
DINIKFCCNFP  DEVKRRDFPK  YESNIMSTIN  GYVPESITTL
GFCFDDDTVQW  HFCGVTQNE  ILTIHFTGHS  FIYGRKHDIT
LTLFPMRGES  VTIVMDNVGT  WMLTSMNSSP  RSKKRLRKFR
DVKCIPDDDE  DSYEIFEPPE  STVMATRMKH  DRLEPEDDEES
DADYDYQNRN  AAALGIRFSR  NSSLNQEEEEE  FNLTLALALEN
GETEVSSNSTD  IIVSGNYSSP  SNISKFTVNN  LAEPQKAPSH
QQATTAGSPL  RLHIGKNSVL  NSSTAEHSSP  YSEDPIEDPL
QPDVTGIRLL  SLGAGEFKSQ  EHAHKGPKV  ERDQAAKHRF
SWMKDLLAHKV  GRHLSQDTGS  PSGMRPWEDL  PSQDTGSPSR
MRPWRKDPSSD  LLLLKQSNSS  KILVGRWHLA  SEKGSYEIIQ
DTDEDTAVNN  WLISPQNASR  AWGESTPLAN  KPGKQSGHPK
FRPRVHKSLQ  VRQDGGKSRL  KKSQFLIKTR  KKKKEKTHH
APLSPRTFHHP  LRSEAYNTFS  ERRLKHSVL  HKSNETSLPT
DLNQTLPSMD  FGWIALPDHD  NQNSSNDTGQ  ASCPPGLYQT
VPPEEHYQTF  PIQDPDMHS  TSDPSHRSSS  PELSEMLEYD
RSHKSSFTDTI  SQMSPSEHE  VWQTVISPDL  SQVTLSPELS
QTNLSPDLSHA  TSSLPELQQR  NLSPALGQMP  ISPDLHSTTL
SPDLSHHTLS  LDLSQTNLSP  ELSQTNLSPA  LGQMLSPDL
SHTTSLDFS  QTNLSPELSH  MTLSPELSEQ  NLSPALGQMP
Amino acids 1-28 are a signal peptide; thus, the mature F5 protein corresponds to amino acids 29-2224 of SEQ ID NO: 10. F5 is also known as proaccelerin and labile factor.

[042] "SMOC" is SPARC related modular calcium binding. It is encoded, for example, by NCBI Entrez GenelD NOS: 64093 (SMOC1) and 64094 (SMOC2). An example of the amino acid sequence of the first isoform of SMOC1 is published in the NCBI database under the accession number NP_001030024, and is:

MLPARCARLL TPHLLLVLVQ LSPARGHRTT GPRFLI SDRD
PQCNLHCSRT QPKPICASDG RSYESMCYQ RAKCRDPTLG
WHHRGRCKDA GQSKCRLERA QALEQAKKPQ EAVFVPECGE
An example of the amino acid sequence of the second isoform of SMOC1 is published in the NCBI database under the accession number NP_071420, and is:

```
MLPARCARLL TPHLLLVLVQ LSPARGHRTT GPRFLI SDRD
PQCNLHCSRT QPKPICASDG RSYESMCYQ RAKCRDPTLG
WHHRGRCKDA QSQKCRLERA QALEQAKKQ EAVFVPCEGE
DGSFTQVQCH TYTGYCWCVT PDGKPISGSS VQNKTPVCSG
SVTDKPLSQG NSGRKDDGSK PTPTMETQPV FDGDIEATAPT
LWIKHLVIKD SKLNNTNIRN SEKVYSCDQE RQSALEEAQQ
NPREGIVIPE CAPGGLYKPV QCQSTGYCW CVLVDTGRPL
PGTSTRYVMP SCESDARAKT TEADDPFKDR ELPGCPEGKK
MEFITSSLDA LTDDMVQAIN SAAPTGGGRF SEPDPSTHLE
ERWHWYFSQ LDNSSSNDIN KREMKPFKRY VKKKAPKKKC
ARRFTDYCDL NKDKVI SLPE LKGCLGSVE GRLV
(SEQ ID NO: 12).
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An example of the amino acid sequence of SMOC2 is published in the NCBI database under the accession number NP_071421, and is:

```
MLLPQLCWLP LLAGLPPVVP AQKFSALTFL RVQDKKDRC
SLDCAGSPQK PLCASDGRTF LSRCEFQRAK CKDPQLEIAY
RGNCKDVSRC VAERKYTQE Q ARKEOFQVFIF PECNDGTYYS
QVQCHSYTGY CWCVTPNGRP ISGTAHAKT FRCGSVNEK
LPQREGTGTKT VSLQIFSVLN SDDAAAPALE TQPQGDEEDI
ASRYPTLWTE QVKSRQNKTN KNSVSSCDQE HQSALEEAQK
PKNDNWI PE CAHGGLYKPV QCZHPSTGYCW CVLVDTGRPI
```
Human SMOC2 has also been reported to include an alternate splice acceptor site, resulting in the expression of two isoforms of human SMOC2. (Vannahme et al. (2003) BIOCHEM. J. 373: 805-14.) The amino acid sequence of the second isoform is:

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[045] Unless otherwise indicated, "SMOC" refers to both SMOC1 and SMOC2, including any isoforms. SMOC2 is also known as smooth muscle associated protein 2 and secreted modular calcium binding protein 2. It can potentiate angiogenesis and endothelial cell migration, and antibodies to this protein have been produced. (Rocnick et al., (2006) J. BIOL. CHEM. 281 :22855-64).

[046] "LTF" is lactotransferrin. It is encoded, for example, by NCBI Entrez GenelD: 4057. An example of the amino acid sequence is published in the NCBI database under the accession number NP_002334, and is:

| M | K | L | V | F | L | V | L | L | F | L | G | A | L | G | C | L | A | G | R | R | S | V | Q | W | C | A | V | S | Q | P | E | A | T | K | C | F | Q |
| W | Q | R | N | M | R | K | V | R | G | P | V | S | C | I | K | R | D | S | P | I | Q | C | I | Q | A | I | A | E | N | R | A | D | A | V | T | L | D | G |
[048] "ACPP" is acid phosphatase, prostate. It is encoded, for example, by NCBI Entrez GenelD: 55. An example of the amino acid sequence is published in the NCBI database under the accession number NP_001090, and is:

MRAAPLLLAR AASLSLGFLLF LLFFWLDRE SV LAKELKVFVTL
VFRHGRDSPI DTFTDPIKE SSWPGFGQQL TQLGMEQHVE
LELYIRKRYR KFLNEXYKHE QVYIRSTVD RTLMSAMTNL
AALFPEGSSW IWNLPILLWQP IPVHTVPLSE DQLLTLFPFRN
CPRFQLELESE TLKSEEFQKR LHPYKDFIAT LGKLSGLHQQ
DLFIWSKVY DPLYCESVHN FTILPSWATED TMTKRELSE
LSLLSLYGILH QKEKSRLQ0 GVLVNEILNH MKRATGPSY
KKLIMYSAHD TTVSGLQMAL DVYNGLLPYPY ASCHLTELYP
EKGEYFVEMY YRNETQHEP Y PLMLPGCSPS CplerfaeLV

GPVIPQDWST ECMTTNSHQG TEDSTD (SEQ ID NO: 16).

Amino acids 1-32 are a signal peptide. The mature protein corresponds to amino acids 33-386 of SEQ ID NO: 16. ACPP is also known as PAP.
"TGM4" is transglutaminase 4 (prostate). It is encoded, for example, by NCBI Entrez GenelD: 7047. An example of the amino acid sequence is published in the NCBI database under the accession number NPJ303232, and is:

MMDASKELQV LHIDFLNQDN AVSHHTEFQ TSSPVFRRGQ
VFHLRLVLNQ PLQSYHQLKL EFSTGFNPSI AKHTLWLDP
RTPSDHYNWQ ATLQNESGKE VTVAVTSSPN AILGKYQLNV
KTGNHILKSE ENILYLLFN PWCEDMVFM P DEDERKEYIL
NDTGCHYVGA ARSIKCKPWN FGQFEKNVLD CCISLLTESS
LKPTDRRDLP LVRAMCAMM SFEKGQGVILI GNWTGDYEPP
TAPYKWTGSA PILQQYNTK QAVCFGQCWV FAGILTTVLR
ALGIPARSVT GFDSAHDTER NLTVDTYVNE NGKKI'TSMTH
DSVWNFHVWT DAWMKRPDLP KGYDGWQAVD ATPQERSQGV
FCCGPSPLTA IRKGDIFIV Y DTRVFSEVN GDLRIWLVKM
VNGQEELHVI S METTSIKN ISTKAVGQRDR RRDITYEYKY
PEGSEERQV MDHAFLLLSS EREHRPVKE NFKLHMSVQSD
DVLLGNSVF NTVLKRKATA LQNVNILGSF ELQLYTGKKM
AKLCDLNKTS QIQGQVSEVT LTLDSKYIN SLAILDEPV
IRGFIIEAV ESEKEIMASV FTSFQYPEFS IELP NTGRIG
Q LLVNCNFK NTLAIPLTDV KFSLESLGIS SLQTS HGTV
QPGETIQSQI KCTPIKTGPK KFIVKLSSQK VKEINAQKIV LITK
(SEQ ID NO: 17).

"MSMB" is microseminoprotein, beta. There are two alternatively spliced transcript variants encoding different isoforms. The MSMB gene is set forth, for example, in NCBI Entrez GenelD: 4477. An example of the amino acid sequence is published in the NCBI database under the accession number NP_002434, and is:

MNVLLGSWI FATFVTLCNA SCYFIPNEG V PGDSTRKCMD
LKGKNKHPINS EWQTDNCETC TCYETEISCC TLVSTPGYD
KDNCQRIFFK EDCKYIWEK KDPKKTCSVS EWII
(SEQ ID NO: 18).

The amino acid sequence of isoform b is published in the NCBI database under the accession number NP_619540, and is:
Amino acids 1-20 of each isoform encode a signal peptide, so that these amino acids are not present in the mature proteins. MSMB is also known as seminal plasma beta-inhibin.

[051] "WIF1" is WNT inhibitory factor 1. It is encoded, for example, by NCBI Entrez GeneID: 11197. An example of the amino acid sequence is published in the NCBI database under the accession number NP_009122, and is:

MARRSAFPAA ALWLWS ILLC LLALRAEAGP PQEESLYLNI
DAHQARVLIG FEEDILIVSE GKMAPPHTDF RKAQQRMPAI
PVNIHSMNFT WQAAGQAEYF YEFLSLRSLD KGIMADPTVN
VPLLGTVPHK ASWQVGFPFC LGKQDGVAAF EVDVIVMNSE
GNTILQTPQN AIFFKTCQQA ECPGGCRNGG FCNERRICEC
PDGFHGPHEC KALCTPRCMN GGLCVTPGFC ICPPPFYGVN
CDKANCSTTC FNGGTCFYPG KCICPPGLEG EQCEISKCPQ
PCRNGGKCIQ KSKCKCSKGY QGDLCSPKVC EPCCGAHGTCC
HEPNKCQCQE GWHGRHCNKR YEASLHMLAR PAGAQLRQHT
PSLKKAEERR DPESNYiw (SEQ ID NO: 20).

Amino acids 1-28 are a signal peptide. The mature protein therefore corresponds to amino acids 29-379 of SEQ ID NO: 20.

[052] "OLFM4" is olfactomedin 4. It is encoded, for example, by NCBI Entrez GeneID: 10562. An example of the amino acid sequence is published in the NCBI database under the accession number NP_006409, and is:

MRPGLSFLLA LLFFLGQQAAG DLGDVGPPIP SFGSFSFPGV
DSSSSSSSSS RSGSSSSRSR GSGGSVSQLF SNFTGSDDDR
GTCQCSVSLP DTPFPVDRVE RLEFTAHVLS QKEKELSKV
REYQLISVY EKKLLNLTIR DIMEKDTIS YTESTFELIG
VEVKEEMKLV IQLKEFSGGS SEIVDQLEVE IRNMTLLVEK
LETLDKNNVL AIRREIVALK TKLKECEASK DQNTPWHP
PDDGSCGHGG WNI SKPSW QLPWRGFSYL YGAWRDYSP
QHPNKGLYWV APLNTDGRLL EYRVLNTLD DLLFYINARE
LRITYGQGSG TAVYNNNMYV NMYTGNIAV VNLSTNTIAV
Amino acids 1-20 are a signal peptide. Thus, the mature protein corresponds to amino acids 21-510 of SEQ ID NO: 21.

[053] "PH 5" is peptidase inhibitor 15. It is encoded, for example, by NCBI Entrez GenelD: 51050. An example of the amino acid sequence is published in the NCBI database under the accession number NP_056970, and is:

```
TQTLPNAAYN NRFSYANVAW QDIDFAVDEN GLWVIYSTEA
STGNMVISKL NDTLQVLNWT WYTKQYKPSA SNAFMVCGVL
YATRTMNTRT EEIFYYYDTN TGKEKLDIV MHKMQEKVQS
INYNPFDKQL YVYNDGYLLN YDLSQLQKPQ (SEQ ID NO: 21).
```

The mature protein corresponds to amino acids 26-258. Amino acids 1-25 are a signal sequence.

[054] "PDGFD" is platelet-derived growth factor D. It is encoded, for example, by NCBI Entrez GenelD: 80310. There are two splice variants for this gene. An example of the amino acid sequence of isoform 1 is published in the NCBI database under the accession number NP_079484, and is:

```
MHRLIFVYTL ICANFCSCRD TSATPQSASI KALRANLRR
DESNHLTDLY RRDETIQVKG NGYVQSPRFP NSYPRNLVT
WRLHSQENTR IQLVFDNQFG LEEAENDICR YDFVEVEDIS
ETSTIIIRGW CGHKEVPFRI KSRTNQIKIT FKSDDYFVAK
PGFKIYYSLL EDFQPAAASE TNWESVTSSI SGVSYNSPSV
TDPTLIAAL DKKIAEDFTV EDLLKFNPFE SWQEDLLENMY
LDTPRYYGRS YHDRSKVDL DRLNDAKRY SCTPRNYSVN
IREELKLANV VFFPRCLLVQ RCGNCGCGT VNWRSTCNNS
GKTVVKHEYV LQFEPGHIKR GGRAKTMALV DIQLDHHERC
DCicssRPPR (SEQ ID NO: 23).
```
Amino acids 1-23 are signal sequence. Amino acids 24-370 correspond to a mature protein.

[055] An example of the amino acid sequence of isoform 2 is published in the NCBI database under the accession number NP_149126, and is:

```
MHRLIFVYTL ICANFCSCRD TSATPQSAS I KALRNANLRR
DDLYRRDETI QVKGNQYQVS PRFPNSYPRN LLLTWRLSQ
ENTRIQLVFDD NFQGLEEAEN DICYDFVEV EDISETSTII
RGRWCGHEKV PPRIKSRTNQ IKITFKSDDY FVAKPGFKIYL
YSSLLEDQPA AAETNWESV TSSISGVSYN SPSVTDPILI
ADALDKKIAE FDTVEDLLKY FNPESWQEDL ENMYLDTPRY
RGRSYHDRKS KVDLRLNDDD AKRYSCTPRN YSVNIREELK
LANWFFPFRC LLVQRCGGNC GCGTVNWRSC TCNSGRTVKK
YHEVLQFEPG HIKRRGRAKT MALVDIQLDH HERCDCICSS RPPR
(SEQ ID NO: 24).
```

In this isoform, the signal sequence corresponds to amino acids 1-13, while amino acids 14-364 correspond to a mature protein. PDGFD is also known as iris-expressed growth factor.

[056] "CHGA" is chromogranin A. It is encoded, for example, by NCBI Entrez GeneID: 1113. An example of the amino acid sequence is published in the NCBI database under the accession number NP_001266, and is:

```
MRSAAVLALL LCAGQVTALP VNSPMKNDT EVMKCIVEVI
SDTLSKPSPM PVSQECFETL RGERILSIL RHQNLKELQ
DLALQGAKER AHQQKHKSGF EDELSEVLEN QSSQAEKKEA
VEEPSSKIDVM EKREDSEEAE KSGEATDQR PQALPEPMQY
SKAEQQNPQ GEEEEEENQ TNSHPPASLP SQKYPQPAE
GDSEGLSQQG VDREKGLSDE PGWQAKREEE EEEEEEAEAG
EEAVPREEEGP TWLNPHEPL GYKEIKGQES RSEALAVGDQA
GKPQAEAAQD PEGKQEQRHS QQKEEEEEMA WPQGLFRRG
KSQELEQEEE RLSKEWEDSK RWSKMDQILAK ELTAEKRLG
QEEEDNRD LNKLFSRARA YGFRGPGPQQL RRGWRPSSRE
DSLEAGLPLQ VRGYPEEKE EEGSANRRE PQTELESLSAI
EAELEKVHARK LQALRGG (SEQ ID NO: 25).
```
CHGA is the precursor to several polypeptides. When only the signal peptide (amino acids 1-18 of SEQ ID NO: 25) is removed, the polypeptide is called chromogranin A. It corresponds to amino acids 19-457 of SEQ ID NO: 25. Amino acids 19-131 of SEQ ID NO: 25 correspond to the polypeptide called beta-granin. Vasostatin corresponds to amino acids 19-94 of SEQ ID NO: 25. Amino acids 142-161 of SEQ ID NO: 25 correspond to chromostatin. Pancreastatin is the name given to the polypeptide corresponding to amino acids 268-320 of SEQ ID NO: 25. Other polypeptides contained within SEQ ID NO: 22 include WE-14 (amino acids 342-355), parastatin (amino acids 374-445), and GE-25 (amino acids 393-417). CHGA is also known as parathyroid secretory protein 1.

[057] "CAV1" is caveolin 1. It is encoded, for example, by NCBI Entrez GenelID: 857. An example of the amino acid sequence is published in the NCBI database under the accession number NP_001744, and is:

```
MSGGKYVDSE GHLYTVPIRE QGNIYKPNK AMADESEKQ
VYDAHTKEID LVNRDPKHLN DDWKIDFED VIAEPEGTHS
FDGIWKASFT TFTVIKYWY RLLSALFGIP MALIWIYFA
ILSFLHIWAV VPCIKSFLIE IQCISRVYSI YVHTVCDPLF
EAVGKIFSNV RINLQKEI (SEQ ID NO: 26).
```

Although caveolin is usually an integral membrane protein, it can be found in the medium of cultured human prostate cancer cells, which overexpress this protein. (See, e.g., Tahir et al., (2001) CANCER RES. 61: 3882-85.) The mechanism by which secretion of caveolin occurs is unclear. CAV1 is also known as caveolae protein.

[058] "RLN1" is relaxin 1. It is encoded, for example, by NCBI Entrez GenelID: 6013. Alternative splice forms exists, but these have not been characterized. The encoded protein is synthesized as a single-chain polypeptide but the active form consists of an A chain and a B chain linked by disulfide bonds; however, the exact cleavage sites within the prepropolypeptide have not been described. An example of the amino acid sequence is published in the NCBI database under the accession number NP_008842, and is:

```
MPRLFLFHLL EFCLLLNQFS RAVAACKWDD VIKLCGRELV
RAQIAICGMS TWSKRSLSQE DAPQTPRPVA EIVPSFINKD
```
"IGFBP7" is insulin-like growth factor binding protein 7. It is encoded, for example, by GenelD: 3490. An example of the amino acid sequence is published in the NCBI database under the accession number NP_001544, and is:

```
MERPSLRALL LGAAGLLLLL LPLSSSSSSD TCGPCEPASC
PPLPPLGCLL GETRDACGCC PMCARGEGEF CGGGAGRGY
CAPGMECVKS RKRRKKGAGA AAGPGVSGV CVCKSYPVCC
GSDGTTPSGL CQLRAASQRA ESRGKAITEQ VKSKGTECQP
SIVTPPKDIW NVTGAQVYLS CEVIGITPVR LIWNKVRGHR
YGVQRTETLLP GDRDNLAIQT RGGPEKHEV TGVFLVSPLSK
EDAGEYECIIA NSRQGQASAS AKITWDALH EIPVKKGEA EL
```

(Amino acids 1-16 of SEQ ID NO: 27 correspond to a signal peptide, while amino acids 17-368 correspond to a propeptide form. The mature protein corresponds to...

"BGN" is biglycan. It is encoded, for example, by NCBI Entrez GenelD: 633. An example of the amino acid sequence is published in the NCBI database under the accession number NP_001702, and is:

```
MWPLWRLVSL LALSQAPLPF QRGFWDFTL D GPAF M N D E
AGADTSGVL DPDSVPTYS AMCPGCHCH L RW QCSDLG
LKSVPKEIISP DTLIQLQNN DISELRKDDF KGLQHLYALV
LVNNKISKIH EKAFSPLRKL QKLYISKNL VEIPPNLPSS
LVEHLHHDR IRKVPAGVFS GLRMNCIEM GGNPLENSGF
EPAGAFDLKLA NYLRISEAKL TGIPKDLFET LNELHLDHNIK
IQAIELEDLL RYSKLRYGLL GHQRMIEN GSLSFLPTLR
ELHLDDNKLA RVPSDLFLKL LLQWYLHSN NI TKVGNDFD
CPMFGVFKRA YYNGISLFFN PVPYHEEVQPA TFRCVTDLRA
IQFGNYKK (SEQ ID NO: 29).
```

(Amino acids 1-16 of SEQ ID NO: 29 correspond to a signal peptide, while amino acids 17-368 correspond to a propeptide form. The mature protein corresponds to...
amino acids 38-368 of SEQ ID NO: 29. BGN is also known as dermatan sulphate proteoglycan I.

[061] "IL6" is interleukin 6. It is encoded, for example, by NCBI Entrez GeneID: 3569. An example of the amino acid sequence is published in the NCBI database under the accession no. NP_000591, and is:

MNSFSTSAFG
PVAFSLGLLL
VLPAAFPAPV
PPGEDSKDVA


[062] "VEGF" is vascular endothelial growth factor. It is encoded, for example, by NCBI Entrez GeneID: 7422. An example of the amino acid sequence is published in the NCBI database under the accession no. AAH65522, and is:

AAASRGQGPE
PAPGGGVEGV
GARGVALKLF
VQLLGCSRF

Different isoforms of VEGF are associated with different stages of prostate cancer (Kaushal et al. (2005) CLIN CANCER RES. 11:584-93), and VEGF may control the...

[063] "FMOD" is fibromodulin. It is encoded, for example, by NCBI Entrez GeneID: 2331. An example of the amino acid sequence is published in the NCBI database under the accession number NP_002014, and is:

```
MQWTSLLLLA GLFSLSQAQY EDDPHWWFH Y LRSQQSTYYD
PYDPYPYETY EPPYGVDEG PAYTYGFSSP PDPRDCPQEC
DCPPNFTAM YCDNRLNKL Y LFFVPSRMKYV YFQNNQITSI
QEGVFDNATG LLWIALHGNQ ITSĐKVGRKV FSKLRLHLRL
YLDHNNLTRM PGPLRSLRE LHLDHNQISR VFPNNALEGLE
NLTALYLOHN EIQEVGSSMR GLRLILDDL SYNHLRKKVPD
GLPSALEQLY MHHNNVYTVP DSYFRAFPLK LYVRLSHNSL
TNNGLASNTF NSSSLLEDL SYNQLQKIPP VNTNLENYL
QGNRINEFSI SSFCTWDW NFSKLQVRLR DGNEIKRSAM
PADAPLCLRL ASLIEI (SEQ ID NO: 32).
```

There is a signal peptide corresponding to amino acids 1-18 of SEQ ID NO: 32, while amino acids 19-376 correspond to a mature protein.

[064] "AGR2" is anterior gradient 2 homolog (Xenopus laevis). It is encoded, for example, by NCBI Entrez GeneID: 10551. An example of the amino acid sequence is published in the NCBI database under the accession number NP_006399, and is:

```
MEKIPVSAFL LLVALSYTLA RDTTVKPGAK KDTKDSRPKL
PQTLSRGWGD QLIWTQTYEE ALYKSKTSNK PLMIHHHLDDE
CPHSQALKK VAFENKEIQKL AEQFVLLNLV YETTKHLSP
DGQYVPRIMF VGPSLTVRAD ITGRYNSRLY AYEPADTALL
LDNMKKALKL LKTEL (SEQ ID NO: 33).
```

[065] "SERPINA3" is serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3. It is encoded, for example, by NCBI Entrez GeneID: 12. SERPINA3 encodes a plasma protease inhibitor and member of the serine protease inhibitor class. There are a variety of polymorphisms that appear to be tissue specific and influence protease targeting. An example of the amino
acid sequence is published in the NCBI database under the accession number NP_001076, and is:

```
MERMLPLLAL GLLAAGFCPA VLCHPNS PLD EENLTQENQD
RGTHVDLGLA SANVDFAFSL YKQLVLKAPD KNVIFSPLSI
STALAFSLG AHNTTLTEIL KGLKFNLTEQ SEAEIHQSFQ
HLLRTLQNSS DELQLSMMNA MFVKEQLSLL DRFTEDAKRL
YGSEAFATDF QDSSAAAKKI NDYVKNGTRG KITDLKIDLD
SQTMMVLVNY IFFKAKWEMP FDPQDTHQSR FYLSKKKWVM
VPMMSLHHHT IPYFRDEELS CTWELKYTG NASALFILPD
QDKMEEVEAM LLPETLKRWR DSELEFREIG LYLPKSISR
DYNLNDILLQ LGIEEAFTSK ADLSGITGAR NLAVSQWHK
AVLVDFEEGT EASAATAVKI TLSALVETR TIVRFNRFKL
MIIVPTDTQN IFFMSKVTNP KQA (SEQ ID NO: 34).
```

The signal peptide corresponds to amino acids 1-25 of SEQ ID NO: 34, while amino acids 26-423 correspond to the mature protein. SERPINA3 is also known as alpha-1-antichymotrypsin.

[066] "AZGP1" is alpha-2-glycoprotein 1, zinc. It is encoded, for example, by NCBI Entrez GenelID: 563. An example of the amino acid sequence is published in the NCBI database under the accession number NP_001176, and is:

```
MVRMVPVLLS LLLLGPAAVP QENQDGrysL TYIYTGLS Kh
VEDVPAFQAL GSLNDLQFFR YNSKDRKSQG MGLWRQVEGM
EDWKQDSQLQ KAREDFMET LKDIVEYNYND SNGSHVLQGR
FGCEIENRNS SGAFWKYYD GKDYEIFNKE IPAWVFPDPA
AQITKQKWEA EPVYVQRAKA YLLEECPATL RKYLKYSKNI
LDRODPPSW VTSHQAPGKEK KKLKCLAYDF YPGKIDVHWT
RAGEVQEEPGL RDVLHNGNG TYQSWVWAV PPQDTAPYSC
HVQHSSLAQP LWPEAS (SEQ ID NO: 35).
```

[067] "FAM3B" is family with sequence similarity 3, member B. It is encoded, for example, by NCBI Entrez GenelID: 54097. An example of the amino acid sequence is published in the NCBI database under the accession number NP_478066, and is:
MRPLAGGLLK WFWFASLC AWYSGYLLAE LIPDAPLSSA
AYSIRSIGER PVLKAPVPKR QKCDHWTPCP SDTYAYRRLLS
GGGRSKYAKI CFEDNLLMGE QLGNVARGIN IAIVNYVTGN
VTATRCFDMY EGDNSSPMTK FIQSAAPKSL LFMVYDDGS
TRLNNDAKNA IEALGSKEIR NMRKFRSSWVF IAAKGLELPS
EIQREKINHSV DAKNNRYSGW PAEIQIEGCI PKERS
(SEQ ID NO: 36).

FAM3B is also known as cytokine-like protein 2-21 and pancreatic derived factor.

[068] "CD164" is CD164 antigen, sialomucin. It is encoded, for example, by NCBI Entrez GenelD: 8763. Splice variants exist in which either exon 4 or exon 5 is lacking. (Doyonnas et al., (2000) J. IMMUNOL. 165: 840-51.) An example of an amino acid sequence containing both exon 4 and exon 5 is published in the NCBI database under the accession number NP_006007, and is:

```
MSRLSRSLLW AATCLGVLCV LSADKNNTQH PNVTTLAPIS
NVTSAPVTSL PLVTTPAPET CEGRNSCVSC FNVSWNNTCC
FWIECKDESY CSHNSTVSDC QVGNTTDFFCS VSTATPVPTA
NSTAKPTVQP SPSTTSKTVT TSGTTNNTVT PTSQPVRKST
FDAASFIGGI VLVLGVQAVI FFLYKFCKSK ERFYHTL
(SEQ ID NO: 37).
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The isoform lacking exon 4 has the same amino acid sequence as SEQ ID NO: 37, except that amino acids 111-123 are deleted. (Doyonnas et al.) The isoform lacking exon 5 has the same amino acid sequence as SEQ ID NO: 37, except that amino acids 124-142 are deleted. (Doyonnas et al.) A soluble variant has also been described. (Masuzawa et al., (1992) J. BIOL. CHEM. 112: 609-15.) An example of the amino acid sequence of soluble CD164 is:

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MSRLSRSLLW AATCLGVLCV LSADKNNTQH PNVTTLAPIS
NVTSAPVTSL PLVTTPAPET CEGRNSCVSC FNVSWNNTCC
FWIECKDESY CSHNSTVSDC QVGNTTDFFCS VSTATPVPTA
NSTAKPTVQP SPSTTSKTVT TSGTTNNTVT PTSQPVRKST
FDAASFIGGI VLVLGVQAVI FFLYKFCKSK ERFYHTL
(SEQ ID NO: 38).
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Antibodies have been described that bind various forms of CD164. (Doyonnas et al.; Masuzawa et al.)
The term "protein" is used interchangeably with the terms "peptide" and "polypeptide" and refers to any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation), or source (e.g., species).

The term "antibody" refers to an immunoglobulin or fragment thereof, and encompasses any polypeptide comprising an antigen-binding fragment or an antigen-binding domain. The term includes but is not limited to polyclonal, monoclonal, monospecific, polyspecific, humanized, human, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, grafted, and in vitro generated antibodies. Unless preceded by the word "intact", the term "antibody" includes antibody fragments such as Fab, F(\text{ab}')_2, Fv, scFv, Fd, dAb, and other antibody fragments that retain antigen-binding function. Unless otherwise specified, an antibody is not necessarily from any particular source, nor is it produced by any particular method.

The terms "specific interaction," "specific binding," or the like, mean that two molecules form a complex that is relatively stable under physiologic conditions. The term is also applicable where, e.g., an antigen-binding domain is specific for a particular epitope, which is carried by a number of antigens, in which case the specific binding member carrying the antigen-binding domain will be able to bind to the various antigens carrying the epitope. Specific binding is characterized by a high affinity and a low to moderate capacity. Nonspecific binding usually has a low affinity with a moderate to high capacity. Typically, the binding is considered specific when the affinity constant $K_a$ is higher than $10^6 \text{M}^{-1}$, more preferably higher than $10^7 \text{M}^{-1}$, and most preferably $10^8 \text{M}^{-1}$. If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. Such conditions are known in the art, and a skilled artisan using routine techniques can select appropriate conditions. The conditions are usually defined in terms of concentration of antibodies, ionic strength of the solution, temperature, time allowed for binding, concentration of non-related molecules (e.g., serum albumin, milk casein), etc.

The term "detectably labeled" refers to any means for marking and identifying the presence of a molecule, e.g., an antibody or other protein. Methods for labeling a molecule are well known in the art. Labels can be either detectable
or functional labels, and include radiolabels (e.g., $^{131}I$, $^{125}I$, $^{35}S$, and $^{99}Tc$), enzymatic labels (e.g., horseradish peroxidase or alkaline phosphatase), chemiluminescent labels, and other chemical moieties (e.g., biotin).

[073] The term "isolated" refers to a molecule that is substantially free of its natural environment. Any amount of that molecule elevated over the naturally occurring levels due to any manipulation, e.g., over expression, partial purification, etc., is encompassed with the definition. With regard to partially purified compositions only, the term refers to an isolated compound that is at least 50-70%, 70-90%, 90-95% (w/w), or more pure.

[074] A "moderate risk" prostate cancer is cancer in which the patient has, for example, no PSA recurrence, a Gleason score of 6-7, T2a-T3b stage, no seminal vesicle invasion, and well or moderate tumor differentiation.

[075] A "high risk" prostate cancer is cancer in which the patient has, for example, PSA recurrence, a Gleason score of 8-9, T3c stage, seminal vesicle invasion, and poor tumor differentiation.

II. Secreted Protein Biomarkers

[076] The disclosure describes the identification and validation of diagnostic and prognostic biomarkers for prostate cancer, including NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, PU5, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164. These diagnostic biomarkers can be used to diagnose individuals with prostate cancer. These prognostic biomarkers can be used to identify prostate cancer patients who are at risk of developing advanced disease by differentiating between aggressive and non-aggressive courses of cancer development.

[077] Each of the secreted proteins, their fragments, or other derivatives, can be detected using antibodies or other binding proteins. Commercially available antibodies to a particular secreted protein can be used. Alternatively, the secreted protein, a fragment, or a derivative thereof, can be used as an immunogen to generate antibodies that specifically bind that secreted protein. Such antibodies include, but are not limited to, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, single chain antibodies, and antibody fragments. The
antibodies may be from mice, rats, rabbits, hamsters, goats, llamas, humans, or other species.

[078] Various procedures known in the art can be used for the production of polyclonal antibodies to one or more epitopes of a secreted protein. Rabbits, mice, rats, goats, llamas, etc. can be immunized with the native protein, a synthetic version of the protein, or a derivative (e.g., fragment) of the protein. Various adjuvants may be used to increase the immunological response, depending on the host species. Examples of adjuvants include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

[080] Antibody fragments can also be generated using known techniques. Fragments include but are not limited to: F(ab')\textsubscript{2} fragments, which can be produced by pepsin digestion of the antibody molecule; Fab' fragments, which can be generated by reducing the disulfide bridges of the F(ab')\textsubscript{2} fragment; Fab fragments, which can be generated by treating the antibody molecule with papain and a reducing agent; and Fv fragments, including single chain Fv (scFv) fragments.

[081] Following the production of antibodies by, for example, hybridoma technology, screening for the desired antibody can be accomplished by techniques known in the art, e.g., ELISA, and involve no more than routine techniques (e.g., Antibodies: A Laboratory Manual, eds. Harlow et al., Cold Spring Harbor Laboratory, 1988; Current Protocols in Immunology, Chpt. 2; eds. Colligan et al., National Institutes of Health, Published by John Wiley & Sons, Inc., 2006).

[082] When it is necessary to produce an antibody to a secreted protein biomarker, that particular secreted protein, its fragment, or other derivative, can be produced using standard techniques. As noted in the definitions section, nucleic acids encoding the various secreted protein biomarkers are known. Methods of manipulating nucleic acids to express proteins are well known in the art, and include those described in Molecular Cloning, A Laboratory Manual (2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor) and Current Protocols in Molecular Biology (Eds. Aufubel, Brent, Kingston, More, Feidman, Smith and Stuhl, Greene Publ. Assoc, Wiley-Interscience, NY, N.Y., 1992).

[083] Generally, in order to express each secreted protein biomarker, a suitable cell line is transformed with a DNA sequence encoding that protein under the control of known regulatory sequences. The transformed host cells are cultured and the protein recovered and isolated from the culture medium. The isolated expressed proteins are substantially free from other proteins with which they are co-produced as well as from other contaminants. Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO), the monkey kidney COS-1 cell line, or mammalian CV-1 cells. The selection of suitable mammalian host cells and methods for transformation, culturing, amplification, screening, product production and purification are known in the art.

[084] Bacterial cells may also be used as suitable hosts for expression of the secreted proteins. For example, the various strains of *E. coli* (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis, Pseudomonas*, other bacilli and the like may also be used. For expression of a protein in bacterial cells, DNA encoding the propeptide is generally not necessary.

[085] Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the secreted protein biomarkers. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, *e.g.*, Miller *et al.*, *Genetic Engineering*, 8:277-298 (Plenum Press 1986).

[086] In some embodiments, a secreted protein biomarker is expressed using a vector that contains a full length DNA sequence encoding the protein and appropriate expression control sequences. Expression control sequences for such vectors are known to those skilled in the art and may be selected depending upon the host cells. Such selection is routine. In other embodiments, the secreted protein biomarker is expressed as a fusion protein comprising the protein sequence of the biomarker and, for example, a tag to stabilize the resulting fusion protein or to simplify purification of the secreted protein biomarker. Such tags are known in the art. Representative examples include sequences which encode a series of histidine residues, the epitope tag FLAG, the Herpes simplex glycoprotein D, beta-galactosidase, maltose binding protein, streptavidin tag or glutathione S-transferase.

