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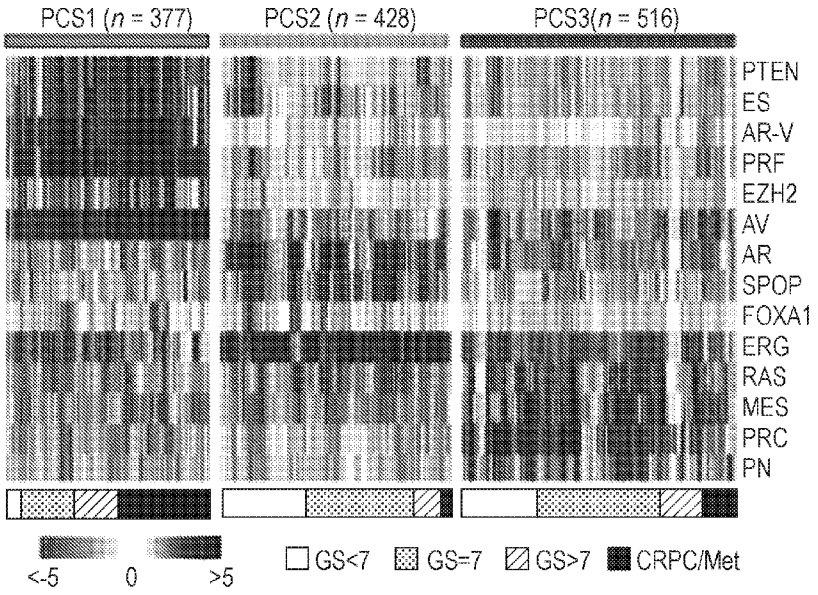
(54) Title: METHOD OF CLASSIFYING AND DIAGNOSING CANCER

FIG. 2C



(57) Abstract: The invention provides various methods for classifying prostate cancers into subtypes. The classification methods may be used to diagnose or prognose prostate cancers. In one embodiment, the subtypes are PCS1, PCS2, or PCS3. In one embodiment, the PCS1 subtype is most likely to progress to metastatic disease or prostate cancer specific mortality when compared to the PCS2 subtype or PCS3 subtype. In one embodiment, the PCS1 subtype is resistant to enzalutamide.

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METHOD OF CLASSIFYING AND DIAGNOSING CANCER

GOVERNMENT RIGHTS

[0001] This invention was made with government support under DK087806, CA143777, and CA098912 awarded by the National Institutes of Health and under W81XWH-14-1-0152 awarded by the Department of Defense. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] The invention relates to medicine and oncology, for example, methods, compositions and kits for classifying cancers and methods, compositions and kits for treating cancers.

BACKGROUND

[0003] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0004] Prostate cancer (PC) is a heterogeneous disease. Currently defined molecular subtypes are based on gene translocations, gene expression, mutations, and oncogenic signatures. In other cancer types, such as breast cancer, molecular classifications predict survival and are routinely used to guide treatment decisions. However, the heterogeneous nature of prostate cancer, and the relative paucity of redundant genomic alterations that drive progression, or that can be used to assess likely response to therapy, have hindered attempts to develop a classification system with clinical relevance.

[0005] Recently, molecular lesions in aggressive prostate cancer have been identified. For example, overexpression of the androgen receptor (*AR*) due to gene amplification has been observed in castration-resistant prostate cancer (CRPC). Presence of AR variants (AR-V) that do not require ligand for activation have been reported in a large percentage of CRPCs and have been correlated with resistance to AR-targeted therapy. The oncogenic function of enhancer of zeste homolog 2 (*EZH2*) was found in cells of CRPC, and recurrent mutations in the speckle-type POZ protein (*SPOP*) gene occur in approximately 15% of prostate cancers. Expression signatures related to these molecular lesions have also been developed to predict

patient outcomes. While, in principle, signature-based approaches could be used independently in small cohorts, there is a potential for an increase in diagnostic or prognostic accuracy if signatures reflecting gene expression perturbations relevant to prostate cancer could be applied to large cohorts containing thousands of clinical specimens.

[0006] Here we present the results of an integrated analysis of an unprecedentedly large set of transcriptome data, including from over 4,600 clinical prostate cancer specimens. This study revealed that RNA expression data can be used to categorize prostate cancer tumors into 3 distinct subtypes, based on molecular pathway representation encompassing molecular lesions and cellular features related to prostate cancer biology. Application of this sub-typing scheme to 10 independent cohorts and a wide range of preclinical prostate cancer models strongly suggest that the subtypes we define originate from inherent differences in prostate cancer origins and/or biological features. We provide evidence that this novel prostate cancer classification scheme can be useful for detection of aggressive tumors using tissue as well as blood from patients with progressing disease. It also provides a starting point for development of subtype-specific treatment strategies and companion diagnostics

[0007] As such, for an informed clinical decision, there still exists a great need for methods, compositions and kits that can categorize/classify/stratify/subtype PC and methods, compositions and kits that can treat PC.

SUMMARY OF THE INVENTION

[0008] The following embodiments and aspects thereof are described and illustrated in conjunction with compositions, methods, systems, and kits which are meant to be exemplary and illustrative, not limiting in scope.

[0009] Various embodiments of the present invention provide a method for classifying prostate cancer into subtypes, comprising: a) obtaining a sample from a subject; b) assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; c) determining the presence of an expression pattern of the one or more genes associated with the subtype in the sample based on the detected changes; and d) classifying the cancer in the subject into the subtype if the expression pattern of the one or more genes associated with the subtype is detected in the sample. In some embodiments, the subtype is PCS1, PCS2, or PCS3. In some embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes listed in Table 1. In some

embodiments, the genes are STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In some embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In some embodiments, the sample is a tissue sample or blood. In some embodiments, the sample is a prostate tissue or blood circulating tumor cells. In some embodiments, the blood circulating tumor cells are classified into the PCS1 subtype. In some embodiments, the PCS1 subtype is resistant to enzalutamide. In some embodiments, the PCS1 subtype is characterized in that it has an increased probability of progressing to metastatic disease or prostate cancer specific mortality when compared to the PCS2 subtype or PCS3 subtype. In some embodiments, wherein the PCS1 subtype has increased expression levels in STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, and KNTC1 genes; and decreased expression levels in RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45 genes. In some embodiments, the PCS2 subtype has increased expression levels in RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2 genes; and decreased expression levels in STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45 genes. In some embodiments, the PCS3 subtype has increased expression levels in CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45 genes; and decreased expression levels in STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2 genes. In some embodiments, the subtype is PCS1, and the method further comprises administering to the subject a therapeutically effective amount of one or more DNA damaging agents selected from cisplatin, PARP inhibitors, or combinations thereof. In some embodiments, the subtype is PCS2, and the method further comprises

administering to the subject a therapeutically effective amount of an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, an androgen synthesis inhibitor, enzalutamide, a mitotic inhibitor, or docetaxel, or combinations thereof. In some embodiments, the subtype is PCS3, and the method further comprises administering to the subject a therapeutically effective amount of dasatinib or docetaxel, or combinations thereof.

[0010] Various embodiments of the present invention provide a method for prognosing a cancer in a subject, comprising; a) obtaining a sample from the subject; b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values; c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; and d) prognosing the cancer in the subject. In some embodiments, the subtype is PCS1, and the cancer is prognosed with a poor clinical outcome. In some embodiments, the poor clinical outcome comprises lower metastasis-free survival, higher risk of metastatic progression, higher rate of cancer specific mortality, lower overall survival, or more aggressive form of cancer, or a combination thereof.

[0011] Various embodiments of the present invention provide a method for treating, preventing, reducing the likelihood of having, reducing the severity of and/or slowing the progression of a cancer in a subject, comprising: a) obtaining a sample from the subject; b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values; c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; and d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of and/or slowing the progression of the cancer. In some embodiments, the subtype is PCS1, and the administered therapeutic agent is one or more DNA damaging agents selected from cisplatin, PARP inhibitors, or combinations thereof. In some embodiments, the subtype is PCS1, and the administered therapeutic agent is a mitotic inhibitor. In some embodiments, the subtype is PCS1, and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof. In some embodiments, the subtype is PCS2, and the administered therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In some embodiments, the subtype is PCS2, and the administered therapeutic agent is enzalutamide, or a functional equivalent, analog, derivative or salt of enzalutamide, or a combination thereof. In some embodiments, the subtype is PCS2, and the

administered therapeutic agent is a mitotic inhibitor. In some embodiments, the subtype is PCS2, and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof. In some embodiments, the subtype is PCS3, and the administered therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof. In some embodiments, the subtype is PCS3, and the administered therapeutic agent is dasatinib, or a functional equivalent, analog, derivative or salt of dasatinib, or a combination thereof. In some embodiments, the subtype is PCS3 and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

[0012] Various embodiments of the present invention provide a method for treating, preventing, reducing the likelihood of having, reducing the severity of and/or slowing the progression of a cancer in a subject, comprising; a) obtaining a sample from the subject; b) assaying the sample to detect a marker for a subtype of the cancer; c) detecting the marker for the subtype in the sample; and d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of and/or slowing the progression of the cancer. In some embodiments, the marker for the subtype comprises: a) an increased expression level in one, two, three, four, five, six, or more, or all of the PCS1 SEGs (SubtypeID = 1) listed in Table 1; and/or b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS1 SEGs (SubtypeID $\neq$ 1) listed in Table 1. In some embodiments, the marker for the subtype comprises: a) an increased expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, and KNTC1; and/or b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In some embodiments, the marker for the subtype comprises: a) an increased expression level in one, two, three, four, five, six, or more, or all of the PCS2 SEGs (SubtypeID=2) listed in Table 1; and/or b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS2 SEGs (SubtypeID $\neq$ 2) listed in Table 1. In some embodiments, the marker for the subtype comprises: a) an increased expression level in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2; and/or b) a decreased or

insignificantly changed expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In some embodiments, the marker for the subtype comprises: a) an increased expression level in one, two, three, four, five, six, or more, or all of the PCS3 SEGs (SubtypeID=3) listed in Table 1; and/or b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS3 SEGs (SubtypeID $\neq$ 3) listed in Table 1. In some embodiments, the marker for the subtype comprises: a) an increased expression level in one, two, three, four, five, six, or more, or all of CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45; and/or b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2.

[0013] Various embodiments of the present invention provide a method for classifying a prostate cancer into a prostate cancer subtype, comprising: a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens; b) converting the pathway activation gene expression signatures into pathway activation profiles; c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to the prostate cancer subtype; and d) classifying the prostate cancer into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the prostate cancer. In some embodiments, the pathway activation profiles are selected from PTEN, ES, AR-V, PRF, EZH2, AV, AR, SPOP, FOXA1, ERG, RAS, MES, PRC, and PN. In some embodiments, the prostate cancer subtype is PCS1, PCS2, or PCS3. In some embodiments, the PCS1 subtype comprises pathway activation profiles PTEN, ES, AR-V, PRF, EZH2, or AV, or combinations thereof; the PCS2 subtype comprises pathway activation profiles AR, SPOP, FOXA1, or ERG, or combinations thereof; and the PCS3 subtype comprises pathway activation profiles RAS, MES, PRC, or PN, or combinations thereof. In some embodiments, determining pathway activation gene expression signatures in the prostate cancer specimens comprises: a) obtaining a first prostate cancer dataset, wherein the first prostate cancer dataset comprises gene expression profiles; b) selecting a second prostate cancer dataset from the first prostate dataset, wherein the

second prostate cancer dataset is numerically smaller than the first prostate cancer dataset; c) normalizing the second prostate cancer dataset; d) removing gene expression profiles for benign prostate tissues; and e) normalizing the gene expression profiles to obtain a merged dataset comprising the pathway activation gene expression signatures. In some embodiments, the gene expression profiles comprise gene expression profiles for benign prostate tissues and gene expression profiles for malignant prostate tissues. In some embodiments, the malignant prostate tissues are primary tumors, metastatic prostate cancers, or castration resistant prostate cancers, or combinations thereof. In some embodiments, normalizing the second prostate cancer dataset is performed using a quantile method. In some embodiments, normalizing the gene expression profiles is performed using median centering and quantile scaling. In some embodiments, converting the pathway activation gene expression signatures into pathway activation profiles comprises: a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization; b) calculating Z-scores for the pathway activation gene expression signatures; and c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures. In some embodiments, grouping the pathway activation profiles into independent clusters comprises, determining a number of independent clusters by applying a consensus non-negative matrix factorization clustering method.

[0014] Various embodiments of the present invention provide a method for classifying a prostate cancer in a subject, comprising: a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens; b) converting the pathway activation gene expression signatures into pathway activation profiles; c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to a prostate cancer subtype; d) obtaining a sample from the subject; e) determining a pathway activation profile in the sample; and f) classifying the prostate cancer in the subject into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the sample. In some embodiments, the pathway activation profiles are selected from PTEN, ES, AR-V, PRF, EZH2, AV, AR, SPOP, FOXA1, ERG, RAS, MES, PRC, and PN. In some embodiments, the prostate cancer subtype is PCS1, PCS2, or PCS3. In some embodiments, the PCS1 subtype comprises pathway activation profiles PTEN, ES, AR-V, PRF, EZH2, or AV, or combinations thereof; the PCS2 subtype comprises pathway activation profiles AR, SPOP, FOXA1, or ERG, or combinations thereof; and the PCS3

subtype comprises pathway activation profiles RAS, MES, PRC, or PN, or combinations thereof. In some embodiments, the PCS1 subtype is characterized in that it has an increased probability of progressing to metastatic disease or prostate cancer specific mortality when compared to the PCS2 subtype or PCS3 subtype. In some embodiments, determining pathway activation gene expression signatures in the prostate cancer specimens comprises: a) obtaining a first prostate cancer dataset, wherein the first prostate cancer dataset comprises gene expression profiles; b) selecting a second prostate cancer dataset from the first prostate dataset, wherein the second prostate cancer dataset is numerically smaller than the first prostate cancer dataset; c) normalizing the second prostate cancer dataset; d) removing gene expression profiles for benign prostate tissues; and e) normalizing the gene expression profiles to obtain a merged dataset comprising the pathway activation gene expression signatures. In some embodiments, the gene expression profiles comprise gene expression profiles for benign prostate tissues and gene expression profiles for malignant prostate tissues. In some embodiments, the malignant prostate tissues are primary tumors, metastatic prostate cancers, or castration resistant prostate cancers, or combinations thereof. In some embodiments, normalizing the second prostate cancer dataset is performed using a quantile method. In some embodiments, normalizing the gene expression profiles is performed using median centering and quantile scaling. In some embodiments, converting the pathway activation gene expression signatures into pathway activation profiles comprises: a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization; b) calculating Z-scores for the pathway activation gene expression signatures; and c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures. In some embodiments, grouping the pathway activation profiles into independent clusters comprises, determining a number of independent clusters by applying a consensus non-negative matrix factorization clustering method. In some embodiments, the sample is a tissue sample or blood. In some embodiments, the sample is a prostate tissue or blood circulating tumor cells. In some embodiments, the blood circulating tumor cells are classified into the PCS1 subtype. In some embodiments, the method further comprises identifying the cancer as having resistance to enzalutamide.

BRIEF DESCRIPTION OF DRAWINGS

[0015] Fig. 1A-Fig. 1E illustrate, in accordance with various embodiments of the present invention, integration of prostate cancer transcriptome and quality control. **Fig. 1A**,

schematic showing the process of collecting and merging prostate cancer transcriptomes. **Fig. 1B**, clinical composition of 2,115 prostate cancer cases. **Fig. 1C**, MDS of merged expression profiles after MCQ or XPN correction in the DISC cohort. Dots with different colors and/or shading represent different batches or datasets. **Fig. 1D**, hierarchical clustering illustrates the sample distribution of uncorrected (top), corrected by MCQ (middle), and corrected by XPN (bottom). Different colors and/or shading on "Batches" rows represent different batches or datasets from the individual studies. **Fig. 1E**, MDS of pathway activation profiles in the DISC cohort shows distribution of the samples from same batches. Dots with different colors and/or shading represent different batches or datasets.

[0016] Fig. 2A-Fig. 2J illustrate, in accordance with various embodiments of the present invention, Identification and validation of novel prostate cancer subtypes. **Fig. 2A,** consensus matrix depicts robust separation of tumors into three subtypes. **Fig. 2B,** changes of cophenetic coefficient and silhouette score at rank 2 to 6. **Fig. 2C,** pathway activation profiles of 1,321 tumors defines three prostate cancer subtypes. **Fig. 2D,** score plot of PCA for benign and three subtypes. **Fig. 2E** and **Fig. 2F,** the three subtypes were recognized in 10 independent cohorts. **Fig. 2G** and **Fig. 2H,** correlation of pathway activation profiles in 8 prostate cancer cell lines from the CCLE and 11 prostate cancer mouse models and probability from the pathway classifier. **Fig. 2I** depicts the pathway activation scores. **Fig. 2J** depicts the Z score of benign signature.

[0017] Fig. 3A – Fig. 3H(i) – (x) illustrate, in accordance with various embodiments of the present invention, comparison of the PCS subtypes with previously described subtypes. **Fig. 3A**, distribution of TCGA tumors (n = 333) using the PCS subtypes compared with TCGA subtypes. **Fig. 3B**, relationship between PCS subtyping and TCGA subtypes. **Fig. 3C**, distribution of GRID tumors (n = 1,626) using PCS categories compared with Tomlins subtypes. **Fig. 3D**, relationship between PCS subtyping and Tomlins subtypes. **Fig. 3E** and **Fig. 3F**, association of metastasis-free survival using Tomlins subtypes and using the PCS subtypes in the GRID tumors. **Fig. 3G**, metastasis-free survival in tumors of GS $\leq$ 7 (left) and GS $\geq$ 8 (right). **Fig. 3H(i) - (x)** depicts the correlation of the subtypes with clinical outcomes in independent cohorts.

[0018] Fig. 4A-Fig. 4E illustrates, in accordance with various embodiments of the present invention, genes enriched in each of the three subtypes are associated with luminal and basal cell features. **Fig. 4A**, relative gene expression (left) and pathway inclusion (right) of SEGs

are displayed. **Fig. 4B**, cellular processes enriched by each of the three subtype enriched genes (SEGs) ($P < 0.05$). **Fig. 4C**, expression of the luminal and basal markers in the three subtypes. **Fig. 4D**, enrichment of basal stem cell signature. **Fig. 4E**, correlation of pathway activities between samples from human and mouse prostate (left) and probability from the pathway classifier (right).

[0019] Fig. 5A-Fig. 5D illustrates, in accordance with various embodiments of the present invention, a 37-gene classifier employed in patient tissues and CTCs. **Fig. 5A,** heatmap displays the mean expression pattern of the 37-gene panel in the three subtypes from the DISC cohort. **Fig. 5B,** hierarchical clustering of 77 CTCs obtained from CRPC patients by gene expression of the 37-gene panel. Bar plot in the bottom displays probability of PCS assignment from application of the classifier. **Fig. 5C**, schematic showing process of gene selection from 428 SEGs. **Fig. 5D**, graph showing comparison of mean squared errors (MSE) of 428 genes and 37 genes.

DETAILED DESCRIPTION OF THE INVENTION

[0020] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Allen *et al.*, *Remington: The Science and Practice of Pharmacy 22nd ed.*, Pharmaceutical Press (September 15, 2012); Hornyak *et al.*, *Introduction to Nanoscience and Nanotechnology*, CRC Press (2008); Singleton and Sainsbury, *Dictionary of Microbiology and Molecular Biology 3rd ed., revised ed.*, J. Wiley & Sons (New York, NY 2006); Smith, *March's Advanced Organic Chemistry Reactions, Mechanisms and Structure 7th ed.*, J. Wiley & Sons (New York, NY 2013); Singleton, *Dictionary of DNA and Genome Technology 3rd ed.*, Wiley-Blackwell (November 28, 2012); and Green and Sambrook, *Molecular Cloning: A Laboratory Manual 4th ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application. For references on how to prepare antibodies, see Greenfield, *Antibodies A Laboratory Manual 2nd ed.*, Cold Spring Harbor Press (Cold Spring Harbor NY, 2013); Köhler and Milstein, *Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion*, Eur. J. Immunol. 1976 Jul, 6(7):511-9; Queen and Selick, *Humanized immunoglobulins*, U. S. Patent No. 5,585,089 (1996 Dec); and Riechmann *et al.*, *Reshaping human antibodies for therapy*, Nature 1988 Mar 24, 332(6162):323-7.

[0021] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention. Indeed, the present invention is in no way limited to the methods and materials described. For convenience, certain terms employed herein, in the specification, examples and appended claims are collected here.

[0022] Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. Unless explicitly stated otherwise, or apparent from context, the terms and phrases below do not exclude the meaning that the term or phrase has acquired in the art to which it pertains. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The definitions and terminology used herein are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims.

[0023] As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.).

[0024] Unless stated otherwise, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of claims) can be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and

all examples, or exemplary language (for example, "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the application and does not pose a limitation on the scope of the application otherwise claimed. The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example." No language in the specification should be construed as indicating any non-claimed element essential to the practice of the application.

[0025] As used herein, the terms "treat," "treatment," "treating," or "amelioration" when used in reference to a disease, disorder or medical condition, refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, reverse, alleviate, ameliorate, inhibit, lessen, slow down or stop the progression or severity of a symptom or condition. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of a condition. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of a disease, disorder or medical condition is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Also, "treatment" may mean to pursue or obtain beneficial results, or lower the chances of the individual developing the condition even if the treatment is ultimately unsuccessful. Those in need of treatment include those already with the condition as well as those prone to have the condition or those in whom the condition is to be prevented.

[0026] "Beneficial results" or "desired results" may include, but are in no way limited to, lessening or alleviating the severity of the disease condition, preventing the disease condition from worsening, curing the disease condition, preventing the disease condition from developing, lowering the chances of a patient developing the disease condition, decreasing morbidity and mortality, and prolonging a patient's life or life expectancy. As non-limiting examples, "beneficial results" or "desired results" may be alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (i.e., not worsening) state of a tumor, delay or slowing of a tumor, and amelioration or palliation of symptoms associated with a tumor.

[0027] "Disorders", "diseases", "conditions" and "disease conditions," as used herein may include, but are in no way limited to any form of malignant neoplastic cell proliferative disorders or diseases. Examples of such disorders include but are not limited to cancer and tumor.

[0028] A "cancer" or "tumor" as used herein refers to an uncontrolled growth of cells which interferes with the normal functioning of the bodily organs and systems, and/or all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. A subject that has a cancer or a tumor is a subject having objectively measurable cancer cells present in the subject's body. Included in this definition are benign and malignant cancers, as well as dormant tumors or micrometastasis. Cancers which migrate from their original location and seed vital organs can eventually lead to the death of the subject through the functional deterioration of the affected organs. As used herein, the term "invasive" refers to the ability to infiltrate and destroy surrounding tissue.

[0029] As used herein, the term "administering," refers to the placement an agent as disclosed herein into a subject by a method or route which results in at least partial localization of the agents at a desired site. "Route of administration" may refer to any administration pathway known in the art, including but not limited to aerosol, nasal, via inhalation, oral, transmucosal, transdermal, parenteral, enteral, topical or local. "Parenteral" refers to a route of administration that is generally associated with injection, including intracranial, intraventricular, intrathecal, epidural, intradural, intraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Via the parenteral route, the compositions may be in the form of solutions or suspensions for infusion or for injection, or as lyophilized powders. Via the enteral route, the pharmaceutical compositions can be in the form of tablets, gel capsules, sugar-coated tablets, syrups, suspensions, solutions, powders, granules, emulsions, microspheres or nanospheres or lipid vesicles or polymer vesicles allowing controlled release. Via the topical route, the pharmaceutical compositions can be in the form of aerosol, lotion, cream, gel, ointment, suspensions, solutions or emulsions. In accordance with the present invention, "administering" can be self-administering. For example, it is considered as "administering" that a subject consumes a composition as disclosed herein.

[0030] The term "sample" or "biological sample" as used herein denotes a sample taken or isolated from a biological organism, e.g., a tumor sample from a subject. Exemplary biological samples include, but are not limited to, cheek swab; mucus; whole blood, blood, serum; plasma; urine; saliva; semen; lymph; fecal extract; sputum; other body fluid or biofluid; cell sample; tissue sample; tumor sample; and/or tumor biopsy etc. The term also includes a mixture of the above-mentioned samples. The term "sample" also includes untreated or pretreated (or pre-processed) biological samples. In some embodiments, a sample can comprise one or more cells from the subject. In some embodiments, a sample can be a tumor cell sample, e.g. the sample can comprise cancerous cells, cells from a tumor, and/or a tumor biopsy.

[0031] As used herein, a "subject" means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomologous monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, and canine species, e.g., dog, fox, wolf. The terms, "patient", "individual" and "subject" are used interchangeably herein. In an embodiment, the subject is mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but are not limited to these examples. In addition, the methods described herein can be used to treat domesticated animals and/or pets. In one embodiment, the subject is human.

[0032] "Mammal" as used herein refers to any member of the class Mammalia, including, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be included within the scope of this term.

[0033] A subject can be one who has been previously diagnosed with or identified as suffering from or having a condition in need of treatment (e.g., prostate cancer) or one or more complications related to the condition, and optionally, have already undergone treatment for the condition or the one or more complications related to the condition. Alternatively, a subject can also be one who has not been previously diagnosed as having a

condition or one or more complications related to the condition. For example, a subject can be one who exhibits one or more risk factors for a condition or one or more complications related to the condition or a subject who does not exhibit risk factors. A "subject in need" of treatment for a particular condition can be a subject suspected of having that condition, diagnosed as having that condition, already treated or being treated for that condition, not treated for that condition, or at risk of developing that condition.

[0034] The term "statistically significant" or "significantly" refers to statistical evidence that there is a difference. It is defined as the probability of making a decision to reject the null hypothesis when the null hypothesis is actually true. The decision is often made using the p-value.

[0035] As used herein, "variants" can include, but are not limited to, those that include conservative amino acid mutations, SNP variants, splicing variants, degenerate variants, and biologically active portions of a gene. A "degenerate variant" as used herein refers to a variant that has a mutated nucleotide sequence, but still encodes the same polypeptide due to the redundancy of the genetic code.

[0036] The term "functional" when used in conjunction with "equivalent", "analog", "derivative" or "variant" or "fragment" refers to an entity or molecule which possess a biological activity that is substantially similar to a biological activity of the entity or molecule of which it is an equivalent, analog, derivative, variant or fragment thereof.

[0037] As used herein, the term "antiandrogen" (also interchangeably called as androgen signaling inhibitor or blocker) refers to any agent that inhibits the androgen signaling, including inhibition of any molecular signaling steps upstream or downstream of androgen. An antiandrogen can be a small molecule; a nucleic acid such as siRNA, shRNA, and miRNA; a nucleic acid analogue such as PNA, pc-PNA, and LNA; an aptamer; a ribozyme; a peptide; a protein; an avimer; or an antibody, or variants and fragments thereof. Antiandrogens prevent androgens from expressing their biological effects on responsive cells, tissues and organs. Antiandrogens alter the androgen pathway by inhibiting androgen receptors (ARs) or suppressing androgen production. Examples of antiandrogens include but are not limited to AR ligands such as AR antagonists and selective AR modulators (SARMs), and androgen synthesis inhibitors such as enzyme inhibitors and antigonadotropins. Examples of AR antagonists include but are not limited to flutamide, nilutamide, bicalutamide, enzalutamide, apalutamide, cyproterone acetate, megestrol acetate,

chlormadinone acetate, spironolactone, canrenone, drospirenone, ketoconazole, topilutamide (fluridil), and cimetidine. Examples of SARMs include but are not limited to andarine and enobosarm (ostarine). Examples of enzyme inhibitors include but are not limited to 5α-reductase inhibitors (e.g., finasteride, dutasteride, alfatradiol, and saw palmetto extract), CYP17A1 (e.g., 17α-hydroxylase/17,20-lyase) inhibitors (e.g., cyproterone acetate, spironolactone, danazol, gestrinone, ketoconazole, and abiraterone acetate), 3β-Hydroxysteroid dehydrogenase inhibitors (e.g., danazol, gestrinone, and abiraterone acetate), 17β-Hydroxysteroid dehydrogenase inhibitors (e.g., danazol and simvastatin), CYP11A1 (cholesterol side-chain cleavage enzyme) inhibitors (e.g., aminoglutethimide and danazol), and HMG-CoA reductase inhibitors (e.g., statins such as atorvastatin, simvastatin). Examples of antigonadotropins include but are not limited to progestogens (e.g., progesterone, cyproterone acetate, medroxyprogesterone acetate, megestrol acetate, chlormadinone acetate, spironolactone, and drospirenone), estrogens (e.g., estradiol, ethinyl estradiol, diethylstilbestrol, and conjugated equine estrogens), GnRH analogues such as GnRH agonists (e.g., buserelin, deslorelin, gonadorelin, goserelin, histrelin, leuprorelin, nafarelin, and triptorelin) and GnRH antagonists (e.g., abarelix, cetrorelix, degarelix, and ganirelix), and anabolic steroids (e.g., nandrolone and oxandrolone).

[0038] As used herein, the term "Src signaling inhibitor" (also interchangeably called as Src signaling blocker, Src inhibitor, Src blocker, anti-Src agent, reagent, molecule, compound, or drug) refers to any agent that inhibits the Src signaling, including inhibition of any molecular signaling steps upstream or downstream of Src. A Src signaling inhibitor can be a small molecule; a nucleic acid such as siRNA, shRNA, and miRNA; a nucleic acid analogue such as PNA, pc-PNA, and LNA; an aptamer; a ribozyme; a peptide; a protein; an avimer; or an antibody, or variants and fragments thereof. Examples of Src signaling inhibitor include but are not limited to Src family tyrosine kinase inhibitor and Bcr-Abl tyrosine kinase inhibitor. Examples of Bcr-Abl tyrosine kinase inhibitor include but are not limited to imatinib, bafetinib, nilotinib, dasatinib, bosutinib, ponatinib, and 1,3,4 thiadiazole derivatives such as substance 14.

[0039] As used herein, the term "mitotic inhibitor" or "mitotic blocker" refers to any agent that inhibits mitosis or cell division, including inhibition of any molecular signaling steps involved in mitosis or cell division. A mitotic inhibitor can be a small molecule; a nucleic acid such as siRNA, shRNA, and miRNA; a nucleic acid analogue such as PNA, pc-PNA, and LNA; an aptamer; a ribozyme; a peptide; a protein; an avimer; or an antibody, or variants

and fragments thereof. Mitotic inhibitors interfere with the assembly and disassembly of tubulin into microtubule polymers, which are structures that pull the cell apart when it divides. Examples of mitotic inhibitors include but are not limited to taxanes, vinca alkaloids, colchicine, podophyllotoxin, and griseofulvin. Examples of taxanes include but are not limited to paclitaxel, docetaxel, and cabazitaxel. Examples of vinca alkaloids include but are not limited to vinblastine, vincristine, vindesine, and vinorelbine.

[0040] As used herein, the terms "categorizing", "classifying", "stratifying", "subtyping", and "subgrouping" are interchangeable. As used herein, the terms "category", "class", "strata", "subtype", and "subgroup" are interchangeable. As used herein in, the terms "profile", "pattern", and "signature" are interchangeable. For example, "expression profile", "expression pattern", and "expression signature" are interchangeable, and "pathway activation profile", "pathway activation pattern", and "pathway activation signature" are interchangeable.

[0041] As used herein, the terms "computed" and "calculated" are interchangeable. As used herein, the terms "computing" and "calculating" are interchangeable.

[0042] In various embodiments of the present invention, the inventors describe an integrated approach involving an atypically large set of transcriptome data from over 4,600 clinical prostate cancer (PC) specimens via analysis based on pathway activation in order to identify clinically relevant prostate cancer subtypes. This approach has resulted in three distinct prostate cancer subtypes. The inventors validated the three subtypes and their prognostic significance using data from the independent patient series and various prostate cancer models. By further analyzing the gene expression profiles of the three subtypes, the inventors identified genes enriched in each of the three prostate cancer subtypes, which are associated with cell types of origin of the prostate cancer, and investigated potential therapeutic implications of the subtypes. Finally, the inventors present a 37 gene panel that can classify prostate cancer in patients into the subtypes for preclinical, clinical, and translational applications. The inventors present evidence that this new prostate cancer classification scheme may improve prognostic accuracy of evaluation of low grade tumors and may enable the development of subtype-specific therapies and companion diagnostics.