**III. Diagnostic Uses and Kits**

[087] In one embodiment, the disclosure provides methods for diagnosing prostate cancer comprising screening biological samples for one or more of the secreted protein biomarkers of a diagnostic panel. In particular, the disclosure describes methods of diagnosing prostate cancer comprising screening biological samples for at least one of the secreted proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15.
In certain embodiments, the method of diagnosing prostate cancer in a subject comprises detecting or measuring in a biological sample from the subject the expression of any one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen secreted proteins chosen from NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15, and correlating the expression level of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PH5 with the presence of prostate cancer or a higher predisposition to develop prostate cancer in the subject, wherein an increased expression level of any one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC or a decreased expression of any one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15 correlates with the presence of prostate cancer or a higher predisposition to develop prostate cancer in the subject.

The skilled artisan will understand how to correlate expression levels or patterns of the one or more secreted proteins with the presence of prostate cancer or a higher predisposition to develop prostate cancer. For example, the expression levels can be quantified such that increased or decreased expression levels relative to a control sample or other standardized value or numerical range indicate the presence of prostate cancer or a higher predisposition to develop prostate cancer.

The increased or decreased expression levels in the methods of the invention may be measured relative to the expression of that secreted protein in a control sample, such as a noncancerous sample from the subject or a sample obtained from a different individual who does not have cancer. Expression of a secreted protein may also be normalized by comparing it to the expression of other cancer-specific markers. For example, in prostate cancer, a prostate-cell specific marker, such as PSA, can be used as a control to compare and/or normalize expression levels of secreted proteins. By way of example, the method of diagnosing prostate cancer can comprise measuring the expression levels of one or more of the secreted proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, and SMOC, where an increased expression level of one or more of the secreted proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, and SMOC of at least
10% as compared to the control sample indicates the presence of prostate cancer. Conversely, by way of example, a decreased expression of one or more of the secreted proteins LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and Pl15 of at least 10% as compared to the control sample indicates the presence of prostate cancer. Although increases and decreases of at least 10% relative to a control can be used in the diagnostic methods, other values may also be used. For example, the increase or decrease may be at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or even 500%. The increase or decrease may also be expressed in terms of statistical significance, such as p < 0.05, p < 0.01, p < 0.005, or p < 0.001, particularly when large numbers of controls are available for comparison.

[091] In some embodiments, PSA, %fPSA, PSA doubling time, PSA velocity, prostate volume, or a combination of these indicators is determined in addition. In other embodiments, the diagnostic assay is conducted with the proviso that PSA, %fPSA, PSA doubling time, PSA velocity, prostate volume, or a combination thereof, are not determined or measured at the same time. Similarly, the diagnostic assay can be conducted on a biological sample with the proviso that either PSA or %fPSA, or both, are measured using a different biological sample.

[092] In various embodiments, the biological samples can be tissue, including prostate tissue, or a biofluid. Examples of biofluids include serum, plasma, whole blood, urine, saliva, and prostatic fluid. In some embodiments, the biological sample is post-digital rectal exam (post-DRE) urine.

[093] Biological samples can be screened using methods known in the art, including, for example, antibody-based assays such as reverse phase protein arrays or ELISA. In particular, such an immunoassay is carried out by a method comprising contacting a sample with at least two antibodies, wherein each antibody binds to a different protein in the diagnostic panel. The binding of the antibody to the protein is then detected or measured using techniques known in the art.

[094] The immunoassays which can be used include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA, protein arrays, reverse-phase protein arrays, immunoprecipitation assays, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent
immunoassays, protein A immunoassays, to name but a few. ELISAs are particularly simple assays that can be used to detect or measure expression levels of proteins.

[095] Kits for diagnostic use are also provided. Each kit provides a combination of reagents, where each reagent detects or measures the level of one secreted protein in the diagnostic panel. The reagent can be an antibody or binding protein that binds to a secreted protein of the diagnostic panel, or it can be a substrate for the secreted protein if the secreted protein is an enzyme. Each reagent can be detectably labeled, or a second reagent can be included that detects binding of the antibody or binding protein to the secreted protein. Examples of such second reagents include labeled antibodies that bind the light chain or heavy chain of the anti-secreted protein antibody (e.g., alkaline phosphatase labeled anti-kappa or anti-IgG antibodies).

[096] The antibody, binding protein, or substrate that is used to detect or measure the corresponding secreted protein of the diagnostic panel is considered a primary reagent. In certain embodiments, the diagnostic kit comprises primary reagents that detect or measure levels of one or more of the secreted proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15. In particular, the diagnostic kit comprises primary reagents for detecting or measuring the expression of any one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen secreted proteins chosen from NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15.

[097] A kit can also comprise a purified, predetermined amount of each secreted protein to be detected or measured using the kit. These purified secreted proteins serve as standards or controls. The kit can also comprise one or more components for detecting the primary reagent, including components described in the examples of this application or known in the art.

[098] The nucleic acids, polypeptides, and antibodies for use in diagnosing and prognosing prostate cancer are generally formulated with a pharmaceutically acceptable carrier. When a nucleic acid, polypeptide, or antibody is part of a kit, an
agent that reduces or inhibits the growth of microorganisms, such as sodium azide, can optionally be included in the formulation.

[0099] Thus, the disclosure provides diagnostic methods and kits for detecting one or more of the secretory proteins NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15.

[0100] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise NPY and SPOCK and optionally at least one of CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise NPY and CRISP3 and optionally at least one of SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise NPY and PLA2G7 and optionally at least one of SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise NPY and TMEFF2 and optionally at least one of SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise NPY and F5 and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise NPY and SMOC and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.

[0101] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise NPY and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise NPY and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise NPY and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise NPY and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise NPY and WIFI and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15. In another embodiment, the
secretory proteins to be detected comprise NPY and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[0102] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise NPY, at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15.

[0103] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise SPOCK and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15.

[0104] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise PI15 and optionally at least one of NPY, CRISP3, PLAG2, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise SPOCK and CRISP3 and optionally at least one of NPY, PLAG2, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise SPOCK and PLA2G7 and optionally at least one of NPY, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise SPOCK and TMEFF2 and optionally at least one of NPY, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise SPOCK and F5 and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise SPOCK and SMOC and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, or F5.

[0115] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise SPOCK and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SPOCK and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SPOCK and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SPOCK and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SPOCK and WIF1 and optionally at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SPOCK and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15.
In another embodiment, the secretory proteins to be detected comprise SPOCK and PU5 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[0105] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise SPOCK, at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15.

[0106] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise CRISP3 and NPY and optionally at least one of SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise CRISP3 and SPOCK and optionally at least one of NPY, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise CRISP3 and PLA2G7 and optionally at least one of NPY, SPOCK, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise CRISP3 and TMEFF2 and optionally at least one of NPY, SPOCK, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise CRISP3 and F5 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise CRISP3 and SMOC and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, or F5.

[0107] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise CRISP3 and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise CRISP3 and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PI5. In another embodiment, the secretory proteins to be detected comprise CRISP3 and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PI5. In another embodiment, the secretory proteins to be detected comprise CRISP3 and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise CRISP3 and WIF1 and optionally at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PI5. In another embodiment, the secretory proteins to be detected comprise CRISP3
and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15. In another embodiment, the secretory proteins to be detected comprise CRISP3 and PM5 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[0108] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise CRISP3, at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PM5.

[0109] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise PLA2G7 and NPY and optionally at least one of SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and SPOCK and optionally at least one of NPY, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and CRISP3 and optionally at least one of NPY, SPOCK, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and F5 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and SMOC and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, or F5.

[0110] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise PLA2G7 and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and WIF1 and optionally at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PM5.
In another embodiment, the secretory proteins to be detected comprise PLA2G7 and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15. In another embodiment, the secretory proteins to be detected comprise PLA2G7 and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[0111] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise PLA2G7, at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15.

[0112] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise TMEFF2 and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, or SMOC. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, or F5.

[0113] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise TMEFF2 and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise
TMEFF2 and WIF1 and optionally at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PI15. In another embodiment, the secretory proteins to be detected comprise TMEFF2 and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[01 14] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise TMEFF2, at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15.

[01 15] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise F5 and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise F5 and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise F5 and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise F5 and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise F5 and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, or SMOC. In another embodiment, the secretory proteins to be detected comprise F5 and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, or TMEFF2.

[01 16] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise F5 and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise F5 and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise F5 and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise F5 and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the
secretory proteins to be detected comprise F5 and WIF1 and optionally at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise F5 and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PM5. In another embodiment, the secretory proteins to be detected comprise F5 and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[01 17] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise F5, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15.

[01 18] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise SMOC and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, or F5. In another embodiment, the secretory proteins to be detected comprise SMOC and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, or F5. In another embodiment, the secretory proteins to be detected comprise SMOC and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, or F5. In another embodiment, the secretory proteins to be detected comprise SMOC and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, or F5. In another embodiment, the secretory proteins to be detected comprise SMOC and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, or F5. In another embodiment, the secretory proteins to be detected comprise SMOC and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, or TMEFF2.

[01 19] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise SMOC and LTF and optionally at least one of ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SMOC and ACPP and optionally at least one of LTF, TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise SMOC and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise SMOC and MSMB and optionally at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or PM5. In another embodiment,
the secretory proteins to be detected comprise SMOC and WIF1 and optionally at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise SMOC and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or PM5. In another embodiment, the secretory proteins to be detected comprise SMOC and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, WIF1, or OLFM4.

[0120] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise SMOC, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5, and at least one of LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, or PI15.

[0121] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise LTF and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise LTF and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise LTF and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise LTF and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise LTF and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise LTF and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise LTF and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.

[0122] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise LTF and ACPP and optionally at least one of TGM4, MSMB, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise LTF and TGM4 and optionally at least one of ACPP, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise LTF and MSMB and optionally at least
one of ACPP, TGM4, WIF1, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise LTF and WIFI and optionally at least one of ACPP, TGM4, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise LTF and OLFM4 and optionally at least one of ACPP, TGM4, MSMB, WIFI, or PI15. In another embodiment, the secretory proteins to be detected comprise LTF and PI15 and optionally at least one of ACPP, TGM4, MSMB, WIFI, OLFM4, or PI15.

[0123] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise LTF, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of ACPP, TGM4, MSMB, WIFI, OLFM4, or PI15.

[0124] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise ACPP and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise ACPP and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise ACPP and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise ACPP and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise ACPP and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise ACPP and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise ACPP and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.

[0125] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise ACPP and LTF and optionally at least one of TGM4, MSMB, WIFI, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise ACPP and TGM4 and optionally at least one of LTF, MSMB, WIFI, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise ACPP, TGM4, and optionally at least one of LTF, MSMB, WIFI, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise ACPP, TGM4, MSMB, WIFI, OLFM4, or PI15.
proteins to be detected comprise ACPP and MSMB and optionally at least one of
LTF, TGM4, WIF1, OLFM4, or PM5. In another embodiment, the secretory
proteins to be detected comprise ACPP and WIF1 and optionally at least one of
LTF, TGM4, MSMB, OLFM4, or PI15. In another embodiment, the secretory
proteins to be detected comprise ACPP and OLFM4 and optionally at least one of
LTF, TGM4, MSMB, WIF1, or PI15. In another embodiment, the secretory proteins
to be detected comprise ACPP and PM5 and optionally at least one of LTF, TGM4,
MSMB, WIF1, or OLFM4.

[0126] In yet another embodiment of the diagnostic methods and kits, the
secretory proteins to be detected comprise ACPP, at least one of NPY, SPOCK,
CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, TGM4, MSMB,
WIF1, OLFM4, or PI15.

[0127] In one embodiment of the diagnostic methods and kits, the secretory
proteins to be detected comprise TGM4 and NPY and optionally at least one of
SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the
secretory proteins to be detected comprise TGM4 and SPOCK and optionally at
least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another
embodiment, the secretory proteins to be detected comprise TGM4 and CRISP3
and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In
another embodiment, the secretory proteins to be detected comprise TGM4 and
PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or
SMOC. In another embodiment, the secretory proteins to be detected comprise
TGM4 and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3,
PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be
detected comprise TGM4 and F5 and optionally at least one of NPY, SPOCK,
CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory
proteins to be detected comprise TGM4 and SMOC and optionally at least one of
NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.