Classification System/Classification Method

[0043] In various embodiments, the present invention provides a method for classifying a prostate cancer into a prostate cancer subtype, comprising: a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens; b) converting the pathway activation gene expression signatures into pathway activation profiles; c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to the prostate cancer subtype; and d) classifying the prostate cancer into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the prostate cancer. In some embodiments, determining pathway activation gene expression signatures in the prostate cancer specimens comprises, a) obtaining a first prostate cancer dataset, wherein the first prostate cancer dataset comprises gene expression profiles (for example as shown in **Fig. 1A** "50 PC Datasets"); b) selecting a second prostate cancer dataset from the first prostate dataset, wherein the second prostate cancer dataset is numerically smaller than the first prostate cancer dataset (for example as shown in **Fig. 1A** "38 PC Datasets") ; c) normalizing the second prostate cancer dataset; d) removing gene expression profiles for benign prostate tissues; and e) normalizing the gene expression profiles to obtain a merged dataset comprising the pathway activation gene expression signatures. In some embodiments, the gene expression profiles comprise gene expression profiles for benign prostate tissues and gene expression profiles for malignant prostate tissues. In some embodiments, the malignant prostate tissues are primary tumors, metastatic prostate cancers, or castration resistant prostate cancers, or combinations thereof. In some embodiments, the second prostate cancer dataset is performed using a quantile method (Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–93). In some embodiments, the normalizing the gene expression profiles is performed using median centering and quantile scaling (You S, Cho CS, Lee I, Hood L, Hwang D, Kim WU. A systems approach to rheumatoid arthritis. PLoS One 2012;7:e51508). In some embodiments, converting the pathway activation gene expression signatures into pathway activation profiles comprises, a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization; b) calculating Z-scores for the pathway activation gene expression signatures; and c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures.

In some embodiments, grouping the pathway activation profiles into independent clusters comprises, determining a number of independent clusters by applying a consensus non-negative matrix factorization clustering method. In some embodiments, the pathway activation profiles obtained from the classification method described herein are selected from but not limited to PTEN, ES, AR-V, PRF, EZH2, AV, AR, SPOP, FOXA1, ERG, RAS, MES, PRC, and PN. In some embodiments, the prostate cancer subtype is PCS1, PCS2, or PCS3. In some embodiments, the PCS1 subtype comprises pathway activation profiles PTEN, ES, AR-V, PRF, EZH2, or AV, or combinations thereof; the PCS2 subtype comprises pathway activation profiles AR, SPOP, FOXA1, or ERG, or combinations thereof; and the PCS3 subtype comprises pathway activation profiles RAS, MES, PRC, or PN, or combinations thereof.

[0044] In various embodiments, the present invention provides a method for classifying a prostate cancer in a subject, comprising: a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens; b) converting the pathway activation gene expression signatures into pathway activation profiles; c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to a prostate cancer subtype; d) obtaining a sample from the subject; e) determining a pathway activation profile in the sample; and f) classifying the prostate cancer in the subject into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the sample. In some embodiments, determining pathway activation gene expression signatures in the prostate cancer specimens comprises: a) obtaining a first prostate cancer dataset, wherein the first prostate cancer dataset comprises gene expression profiles (for example as shown in **Fig. 1A** "50 PC Datasets"); b) selecting a second prostate cancer dataset from the first prostate dataset, wherein the second prostate cancer dataset is numerically smaller than the first prostate cancer dataset (for example as shown in **Fig. 1A** "38 PC Datasets"); c) normalizing the second prostate cancer dataset; d) removing gene expression profiles for benign prostate tissues; and e) normalizing the gene expression profiles to obtain a merged dataset comprising the pathway activation gene expression signatures. In some embodiments, the gene expression profiles comprise gene expression profiles for benign prostate tissues and gene expression profiles for malignant prostate tissues. In some embodiments, the malignant prostate tissues are primary tumors, metastatic prostate cancers, or castration resistant prostate cancers, or combinations thereof. In some embodiments, normalizing the second prostate cancer dataset is performed using a quantile

method (Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–93). In some embodiments, normalizing the gene expression profiles is performed using median centering and quantile scaling (You S, Cho CS, Lee I, Hood L, Hwang D, Kim WU. A systems approach to rheumatoid arthritis. PLoS One 2012;7:e51508). In some embodiments, converting the pathway activation gene expression signatures into pathway activation profiles comprises, a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization; b) calculating Z-scores for the pathway activation gene expression signatures; and c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures. In some embodiments, grouping the pathway activation profiles into independent clusters comprises, determining a number of independent clusters by applying a consensus non-negative matrix factorization clustering method. In some embodiments, the pathway activation profiles obtained from the classification method described herein are selected from but not limited to PTEN, ES, AR-V, PRF, EZH2, AV, AR, SPOP, FOXA1, ERG, RAS, MES, PRC, and PN. In some embodiments, the prostate cancer subtype is PCS1, PCS2, or PCS3. In some embodiments, the PCS1 subtype comprises pathway activation profiles PTEN, ES, AR-V, PRF, EZH2, or AV, or combinations thereof; the PCS2 subtype comprises pathway activation profiles AR, SPOP, FOXA1, or ERG, or combinations thereof; and the PCS3 subtype comprises pathway activation profiles RAS, MES, PRC, or PN, or combinations thereof. In some embodiments, the PCS1 subtype is characterized in that it has an increased probability of progressing to metastatic disease and/or prostate cancer specific mortality when compared to the PCS2 subtype or PCS3 subtype. In some embodiments, the sample is a tissue sample or blood. In some embodiments, the sample is a prostate tissue or blood circulating tumor cells. In some embodiments, the blood circulating tumor cells are classified into the PCS1 subtype. In some embodiments, the method further comprises identifying the cancer as having resistance to enzalutamide. In one embodiment, PCS1 subtype prostate cancer is resistant to enzalutamide.

[0045] In various embodiments, the present invention provides a method for classifying prostate cancer into subtypes, comprising: a) obtaining a sample from a subject; b) assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; c) determining the presence of an expression pattern of the one or more

genes associated with the subtype in the sample based on the detected changes; and d) classifying the cancer in the subject into the subtype if the expression pattern of the one or more genes associated with the subtype is detected in the sample. In some embodiments, the subtype is PCS1, PCS2, or PCS3. In some embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes listed in **Table 1**. In some embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In some embodiments, the sample is a tissue sample or blood. In some embodiments, the sample is a prostate tissue or blood circulating tumor cells. In some embodiments, the blood circulating tumor cells are classified into the PCS1 subtype. In some embodiments, the method further comprises identifying the cancer as having resistance to enzalutamide. In one embodiment, PCS1 subtype prostate cancer is resistant to enzalutamide

Diagnostic and Prognostic Methods

[0046] Various embodiments of the present invention provide a method for classifying a cancer into one or more subtypes in a subject having or suspected of having the cancer. The method comprises: obtaining a sample from the subject; assaying the sample to detect changes in gene expression in one or more pathways relative to reference samples or values; computing activity scores (as described herein) of the one or more pathways based on the detected changes in the gene expression; determining, in the sample, a pathway activation profile of the one or more pathways associated with the subtype of the cancer based on the computed activity scores of the one or more pathways; and classifying a cancer into the subtype in the subject if the pathway activation profile associated with the subtype is detected in the sample. In one embodiment, computing activity scores, as described herein, comprises a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization; b) calculating Z-scores for the pathway activation gene expression signatures; and c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures. In one embodiment, the change in the gene expression is an increase in the gene expression level of the one or more genes in the pathway. In another embodiment, the change in the gene expression is a decrease in the gene

expression level of the one or more genes in the pathway. In one embodiment, the cancer is prostate cancer. In some embodiments, the prostate cancer subtypes are PCS1, PCS2 or PCS3 as described herein. In various embodiments, the activity scores are computed as described herein.

[0047] Various embodiments of the present invention provide a method for classifying a cancer in a subject having or suspected of having the cancer. The method comprises: obtaining a sample from the subject; assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; determining the presence of gene expression patterns of the one or more genes associated with the subtype in the sample based on the detected changes; and classifying the cancer in the subject into the subtype if the gene expression pattern of the one or more genes associated with the subtype is detected in the sample. In one embodiment, the change in the gene expression is an increase in expression level of the gene. In another embodiment, the change in the gene expression is a decrease in gene expression level of the gene. In one embodiment, the cancer is prostate cancer. In some embodiments, the prostate cancer subtypes are PCS1, PCS2 or PCS3 as described herein.

[0048] In some embodiments, provided herein are methods for prognosing prostate cancer in a subject having or suspected of having prostate cancer. The methods comprise classifying the cancer comprising: obtaining a sample from the subject; assaying the sample to detect changes in gene expression in one or more pathways relative to reference samples or values; computing activity scores (as described herein) of the one or more pathways based on the detected changes in the gene expression; determining, in the sample, the pathway activation profile of the one or more pathways associated with the subtype of the cancer based on the computed activity scores of the one or more pathways; and classifying the cancer into the subtype in the subject if the pathway activation profile associated with the subtype is detected in the sample. In one embodiment, the subject has PCS1 prostate cancer subtype. In an embodiment, the PCS1 subtype is associated with poor prognosis. In one embodiment, the change in the gene expression is an increase in the gene expression level of the one or more genes in the pathway. In another embodiment, the change in the gene expression is a decrease in the gene expression level of the one or more genes in the pathway.

[0049] In some embodiments, provided herein are methods for prognosing prostate cancer in a subject having or suspected of having prostate cancer. The methods comprise classifying

the cancer comprising: obtaining a sample from the subject; assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; determining the presence of gene expression patterns of the one or more genes associated with the subtype in the sample based on the detected changes; and classifying the cancer in the subject into the subtype if the gene expression pattern of the one or more genes associated with the subtype is detected in the sample. In one embodiment, the subject has PCS1 prostate cancer subtype. In an embodiment, the PCS1 subtype is associated with poor prognosis. In one embodiment, the change in the gene expression is an increase in the gene expression level of the one or more genes in the pathway. In another embodiment, the change in the gene expression is a decrease in the gene expression level of the one or more genes in the pathway.

[0050] In various embodiments, the cancer is prostate cancer (PC), low grade PC, high grade PC, benign PC, aggressive PC, primary PC, secondary PC, luminal PC, basal PC, metastatic PC, castration-resistant PC (CRPC), recurrent PC, or non-recurrent PC, or a combination thereof.

[0051] In various embodiments, the subtype of prostate cancer is PCS1, PCS2, or PCS3 as described herein.

[0052] In various embodiments, the one or more pathways comprise one, two, three, four, five, six, or more, or all of the pathways listed in **Table 4** (namely, AR inducible pathway, AR-Variant inducible pathway, PTEN-null inducible pathway, ERG-fusion inducible pathway, FOXA1 inducible pathway, SPOP-mutation inducible pathway, EZH2-solo inducible pathway, Polycomb repression pathway, RAS activation pathway, Stemness pathway, Aggressive Variant pathway, Pro-neural pathway, Mesenchymal pathway, and Proliferation pathway). In various embodiments, non-limiting examples of pathway activation profile for PCS1 subtype, pathway activation profile for PCS2 subtype, and pathway activation profile for PCS3 subtype, are shown in **Fig. 2.** In one embodiment, pathways PTEN, ES, AR-V, PRF, EZH2 and AV are activated in prostate cancer subtype PCS1. In another embodiment, pathways AR, SPOP, FOXA1 and ERG are activated in prostate cancer subtype PCS2. In a further embodiment, pathways RAS, MES, PRC and PN are activated in prostate cancer subtype PCS3. In some embodiments, the sample is a blood sample or a prostate tissue sample.

[0053] Non-limiting examples of the gene expression pattern of PCS1 subtype, gene expression pattern of PCS2 subtype, and gene expression pattern of PCS3 subtype are shown in **Fig. 5** or **Table 1**. In some embodiments, the gene expression pattern for the PCS1 subtype comprises increased gene expression in one, two, three, four, five, six, or more, or all of the PCS1 SEGs (SubtypeID = 1) listed in **Fig. 5** or **Table 1** and/or decreased gene expression in one, two, three, four, five, six, or more, or all of the non-PCS1 SEGs (SubtypeID ≠ 1) listed in **Fig. 5** or **Table 1**. In some embodiments, the gene expression pattern for PCS2 subtype comprises increased gene expression in one, two, three, four, five, six, or more, or all of the PCS2 SEGs (SubtypeID = 2) listed in **Fig. 5** or **Table 1** and/or decreased gene expression in one, two, three, four, five, six, or more, or all of the non-PCS2 SEGs (SubtypeID ≠ 2) listed in **Fig. 5** or **Table 1**. In some embodiments, the gene expression pattern for PCS3 subtype comprises increased gene expression in one, two, three, four, five, six, or more, or all of the PCS3 SEGs (SubtypeID = 3) listed in **Fig.5** or **Table 1** and/or decreased gene expression in one, two, three, four, five, six, or more, or all of the non-PCS3 SEGs (SubtypeID ≠ 3) listed in **Fig. 5** or **Table 1**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes with more than 80%, 85%, 90%, 95%, or 99% consistency listed in **Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes with about 100% consistency listed in **Table 1 or Fig. 5**.

[0054] **Table 1**: Gene expression patterns in PCS1, PCS2 and PCS3 subtypes.

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
1	699	BUB1	1	0.732878	-0.29233	-0.35893	0.6
2	24137	KIF4A	1	0.796567	-0.35685	-0.35413	0.5
3	890	CCNA2	1	0.704881	-0.23407	-0.38855	0.9
4	1062	CENPE	1	0.607498	-0.25037	-0.29012	0.7
5	1164	CKS2	1	1.036744	-0.25972	-0.64929	0.9
6	9787	DLGAP5	1	0.831705	-0.3142	-0.42348	0.9
7	11004	KIF2C	1	0.736702	-0.37172	-0.28916	0.5
8	701	BUB1B	1	0.742463	-0.22647	-0.42774	0.7
9	983	CDK1	1	0.965364	-0.30454	-0.54688	0.7
10	990	CDC6	1	0.616512	-0.16806	-0.37357	0.9
11	1058	CENPA	1	0.70422	-0.33929	-0.29117	0.4
12	9493	KIF23	1	0.609925	-0.32145	-0.22679	0.9
13	891	CCNB1	1	0.79608	-0.15555	-0.53894	0.9
14	991	CDC20	1	0.918191	-0.45797	-0.3653	0.8
15	1063	CENPF	1	1.176024	-0.4504	-0.59316	1
16	3161	HMMR	1	0.916977	-0.28956	-0.51921	0.9
17	6241	RRM2	1	0.96256	-0.26336	-0.58237	0.9

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
18	6790	AURKA	1	0.789153	-0.26199	-0.43506	0.6
19	9133	CCNB2	1	0.868887	-0.19926	-0.5611	0.9
20	9232	PTTG1	1	1.163149	-0.54816	-0.49218	0.6
21	9735	KNTC1	1	0.610572	-0.25666	-0.28696	1
22	9928	KIF14	1	0.580428	-0.31983	-0.20302	0.3
23	11130	ZWINT	1	0.903893	-0.18787	-0.60157	0.8
24	51203	NUSAP1	1	1.088921	-0.32751	-0.63161	0.9
25	113130	CDCA5	1	0.68834	-0.30251	-0.31141	0.4
26	259266	ASPM	1	0.912815	-0.37851	-0.4338	0.7
27	4173	MCM4	1	0.661987	-0.24561	-0.34118	1
28	9768	KIAA0101	1	1.067884	-0.26787	-0.66846	0.8
29	22974	TPX2	1	1.099269	-0.394	-0.57929	0.9
30	29128	UHRF1	1	0.748383	-0.35395	-0.31552	0.3
31	51514	DTL	1	0.687434	-0.35548	-0.26189	0.6
32	332	BIRC5	1	0.926629	-0.40355	-0.4226	0.7
33	1894	ECT2	1	0.65386	0.150249	-0.69846	0.9
34	2171	FABP5	1	0.590057	-0.08456	-0.42775	0.3
35	4001	LMNB1	1	0.691259	-0.25556	-0.35711	0.8
36	7153	TOP2A	1	1.212938	-0.33307	-0.73275	0.9
37	7272	TTK	1	0.785224	-0.1954	-0.49297	0.9
38	7298	TYMS	1	0.717222	-0.33868	-0.30287	0.8
39	8318	CDC45	1	0.602291	-0.24965	-0.28632	0.8
40	9088	PKMYT1	1	0.607746	-0.36834	-0.18178	0.3
41	9833	MELK	1	1.008142	-0.3543	-0.53775	0.9
42	10112	KIF20A	1	0.877737	-0.37613	-0.40594	0.5
43	11113	CIT	1	0.58729	-0.34989	-0.18123	0.6
44	54845	ESRP1	1	0.610241	0.232201	-0.7365	0.5
45	55355	HJURP	1	0.656315	-0.23448	-0.34656	0.7
46	64151	NCAPG	1	0.872433	-0.34576	-0.42933	0.8
47	79019	CENPM	1	0.590031	-0.30965	-0.2206	0.4
48	81831	NETO2	1	0.60986	0.161958	-0.67154	0.7
49	55502	HES6	1	0.604261	-0.26576	-0.27318	0.3
50	2146	EZH2	1	1.006638	-0.20229	-0.67633	0.9
51	7366	UGT2B15	1	0.609459	-0.43442	-0.12244	0.4
52	54443	ANLN	1	0.695782	-0.32235	-0.29952	0.8
53	54892	NCAPG2	1	0.611082	-0.11711	-0.4158	0.8
54	56992	KIF15	1	0.699147	-0.31196	-0.31197	0.6
55	83540	NUF2	1	0.753009	-0.31231	-0.35779	0.6
56	213	ALB	1	0.631156	-0.3166	-0.24945	0.6
57	367	AR	1	0.739025	-0.08519	-0.55479	0.4
58	2305	FOXM1	1	0.692848	-0.34179	-0.27913	1
59	3148	HMGB2	1	0.594215	-0.17765	-0.34565	0.9
60	3832	KIF11	1	0.602635	-0.2067	-0.32613	1
61	3925	STMN1	1	0.755844	-0.19839	-0.46504	1
62	4288	MKI67	1	0.634432	-0.17544	-0.38214	1
63	7083	TK1	1	0.835438	-0.48747	-0.26725	0.7
64	9055	PRC1	1	0.881146	-0.29139	-0.48683	0.9
65	9134	CCNE2	1	0.600059	-0.17521	-0.3529	0.9
66	9156	EXO1	1	0.604351	-0.30764	-0.23472	0.5
67	10024	TROAP	1	0.722668	-0.39012	-0.26021	0.5

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
68	10460	TACC3	1	0.618949	-0.37565	-0.18465	0.8
69	11065	UBE2C	1	1.164182	-0.46906	-0.56584	0.8
70	29089	UBE2T	1	0.89392	-0.3859	-0.41081	0.8
71	29127	RACGAP1	1	0.748508	-0.24041	-0.4201	0.3
72	55143	CDCA8	1	0.619341	-0.26427	-0.28748	0.5
73	55165	CEP55	1	0.697617	-0.28474	-0.3357	0.6
74	55872	PBK	1	0.895022	-0.33544	-0.45818	0.5
75	79682	MLF1IP	1	0.800021	-0.16748	-0.53133	0.7
76	374393	FAM111B	1	0.581026	-0.18703	-0.32571	0.8
77	3223	HOXC6	1	0.632505	0.210087	-0.73522	0.2
78	1033	CDKN3	1	0.868086	-0.28547	-0.48109	0.9
79	1951	CELSR3	1	0.659384	-0.39411	-0.20231	0.4
80	6472	SHMT2	1	0.599045	-0.03074	-0.48497	0.9
81	6696	SPP1	1	0.841317	-0.36701	-0.38312	0.8
82	8438	RAD54L	1	0.617831	-0.32054	-0.23441	0.5
83	10615	SPAG5	1	0.785096	-0.31031	-0.38713	0.7
84	10721	POLQ	1	0.580921	-0.2822	-0.23806	0.5
85	29923	HILPDA	1	0.796377	-0.30733	-0.39953	0.5
86	51155	HN1	1	0.63131	-0.13259	-0.41889	0.8
87	8611	PPAP2A	2	-0.23329	0.729885	-0.47171	0.9
88	10551	AGR2	2	-0.58473	0.974231	-0.39544	0.3
89	4824	NKX3-1	2	-0.30631	0.58501	-0.27584	0.8
90	4072	EPCAM	2	0.348825	0.629971	-0.87852	0.9
91	5865	RAB3B	2	-0.1764	0.894862	-0.67225	1
92	6480	ST6GAL1	2	-0.55638	0.691335	-0.15942	0.8
93	23671	TMEFF2	2	0.14689	0.789374	-0.85218	0.7
94	262	AMD1	2	-0.32478	0.656896	-0.32617	1
95	10040	TOM1L1	2	-0.0284	0.610534	-0.53744	0.4
96	384	ARG2	2	-0.44676	0.625144	-0.19244	0.8
97	776	CACNA1D	2	0.128888	0.628	-0.68827	0.9
98	2982	GUCY1A3	2	-0.08874	0.654917	-0.52657	1
99	6675	UAP1	2	-0.00443	0.68233	-0.62404	1
100	354	KLK3	2	-0.56351	0.737691	-0.19597	0.9
101	2153	F5	2	0.264994	0.773606	-0.93886	0.3
102	3109	HLA-DMB	2	-0.4297	0.833321	-0.39861	0.8
103	3781	KCNN2	2	-0.01902	0.83366	-0.75078	0.7
104	10257	ABCC4	2	-0.03837	0.840833	-0.74081	1
105	27347	STK39	2	-0.13459	0.622779	-0.45773	1
106	57630	SH3RF1	2	0.046684	0.601567	-0.59352	0.9
107	445347	TARP	2	-0.14311	0.940252	-0.74254	0.7
108	1298	COL9A2	2	-0.19489	0.673584	-0.45281	0.3
109	1803	DPP4	2	-0.86264	0.714411	0.081739	0.8
110	2690	GHR	2	-0.42541	0.656978	-0.24002	0.8
111	4646	MYO6	2	0.07681	0.904504	-0.89807	0.8
112	81035	COLEC12	2	-0.08649	0.589295	-0.46813	0.9
113	55	ACPP	2	-1.23756	0.79755	0.326462	0.8
114	220	ALDH1A3	2	-0.75251	0.874735	-0.16014	1
115	288	ANK3	2	-0.17705	0.584709	-0.38631	1
116	1718	DHCR24	2	-0.10366	0.660574	-0.519	1
117	1824	DSC2	2	-0.17065	0.73219	-0.5275	1

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
118	2078	ERG	2	-0.47748	1.143003	-0.64262	0.8
119	2152	F3	2	-0.76862	0.700003	0.014445	0.9
120	2181	ACSL3	2	-0.15867	0.77747	-0.57943	1
121	2331	FMOD	2	-0.96767	0.847818	0.048977	0.7
122	2650	GCNT1	2	-0.09738	0.819383	-0.67051	0.8
123	2705	GJB1	2	-0.16346	0.677957	-0.48376	0.9
124	3249	HPN	2	0.232825	0.713752	-0.85622	0.9
125	3817	KLK2	2	-0.52028	0.61895	-0.12375	1
126	3936	LCP1	2	-0.57643	0.625152	-0.08135	0.9
127	4070	TACSTD2	2	-0.68312	0.710865	-0.06881	0.9
128	4477	MSMB	2	-1.6707	0.865118	0.635396	0.4
129	4604	MYBPC1	2	-0.6832	0.713151	-0.07084	0.7
130	5238	PGM3	2	-0.11715	0.676376	-0.52198	1
131	5530	PPP3CA	2	-0.0101	0.612551	-0.55497	0.8
132	6652	SORD	2	-0.41587	0.643562	-0.23585	0.5
133	6695	SPOCK1	2	-0.43179	0.958522	-0.51201	1
134	7113	TMPRSS2	2	-0.34717	0.625653	-0.27823	0.9
135	7941	PLA2G7	2	-0.26875	1.197653	-0.87174	0.7
136	8671	SLC4A4	2	-0.37296	0.703932	-0.32816	1
137	9073	CLDN8	2	-0.16713	0.825686	-0.61655	0.8
138	10269	ZMPSTE24	2	-0.04795	0.611414	-0.5215	0.9
139	10321	CRISP3	2	-0.15696	1.017958	-0.80218	0.6
140	10611	PDLIM5	2	0.136575	0.591529	-0.6613	1
141	10788	IQGAP2	2	-0.31507	0.907259	-0.56485	1
142	10954	PDIA5	2	-0.08748	0.581675	-0.46027	1
143	23316	CUX2	2	-0.43357	0.605124	-0.18532	0.5
144	23327	NEDD4L	2	-0.06212	0.646069	-0.54125	0.9
145	25800	SLC39A6	2	-0.06339	0.629034	-0.52448	0.9
146	51109	RDH11	2	-0.38407	0.588355	-0.2123	0.9
147	51313	FAM198B	2	-0.16945	0.591079	-0.39869	0.7
148	51365	PLA1A	2	-0.12517	0.825681	-0.65249	0.5
149	57600	FNIP2	2	-0.12172	0.741522	-0.57801	0.4
150	58511	DNASE2B	2	-0.06995	0.682209	-0.56779	0.7
151	59084	ENPP5	2	-0.27359	0.584764	-0.30365	0.9
152	60481	ELOVL5	2	-0.11911	0.62122	-0.46955	0.9
153	79054	TRPM8	2	-0.51799	0.886222	-0.37164	0.9
154	79689	STEAP4	2	-0.2624	0.780323	-0.49318	0.9
155	116285	ACSM1	2	0.164289	0.722582	-0.80563	0.8
156	130733	TMEM178A	2	-0.68877	0.848187	-0.19032	0.3
157	143503	OR51E1	2	-0.12499	0.640844	-0.48257	0.7
158	148327	CREB3L4	2	-0.18542	0.620886	-0.41244	0.9
159	151258	SLC38A11	2	-0.19184	0.589014	-0.37761	0.3
160	9185	REPS2	2	-0.05421	0.646709	-0.54861	1
161	2203	FBP1	2	-0.36904	0.713318	-0.34016	0.7
162	7782	SLC30A4	2	-0.49281	0.677853	-0.20148	0.8
163	10481	HOXB13	2	-0.03619	0.610781	-0.531	0.8
164	11001	SLC27A2	2	0.077893	0.581359	-0.60166	0.4
165	57535	KIAA1324	2	-0.59729	0.836886	-0.2583	0.8
166	120224	TMEM45B	2	0.173249	0.677234	-0.77158	0.5
167	306	ANXA3	2	-0.91397	0.917548	-0.0612	0.8

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
168	957	ENTPD5	2	-0.15434	0.696438	-0.50857	0.9
169	2346	FOLH1	2	0.029609	0.925683	-0.87712	0.9
170	3081	HGD	2	-0.56597	0.716772	-0.17462	0.2
171	4744	NEFH	2	-1.37688	0.580045	0.645966	0.3
172	4852	NPY	2	-1.11902	1.599439	-0.51294	0.6
173	5320	PLA2G2A	2	-0.88085	0.83274	-0.01154	0.7
174	5874	RAB27B	2	-0.39877	0.594925	-0.20575	1
175	6296	ACSM3	2	0.000189	0.65262	-0.60066	0.6
176	6558	SLC12A2	2	-0.41436	0.740473	-0.32632	1
177	6646	SOAT1	2	-0.12756	0.602482	-0.44507	0.9
178	7103	TSPAN8	2	-0.4271	0.629825	-0.21359	0.6
179	9375	TM9SF2	2	-0.24777	0.586955	-0.32779	1
180	9413	FAM189A2	2	-0.51959	0.580311	-0.08879	1
181	10103	TSPAN1	2	-0.41665	0.716401	-0.30221	1
182	11013	TMSB15A	2	-0.035	0.850727	-0.75279	0.6
183	23600	AMACR	2	0.188227	1.177096	-1.24435	0.8
184	25874	MPC2	2	0.11509	0.59419	-0.64534	0.6
185	26503	SLC17A5	2	-0.08013	0.590589	-0.47476	0.9
186	26872	STEAP1	2	0.064834	0.6005	-0.60809	0.6
187	26996	GPR160	2	0.168502	0.821046	-0.89984	0.6
188	27249	MMADHC	2	-0.31034	0.661875	-0.34312	0.8
189	51084	CRYL1	2	-0.31716	0.619291	-0.29809	0.9
190	51170	HSD17B11	2	-0.05529	0.601338	-0.50594	0.4
191	51280	GOLM1	2	-0.31212	0.913923	-0.57351	1
192	51302	CYP39A1	2	-0.2926	0.623607	-0.32311	0.7
193	51635	DHRS7	2	-0.37222	0.742384	-0.36418	0.9
194	51809	GALNT7	2	-0.11074	0.779964	-0.62279	0.9
195	54431	DNAJC10	2	-0.13587	0.76741	-0.58971	0.9
196	54502	RBM47	2	-0.20937	0.585444	-0.35931	0.9
197	55790	CSGALNACT1	2	-0.57552	0.876535	-0.31343	0.9
198	56165	TDRD1	2	-0.40284	1.093566	-0.66108	0.6
199	64094	SMOC2	2	-0.49596	0.621265	-0.14672	0.8
200	80110	ZNF614	2	-0.04913	0.607409	-0.5168	0.8
201	80157	CWH43	2	-0.35465	0.613516	-0.26066	0.8
202	81285	OR51E2	2	-0.51407	1.196625	-0.66061	0.9
203	84419	C15orf48	2	-0.4575	0.606869	-0.16642	0.4
204	84899	TMTC4	2	-0.07848	0.659873	-0.53993	0.9
205	90701	SEC11C	2	-0.2865	0.74191	-0.43719	0.8
206	92292	GLYATL1	2	-0.06208	0.704136	-0.59471	0.8
207	131034	CPNE4	2	-0.29035	0.788477	-0.47674	0.7
208	219595	FOLH1B	2	0.156082	0.635452	-0.71843	0.3
209	284370	ZNF615	2	-0.08794	0.586175	-0.46401	0.7
210	70	ACTC1	3	-1.02191	-0.1473	1.011081	0.8
211	72	ACTG2	3	-1.76535	0.320045	1.218031	0.8
212	477	ATP1A2	3	-0.8676	-0.16949	0.899292	0.9
213	5919	RARRES2	3	-0.66338	-0.29374	0.83865	0.9
214	2919	CXCL1	3	-0.45737	-0.23973	0.612444	0.7
215	5239	PGM5	3	-1.25303	-0.00661	1.079647	0.9
216	6876	TAGLN	3	-0.94824	-0.04705	0.855727	0.8
217	7881	KCNAB1	3	-0.51165	-0.16622	0.591319	0.8

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
218	10418	SPON1	3	-0.54973	-0.20797	0.662352	0.9
219	284	ANGPT1	3	-0.69304	-0.16956	0.749792	0.7
220	1674	DES	3	-1.31754	-0.07009	1.193337	1
221	1805	DPT	3	-0.61865	-0.27012	0.778597	0.7
222	2354	FOSB	3	-1.03176	0.277239	0.628891	0.6
223	2568	GABRP	3	-0.3939	-0.27995	0.595074	0.8
224	4638	MYLK	3	-1.43663	0.279998	0.97324	0.8
225	4660	PPP1R12B	3	-0.75727	0.013151	0.636714	0.9
226	4681	NBL1	3	-0.57551	-0.18859	0.666611	0.6
227	4921	DDR2	3	-0.61766	-0.05683	0.581486	0.7
228	5918	RARRES1	3	-0.67217	-0.1758	0.737655	0.7
229	5947	RBP1	3	-0.2789	-0.37145	0.580736	0.6
230	7047	TGM4	3	-0.70809	-0.12198	0.718912	0.5
231	7169	TPM2	3	-1.14192	-0.14729	1.113893	0.8
232	9510	ADAMTS1	3	-0.57365	-0.17346	0.651093	0.7
233	10563	CXCL13	3	-0.217	-0.51526	0.660028	0.8
234	3371	TNC	3	-0.57749	-0.12098	0.606099	0.8
235	4684	NCAM1	3	-0.27293	-0.41903	0.619395	0.9
236	59	ACTA2	3	-1.07121	0.044251	0.877075	0.8
237	290	ANPEP	3	-0.86125	0.065063	0.67803	0.4
238	467	ATF3	3	-0.81384	0.106187	0.599576	0.5
239	1288	COL4A6	3	-0.67553	-0.23058	0.790939	0.8
240	1410	CRYAB	3	-0.72445	-0.39396	0.983195	0.5
241	2294	FOXF1	3	-0.64025	-0.18804	0.721573	0.9
242	2316	FLNA	3	-0.80011	-0.05759	0.73851	0.8
243	2920	CXCL2	3	-0.45536	-0.23965	0.610645	0.6
244	3678	ITGA5	3	-0.50666	-0.28354	0.694985	0.8
245	3679	ITGA7	3	-0.57694	-0.17511	0.655426	1
246	3872	KRT17	3	-0.59298	-0.21969	0.710193	0.8
247	4118	MAL	3	-0.30253	-0.40273	0.629763	0.8
248	4629	MYH11	3	-1.54975	0.135351	1.203251	0.8
249	5179	PENK	3	-0.41603	-0.40585	0.729874	0.8
250	5268	SERPINB5	3	-0.49718	-0.18633	0.597424	0.8
251	5376	PMP22	3	-0.58417	-0.22982	0.711969	0.7
252	5730	PTGDS	3	-1.00841	-0.02793	0.889679	1
253	6277	S100A6	3	-0.63266	-0.22145	0.745817	0.7
254	6387	CXCL12	3	-0.45774	-0.21218	0.587415	0.9
255	6525	SMTN	3	-0.73332	-0.20648	0.818281	0.9
256	6716	SRD5A2	3	-1.01803	0.009175	0.863785	0.9
257	7168	TPM1	3	-0.88168	0.135165	0.631035	0.8
258	7538	ZFP36	3	-1.11312	0.392642	0.592412	0.6
259	8013	NR4A3	3	-0.64995	-0.03142	0.585773	0.7
260	8406	SRPX	3	-0.57258	-0.14163	0.620886	0.8
261	8854	ALDH1A2	3	-0.78346	-0.02715	0.696231	0.9
262	8870	IER3	3	-0.52628	-0.236	0.668058	0.9
263	9021	SOCS3	3	-0.76567	-0.01766	0.672261	1
264	9260	PDLIM7	3	-0.48836	-0.24626	0.64501	0.5
265	9506	PAGE4	3	-1.38822	0.087132	1.109223	0.8
266	10398	MYL9	3	-1.13266	-0.159	1.116742	0.8
267	10580	SORBS1	3	-0.98189	0.011495	0.830685	0.8