[0128] In another embodiment of the diagnostic methods and kits, the
secretory proteins to be detected comprise TGM4 and LTF and optionally at least
one of ACPP, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the
secretory proteins to be detected comprise TGM4 and ACPP and optionally at least
one of LTF, MSMB, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise TGM4 and MSMB and optionally at least one of LTF, ACPP, WIF1, OLFM4, or PM5. In another embodiment, the secretory proteins to be detected comprise TGM4 and WIF1 and optionally at least one of LTF, ACPP, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise TGM4 and OLFM4 and optionally at least one of LTF, ACPP, MSMB, WIF1, or PM5. In another embodiment, the secretory proteins to be detected comprise TGM4 and PI15 and optionally at least one of LTF, ACPP, MSMB, WIFI, or OLFM4.

[0129] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise TGM4, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, MSMB, WIFI, OLFM4, or PI15.

[0130] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise MSMB and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise MSMB and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise MSMB and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise MSMB and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise MSMB and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise MSMB and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise MSMB and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.

[0131] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise MSMB and LTF and optionally at least one of ACPP, TGM4, WIF1, OLFM4, or PM5. In another embodiment, the
secretory proteins to be detected comprise MSMB and ACPP and optionally at least one of LTF, TGM4, WIF1, OLFM4, or PIM5. In another embodiment, the secretory proteins to be detected comprise MSMB and TGM4 and optionally at least one of LTF, ACPP, WIF1, OLFM4, or P115. In another embodiment, the secretory proteins to be detected comprise MSMB and WIF1 and optionally at least one of LTF, ACPP, TGM4, OLFM4, or P115. In another embodiment, the secretory proteins to be detected comprise MSMB and WIF1 and optionally at least one of LTF, ACPP, TGM4, WIF1, or P115. In another embodiment, the secretory proteins to be detected comprise MSMB and P115 and optionally at least one of LTF, ACPP, TGM4, WIF1, or OLGM4.

[0132] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise MSMB, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, WIF1, OLFM4, or P115.

[0133] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise WIF1 and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise WIF1 and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise WIF1 and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise WIF1 and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise WIF1 and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise WIF1 and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise WIF1 and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.

[0134] In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise WIF1 and LTF and optionally at least
one of ACPP, TGM4, MSMB, OLFM4, or PU5. In another embodiment, the secretory proteins to be detected comprise WIF1 and ACPP and optionally at least one of LTF, TGM4, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise WIF1 and TGM4 and optionally at least one of LTF, ACPP, MSMB, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise WIF1 and MSMB and optionally at least one of LTF, ACPP, TGM4, OLFM4, or PI15. In another embodiment, the secretory proteins to be detected comprise WIF1 and OLFM4 and optionally at least one of LTF, ACPP, TGM4, MSMB, or PI15. In another embodiment, the secretory proteins to be detected comprise WIF1 and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, or OLFM4.

[0135] In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise WIF1, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, OLFM4, or PI15.

[0136] In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise OLFM4 and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise OLFM4 and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise OLFM4 and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise OLFM4 and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise OLFM4 and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise OLFM4 and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected comprise OLFM4 and SMOC and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or F5.
In another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise OLMF4 and LTF and optionally at least one of ACPP, TGM4, MSMB, WIFI, or PI15. In another embodiment, the secretory proteins to be detected comprise OLMF4 and ACPP and optionally at least one of LTF, TGM4, MSMB, WIFI, or PI15. In another embodiment, the secretory proteins to be detected comprise OLMF4 and TGM4 and optionally at least one of LTF, ACPP, MSMB, WIFI, or PI15. In another embodiment, the secretory proteins to be detected comprise OLMF4 and MSMB and optionally at least one of LTF, ACPP, TGM4, WIFI, or PI15. In another embodiment, the secretory proteins to be detected comprise OLMF4 and WIFI and optionally at least one of LTF, ACPP, TGM4, MSMB, or PI15. In another embodiment, the secretory proteins to be detected comprise OLMF4 and PI15 and optionally at least one of LTF, ACPP, TGM4, MSMB, or WIFI.

In yet another embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise OLMF4, at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4, MSMB, WIFI, or PI15.

In one embodiment of the diagnostic methods and kits, the secretory proteins to be detected comprise PM5 and NPY and optionally at least one of SPOCK, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PI15 and SPOCK and optionally at least one of NPY, CRISP3, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PI15 and CRISP3 and optionally at least one of NPY, SPOCK, PLA2G7, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PI15 and PLA2G7 and optionally at least one of NPY, SPOCK, CRISP3, TMEFF2, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PI15 and TMEFF2 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, F5, or SMOC. In another embodiment, the secretory proteins to be detected comprise PI15 and F5 and optionally at least one of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, or SMOC. In another embodiment, the secretory proteins to be detected
comprise PI15 and SMOC and optionally at least one of NPY, SPOCK, CRISP3,
PLA2G7, TMEFF2, or F5.

[0140] In another embodiment of the diagnostic methods and kits, the
secretory proteins to be detected comprise PI15 and LTF and optionally at least
one of ACPP, TGM4, MSMB, WIF1, or OLMF4. In another embodiment, the
secretory proteins to be detected comprise PI15 and ACPP and optionally at least
one of LTF, TGM4, MSMB, WIF1, or OLMF4. In another embodiment, the
secretory proteins to be detected comprise PU5 and TGM4 and optionally at least
one of LTF, ACPP, MSMB, WIF1, or OLMF4. In another embodiment, the
secretory proteins to be detected comprise PM5 and MSMB and optionally at least
one of LTF, ACPP, TGM4, WIF1, or OLMF4. In another embodiment, the secretory
proteins to be detected comprise PI15 and WIF1 and optionally at least one of LTF,
ACPP, TGM4, MSMB, or OLMF4. In another embodiment, the secretory proteins
to be detected comprise PI15 and OLMF4 and optionally at least one of LTF,
ACPP, TGM4, MSMB, or WIF1.

[0141] In yet another embodiment of the diagnostic methods and kits, the
secretory proteins to be detected comprise PM5, at least one of NPY, SPOCK,
CRISP3, PLA2G7, TMEFF2, F5, or SMOC, and at least one of LTF, ACPP, TGM4,
MSMB, WIF1 or OLMF4.

IV. Prognostic Uses and Kits

[0142] In one embodiment, the disclosure provides methods for prognosing
prostate cancer comprising screening biological samples for one or more of the
secreted protein biomarkers of a prognostic panel. In particular, the disclosure
describes methods of prognosing prostate cancer comprising screening biological
samples for one or more of the secreted proteins SPOCK, PDGFD, CHGA, CAV1,
RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1,
FAM3B, or CD164.

[0143] In certain embodiments, the method of prognosing prostate cancer in
a subject comprises detecting or measuring in a biological sample from the subject
the expression of any one, two, three, four, five, six, seven, eight, nine, ten, eleven,
twelve, thirteen, or fourteen secreted proteins chosen from SPOCK, PDGFD,
CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3,
AZGP1, FAM3B, and CD164, wherein an increased expression level of any one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF or a decreased expression level of any one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164 correlates with prostate cancer progression or severity, such as whether the prostate cancer is a moderate risk prostate cancer or a high risk prostate cancer, whether the prostate cancer is progressing, regressing, or in remission, or whether survival will be disease-free following radical prostatectomy.

[0144] The skilled artisan will understand how to correlate expression levels or patterns of the one or more secreted proteins with prostate cancer progression or disease severity. For example, the expression levels can be quantified such that increased or decreased expression levels relative to a control sample or other standardized value or numerical range indicate an increased disease severity, such as high risk prostate cancer, or decreased disease-free survival following radical prostatectomy.

[0145] The increased or decreased expression levels in the methods of the invention may be measured relative to the expression of that secreted protein in a control sample, such as a noncancerous sample from the subject or a sample obtained from a different individual who does not have cancer. Expression of a secreted protein may also be normalized by comparing it to the expression of other cancer-specific markers. For example, in prostate cancer, a prostate-cell specific marker, such as PSA, can be used as a control to compare and/or normalize expression levels of secreted proteins. By way of example, the method of prognosing prostate cancer can comprise measuring the expression levels of one or more of the secreted proteins SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, and VEGF, where an increased expression level of one or more of the secreted proteins SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF of at least 10% as compared to the control sample indicates a higher predisposition to develop prostate cancer, prostate cancer progression, or disease severity. Conversely, by way of example, a decreased expression of one or more of the secreted proteins LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 of at least 10% as compared to the control sample indicates a higher
predisposition to develop prostate cancer, prostate cancer progression, or disease severity.

[0146] Although increases and decreases of at least 10% relative to a control can be used in the prognostic methods, other values may also be used. For example, the increase or decrease may be at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or even 500%. The increase or decrease may also be expressed in terms of statistical significance, such as p< 0.05, p< 0.01 , p< 0.005, or p< 0.001.

[0147] In some embodiments, PSA, %fPSA, PSA doubling time, PSA velocity, prostate volume, or a combination of these indicators is determined in addition. In other embodiments, the prognostic assay is conducted with the proviso that PSA, %fPSA, PSA doubling time, PSA velocity, prostate volume, or a combination thereof, are not determined or measured at the same time. Similarly, the prognostic assay can be conducted on a biological sample with the proviso that either PSA or %fPSA, or both, are measured using a different biological sample.

[0148] The biological samples can be screened in the prognostic assays as described for the diagnostic assays. Thus, the biological samples can be tissue, including prostate tissue, or a biofluid. Examples of biofluids include serum, plasma, whole blood, urine, saliva, and prostatic fluid. In some embodiments, the biological sample is post-digital rectal exam (post-DRE) urine.

[0149] Kits for prognostic use are also provided and these kits comprise the same basic components as the diagnostic kits, except that the primary reagents differ. As discussed for the diagnostic kits, the prognostic kits may also comprise standards or controls. In the prognostic kits, the primary reagents detect or measure levels of one or more of the secreted proteins SPOCK, PDGFD, CHGA, CAV1, RLN1 , IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1 , FAM3B, or CD164. In particular, the prognostic kits comprise primary reagents for detecting or measuring the expression of any one two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen secreted proteins chosen from SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164.
Thus, the disclosure provides prognostic methods and kits for detecting one or more of the secretory proteins SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise SPOCK and PDGFD and optionally at least one of CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SPOCK and CHGA and optionally at least one of PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SPOCK and CAV1 and optionally at least one of PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SPOCK and IGFBP7 and optionally at least one of PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SPOCK and BGN and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SPOCK and IL6 and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise SPOCK and VEGF and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, or IL6.

In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise SPOCK and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SPOCK and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SPOCK and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SPOCK and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected
comprise SPOCK and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SPOCK and AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SPOCK and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0153] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise SPOCK, at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0154] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise PDGFD and SPOCK and optionally at least one of CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and CHGA and optionally at least one of SPOCK, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and CAV1 and optionally at least one of SPOCK, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and RLN1 and optionally at least one of SPOCK, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and IGFBP7 and optionally at least one of SPOCK, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and BGN and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and IL6 and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise PDGFD and VEGF and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, or IL6.

[0155] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise PDGFD and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise PDGFD and FMOD
and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise PDGFD and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise PDGFD and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise PDGFD and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise PDGFD and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise PDGFD and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0156] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise PDGFD, at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164.

[0157] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CHGA and SPOCK and optionally at least one of PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CHGA and PDGFD and optionally at least one of SPOCK, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CHGA and CAV1 and optionally at least one of SPOCK, PDGFD, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CHGA and RLN1 and optionally at least one of SPOCK, PDGFD, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CHGA and IGFBP7 and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CHGA and BGN and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CHGA and IL6 and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory
proteins to be detected comprise CHGA and VEGF and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, or IL6.

[0158] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CHGA and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CHGA and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CHGA and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CHGA and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CHGA and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CHGA and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise CHGA and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0159] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CHGA, at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0160] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CAV1 and SPOCK and optionally at least one of PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CAV1 and PDGFD and optionally at least one of SPOCK, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CAV1 and CHGA and optionally at least one of SPOCK, PDGFD, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CAV1 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise
CAV1 and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CAV1 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CAV1 and IL6 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise CAV1 and VEGF and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, or IL6.

[0161] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CAV1 and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CAV1 and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CAV1 and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CAV1 and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CAV1 and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise CAV1 and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise CAV1 and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0162] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CAV1, at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0163] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise RLN1 and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and PDGFD and optionally at
least one of SPOCK, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and IL6 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise RLN1 and VEGF and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, or IL6.