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
268	22943	DKK1	3	-0.37356	-0.29576	0.592195	0.7
269	25802	LMOD1	3	-1.03924	-0.13072	1.010668	0.8
270	30008	EFEMP2	3	-0.36478	-0.32231	0.609104	0.8
271	50859	SPOCK3	3	-0.85638	-0.06028	0.789192	0.6
272	53826	FXYD6	3	-0.54854	-0.3193	0.763775	0.6
273	64093	SMOC1	3	-0.4463	-0.22438	0.588838	0.8
274	284119	PTRF	3	-0.79821	-0.07594	0.753768	1
275	316	AOX1	3	-0.74241	-0.12039	0.746853	0.9
276	390	RND3	3	-0.80498	-0.04926	0.735008	0.8
277	443	ASPA	3	-0.44733	-0.25541	0.618271	0.8
278	493	ATP2B4	3	-0.55513	-0.14277	0.606989	0.8
279	629	CFB	3	-0.63793	-0.05022	0.592778	0.5
280	653	BMP5	3	-0.28977	-0.36387	0.583081	0.8
281	710	SERPING1	3	-0.68451	-0.17802	0.750279	0.7
282	716	C1S	3	-0.81499	-0.02649	0.722641	0.8
283	857	CAV1	3	-0.93403	-0.07806	0.872083	0.7
284	858	CAV2	3	-0.52407	-0.15917	0.595466	0.8
285	894	CCND2	3	-0.51119	-0.15782	0.583186	0.8
286	1066	CES1	3	-0.71488	-0.1904	0.787679	0.3
287	1191	CLU	3	-0.70499	-0.31222	0.891302	0.7
288	1264	CNN1	3	-1.5399	0.018621	1.302214	0.8
289	1291	COL6A1	3	-0.40342	-0.40542	0.718682	1
290	1292	COL6A2	3	-0.532	-0.23995	0.676587	1
291	1307	COL16A1	3	-0.50929	-0.29474	0.707551	1
292	1346	COX7A1	3	-0.80342	-0.23464	0.904251	0.9
293	1465	CSRP1	3	-1.10308	0.122379	0.832492	0.8
294	1577	CYP3A5	3	-0.58063	-0.23187	0.710821	0.9
295	1580	CYP4B1	3	-0.40098	-0.2692	0.591252	0.8
296	1593	CYP27A1	3	-0.56913	-0.21108	0.681836	0.9
297	1672	DEFB1	3	-0.40478	-0.28843	0.6122	0.7
298	1675	CFD	3	-0.57905	-0.30524	0.776983	1
299	1809	DPYSL3	3	-0.69632	-0.07423	0.664887	0.8
300	2192	FBLN1	3	-1.12524	0.032894	0.933816	0.8
301	2202	EFEMP1	3	-0.54151	-0.19884	0.646914	0.7
302	2263	FGFR2	3	-0.66919	-0.08906	0.655293	0.9
303	2273	FHL1	3	-1.11106	-0.01079	0.961858	0.9
304	2274	FHL2	3	-0.83923	-0.02819	0.744972	0.8
305	2318	FLNC	3	-0.74745	-0.29375	0.910692	0.9
306	2564	GABRE	3	-0.71531	-0.17765	0.776322	0.8
307	2619	GAS1	3	-0.7175	-0.11019	0.716131	0.9
308	2934	GSN	3	-0.82124	-0.02295	0.724736	0.9
309	2944	GSTM1	3	-0.56563	-0.22943	0.69573	0.6
310	2946	GSTM2	3	-0.7024	-0.24541	0.827603	0.7
311	2949	GSTM5	3	-0.6071	-0.20369	0.707568	0.8
312	2950	GSTP1	3	-0.81277	-0.30717	0.978992	0.9
313	3397	ID1	3	-0.75067	-0.14742	0.778799	0.9
314	3399	ID3	3	-0.55305	-0.16072	0.621727	0.9
315	3489	IGFBP6	3	-0.75459	-0.26573	0.891019	0.9
316	3491	CYR61	3	-1.00564	0.246674	0.634637	0.8
317	3569	IL6	3	-0.39204	-0.33016	0.639681	0.8

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
318	3764	KCNJ8	3	-0.36509	-0.29554	0.584741	0.8
319	3779	KCNMB1	3	-0.94501	-0.25442	1.043763	0.8
320	3852	KRT5	3	-0.9539	-0.1843	0.986855	0.6
321	3860	KRT13	3	-0.61386	-0.18989	0.700659	0.8
322	3866	KRT15	3	-1.10462	-0.08224	1.022088	0.8
323	3910	LAMA4	3	-0.37227	-0.33086	0.623392	0.8
324	3914	LAMB3	3	-0.59153	-0.23076	0.719138	0.8
325	3934	LCN2	3	-0.70583	-0.19126	0.780723	0.7
326	3956	LGALS1	3	-0.6414	-0.2305	0.761625	0.6
327	4057	LTF	3	-1.09944	0.124029	0.82785	0.8
328	4129	MAOB	3	-0.94227	0.026149	0.783253	0.9
329	4147	MATN2	3	-0.73575	0.051341	0.583135	0.7
330	4211	MEIS1	3	-0.70561	-0.05064	0.651146	0.7
331	4212	MEIS2	3	-0.8253	-0.02687	0.731824	0.7
332	4239	MFAP4	3	-0.70001	-0.19007	0.774641	0.8
333	4920	ROR2	3	-0.49307	-0.18093	0.588929	0.8
334	4969	OGN	3	-0.85745	0.073606	0.666914	0.5
335	5099	PCDH7	3	-0.51994	-0.16927	0.601226	0.8
336	5121	PCP4	3	-1.57069	0.231246	1.132954	0.6
337	5176	SERPINF1	3	-0.64073	-0.25706	0.785494	0.8
338	5348	FXYD1	3	-0.52854	-0.32276	0.749826	0.9
339	5350	PLN	3	-0.85008	0.008146	0.720831	0.6
340	5579	PRKCB	3	-0.39028	-0.29512	0.605936	0.9
341	5648	MASP1	3	-0.44301	-0.22395	0.585617	0.8
342	5764	PTN	3	-0.97907	0.065302	0.778758	0.7
343	5837	PYGM	3	-0.52059	-0.15809	0.591494	0.7
344	6273	S100A2	3	-0.54321	-0.1449	0.598741	0.3
345	6275	S100A4	3	-0.42302	-0.39463	0.725548	0.4
346	6347	CCL2	3	-0.78072	0.006393	0.663023	0.6
347	6376	CX3CL1	3	-0.68342	-0.21166	0.780294	1
348	6401	SELE	3	-0.80088	0.055729	0.634898	0.8
349	6442	SGCA	3	-0.40577	-0.26301	0.589654	0.8
350	6518	SLC2A5	3	-0.51265	-0.21572	0.637716	0.8
351	6563	SLC14A1	3	-0.79401	-0.06416	0.739323	0.7
352	6604	SMARCD3	3	-0.35997	-0.32498	0.607441	1
353	6769	STAC	3	-0.47465	-0.20587	0.596098	0.8
354	6840	SVIL	3	-0.66534	-0.02733	0.595197	0.8
355	7041	TGFB1I1	3	-0.51524	-0.24502	0.666896	1
356	7043	TGFB3	3	-0.56912	-0.2945	0.758593	0.8
357	7077	TIMP2	3	-0.43641	-0.26146	0.614477	0.8
358	7123	CLEC3B	3	-0.33826	-0.3571	0.618388	0.8
359	7145	TNS1	3	-0.84771	-0.08975	0.808877	0.7
360	7205	TRIP6	3	-0.46717	-0.23923	0.620383	0.9
361	7356	SCGB1A1	3	-0.45669	-0.32748	0.692607	0.8
362	7414	VCL	3	-0.60084	-0.11342	0.619151	0.8
363	7732	RNF112	3	-0.37306	-0.28463	0.581531	0.7
364	8309	ACOX2	3	-0.51335	-0.20797	0.631185	0.9
365	8404	SPARCL1	3	-1.20127	0.168951	0.87376	0.8
366	8425	LTBP4	3	-0.53436	-0.15048	0.596288	1
367	8613	PPAP2B	3	-0.67164	-0.03941	0.611715	0.7

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
368	8626	TP63	3	-1.07269	0.025122	0.895937	0.8
369	8639	AOC3	3	-0.71857	-0.13566	0.740477	0.7
370	8654	PDE5A	3	-0.87976	0.091556	0.669517	0.6
371	9843	HEPH	3	-0.45318	-0.27184	0.638407	1
372	10231	RCAN2	3	-0.6427	-0.21565	0.74908	0.8
373	10278	EFS	3	-0.50046	-0.22534	0.636124	0.9
374	10290	SPEG	3	-0.54476	-0.23684	0.684658	1
375	10335	MRVI1	3	-0.6604	-0.15611	0.709458	0.8
376	10406	WFDC2	3	-0.63964	-0.23007	0.759716	0.7
377	10562	OLFM4	3	-1.10279	0.132391	0.823025	0.8
378	10826	FAXDC2	3	-0.48038	-0.22945	0.622698	0.7
379	10974	ADIRF	3	-1.00822	0.114667	0.758309	0.5
380	11030	RBPMS	3	-0.63321	-0.17213	0.700907	0.8
381	11117	EMILIN1	3	-0.41065	-0.27028	0.600521	1
382	11155	LDB3	3	-0.52936	-0.21976	0.655745	0.8
383	11170	FAM107A	3	-0.86714	-0.13489	0.867058	0.9
384	11259	FILIP1L	3	-0.60332	-0.18253	0.684863	0.8
385	11341	SCRG1	3	-0.48197	-0.3457	0.731025	0.8
386	23022	PALLD	3	-0.75108	-0.03353	0.674363	0.8
387	23336	SYNM	3	-1.44993	0.190874	1.066641	0.8
388	23584	VSIG2	3	-0.60002	-0.13924	0.642202	0.8
389	23650	TRIM29	3	-0.8207	-0.18226	0.870858	0.8
390	25959	KANK2	3	-0.55779	-0.14349	0.609928	0.7
391	25984	KRT23	3	-0.75711	-0.14065	0.778091	0.7
392	25999	CLIP3	3	-0.38782	-0.41018	0.709695	1
393	26353	HSPB8	3	-0.91053	-0.16569	0.932582	0.9
394	26577	PCOLCE2	3	-0.73061	-0.11131	0.728395	0.8
395	27122	DKK3	3	-0.70441	-0.0871	0.683669	0.7
396	27129	HSPB7	3	-0.35844	-0.31661	0.598427	0.6
397	29951	PDZRN4	3	-0.8258	-0.00679	0.713775	0.8
398	51285	RASL12	3	-0.56946	-0.30566	0.769151	0.9
399	51676	ASB2	3	-0.56374	-0.16152	0.631615	0.7
400	55679	LIMS2	3	-0.54444	-0.25681	0.702765	0.9
401	58189	WFDC1	3	-0.8631	-0.27908	0.996276	0.9
402	59353	TMEM35	3	-0.73144	-0.05343	0.675843	0.5
403	64091	POPDC2	3	-0.59382	-0.12841	0.626922	0.5
404	79625	NDNF	3	-0.48848	-0.23457	0.634352	0.4
405	79630	C1orf54	3	-0.41683	-0.26077	0.59708	0.5
406	80206	FHOD3	3	-0.50454	-0.22075	0.635398	0.3
407	83643	CCDC3	3	-0.344	-0.31356	0.583248	0.7
408	83716	CRISPLD2	3	-0.70159	-0.02191	0.621259	0.7
409	84417	C2orf40	3	-0.69548	-0.24663	0.822807	0.5
410	84617	TUBB6	3	-0.57282	-0.19141	0.666906	0.9
411	89927	C16orf45	3	-0.4606	-0.22711	0.603603	0.9
412	91624	NEXN	3	-0.889	-0.05783	0.814888	0.7
413	91851	CHRDL1	3	-0.98756	-0.05396	0.895768	0.6
414	93649	MYOCD	3	-0.60736	-0.13002	0.640005	0.8
415	94274	PPP1R14A	3	-0.46415	-0.31571	0.68817	0.8
416	112464	PRKCDBP	3	-0.4874	-0.25772	0.654727	0.3
417	113146	AHNAK2	3	-0.49377	-0.31079	0.709021	0.6

Order	EntrezID	Symbol	SubtypeID	Fold Change			Consistency
				PCS1	PCS2	PCS3	
418	116535	MRGPRF	3	-0.63991	-0.13197	0.669687	0.3
419	118425	PCAT4	3	-0.84039	0.125967	0.604121	0.1
420	126393	HSPB6	3	-0.50742	-0.29286	0.704212	0.9
421	140597	TCEAL2	3	-0.82459	-0.13391	0.829704	0.6
422	146713	RBFOX3	3	-0.60162	-0.10432	0.611441	0.2
423	147906	DACT3	3	-0.51691	-0.16054	0.590597	0.8
424	148741	ANKRD35	3	-0.56905	-0.2048	0.675992	0.7
425	171024	SYNPO2	3	-1.26852	0.265743	0.84232	0.4
426	253827	MSRB3	3	-0.63971	-0.0841	0.625468	0.9
427	387763	C11orf96	3	-0.47854	-0.27227	0.660526	0.4
428	728264	MIR143HG	3	-0.67359	-0.1042	0.672989	0.2

[0055] In various embodiments, the prostate cancer in the subject may be classified into one of PCS1, PCS2 and PCS3 subtypes based on the changes in expression of one or more genes wherein the one or more genes comprise one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In various embodiments, the one or more genes comprise STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and/or C16orf45, or a combination thereof.

[0056] Non-limiting examples of the gene expression pattern for the PCS1 subtype, the gene expression pattern for the PCS2 subtype, and the gene expression pattern for the PCS3 subtype are shown in **Fig. 5** and **Table 1**. In some embodiments, the gene expression pattern for the PCS1 subtype comprises increased expression levels in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, and KNTC1 and/or decreased expression levels in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

[0057] In some embodiments, the gene expression pattern for the PCS2 subtype comprises increased expression levels in one, two, three, four, five, six, or more, or all of RAB3B,

SLC4A4, ANK3, GJB1, and SLC12A2 and/or decreased expression levels in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

[0058] In some embodiments, the gene expression pattern for the PCS3 subtype comprises increased expression levels in one, two, three, four, five, six, or more, or all of CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45; and/or decreased expression levels in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2.

[0059] In one embodiments, the sample is a blood sample and the cancer (for example, prostate cancer) is classified using the methods described herein based on the gene expression and/or pathway activation profiles in the circulating tumor cells (CTCs). In another embodiment, the sample is a tumor tissue sample, for example, prostate tumor sample.

[0060] In various embodiments, the subtype is PCS1, and the subject is prognosed with a poor clinical outcome. In various embodiments, the poor clinical outcome comprises lower metastasis-free survival, higher risk of metastatic progression, higher rate of cancer specific mortality, lower overall survival, or more aggressive form of cancer, or a combination thereof.

[0061] In various embodiments, the subtype is PCS1, and the subject is prognosed with resistance to an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor. In various embodiments, the antiandrogen is flutamide, nilutamide, bicalutamide, enzalutamide, or apalutamide. In some embodiments, the subtype is PCS1, and the subject is prognosed with resistance to enzalutamide.

[0062] In various embodiments, the subtype is PCS1, and the subject is prognosed with resistance to a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor. In various embodiments, the Src signaling inhibitor is imatinib, bafetinib, nilotinib, dasatinib, bosutinib, or ponatinib. In some embodiments, the subtype is PCS1, and the subject is prognosed with resistance to dasatinib.

[0063] In various embodiments, the subtype is PCS1, and the subject is prognosed with resistance to a mitotic inhibitor. In various embodiments, the mitotic inhibitor is taxane, paclitaxel, docetaxel, or cabazitaxel. In some embodiments, the subtype is PCS1, and the subject is prognosed with resistance to docetaxel or taxane.

[0064] Various embodiments of the invention provide methods for personalizing therapies in a subject having or suspected of having prostate cancer, comprising: classifying the cancer by the methods described herein and administering therapies based on the cancer subtypes. In one embodiment, the subtype is PCS1 and the subject is not administered antiandrogen agents. In one embodiment, the subtype is PCS1 and the subject is not administered enzalutamide.

Treatment Methods

[0065] Various embodiments of the present invention provide a method for treating, inhibiting, preventing metastases of, reducing the severity of and/or slowing the progression of a cancer in a subject. In one embodiment, the cancer is prostate cancer. The methods include classifying the cancer by the methods described herein and administering an effective amount of a therapeutic agent so as to treat, inhibit, prevent metastases of and/or slow progression of the cancer in the subject.

[0066] In one embodiment, the methods for treating, inhibiting, preventing metastases of, reducing the severity of and/or slowing the progression of a cancer in a subject comprise: obtaining a sample from the subject; assaying the sample to detect changes in gene expression in one or more pathways relative to reference samples or values; computing activity scores (as described herein) of the one or more pathways based on the detected changes in the gene expression; determining, in the sample, the pathway activation profile of the one or more pathways associated with the subtype of the cancer based on the computed activity scores of the one or more pathways; classifying the cancer into the subtype in the subject if the pathway activation profile associated with the subtype is detected in the sample; and administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, reducing the likelihood of having, reducing the severity of and/or slowing the progression of the cancer.

[0067] In various embodiments, the one or more pathways comprise one, two, three, four, five, six, or more, or all of the pathways listed in **Table 4** (namely, AR inducible pathway, AR-Variant inducible pathway, PTEN-null inducible pathway, ERG-fusion inducible

pathway, FOXA1 inducible pathway, SPOP-mutation inducible pathway, EZH2-solo inducible pathway, Polycomb repression pathway, RAS activation pathway, Stemness pathway, Aggressive Variant pathway, Pro-neural pathway, Mesenchymal pathway, and Proliferation pathway). In various embodiments, non-limiting examples of PCS1's pathway activation profile, PCS2's pathway activation profile, and PCS3's pathway activation profile are shown in **Fig. 2**.

[0068] In another embodiment, the methods for treating, inhibiting, preventing metastases of, reducing the severity of and/or slowing the progression of a cancer in a subject comprise obtaining a sample from the subject; assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; determining the presence of gene expression pattern of the one or more genes associated with the subtype in the sample based on the detected changes; classifying the cancer in the subject into the subtype if the gene expression pattern of the one or more genes associated with the subtype is detected in the sample; and administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, reducing the likelihood of having, reducing the severity of and/or slowing the progression of the cancer.

[0069] In various embodiments, the one or more genes comprise one or more subtype enriched genes (SEGs), for examples, those genes listed in **Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes listed in **Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes with more than 80%, 85%, 90%, 95%, or 99% consistency listed in **Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes with about 100% consistency listed in **Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the PCS1 SEGs (SubtypeID = 1) listed **in Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the PCS2 SEGs (SubtypeID = 2) listed in **Table 1 or Fig. 5**. In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of the PCS3 SEGs (SubtypeID = 3) listed in **Table 1 or Fig. 5**. In various embodiments, non-limiting examples of PCS1's expression pattern, PCS2's expression pattern, and PCS3's expression pattern are shown in **Table 1 or Fig. 5.**

[0070] In various embodiments, the one or more genes comprise one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In various embodiments, the one or more genes comprise STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and/or C16orf45, or a combination thereof. In various embodiments, non-limiting examples of PCS1's expression pattern, PCS2's expression pattern, and PCS3's expression pattern are shown in **Fig. 5** or **Table 1**.

[0071] Various embodiments of the present invention provide a method for treating, preventing, reducing the likelihood of having, reducing the severity of and/or slowing the progression of a cancer in a subject. The method comprises: obtaining a sample from the subject; assaying the sample to detect a marker for a subtype of the cancer; detecting the marker for the subtype in the sample; and administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of and/or slowing the progression of the cancer.

[0072] In various embodiments, the marker comprises one or more subtype enriched genes (SEGs), for examples, those genes listed in **Table 1 or Fig. 5**. In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of the genes listed in **Table 1 or Fig. 5**. In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of the genes with more than 80%, 85%, 90%, 95%, or 99% consistency listed in **Table 1 or Fig. 5**. In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of the genes with about 100% consistency listed in **Table 1 or Fig. 5**. In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of the PCS1 SEGs (SubtypeID = 1) listed in **Table 1 or Fig. 5**. In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of the PCS2 SEGs (SubtypeID = 2) listed in **Table 1 or Fig. 5**. In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of the PCS3 SEGs (SubtypeID = 3) listed in **Table 1 or Fig. 5**.

[0073] In various embodiments, the marker comprises one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45. In various embodiments, the marker comprises STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and/or C16orf45, or a combination thereof.

[0074] In various embodiments, non-limiting examples of PCS1's marker expression changes, PCS2's marker expression changes, and PCS3's marker expression changes are shown in **Fig. 5** or **Table 1**.

[0075] In various embodiments, the marker for the subtype comprises an increased expression level in one, two, three, four, five, six, or more, or all of the PCS1 SEGs (SubtypeID = 1) listed in **Table 1** or **Fig. 5**, and/or a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS1 SEGs (SubtypeID $\neq$ 1) listed in **Table 1** or **Fig. 5**.

[0076] In various embodiments, the marker for the subtype comprises an increased expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, and KNTC1; and/or a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

[0077] In various embodiments, the marker for the subtype comprises an increased expression level in one, two, three, four, five, six, or more, or all of the PCS2 SEGs (SubtypeID=2) listed in **Table 1** or **Fig. 5**, and/or a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS2 SEGs (SubtypeID $\neq$ 2) listed in **Table 1** or **Fig. 5**.

[0078] In various embodiments, the marker for the subtype comprises an increased expression level in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2; and/or a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

[0079] In various embodiments, the marker for the subtype comprises an increased expression level in one, two, three, four, five, six, or more, or all of the PCS3 SEGs (SubtypeID=3) listed in **Table 1** or **Fig. 5**, and/or a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS3 SEGs (SubtypeID $\neq$ 3) listed in **Table 1** or **Fig. 5**.

[0080] In various embodiments, the marker for the subtype comprises an increased expression level in one, two, three, four, five, six, or more, or all of CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45; and/or a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2.

[0081] In various embodiments, the cancer is prostate cancer (PC), low grade PC, high grade PC, benign PC, aggressive PC, primary PC, secondary PC, luminal PC, basal PC, metastatic PC, castration-resistant PC (CRPC), recurrent PC, or non-recurrent PC, or a combination thereof.

[0082] In various embodiments, the therapeutic agent is a nucleic acid, DNA, RNA, peptide, protein, antibody, aptamer, or small molecule, or a combination thereof. In various embodiments, the therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In various embodiments, the antiandrogen is flutamide, nilutamide, bicalutamide, enzalutamide, or apalutamide, or any of their functional equivalents, analogs, derivatives or salts. In various embodiments, the therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof. In various embodiments, the Src signaling inhibitor is imatinib, bafetinib, nilotinib, dasatinib, bosutinib,

or ponatinib, or any of their functional equivalents, analogs, derivatives or salts. In various embodiments, the therapeutic agent is a mitotic inhibitor. In various embodiments, the mitotic inhibitor is taxane, paclitaxel, docetaxel, or cabazitaxel, or any of their functional equivalents, analogs, derivatives or salts.

[0083] In various embodiments, the subtype is PCS1, PCS2, or PCS3.

[0084] In various embodiments, the subtype is PCS1, and the administered therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In some embodiments, the subtype is PCS1, and the administered therapeutic agent is a mitotic inhibitor. In some embodiments, the subtype is PCS1, and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

[0085] In one embodiment the subtype is PCS1 and the subject is administered DNA damaging agents including but not limited to cisplatin and poly ADP ribose polymerase (PARP) inhibitors.

[0086] In one embodiment, the subtype is PCS1 and the subject is not administered an antiandrogen agent. In one embodiment, the subtype is PCS1 and the subject is not administered enzalutamide.

[0087] In further embodiments, the subtype is PCS1, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor. In some embodiments, the subtype is PCS1, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving dasatinib, or a functional equivalent, analog, derivative or salt of dasatinib.

[0088] In further embodiments, the subtype is PCS1, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving a mitotic inhibitor. In some embodiments, the subtype is PCS1, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel.

[0089] In various embodiments, the subtype is PCS2, and the administered therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an

androgen synthesis inhibitor, or a combination thereof. In some embodiments, the subtype is PCS2, and the administered therapeutic agent is enzalutamide, or a functional equivalent, analog, derivative or salt of enzalutamide, or a combination thereof.

[0090] In further embodiments, the subtype is PCS2, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor. In some embodiments, the subtype is PCS2, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving dasatinib, or a functional equivalent, analog, derivative or salt of dasatinib.

[0091] In various embodiments, the subtype is PCS2, and the administered therapeutic agent is a mitotic inhibitor. In some embodiments, the subtype is PCS2, and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

[0092] In further embodiments, the subtype is PCS3, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor. In some embodiments, the subtype is PCS3, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving enzalutamide, or a functional equivalent, analog, derivative or salt of enzalutamide.

[0093] In various embodiments, the subtype is PCS3, and the administered therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, c-Kit receptor inhibitors, ephrin receptor inhibitors or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof. In some embodiments, the subtype is PCS3, and the administered therapeutic agent is dasatinib, or a functional equivalent, analog, derivative or salt of dasatinib, or a combination thereof.

[0094] In further embodiments, the subtype is PCS3, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving a mitotic inhibitor. In some embodiments, the subtype is PCS3, and the method comprises instructing, directing, or informing the subject not to receive or preventing the subject from receiving docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel.

[0095] In various embodiments, the present invention provides a method for treating PCS1 in a subject. The method comprises: providing a therapeutic agent; and administering a therapeutically effective amount of the therapeutic agent to the subject, thereby treating PCS1 in the subject. In some embodiments, the therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In some embodiments, the therapeutic agent is a mitotic inhibitor.

[0096] In various embodiments, the present invention provides a method for treating PCS2 in a subject. The method comprises: providing a therapeutic agent; and administering a therapeutically effective amount of the therapeutic agent to the subject, thereby treating PCS2 in the subject. In some embodiments, the therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In some embodiments, the therapeutic agent is a mitotic inhibitor.

[0097] In various embodiments, the present invention provides a method for treating PCS3 in a subject. The method comprises: providing a therapeutic agent; and administering a therapeutically effective amount of the therapeutic agent to the subject, thereby treating PCS3 in the subject. In some embodiments, the therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof.

[0098] In various embodiments, the present invention provides a method for treating a cancer subtype in a subject. The method comprises: ordering a diagnostic test to determine if the subject has a cancer subtype; and administering a therapeutically effective amount of a therapeutic agent to the subject who has been diagnosed with the cancer subtype, thereby treating the cancer subtype in the subject. In various embodiments, the cancer subtype is PCS1, PCS2, or PCS3. In some embodiments, the diagnostic test is performed via methods as described in the present invention. In various embodiments, the method may further comprise providing the therapeutic agent.

[0099] In various embodiments, the present invention provides a method for treating PCS1 in a subject. The method comprises ordering: a diagnostic test to determine if the subject has PCS1; and administering a therapeutically effective amount of a therapeutic agent to the subject who has been diagnosed with PCS1, thereby treating PCS1 in the subject. In some embodiments, the diagnostic test is performed via methods as described in the present invention. In various embodiments, the method may further comprise providing the

therapeutic agent. In some embodiments, the therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In some embodiments, the therapeutic agent is a mitotic inhibitor.

[0100] In various embodiments, the present invention provides a method for treating PCS2 in a subject. The method comprises ordering: a diagnostic test to determine if the subject has PCS2; and administering a therapeutically effective amount of a therapeutic agent to the subject who has been diagnosed with PCS2, thereby treating PCS2 in the subject. In some embodiments, the diagnostic test is performed via methods as described in the present invention. In various embodiments, the method may further comprise providing the therapeutic agent. In some embodiments, the therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In some embodiments, the therapeutic agent is a mitotic inhibitor.

[0101] In various embodiments, the present invention provides a method for treating PCS3 in a subject. The method comprises ordering: a diagnostic test to determine if the subject has PCS3; and administering a therapeutically effective amount of a therapeutic agent to the subject who has been diagnosed with PCS3, thereby treating PCS3 in the subject. In some embodiments, the diagnostic test is performed via methods as described in the present invention. In various embodiments, the method may further comprise providing the therapeutic agent. In some embodiments, the therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof.

[0102] Various embodiments of the present invention provide a method of selecting and/or excluding a therapeutic agent for a subject with a cancer. The method comprises: providing a subject with a cancer classified into a subtype utilizing a classification method disclosed herein; and selecting for the subject a therapeutic agent that specifically benefits the subtype and/or excluding for the subject a therapeutic agent that does not benefit the subtype. In accordance with the present invention, "selecting" a therapy may be used interchangeably with "choosing", "ordering", or "prescribing" a therapy.

[0103] Various embodiments of the present invention provide a method of selecting a therapeutic agent for a subject with a cancer. The method comprises: providing a subject

with a cancer classified into a subtype utilizing a classification method disclosed herein; and selecting for the subject a therapeutic agent that specifically benefits the subtype.

[0104] Various embodiments of the present invention provide a method of excluding a therapeutic agent for a subject with a cancer. The method comprises: providing a subject with a cancer classified into a subtype utilizing a classification method disclosed herein; and excluding for the subject a therapeutic agent that does not benefit the subtype.

[0105] In various embodiments, the subtype is PCS1, and the selected therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In various embodiments, the subtype is PCS1, and the selected therapeutic agent is a mitotic inhibitor. In various embodiments, the subtype is PCS2, and the selected therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In various embodiments, the subtype is PCS2, and the selected therapeutic agent is a mitotic inhibitor. In various embodiments, the subtype is PCS3, and the selected therapeutic agent a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof. In some embodiments, the method further comprises instructing, directing, or informing the subject to receive the selected therapeutic agent. In some embodiments, the method further comprises administering the selected therapeutic agent to the subject.

[0106] In various embodiments, the subtype is PCS1, and the excluded therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof. In various embodiments, the subtype is PCS1, and the excluded therapeutic agent is a mitotic inhibitor. In various embodiments, the subtype is PCS2, and the excluded therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof. In various embodiments, the subtype is PCS3, and the excluded therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof. In various embodiments, the subtype is PCS3, and the excluded therapeutic agent is a mitotic inhibitor. In some embodiments, the method further comprises instructing, directing, or informing the subject not to receive the excluded therapeutic agent. In some embodiments, the method further comprises preventing the subject from receiving the excluded therapeutic agent.

[0107] In various embodiments, the antiandrogen is flutamide, nilutamide, bicalutamide, enzalutamide, or apalutamide, or any of their functional equivalents, analogs, derivatives or salts. In some embodiments, the antiandrogen is enzalutamide, a functional equivalent, analog, derivative or salt of enzalutamide, or a combination thereof. In various embodiments, the Src signaling inhibitor is imatinib, bafetinib, nilotinib, dasatinib, bosutinib, or ponatinib, or any of their functional equivalents, analogs, derivatives or salts. In some embodiments, the Src signaling inhibitor is dasatinib, a functional equivalent, analog, derivative or salt of dasatinib, or a combination thereof. In various embodiments, the mitotic inhibitor is taxane, paclitaxel, docetaxel, or cabazitaxel, or any of their functional equivalents, analogs, derivatives or salts. In some embodiments, the mitotic inhibitor is docetaxel, a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

[0108] Typical dosages of a therapeutically effective amount of a therapeutic agent disclosed herein can be in the ranges recommended by the manufacturer where known therapeutic molecules or compounds are used, and also as indicated to the skilled artisan by the *in vitro* responses in cells or *in vivo* responses in animal models. Such dosages typically can be reduced by up to about an order of magnitude in concentration or amount without losing relevant biological activity. The actual dosage can depend upon the judgment of the physician, the condition of the patient, and the effectiveness of the therapeutic method based, for example, on the *in vitro* responsiveness of relevant cultured cells or histocultured tissue sample, or the responses observed in the appropriate animal models. In various embodiments, the therapeutic agent may be administered once a day (SID/QD), twice a day (BID), three times a day (TID), four times a day (QID), or more, so as to administer an effective amount of the therapeutic agent to the subject, where the effective amount is any one or more of the doses described herein.