[0164] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise RLN1 and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise RLN1 and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise RLN1 and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise RLN1 and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise RLN1 and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise RLN1 and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise RLN1 and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0165] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise RLN1, at least one of SPOCK, PDGFD,
CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0166] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise IGFBP7 and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and IL6 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and VEGF and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, or IL6.

[0167] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise IGFBP7 and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to
be detected comprise IGFBP7 and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise IGFBP7 and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0168] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise IGFBP7, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0169] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise BGN and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and IL6 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, or VEGF. In another embodiment, the secretory proteins to be detected comprise BGN and VEGF and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, or IL6.

[0170] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise BGN and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise BGN and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise BGN and
AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise BGN and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise BGN and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise BGN and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise BGN and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0171] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise BGN, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0172] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise IL6 and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, or VEGF. In another embodiment, the secretory proteins to be detected comprise IL6 and VEGF and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, or BGN.
[0173] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise IL6 and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IL6 and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IL6 and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IL6 and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IL6 and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise IL6 and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise IL6 and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0174] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise IL6, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0175] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise VEGF and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, or IL6. In another embodiment, the secretory proteins to be detected comprise VEGF and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, or IL6. In another embodiment, the secretory proteins to be detected comprise VEGF and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, or IL6. In another embodiment, the secretory proteins to be detected comprise VEGF and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, or IL6. In another embodiment, the secretory proteins to be detected comprise VEGF and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, or IL6. In another embodiment, the secretory proteins to be
detected comprise VEGF and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, or IL6. In another embodiment, the secretory proteins to be detected comprise VEGF and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, or IL6. In another embodiment, the secretory proteins to be detected comprise VEGF and IL6 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, or BGN.

[0176] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise VEGF and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise VEGF and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise VEGF and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise VEGF and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise VEGF and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise VEGF and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise VEGF and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0177] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise VEGF, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, or IL6, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0178] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise LTF and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise LTF and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise
LTF and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise LTF and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise LTF and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise LTF and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise LTF and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

[0179] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise LTF and FMD and optionally at least one of AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise LTF and AGR2 and optionally at least one of FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise LTF and SERPINA3 and optionally at least one of FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise LTF and AZGP1 and optionally at least one of FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise LTF and FAM3B and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise LTF and CD164 and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

[0180] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise LTF, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of FMOD, AGR2, SERPINA3, AZGP1, FAM3B, or CD164.

[0181] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise FMOD and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FMOD and PDGFD
and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FMOD and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FMOD and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FMOD and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FMOD and FMOD and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FMOD and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

[0182] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise FMOD and LTF and optionally at least one of AGR2, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise FMOD and AGR2 and optionally at least one of LTF, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise FMOD and AGR2 and optionally at least one of LTF, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise FMOD and AZGP1 and optionally at least one of LTF AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise FMOD and FAM3B and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise FMOD and CD164 and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, or FAM3B.

[0183] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise FMOD, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, AGR2, SERPINA3, AZGP1, or FAM3B, or CD164.

[0184] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise AGR2 and SPOCK and optionally at least one of
PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AGR2 and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AGR2 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AGR2 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AGR2 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AGR2 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

[0185] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise AGR2 and LTF and optionally at least one of FMOD, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AGR2 and FMOD and optionally at least one of LTF, SERPINA3, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AGR2 and SERPINA3 and optionally at least one of LTF, FMOD, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AGR2 and AZGP1 and optionally at least one of LTF, FMOD, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AGR2 and FAM3B and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise AGR2 and CD164 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, or FAM3B.

[0186] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise AGR2, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, SERPINA3, AZGP1, FAM3B, or CD164.
In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise SERPINA3 and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise SERPINA3 and LTF and optionally at least one of FMOD, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and FMOD and optionally at least one of LTF, AGR2, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and AGR2 and optionally at least one of LTF, FMOD, AZGP1, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and AZGP1 and optionally at least one of LTF, FMOD, AGR2, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and FAM3B and optionally at least one of LTF, FMOD, AGR2, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise SERPINA3 and CD164 and optionally at least one of LTF, FMOD, AGR2, AZGP1, or FAM3B.

In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise SERPINA3, at least one of SPOCK,
[0190] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise AZGP1 and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AZGP1 and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AZGP1 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AZGP1 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AZGP1 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AZGP1 and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise AZGP1 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

[0191] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise AZGP1 and LTF and optionally at least one of FMOD, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AZGP1 and FMOD and optionally at least one of LTF, AGR2, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AZGP1 and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AZGP1 and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, FAM3B, or CD164. In another embodiment, the secretory proteins to be detected comprise AZGP1 and FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, or CD164. In another embodiment, the secretory proteins to be detected comprise AZGP1 and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, or FAM3B.
[0192] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise AZGP1, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, FAM3B, or CD164.

[0193] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise FAM3B and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FAM3B and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FAM3B and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FAM3B and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FAM3B and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FAM3B and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise FAM3B and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

[0194] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise FAM3B and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise FAM3B and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise FAM3B and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise FAM3B and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, or CD164. In another embodiment, the secretory proteins to be detected comprise FAM3B and AZGP1 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, or CD164. In
another embodiment, the secretory proteins to be detected comprise FAM3B and CD164 and optionally at least one of LTF, FMOD, AGR2, SERPINA3, or AZGP1.

[0195] In yet another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise FAM3B, at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of LTF, FMOD, AGR2, SERPINA3, AZGP1, or CD164.

[0196] In one embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CD164 and SPOCK and optionally at least one of PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CD164 and PDGFD and optionally at least one of SPOCK, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CD164 and CHGA and optionally at least one of SPOCK, PDGFD, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CD164 and CAV1 and optionally at least one of SPOCK, PDGFD, CHGA, RLN1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CD164 and RLN1 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, IGFBP7, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CD164 and IGFBP7 and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, BGN, IL6, or VEGF. In another embodiment, the secretory proteins to be detected comprise CD164 and BGN and optionally at least one of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, IL6, or VEGF.

[0197] In another embodiment of the prognostic methods and kits, the secretory proteins to be detected comprise CD164 and LTF and optionally at least one of FMOD, AGR2, SERPINA3, AZGP1, or FAM3B. In another embodiment, the secretory proteins to be detected comprise CD164 and FMOD and optionally at least one of LTF, AGR2, SERPINA3, AZGP1, or FAM3B. In another embodiment, the secretory proteins to be detected comprise CD164 and AGR2 and optionally at least one of LTF, FMOD, SERPINA3, AZGP1, or FAM3B. In another embodiment, the secretory proteins to be detected comprise CD164 and SERPINA3 and optionally at least one of LTF, FMOD, AGR2, AZGP1, or FAM3B. In another
embodiment, the secretory proteins to be detected comprise CD164 and AZGP1
and optionally at least one of LTF, FMOD, AGR2, SERPINA3, or FAM3B. In
another embodiment, the secretory proteins to be detected comprise CD164 and
FAM3B and optionally at least one of LTF, FMOD, AGR2, SERPINA3, or AZGP1.

[0198] In yet another embodiment of the prognostic methods and kits, the
secretory proteins to be detected comprise CD164, at least one of SPOCK,
PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, or VEGF, and at least one of
LTF, FMOD, AGR2, SERPINA3, AZGP1, or FAM3B.

V. Artificial Neural Network Models

[0199] In addition to providing methods and kits for detecting or measuring
one or more of the secreted proteins of the diagnostic and prognostic panels,
alternative neural networks trained with the resulting data are also described. ANN
models are well-suited for studies using multiple biomarkers. (Stephan et al.,
ANN models are particularly advantageous when the data sets are large because
they have the ability to resolve complex, non-linear relationships among variables
(e.g. individual protein expression level, clinical factors) without the need to make
prior assumptions.

[0200] The application of ANN to biological problems is routine in the art.
(See, e.g., Itchhaporia et al., (1996) J. AM. COLL. CARDIOL. 28: 515-21; Babaian et
Various software, such as the MATLAB Neural Network Toolbox™ software
package (The Mathworks, Natick, MA), can be used as a basis for building ANN
models. Although the same software package is used to develop various models,
the resulting models differ, however, because different inputs and different
numbers of inputs are used, thereby determining the application of the ANN and
the sensitivity and specificity of the model. Input selection and ANN training are
therefore unique to each model.

[0201] The disclosure provides ANN models in which the input data are
based upon the presence or levels of one or more secreted biomarkers from Table
1 and/or Table 2 in a biofluid of the patient. This approach is advantageous for
several reasons. As already mentioned, secreted biomarkers can be measured in
biofluids, which are much easier to obtain than is a biopsy sample. In addition, unlike potentially subjective clinical criteria such as digital rectal exam results and Gleason scoring, secreted biomarkers can be measured objectively using standardized assays, such as ELISA. This helps to minimize variation between different users. Further, combining biomarkers helps to minimize the impact of outlier input data on the output.

[0202] Accordingly, the disclosure provides a computer-implemented method for diagnosing prostate cancer comprising:
identifying one or more of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5 as parameters;
receiving initial data values for the identified parameters from a patient with a known disease status;
training an artificial neural network using the parameters and received initial data;
modifying a template using learning gained by the artificial neural network; and
using the modified template to diagnose a new patient based on new input values.

[0203] In one embodiment, each of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5 is identified as a parameter.

[0204] The disclosure also provides a computer readable medium including computer code for causing a processor to perform the steps of:
identifying one or more of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5 as parameters;
receiving initial data values for the identified parameters from a patient with a known disease status;
training an artificial neural network using the parameters and received initial data;
modifying a template using learning gained by the artificial neural network; and
using the modified template to diagnose a new patient based on new input values.

[0205] For the biomarker panel ANN models, initial model development can utilize established clinical factors, such as PSA, %fPSA, PSA doubling time, PSA velocity, PSA recurrence, and prostate volume to find an optimum number of hidden layers. The ANN models can then be trained using biomarker data that is associated with a known clinical outcome. To avoid overfitting, Bayesian
regularization developed by Finne et al. (2000) UROLOGY 56: 418-22) can be used during the training run.

[0206] In some embodiments, diagnostic ANN are trained with data, optionally normalized or scaled, for one or more of the secreted protein biomarkers NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5. Training may optionally further comprise inclusion of data for one or more of PSA, %fPSA, PSA doubling time, PSA velocity, PSA recurrence, prostate volume, digital rectal exam results, PSA derivative data, or pathology results (including Gleason score). By using multiple, rather than single, inputs, the models learn to recognize outliers in the data set and so give such outlying data less weight. Combinations of biomarkers, therefore, minimize the impact of outlying data for one or a small subset of biomarkers.

[0207] In other embodiments, prognostic ANN are trained in the same manner as diagnostic ANN, but the input data, optionally normalized or scaled, is for one or more of the secreted protein biomarkers SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164. Here, too, training may optionally further comprise inclusion of data for one or more of PSA, %fPSA, PSA doubling time, PSA velocity, PSA recurrence, prostate volume, digital rectal exam results, PSA derivative data, or pathology results (including Gleason score).

[0208] Accordingly, the disclosure also provides a computer-implemented method for prognosing prostate cancer comprising:
identifying one or more of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 as parameters;
receiving initial data values for the identified parameters from a patient with a known disease status;
training an artificial neural network using the parameters and received initial data;
modifying a template using learning gained by the artificial neural network; and
using the modified template to prognose a new patient based on new input values.
[0209] In one embodiment, each of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 is identified as a parameter.

[0210] In still another embodiment, the disclosure provides a computer readable medium including computer code for causing a processor to perform the steps of:
identifying one or more of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 as parameters;
receiving initial data values for the identified parameters from a patient with a known disease status;
training an artificial neural network using the parameters and received initial data;
modifying a template using learning gained by the artificial neural network; and
using the modified template to prognose a new patient based on new input values.

[0211] Trained ANN models are tested by asking whether the model can correctly predict the clinical outcome using patient data other than that with which the ANN model was trained, but having a known clinical outcome. After training, the model output from 0 (control or moderate risk) to 1 (cancer or high risk) can be calculated in blind fashion by the average error of all N predictions (a validation group). Based on the output values, the receiver operating characteristic (ROC) curve can be built to calculate the outcome of clinical prediction: specificity and sensitivity of prostate cancer. The inclusion of clinical factors, such as one or more of PSA, %fPSA, PSA doubling time, PSA velocity, PSA recurrence, prostate volume, digital rectal exam, PSA derivative data, or pathology results (including Gleason score), can be used to further optimize the performance of ANN models.

[0212] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. Moreover, advantages described in the body of the specification, if not included in the claims, are not per se limitations to the claimed invention.
[0213] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Moreover, it must be understood that the invention is not limited to the particular embodiments described, as such may, of course, vary. Further, the terminology used to describe particular embodiments is not intended to be limiting, since the scope of the present invention will be limited only by its claims. The claims do not encompass embodiments in the public domain.

[0214] With respect to ranges of values, the invention encompasses each intervening value between the upper and lower limits of the range to at least a tenth of the lower limit's unit, unless the context clearly indicates otherwise. Further, the invention encompasses any other stated intervening values. Moreover, the invention also encompasses ranges excluding either or both of the upper and lower limits of the range, unless specifically excluded from the stated range.

[0215] Unless defined otherwise, the meanings of all technical and scientific terms used herein are those commonly understood by one of ordinary skill in the art to which this invention belongs. One of ordinary skill in the art will also appreciate that any methods and materials similar or equivalent to those described herein can also be used to practice or test the invention. Further, all publications mentioned herein are incorporated by reference in their entireties.

[0216] It must be noted that, as used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a subject polypeptide" includes a plurality of such polypeptides and reference to "the agent" includes reference to one or more agents and equivalents thereof known to those skilled in the art, and so forth.

[0217] Further, all numbers expressing quantities of ingredients, reaction conditions, % purity, polypeptide and polynucleotide lengths, and so forth, used in the specification and claims, are modified by the term "about," unless otherwise indicated. Accordingly, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties of the present invention. At the very least, and not as an attempt to limit the
application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits, applying ordinary rounding techniques. Nonetheless, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors from the standard deviation of its experimental measurement.

[0218] The specification is most thoroughly understood in light of the references cited herein. Each of these references is hereby incorporated by the reference in its entirety.

EXAMPLES

Example 1: Identification of Secreted Proteins for use as Biomarkers

[0219] Biofluids are complex mixtures of proteins. It is therefore difficult to discover biomarker candidates using a biofluid as the starting material. To overcome this obstacle, we focused on proteins encoded by genes that we have previously identified as having an expression signature in prostate tumor tissue that varied from that in benign matched tissue using an epithelial cell transcriptome from 50 matched pairs of laser capture micro-dissected benign and malignant prostate epithelial cells. (See copending application no. PCT/US05/15926, incorporated herein by reference.) From among those genes, a unique panel of secreted proteins having altered expression in prostate tumor cells as compared to benign prostate cells were selected as biomarkers for diagnosing prostate cancer.

[0220] Table 1 presents those secreted proteins selected as diagnostic biomarkers for prostate cancer:

Table 1:

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>ID</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NPY</td>
<td>4852</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6695,</td>
<td>sparc/osteonectin, cwcv and kazal-like domains</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9806,</td>
<td>proteoglycan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50859</td>
<td>(testican) 1, 2, and 3</td>
</tr>
<tr>
<td>3</td>
<td>CRISP3</td>
<td>10321</td>
<td>cysteine-rich secretory protein 3</td>
</tr>
</tbody>
</table>
A unique panel of secreted proteins that are prognostic biomarkers for prostate cancer was also selected. Alterations in the expression patterns of these proteins distinguish prostate tumor cells from benign prostate cells when comparing moderate risk prostate cancer samples to high risk prostate cancer samples.

Table 2 presents those secreted proteins selected as prognostic biomarkers for prostate cancer:

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>ID</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>PLA2G7</td>
<td>7941</td>
<td>phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)</td>
</tr>
<tr>
<td>5</td>
<td>TMEFF2</td>
<td>23671</td>
<td>transmembrane protein with EGF-like and two follistatin-like domains 2</td>
</tr>
<tr>
<td>6</td>
<td>F5</td>
<td>2153</td>
<td>coagulation factor V (proaccelerin, labile factor)</td>
</tr>
<tr>
<td>7</td>
<td>SMOC</td>
<td>64093 64094</td>
<td>SPARC related modular calcium binding 1 and 2</td>
</tr>
</tbody>
</table>

**Down regulated proteins in prostate cancer tissue**

<table>
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<tr>
<th>No.</th>
<th>Gene</th>
<th>ID</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>LTF</td>
<td>4057</td>
<td>lactotransferrin</td>
</tr>
<tr>
<td>9</td>
<td>ACPP</td>
<td>55</td>
<td>acid phosphatase, prostate, PAP</td>
</tr>
<tr>
<td>10</td>
<td>TGM4</td>
<td>7047</td>
<td>transglutaminase 4 (prostate)</td>
</tr>
<tr>
<td>11</td>
<td>MSMB</td>
<td>4477</td>
<td>microseminoprotein, beta-</td>
</tr>
<tr>
<td>12</td>
<td>WIF1</td>
<td>11197</td>
<td>WNT inhibitory factor 1</td>
</tr>
<tr>
<td>13</td>
<td>OLFM4</td>
<td>10562</td>
<td>olfactomedin 4</td>
</tr>
<tr>
<td>14</td>
<td>PI15</td>
<td>51050</td>
<td>peptidase inhibitor 13</td>
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</table>

[0221] A unique panel of secreted proteins that are prognostic biomarkers for prostate cancer was also selected. Alterations in the expression patterns of these proteins distinguish prostate tumor cells from benign prostate cells when comparing moderate risk prostate cancer samples to high risk prostate cancer samples.

[0222] Table 2 presents those secreted proteins selected as prognostic biomarkers for prostate cancer:

Table 2:

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>ID</th>
<th>Name</th>
</tr>
</thead>
</table>

**Up regulated proteins in metastatic prostate cancer**

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>ID</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SPOCK</td>
<td>6695, 9806, 50859</td>
<td>sparco/osteonectin, cwcv and kaval-like domains proteoglycan (testican) 1, 2, and 3</td>
</tr>
<tr>
<td>2</td>
<td>PDGFD</td>
<td>80310</td>
<td>platelet derived growth factor D</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>CHGA</td>
<td>1113</td>
<td>chromogranin A (parathyroid secretory protein 1)</td>
</tr>
<tr>
<td>4</td>
<td>CAV1</td>
<td>857</td>
<td>caveolin 1, caveolae protein, 22kDa</td>
</tr>
<tr>
<td>5</td>
<td>RLN1</td>
<td>6013</td>
<td>relaxin 1</td>
</tr>
<tr>
<td>6</td>
<td>IGFBP7</td>
<td>3490</td>
<td>insulin-like growth factor binding protein 7</td>
</tr>
<tr>
<td>7</td>
<td>BGN</td>
<td>633</td>
<td>biglycan</td>
</tr>
<tr>
<td>8</td>
<td>IL6</td>
<td>3569</td>
<td>interleukin 6 (interferon, beta 2)</td>
</tr>
<tr>
<td>9</td>
<td>VEGF</td>
<td>7422</td>
<td>vascular endothelial growth factor</td>
</tr>
</tbody>
</table>

**Down regulated proteins in metastatic prostate cancer**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>LTF</td>
<td>4057</td>
<td>lactotransferrin</td>
</tr>
<tr>
<td>11</td>
<td>FMOD</td>
<td>2331</td>
<td>fibromodulin</td>
</tr>
<tr>
<td>12</td>
<td>AGR2</td>
<td>10551</td>
<td>anterior gradient 2 homolog (Xenopus laevis)</td>
</tr>
<tr>
<td>13</td>
<td>SERPINA3</td>
<td>12</td>
<td>serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3</td>
</tr>
<tr>
<td>14</td>
<td>AZGP1</td>
<td>563</td>
<td>alpha-2-glycoprotein 1, zinc</td>
</tr>
<tr>
<td>15</td>
<td>FAM3B</td>
<td>54097</td>
<td>family with sequence similarity 3, member B</td>
</tr>
<tr>
<td>16</td>
<td>CD164</td>
<td>8763</td>
<td>CD164 molecule, sialomucin</td>
</tr>
</tbody>
</table>

[0223] The prognostic panel includes proteins encoded by genes that are differentially expressed in tumors of patients with moderate risk or high risk prostate cancer or who have increased risk for cancer recurrence following radical prostatectomy.

[0224] Figure 1 shows a meta-analysis of gene expression data for the seven most frequently upregulated genes in the diagnostic panel. The individual discriminatory power of the seven most frequently up-regulated genes between normal and prostate tissue is presented in box-plot format, and includes both a
GeneChip® analysis (Affymetrix, Santa Clara, Ca), and an analysis of data extracted from the Oncomine™ database (Compendia Bioscience, Ann Arbor, Mi). Examination of any of these genes resulted in the ability to separate normal tissue from prostate cancer tissue (p < 0.05).

[0225] The secreted proteins listed in Table 1 and Table 2 are present and can be detected in the biofluids of patients. Using a direct ELISA, we detected expression of SPOCK, F5, NPY, CRISP3, PLA2G7, and TMEFF2 protein in serum pooled from controls, patients with prostate cancer (CaP), and patients with metastatic prostate cancer (Met) (Figure 2). We have also validated capture and detection antibody pairs for use in sandwich ELISAs to detect and measure levels of SPOCK, CRISP3, PLA2G7, NPY, F5, and TMEFF2. Appropriate antibody pairs for the capture and detection of the remaining secreted biomarkers can be developed and validated using routine experimentation. Using direct- or sandwich-ELISA, we have confirmed overexpression of SPOCK, PLA2G7, NPY, CRISP3 and F5, protein in serum or plasma of prostate cancer versus control.

[0226] Although PSA is widely used for prostate cancer screening, serum PSA is also frequently elevated in non-malignant conditions and, in practice, only about 25% of men with elevated serum PSA have prostate cancer on tissue biopsy. Thus, although PSA is a very sensitive assay, its specificity is somewhat lacking. We therefore further investigated the specificity of two of our biomarkers, SPOCK1 and PLA2G7, and compared their specificity to that of total PSA (tPSA). Serum levels of both biomarkers were quantitatively measured by ELISA. Figure 3A and 3B show that both SPOCK1 (Fig. 3A) and PLA2G7 (Fig. 3B) can successfully distinguish the prostate cancer (CaP) cohort from the control cohort. The results for each were statistically significant (p< 0.05).

[0227] Separating performance was quantitatively evaluated for PLA2G7 and for total PSA (tPSA) by a linear classifier algorithm (Support Vector Machine (SVM)) and a non-linear classifier algorithm (Artificial Neural Network (ANN)). Performance accuracy and probability for individual markers (tPSA or PLA2G7) and for a panel of tPSA and PLA2G7 in SVM or ANN were further quantitatively evaluated by the area under curve (AUC) in receiver operator characteristic curve analysis. Figures 3C and 3D show the results for two sub-groups of subjects:
those with a PSA range of 0-10 ng/ml (Fig. 3C) and those with a PSA range 0-4 ng/ml (Fig. 3D). As summarized in Figure 3E, PLA2G7 is superior to total PSA in separating the prostate cancer and control cohorts. (Compare the sensitivity cutoffs for PLA2G7 and for tPSA.) The separating effect became even greater when PLA2G7 is combined as a panel with tPSA in terms of AUC for both subgroups. In addition, use of the ANN algorithm improved specificity compared to the SVM algorithm in both groups for either sensitivity cutoff.

[0228] Initial validation experiments used pooled serum. We have also examined expression levels of SPOCK and CRISP3 in the serum of individual samples. Here, we compared expression levels in individual control (PSA < 2 ng/mL, normal digital rectal exam) and prostate cancer (CaP) patients (PSA between 4 and 10 ng/mL, Gleason score of 6-8). Although there is sample-to-sample variation in expression levels, there is a statistically significant difference (p-value < 0.05) in the levels of SPOCK (Figure 4) and CRISP3 (Figure 5) in prostate cancer patients compared to controls. These data are consistent with our analysis of the gene expression in tissue data shown in Figure 1 (p-value < 0.05).