[0109] In various embodiments, the therapeutic agent is administered at about 0.001-0.01, 0.01-0.1, 0.1-0.5, 0.5-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, or 900-1000 mg/m^2, or a combination thereof. In various embodiments, the therapeutic agent is administered at about 0.001-0.01, 0.01-0.1, 0.1-0.5, 0.5-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, or 900-1000 mg/kg, or a combination thereof. In various embodiments, the therapeutic agent is administered once, twice, three or more times. In various embodiments, the therapeutic agent is administered about 1-3 times per day, 1-7 times per week, 1-9 times per month, or 1-12 times per year. In various embodiments, the

therapeutic agent is administered for about 1-10 days, 10-20 days, 20-30 days, 30-40 days, 40-50 days, 50-60 days, 60-70 days, 70-80 days, 80-90 days, 90-100 days, 1-6 months, 6-12 months, or 1-5 years. Here, "mg/kg" refers to mg per kg body weight of the subject, and "mg/m2" refers to mg per m^2 body surface area of the subject. In certain embodiments, the therapeutic agent is administered to a human.

[0110] In various embodiments, the effective amount of the therapeutic agent is any one or more of about 0.001-0.01, 0.01-0.1, 0.1-0.5, 0.5-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, or 900-1000 μg/kg/day, or a combination thereof. In various embodiments, the effective amount of the therapeutic agent is any one or more of about 0.001-0.01, 0.01-0.1, 0.1-0.5, 0.5-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, or 900-1000 $\mu g/m^2$/day, or a combination thereof. In various embodiments, the effective amount of the therapeutic agent is any one or more of about 0.001-0.01, 0.01-0.1, 0.1-0.5, 0.5-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, or 900-1000 mg/kg/day, or a combination thereof. In various embodiments, the effective amount of the therapeutic agent is any one or more of about 0.001-0.01, 0.01-0.1, 0.1-0.5, 0.5-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, or 900-1000 mg/m^2/day, or a combination thereof. Here, "μg/kg/day" or "mg/kg/day" refers to μg or mg per kg body weight of the subject per day, and "μg/m2/day" or "mg/m2/day" refers to μg or mg per m2 body surface area of the subject per day.

[0111] In some embodiments, the therapeutic agent may be administered at the prevention stage of a condition (i.e., when the subject has not developed the condition but is likely to or in the process to develop the condition). In other embodiments, the therapeutic agent may be administered at the treatment stage of a condition (i.e., when the subject has already developed the condition). As a non-limiting example, the target condition is prostate cancer (PC), PCS1, PCS2, or PCS3. In this exemplar situation, the patient may be treated with the methods described herein when the patient has not yet developed PCS1, PCS2, or PCS3, or is likely to develop PCS1, PCS2, or PCS3, or is in the process of developing PCS1, PCS2, or PCS3, or has already developed PCS1, PCS2, or PCS3.

[0112] In accordance with the invention, the therapeutic agent may be administered using the appropriate modes of administration, for instance, the modes of administration recommended

by the manufacturer for each of the therapeutic agent. In accordance with the invention, various routes may be utilized to administer the therapeutic agent of the claimed methods, including but not limited to intravascular, intravenous, intraarterial, intratumoral, intramuscular, subcutaneous, intraperitoneal, intranasal, or oral.

[0113] In various embodiments, the subject is a human. In various embodiments, the subject is a mammalian subject including but not limited to human, monkey, ape, dog, cat, cow, horse, goat, pig, rabbit, mouse and rat.

[0114] In various embodiments, the sample or biological sample is a cancer or tumor sample. In various embodiments, the sample or biological sample comprises a tumor cell or a tumor tissue. In various embodiments, the sample or biological sample comprises a tumor biopsy or a tumor sample.

[0115] In various embodiments, the reference sample is a non-neoplastic sample. In some embodiments, the non-neoplastic sample is obtained from the subject itself. In other embodiments, the non-neoplastic sample is obtained from another individual. In various embodiments, the individual does not have prostate cancer or prostate diseases. In various embodiments, the individual and the subject belong to the same species, for example, human. In various embodiments, the reference value is obtained from one or more non-neoplastic samples.

[0116] In various embodiments, changes (e.g., increases and/or decreases) in gene expression levels relative to reference samples or values are detected by: contacting the sample with detection agents that specifically bind to target genes' mRNAs and/or proteins; and detecting the binding levels between the detection agents and the target genes' mRNAs and/or proteins. In various embodiments, the sample is assayed to detect changes in mRNA expression levels relative to reference samples or values. In various embodiments, the sample is assayed to detect changes in protein expression levels relative to reference samples or values. Proteins can be detected by various techniques such as IHC, Western blots and protein arrays; and genes and mRNA can be detected by genotyping assays, PCR, Reverse transcription PCR, real-time PCR, microarray, DNA sequencing, and RNA sequencing techniques.

[0117] In various embodiments, the detection agents are oligonucleotide probes, nucleic acids, DNAs, RNAs, aptamers, peptides, proteins, antibodies, avimers, or small molecules, or a combination thereof. In various embodiments, changes (e.g., increases and/or decreases) in

gene expression levels relative to reference samples or values are detected by using a microarray. In some embodiments, the microarray is an oligonucleotide microarray, DNA microarray, cDNA microarrays, RNA microarray, peptide microarray, protein microarray, or antibody microarray, or a combination thereof.

[0118] Various embodiments of the present invention also provide a composition for classifying, and/or diagnosing, and/or prognosing, and/or treating cancers and cancer subtypes. In various embodiments, the cancer is prostate cancer (PC), low grade PC, high grade PC, benign PC, aggressive PC, primary PC, secondary PC, luminal PC, basal PC, metastatic PC, castration-resistant PC (CRPC), recurrent PC, or non-recurrent PC, or a combination thereof. In various embodiments, the subtype is PCS1, PCS2, or PCS3. In various embodiments, the composition comprises one or more detection agents that specifically bind to one or more SEGs' mRNAs and/or proteins. In various embodiments, the composition further comprises a biological sample from a subject. In various embodiments, the subject desires a diagnosis on whether he/she has a cancer or a cancer subtype, or desires a classification of his/her cancer in to a cancer subtype, or desires a prognosis of the clinical outcome of his/her cancer, or desires a prognosis of the drug resistance or response of his/her cancer.

Expression Pattern Assay – RNA

[0119] In various embodiments, determining an expression pattern of SEGs in the biological sample comprises assaying mRNA levels. In various embodiments, assaying mRNA levels comprises using RNA sequencing, northern blot, in situ hybridization, hybridization array, serial analysis of gene expression (SAGE), reverse transcription PCR, real-time PCR, real-time reverse transcription PCR, quantitative PCR, or microarray, or a combination thereof.

[0120] In various embodiments, assaying mRNA levels comprises contacting the biological sample with polynucleotide probes capable of specifically hybridizing to mRNA of one or more SEGs and thereby forming probe-target hybridization complexes.

[0121] Hybridization-based RNA assays include, but are not limited to, traditional "direct probe" methods such as, northern blot or in situ hybridization (e.g., Angerer (1987) Meth. Enzymol 152: 649). The methods can be used in a wide variety of formats including, but not limited to, substrate (e.g. membrane or glass) bound methods or array-based approaches. In a typical in situ hybridization assay, cells are fixed to a solid support, typically a glass slide. If

a nucleic acid is to be probed, the cells are typically denatured with heat or alkali. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of labeled probes specific to the nucleic acid sequence encoding the protein. The targets (e.g., cells) are then typically washed at a predetermined stringency or at an increasing stringency until an appropriate signal to noise ratio is obtained. The probes are typically labeled, e.g., with radioisotopes or fluorescent reporters. Preferred probes are sufficiently long so as to specifically hybridize with the target nucleic acid(s) under stringent conditions. The preferred size range is from about 200 bases to about 1000 bases. Hybridization protocols suitable for use with the methods of the invention are described, e.g., in Albertson (1984) EMBO J. 3: 1227-1234; Pinkel (1988) Proc. Natl. Acad. Sci. USA 85: 9138-9142; EPO Pub. No. 430,402; Methods in Molecular Biology, Vol. 33: In situ Hybridization Protocols, Choo, ed., Humana Press, Totowa, N.J. (1994), Pinkel, et al. (1998) Nature Genetics 20: 207-211, and/or Kallioniemi (1992) Proc. Natl Acad Sci USA 89:5321-5325 (1992). In some applications, it is necessary to block the hybridization capacity of repetitive sequences. Thus, in some embodiments, tRNA, human genomic DNA, or Cot-I DNA is used to block non-specific hybridization.

[0122] In various embodiments, assaying mRNA levels comprises contacting the biological sample with polynucleotide primers capable of specifically hybridizing to mRNAs of SEGs listed in **Table 1**, forming primer-template hybridization complexes, and performing a PCR reaction. In some embodiments, the polynucleotide primers comprises about 15-45, 20-40, or 25-35 bp sequences that are identical (for forward primers) or complementary (for reverse primers) to sequences of SEGs listed in **Table 1**. As a non-liming example, the polynucleotide primers for STMN1 (e.g., NM_203401, Homo sapiens stathmin 1 (STMN1), transcript variant 1, mRNA, 1730 bp) can comprise sequences that are identical (for forward primers) or complementary (for reverse primers) to STMN1's bp 1-20, 5-25, 10-30, 15-35, 20-40, 25-45, 30-50, so on and so forth, until the end of STMN, bp 1690-1710, 1695-1715, 1700-1720, 1705-1725, 1710-1730. While not listed here exhaustively because of the space, all these polynucleotide primers for STMN1 and other SEGs listed in **Table 1** can be used in the present invention. In various embodiments, the polynucleotide primers are labeled with radioisotopes or fluorescent molecules. As the labeled primers emit radio or fluorescent signals, the PCR products containing the labeled primers can be detected and analyzed with a variety of imaging equipment.

[0123] Methods of "quantitative" amplification are well known to those of skill in the art. For example, quantitative PCR involves simultaneously co-amplifying a known quantity of a control sequence using the same primers. This provides an internal standard that may be used to calibrate the PCR reaction. Detailed protocols for quantitative PCR are provided in Innis, et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, Inc. N.Y.). Measurement of DNA copy number at microsatellite loci using quantitative PCR anlaysis is described in Ginzonger, et al. (2000) Cancer Research 60:5405-5409. The known nucleic acid sequence for the genes is sufficient to enable one of skill in the art to routinely select primers to amplify any portion of the gene. Fluorogenic quantitative PCR may also be used in the methods of the invention. In fluorogenic quantitative PCR, quantitation is based on amount of fluorescence signals, e.g., TaqMan and sybr green. Other suitable amplification methods include, but are not limited to, ligase chain reaction (LCR) (see Wu and Wallace (1989) Genomics 4: 560, Landegren, et al. (1988) Science 241:1077, and Barringer et al. (1990) Gene 89: 117), transcription amplification (Kwoh, et al. (1989) Proc. Natl. Acad. Sci. USA 86: 1173), self-sustained sequence replication (Guatelli, et al. (1990) Proc. Nat. Acad. Sci. USA 87: 1874), dot PCR, and linker adapter PCR, etc.

Expression Level Assay – Protein

[0124] In various embodiments, determining an expression pattern of SEGs in a biological sample comprises assaying protein levels. In various embodiments, assaying a protein level comprises using western blot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, or mass spectrometry, or a combination thereof.

[0125] In various embodiments, assaying protein levels comprises contacting the biological sample with antibodies capable of specifically binding to proteins encoded by SEGs listed in **Table 1** and thereby forming antigen-antibody complexes. In the methods and assays of the invention, the expression levels of proteins encoded by SEGs listed in **Table 1**, or fragments or variants thereof can be determined using antibodies specific for those individual proteins or fragments or variants thereof and detecting immunospecific binding of each antibody to its respective cognate biomarker protein.

[0126] Antibodies, both polyclonal and monoclonal, can be produced by a skilled artisan either by themselves using well known methods or they can be manufactured by service providers who specialize making antibodies based on known protein sequences. In the

present invention, the protein sequences of SEGs listed in **Table 1** are known and thus production of antibodies against them is a matter of routine.

[0127] For example, production of monoclonal antibodies can be performed using the traditional hybridoma method by first immunizing mice with an antigen which may be an isolated protein of choice or fragment thereof (for example, a protein encode by a SEG listed in **Table 1**, or a fragment thereof or a variant thereof) and making hybridoma cell lines that each produce a specific monoclonal antibody. The antibodies secreted by the different clones are then assayed for their ability to bind to the antigen using, e.g., ELISA or Antigen Microarray Assay, or immuno-dot blot techniques. The antibodies that are most specific for the detection of the protein of interest can be selected using routine methods and using the antigen used for immunization and other antigens as controls. The antibody that most specifically detects the desired antigen and protein and no other antigens or proteins are selected for the processes, assays and methods described herein. The best clones can then be grown indefinitely in a suitable cell culture medium. They can also be injected into mice (in the peritoneal cavity, surrounding the gut) where they produce an antibody-rich ascites fluid from which the antibodies can be isolated and purified. The antibodies can be purified using techniques that are well known to one of ordinary skill in the art.

[0128] Any suitable immunoassay method may be utilized, including those which are commercially available, to determine the expression level of a SEG protein or a variant thereof assayed according to the invention. Extensive discussion of the known immunoassay techniques is not required here since these are known to those of skill in the art. Typical suitable immunoassay techniques include sandwich enzyme-linked immunoassays (ELISA), radioimmunoassays (RIA), competitive binding assays, homogeneous assays, heterogeneous assays, etc.

[0129] For example, in the assays of the invention, “sandwich-type” assay formats can be used. An alternative technique is the "competitive-type" assay. In a competitive assay, the labeled probe is generally conjugated with a molecule that is identical to, or an analog of, the analyte. Thus, the labeled probe competes with the analyte of interest for the available receptive material. Competitive assays are typically used for detection of analytes such as haptens, each hapten being monovalent and capable of binding only one antibody molecule.

[0130] The antibodies can be labeled. In some embodiments, the detection antibody is labeled by covalently linking to an enzyme, label with a fluorescent compound or metal, label

with a chemiluminescent compound. For example, the detection antibody can be labeled with catalase and the conversion uses a colorimetric substrate composition comprises potassium iodide, hydrogen peroxide and sodium thiosulphate; the enzyme can be alcohol dehydrogenase and the conversion uses a colorimetric substrate composition comprises an alcohol, a pH indicator and a pH buffer, wherein the pH indicator is neutral red and the pH buffer is glycine-sodium hydroxide; the enzyme can also be hypoxanthine oxidase and the conversion uses a colorimetric substrate composition comprises xanthine, a tetrazolium salt and 4,5-dihydroxy-1,3-benzene disulphonic acid. In one embodiment, the detection antibody is labeled by covalently linking to an enzyme, label with a fluorescent compound or metal, or label with a chemiluminescent compound.

[0131] Direct and indirect labels can be used in immunoassays. A direct label can be defined as an entity, which in its natural state, is visible either to the naked eye or with the aid of an optical filter and/or applied stimulation, e.g., ultraviolet light, to promote fluorescence. Examples of colored labels which can be used include metallic sol particles, gold sol particles, dye sol particles, dyed latex particles or dyes encapsulated in liposomes. Other direct labels include radionuclides and fluorescent or luminescent moieties. Indirect labels such as enzymes can also be used according to the invention. Various enzymes are known for use as labels such as, for example, alkaline phosphatase, horseradish peroxidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase and urease.

[0132] The antibody can be attached to a surface. Examples of useful surfaces on which the antibody can be attached for the purposes of detecting the desired antigen include nitrocellulose, PVDF, polystyrene, and nylon.