[0229] In addition to serum samples, we have also tested for the presence of two of our diagnostic biomarkers, SMOC1 and F5, in post-DRE (digit rectal exam) urine samples. Using a quantitative ELISA, we compared post-DRE urine from patients with positive ("Pos") or negative ("Neg") biopsy ("Bx") for prostate cancer. Figure 6 presents results for SMOC1 (Fig. 6A, Bx (Pos) N = 15; Bx (Neg) N = 8) and F5 (Fig. 6B, Bx (Pos) N = 15; Bx (Neg) N = 8) in comparison with serum PSA (Fig. 6C, Bx (Pos) N = 15; Bx (Neg) N = 8). Using post-DRE urine, both SMOC1 and F5 separate the biopsy positive (prostate cancer) group from the biopsy negative (control) group (p < 0.05 for both SMOC1 and F5 using Student t-test). SMOC1 correctly distinguished 7 of 15 biopsy positive samples and 6 of 8 biopsy negative samples. Thus, based on these preliminary data, SMOC1 separated the two cohorts with 47% sensitivity and 75% specificity. Similarly, in this preliminary study, F5 distinguished the biopsy positive cohort from the negative cohort with 40% sensitivity and 87% specificity. In contrast, serum PSA levels for two cohorts were similar at their median values (Fig. 6C). A panel including serum PSA and post-DRE urine SMOC1 and F5 data showed combined sensitivity and
specificity of 87% and 87%, respectively. Of note is the improved specificity in predicting biopsy negative outcome: specificity of a panel (SMOC1 and F5) reaches 87%, whereas PSA specificity remains at 25%. Thus, our preliminary data support using SMOC1 and F5 either alone or in combination with each other as diagnostic markers of prostate cancer in urine. Further, SMOC1 and F5, either individually or in combination, should also enhance the specificity of PSA.

Example 2: Combining Biomarkers in a Panel Improves Sensitivity and Specificity

[0230] Ideally, a clinical test should detect prostate cancer with high specificity and sensitivity. By combining individual biomarkers into a panel, it is possible to improve diagnostic power compared to single biomarkers. We tested this panel approach using data for the up-regulated genes presented in Table 1. When we calculated the probability of prostate diagnosis based on the exhaustive combination of these seven genes, we found that the probability of prostate cancer linearly correlated with the frequency of up-regulated genes encoding secretory proteins (linearity constant at 0.9632) (Figure 7). Combining increasing numbers of the prostate cancer overexpressed genes resulted in an increased association with prostate cancer specimens in our tumor versus benign prostate epithelial cell data set (Figure 8). The pie charts illustrate patient distribution by tumor versus benign gene expression ratios for PLA2G7, F5, TMEFF2, SMOC, NPY, SPOCK, and CRISP3. When all seven genes encoding for secretory proteins were combined, these genes exhibited greater than 70% association with prostate cancer.

[0231] This analysis indicates that improved diagnosis of prostate cancer can be achieved if panels comprising two, three, four, five, six, or seven of the proteins listed in Table 1 are used rather than single biomarkers. The inclusion of additional informative proteins, such as the secreted proteins encoded by the down-regulated genes in Table 1 or PSA, can further improve the diagnostic value of the panel. Of course, the fact that the diagnostic value of individual biomarkers can be improved by combining them into panels of more than one biomarker does not negate the fact that many biomarkers will have sufficient sensitivity and specificity for individual use as diagnostic or prognostic markers. Further,
individual biomarkers may be more or less specific or sensitive depending upon the biofluid assayed.

**Example 3: Development of Trained Artificial Neural Network (ANN) Models**


[0233] Accordingly, the results for individual protein biomarkers are used to develop diagnostic and prognostic ANN models using the MATLAB Neural Network Toolbox™ software package (The Mathworks, Natick, MA). Using two or more of the biomarkers listed in Table 1 or Table 2, and various clinical factors (e.g., PSA, %fPSA, PSA doubling time, PSA velocity, PSA recurrence, prostate volume), ANN models for early diagnosis or prognosis of prostate cancer are developed. In initial model development, 3-5 hidden layers are tested to find an optimum number of
layers. The ANN model is then "trained." Data for all but one patient of a data set of N patients with different marker levels are used in the training, then a prediction is made for the remaining patient in the basis of N-1 classifiers. This is referred to as the 'leave-one-out' method. (Stephan et al., (2006) PROSTATE 66: 651-59.) To avoid overfitting, Bayesian regularization within the training run developed by Finne et al. ((2000) UROLOGY 56: 418-22) is used. After training the ANN models, the model output from 0 (control or moderate risk) to 1 (cancer or high risk) is calculated in blind fashion by the average error of all N predictions (a validation group). Based on the output values, the receiver operating characteristic (ROC) curve is built to calculate the outcome of clinical prediction: specificity and sensitivity of prostate cancer. The area under the ROC curves are calculated and compared using in-house user-defined M-file scripts within the MATLAB software package. Changing clinical factors further optimizes the performance of ANN models.

[0234] Control sera are used to develop diagnostic ANN models. By way of example, the control sera are from men having serum PSA ≤2 ng/ml, normal PSA velocity (if data is available), normal digital rectal examination (DRE); no genitourinary symptoms, and optionally a biopsy that is negative for BPH or cancer. These men are age and race matched to the BPH and cancer groups. The BPH group includes patients having LUTS (lower urinary track symptoms), a normal DRE, a biopsy that is negative for cancer, and a PSA of 2-10 ng/ml). The prostate cancer group includes patients with a biopsy that was positive for cancer and a serum PSA of 2-10 ng/ml.

[0235] To develop prognostic ANN models, the prostate cancer patients are grouped into those with biologically aggressive disease (high risk, Gleason Score of 7 or greater) and those with low/moderately aggressive disease (moderate risk, Gleason Score of 6 or less) based on tumor pathology. Patients are also stratified for disease recurrence (biochemical recurrence by rising PSA after radical prostatectomy (RP) and/or clinical metastasis) and recurrence free survival after RP for the evaluation of prognostic markers characterizing disease progression or progression free survival.
We tested this approach using the gene expression data for TMEFF2, NPY, CRISP3, SMOC2, PLA2G7, SPOCK, and F5. The results are shown in Figure 9. After training with normalized gene expression results, the ability of the ANN to correctly classify samples from patients having a well-differentiated (moderate risk) tumor and from patients having a poorly-differentiated (high risk) tumor was tested. The test (Figure 9) was performed to evaluate the representative ANN model using the leave-one-out method. The resulting ANN model was able to correctly classify 90% of the well-differentiated samples and 80% of the poorly differentiated samples. Accordingly, this result shows that data from the diagnostic and prognostic panels of biomarkers can be used to train diagnostic and prognostic ANNs. Such ANN models can in turn utilize data from patient biofluids, such as serum, to diagnose or prognose prostate cancer without the need for biopsy.

Citation of references herein shall not be construed as an admission that such references are prior art.
CLAIMS

What is claimed is:

1. A diagnostic kit for prostate cancer comprising reagents for detecting or measuring two or more secreted proteins chosen from NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15.

2. The diagnostic kit of claim 1, wherein each of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15 is detected or measured.

3. The diagnostic kit of claim 1, wherein at least one of the reagents in the kit is an antibody.

4. A computer-implemented method for diagnosing prostate cancer comprising:
   - identifying two or more of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PU5 as parameters;
   - receiving initial data values for the identified parameters from a patient with a known disease status;
   - training an artificial neural network using the parameters and received initial data;
   - modifying a template using learning gained by the artificial neural network;
   - and
   - using the modified template to diagnose a new patient based on new input values.

5. The method of claim 4, wherein each of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PM5 is identified as a parameter.

6. A computer readable medium including computer code for causing a processor to perform the steps of:
identifying two or more of NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15 as parameters;
receiving initial data values for the identified parameters from a patient with a known disease status;
training an artificial neural network using the parameters and received initial data;
modifying a template using learning gained by the artificial neural network; and
using the modified template to diagnose a new patient based on new input values.

7. A method of diagnosing prostate cancer, comprising:
   (a) detecting or measuring in a biological sample from a patient the expression of two or more secreted proteins chosen from NPY, SPOCK, CRISP3, PLA2G7, TMEFF2, F5, SMOC, LTF, ACPP, TGM4, MSMB, WIF1, OLFM4, and PI15; and
   (b) comparing, for each secreted protein detected or measured in (a), the results obtained in (a) with the expression of the same protein in a control sample,
wherein altered expression of the two or more secreted proteins in the patient sample relative to the control is indicative of prostate cancer.

8. The method of claim 7, wherein expression is measured by ELISA, protein arrays, or a combination of ELISA and protein arrays.

9. The method of claim 7, wherein the biological sample is chosen from serum, plasma, whole blood, urine, and prostatic fluid.

10. The method of claim 7, wherein comparing step (b) utilizes an artificial neural network.

11. The method according to claim 7, wherein SMOC1 is detected or measured and the biological sample is urine.
12. The method according to claim 7, wherein F5 is detected or measured and the biological sample is urine.

13. The method according to claim 7, wherein SMOC1 and F5 are detected or measured and the biological sample is urine.

14. A prognostic kit for prostate cancer comprising reagents for detecting or measuring two or more secreted proteins chosen from SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164.

15. The prognostic kit of claim 14, wherein each of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 is detected or measured.

16. The prognostic kit of claim 14, wherein at least one of the reagents in the kit is an antibody.

17. A computer-implemented method for prognosing prostate cancer comprising:
identifying two or more of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 as parameters;
receiving initial data values for the identified parameters from a patient with a known disease status;
training an artificial neural network using the parameters and received initial data;
modifying a template using learning gained by the artificial neural network;
and
using the modified template to prognose a new patient based on new input values.

18. The method of claim 17, wherein each of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 is identified as a parameter.
19. A computer readable medium including computer code for causing a processor to perform the steps of:
   identifying two or more of SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164 as parameters;
   receiving initial data values for the identified parameters from a patient with a known disease status;
   training an artificial neural network using the parameters and received initial data;
   modifying a template using learning gained by the artificial neural network;
   and
   using the modified template to prognose a new patient based on new input values.

20. A method of prognosing prostate cancer, comprising:
   (a) detecting or measuring in a biological sample from a patient the expression of two or more secreted proteins chosen from SPOCK, PDGFD, CHGA, CAV1, RLN1, IGFBP7, BGN, IL6, VEGF, LTF, FMOD, AGR2, SERPINA3, AZGP1, FAM3B, and CD164; and
   (b) comparing, for each secreted protein detected or measured in (a), the results obtained in (a) with the expression of the same protein in a control sample,
wherein altered expression of the two or more secreted proteins in the patient sample relative to the control is predictive of metastatic prostate cancer or prostate cancer recurrence.

21. The method of claim 20, wherein expression is measured by ELISA, protein arrays, or a combination of ELISA and protein arrays.

22. The method of claim 20, wherein the biological sample is chosen from serum, plasma, whole blood, urine, and prostatic fluid.

23. The method of claim 20, wherein comparing step (b) utilizes an artificial neural network.
Figure 1

Gene

NPY

SPOCK

CRISP3

CPDR database

Oncomine database

Normalized expression unit

Normalized expression units

Normalized expression units

Normalized expression units
Figure 1 (continuation)

PLA2G7

TMEFF2

F5

SMOC2
Figure 3

A. 

B. 

C. 

D. 

E. 

<table>
<thead>
<tr>
<th>Specificities</th>
<th>Group 1*</th>
<th>Group 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity Cutoffs (%)</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>PLA2G7</td>
<td>55%</td>
<td>41%</td>
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<tr>
<td>tPSA</td>
<td>47%</td>
<td>39%</td>
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<tr>
<td>SVM</td>
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<td>59%</td>
</tr>
<tr>
<td>ANN</td>
<td>69%</td>
<td>65%</td>
</tr>
</tbody>
</table>
Figure 5

CRISP3

[Graph showing data for CRISP3 with bars and error bars]

Control (n=11)
CaP (n=20)

[Graph showing box plots for CTL and CaP with whiskers and box]

CTL
CaP
Figure 6

A. 

B. 

C.
Figure 8

- One Gene: 40% CaP, 60% Normal
- Two Genes: 35% CaP, 65% Normal
- Three Genes: 40% CaP, 60% Normal
- Four Genes: 50% CaP, 50% Normal
- Five Genes: 62% CaP, 38% Normal
- Six Genes: 33% CaP, 67% Normal
- Seven Genes: 20% CaP, 80% Normal
Figure 9

Histogram of log(Data)

Normalization

Transfer function

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<tr>
<th></th>
<th>Calculated as WD</th>
<th>Calculated as PD</th>
</tr>
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<td>True WD (90%)</td>
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<td>3</td>
</tr>
<tr>
<td>True PD (80%)</td>
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<td>16</td>
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</table>