[0133] In some embodiments of the processes, assays and methods described herein, detecting the level of antibodies reactive to a SEG protein or a variant thereof includes contacting the sample from the cancer patient with an antibody or a fragment thereof that specifically binds a SEG protein or a variant thereof, forming an antibody-protein complex between the antibody and the SEG protein or the variant thereof present in the sample, washing the sample to remove the unbound antibody, adding a detection antibody that is labeled and is reactive to the antibody bound to the SEG protein or a variant thereof in the sample, washing to remove the unbound labeled detection antibody and converting the label to a detectable signal, wherein the detectable signal is indicative of the level of SEG protein or a variant thereof in the sample from the patient. In some embodiments, the effector

component is a detectable moiety selected from the group consisting of a fluorescent label, a radioactive compound, an enzyme, a substrate, an epitope tag, electron-dense reagent, biotin, digonigenin, hapten and a combination thereof. In some embodiments, the detection antibody is labeled by covalently linking to an enzyme, labeled with a fluorescent compound or metal, labeled with a chemiluminescent compound. The level of the SEG protein may be obtained by assaying a light scattering intensity resulting from the formation of an antibody-protein complex formed by a reaction of the SEG protein in the sample with the antibody, wherein the light scattering intensity of at least 10% above a control light scattering intensity indicates the likelihood of chemotherapy resistance.

Reference Value of Expression Level

[0134] Various methods described herein may compare a SEG's expression level in a subject's biological sample to a pre-determined reference value of the SEG. In various embodiments, a SEG's reference value of expression level is the SEG's median or mean expression level from all tumor samples in the discovery dataset. In various embodiments, a SEG's reference value of expression level is the SEG's median or mean expression level from all PC samples in the discovery dataset. In various embodiments, a SEG's reference value of expression level is the SEG's median or mean expression level from all tumor samples in the validation dataset. In various embodiments, a SEG's reference value of expression level is the SEG's median or mean expression level from all PC samples in the validation dataset. In various embodiments, a SEG's reference value of expression level is the SEG's median or mean expression level from non-cancerous, non-tumorous, or non-neoplastic cells or tissues. In accordance with the present invention, SEGs include but are not limited to those listed in **Table 1**.

[0135] Reference values may be obtained by various methods known in the field. For example, one or more biopsies from one cancer patient' tumor (hereinafter "Tumor-1") may be collected, processed and analyzed to obtain the expression level of one SEG (hereinafter "Gene-1") in this tumor (hereinafter "Expression-Tumor-1-Gene-1"). The same step is used to obtain Gene-1's expression levels in another 10, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more cancer patients' tumors (hereinafter "Tumor-N), that is, "Expression-Tumor-N-Gene-1" (N is 1, 2, 3, 4, 5, 6, 7, ...). Then, Gene-1's median or mean expression level from all tumors may be used as the reference value of Gene-1 (hereinafter "REF-Gene-1"), to which Gene-1's expression in a subject's biological sample is compared to so as to

determine if Gene-1's expression is increased (high) or decreased (low) in the subject's biological sample. In other words, REF-Gene-1 is the median or mean of Expression-Tumor-N-Gene-1. Similar steps may be used to obtain another 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more SEGs' reference values, that is, "REF-Gene-M" (M=1, 2, 3, 4, 5, 6, 7, ...). In various embodiments, SEGs (i.e., Gene-M) are listed in **Table 1**. To determine the expression pattern of SEGs in a subject's biological sample, one may compare one, two, three, four, five, or more SEGs' expression levels to their respective reference values.

[0136] As used herein, "expression pattern", "expression profile" and "expression signature" are exchangeable terms referring to the specific combination or setting of one or more genes' high (increased) expressions and/or low (decreased) expressions relative to reference values. In various embodiments, the expression patterns of prostate cancer subtypes are the specific combinations of SEGs' high and low expressions. For non-limiting example, **Table 1**, **Fig. 4 or Fig. 5** shows the expression patterns of PCS1, PCS2, and PCS3. Among the 37 SEGs shown in **Fig. 5**, those having high expressions relative to reference values are shown as dark gray, and those having low expressions relative to reference values are shown as light gray to white.

[0137] Various statistical methods, for example, a two-tailed student t-test with unequal variation, may be used to measure the differences in expression levels of a SEG between the subject's sample and a reference value of expression level generate by computer algorithm pooling many tumor samples, as described herein, for example, all the PC samples in the discovery dataset and/or validation dataset. Various statistical methods, for example, a two-tailed student t-test with unequal variation, may be used to measure the differences in expression levels of a SEG between the subject's sample and a control sample from a normal/healthy individual. Various statistical methods, for example, a two-tailed student t-test with unequal variation, may be used to measure the differences in expression levels of a SEG between the subject's sample and a reference value of expression level generate by computer algorithm pooling many control samples, as described herein. A significant difference may be achieved where the p value is equal to or less than 0.05.

[0138] In various embodiments, the expression level of a SEG or a variant thereof in the subject as compared to the reference value is higher by at least or about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100%. In various embodiments, the

expression level of a SEG or a variant thereof in the subject as compared to the reference value is lower by at least or about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100%. In various embodiments, the expression level ratio between a SEG or a variant thereof in the subject and the reference value is at least or about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1, or 100:1. In various embodiments, the expression level ratio between the reference value and a SEG or a variant thereof in the subject is at least or about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, 1.9:1, 2:1, 2.1:1, 2.2:1, 2.3:1, 2.4:1, 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1, or 100:1.

[0139] Many variations and alternative elements have been disclosed in embodiments of the present invention. Still further variations and alternate elements will be apparent to one of skill in the art. Among these variations, without limitation, are the selection of constituent modules for the inventive compositions, and the diseases and other clinical conditions that may be diagnosed, prognosed or treated therewith. Various embodiments of the invention can specifically include or exclude any of these variations or elements.

[0140] In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0141] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0142] To provide aspects of the present disclosure, embodiments may employ any number of programmable processing devices that execute software or stored instructions. Physical processors and/or machines employed by embodiments of the present disclosure for any processing or evaluation may include one or more networked (Internet, cloud, WAN, LAN, satellite, wired or wireless (RF, cellular, WiFi, Bluetooth, etc.)) or non-networked general purpose computer systems, microprocessors, filed programmable gate arrays (FPGAs), digital signal processors (DSPs), micro-controllers, smart devices (e.g., smart phones), computer tablets, handheld computers, and the like, programmed according to the teachings of the exemplary embodiments. In addition, the devices and subsystems of the exemplary embodiments can be implemented by the preparation of application-specific integrated circuits (ASICs) or by interconnecting an appropriate network of conventional component circuits. Thus, the exemplary embodiments are not limited to any specific combination of hardware circuitry and/or software.

[0143] Stored on any one or on a combination of computer readable media, the exemplary embodiments of the present disclosure may include software for controlling the devices and subsystems of the exemplary embodiments, for driving the devices and subsystems of the exemplary embodiments, for enabling the devices and subsystems of the exemplary embodiments to interact with a human user, and the like. Such software can include, but is not limited to, device drivers, firmware, operating systems, development tools, applications software, database management software, and the like. Computer code devices of the exemplary embodiments can include any suitable interpretable or executable code mechanism, including but not limited to scripts, interpretable programs, dynamic link libraries (DLLs), Java classes and applets, complete executable programs, and the like. Moreover, processing capabilities may be distributed across multiple processors for better performance, reliability, cost, or other benefits.

[0144] Common forms of computer-readable media may include, for example, a floppy disk, a flexible disk, a hard disk, magnetic tape, any other suitable magnetic medium, a CD-ROM, CDRW, DVD, any other suitable optical medium, punch cards, paper tape, optical mark sheets, any other suitable physical medium with patterns of holes or other optically recognizable indicia, a RAM, a PROM, an EPROM, a FLASH-EPROM, any other suitable memory chip or cartridge, a carrier wave or any other suitable medium from which a computer can read. Such storage media can also be employed to store other types of data, e.g., data organized in a database, for access, processing, and communication by the processing devices.

EXAMPLES

[0145] The invention will be further explained by the following Examples, which are intended to be purely exemplary of the invention, and should not be considered as limiting the invention in any way. The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Experimental Methods

Merging transcriptome datasets and quality control

[0146] To assemble a merged dataset from diverse microarray and high-throughput sequencing platforms, we applied a median-centering method followed by quantile scaling (MCQ; (You S, Cho CS, Lee I, Hood L, Hwang D, Kim WU. A systems approach to rheumatoid arthritis. PLoS One 2012;7:e51508). Briefly, each dataset was normalized using the quantile method (Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–93). Probes or transcripts were assigned to unique genes by mapping NCBI entrez gene IDs. Redundant replications for each probe and transcript were removed by selecting the one with the highest mean expression. Log2 intensities for each gene were centered by the median of all samples in the dataset. Each of the matrices was then transformed into a single vector. The vectors for the matrices were scaled by the quantile

method to avoid a bias toward certain datasets or batches with large variations from the median values. These scaled vectors were transformed back into the matrices. Finally, the matrices were combined by matching the gene IDs in the individual matrices, resulting in a merged dataset of 2,115 samples by 18,390 human genes. To evaluate the MCQ-based normalization strategy, we applied the XPN (cross platform normalization; Shabalin AA, Tjelmeland H, Fan C, Perou CM, Nobel AB. Merging two gene-expression studies via cross-platform normalization. Bioinformatics 2008;24:1154–60) method to the same datasets and compared it with the merged data from MCQ. Multidimensional scaling (MDS) between samples was performed to assess batch effects. The same MCQ approach with the quantile method, or the single channel array normalization (SCAN) method (Piccolo SR, Sun Y, Campbell JD, Lenburg ME, Bild AH, Johnson WE. A single-sample microarray normalization method to facilitate personalized-medicine workflows. Genomics 2012;100:337–44), was also applied for normalization and batch correction of data from the independent cohorts.

Computing pathway activation score

[0147] We used the Z-score method to quantify pathway activation (Levine DM, Haynor DR, Castle JC, Stepaniants SB, Pellegrini M, Mao M, et al. Pathway and gene-set activation measurement from mRNA expression data: the tissue distribution of human pathways. Genome Biol 2006; 7:R93). Briefly, the Z-score was defined by the difference between the error-weighted mean of the expression values of the genes in a gene signature and the error-weighted mean of all genes in a sample after normalization. Z-scores were computed using each signature in the signature collection for each of the samples, resulting in a matrix of pathway activation scores.

Determination of the optimal number of clusters

[0148] Non-negative matrix factorization (NMF) clustering with a consensus approach is useful to elucidate biologically meaningful classes (Carrasco DR, Tonon G, Huang Y, Zhang Y, Sinha R, Feng B, et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell 2006;9:313–25). Thus, we applied the consensus NMF clustering method (Brunet JP, Tamayo P, Golub TR, Mesirov JP. Metagenes and molecular pattern discovery using matrix factorization. Proc Natl Acad Sci U S A 2004;101:4164–9) to identify the optimal number of clusters. NMF was computed 100 times for each rank k from 2 to 6, where k was a presumed number of subtypes in the dataset.

For each k, 100 matrix factorizations were used to classify each sample 100 times. The consensus matrix with samples was used to assess how consistently sample-pairs cluster together. We then computed the cophenetic coefficients and silhouette scores for each k, to quantitatively assess global clustering robustness across the consensus matrix. The maximum peak of the cophenetic coefficient and silhouette score plots determined the optimal number of clusters.

Classification using a 14-pathway classifier

[0149] We constructed a classifier, where a set of predictors consists of 14 pathways, using a naïve Bayes machine learning algorithm. For training the classifier, we used the pathway activation scores and subtype labels of the result of the NMF clustering process. We then computed the misclassification rate using stratified 10-fold cross validation. To assess performance, we adopted a 3-class classification as a 2-class classification (e.g., PCS1 vs. others) and computed the average area under the receiver operating characteristic (ROC) curves from all 3 of 2-class classifications. Finally, we applied the 14-pathway classifier to assign subtypes to the specimens.

Identifying subtype-enriched genes

[0150] Wilcoxon rank-sum test and subsequent false discovery rate (FDR) correction with Storey's method (Storey JD. A direct approach to false discovery rates. J Roy Stat Soc B 2002;64:479–98) were employed to identify differentially expressed genes between the subtypes. Genes were selected with FDR < 0.001 and fold change > 1.5, resulting in 428 subtype-enriched genes (SEG).

Development of a 37-gene diagnostic panel

[0151] A random forest machine learning algorithm was employed to develop a diagnostic gene panel. For parameter estimation and training the model, we used the merged dataset. Initially, the model comprised of the 428 SEGs as a set of predictors and subtype label of the merged dataset was used as a response variable for model training. To verify the optimal leaf size, we compared the mean squared errors (MSE) obtained by classification of leaf sizes of 1 to 50 with 100 trees, resulting in an optimal leaf size of 1 for model training. We then permuted the values for each gene across every sample and measured how much worse MSE became after the permutation. Imposing a cutoff of importance score at 0.5, we selected the 37 genes for subtyping. From the computation of MSE growing 100 trees on 37 genes and on

the 428 SEGs, the 37 genes we chose gave the same MSE as the full set of 428 genes. ROC curve analyses and 10-fold cross-validation were also conducted to assess the performance of a classification ensemble.

Statistical analysis

[0152] We performed principal component analysis (PCA) and MDS for visualizing the samples to assess their distribution using pathway activation profiles. Wilcoxon rank-sum statistics were used to test for significant differences in pathway activation scores between the subtypes. Kaplan–Meier analysis, Cox proportional hazard regression, and the χ^2 test were performed to examine the relationship(s) between clinical variables and subtype assignment. The OR test using dichotomized variables was conducted to investigate relationships between different subtyping schemes. The MATLAB package (Mathworks) and the R package (v.3.1 http://www.r-project.org/) were used for all statistical tests.

A prostate cancer gene expression atlas

[0153] To achieve adequate power for a robust molecular classification of prostate cancer, we initially collected 50 prostate cancer datasets from three public databases: Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo), ArrayExpress (http://www.ebi.ac.uk/arrayexpress), and the UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu) and selected 38 data-sets (**Table 2**), in which the numbers of samples are larger than 10 and where over 10,000 genes were measured (**Fig. 1A**).

[0154] Table 2: List of gene expression datasets included in the analysis of the DISC cohort

Data Source ID.	Total # of Genes in Array	Total # of Samples	# of Benign	# of Primary	# of CRPC/Met
GSE6099	10137	104	52	32	20
GSE6752	12418	31	0	10	21
GSE6956	13020	89	20	69	0
GSE8218	13020	148	71	78	0
GSE32269	13020	51	0	22	29
GSE2443	13020	20	0	20	0
GSE25136	13020	79	0	79	0
GSE7055	13020	57	0	57	0
E-SMDB-2486	13888	112	41	62	9
GSE3933	15468	112	41	62	9
GSE15484	16110	65	13	52	0
GSE6919	16386	160	72	63	25
GSE14206	16548	67	14	53	0
GSE6811	16625	35	0	24	11

Data Source ID.	Total # of Genes in Array	Total # of Samples	# of Benign	# of Primary	# of CRPC/Met
E-MTAB-154	16709	48	0	48	0
GSE12378	17406	39	3	36	0
GSE29079	17406	95	48	47	0
GSE41408	17406	48	0	48	0
GSE30521	17839	23	5	18	0
E-TABM-26	18804	57	13	44	0
GSE8511	18848	41	16	12	13
GSE11682	19075	34	17	17	0
GSE41619	19497	14	0	0	14
GSE35988	19596	119	28	59	32
GSE27616	19751	13	4	5	4
GSE38241	19751	39	21	0	18
TCGA (2013-04-24)	20437	220	44	176	0
GSE3325	20678	19	6	7	6
GSE26910	20678	12	6	6	0
GSE17951	20678	154	81	73	0
GSE32448	20678	80	40	40	0
GSE2109	20678	56	0	56	0
GSE16120	22153	65	14	51	0
GSE21034	22261	179	29	131	19
GSE40272	24013	153	52	101	0
GSE32571	24319	98	39	59	0
GSE29650	24384	30	0	0	30
GSE28680	27317	24	4	20	0

[0155] This collection contains datasets consisting of 2,790 expression profiles of benign prostate tissue, primary tumors, and metastatic or CRPC (CRPC/Met; **Fig. 1B**). We then removed a subset of samples with ambiguous clinical information and generated a single merged dataset by cross study normalization, based on median-centering and the quantile normalization meth¬od (MCQ; You S, Cho CS, Lee I, Hood L, Hwang D, Kim WU. A systems approach to rheumatoid arthritis. PLoS One 2012;7:e51508. The merged dataset consists of 1,321 tumor specimens that we named the Discovery (DISC) cohort. The merged gene expression profiles showed a significant reduction of systematic, dataset-specific bias in comparison with the same dataset corrected by the XPN method, which is also used for merging data from different platforms (Shabalin AA, Tjelmeland H, Fan C, Perou CM, Nobel AB. Merging two gene-expression studies via cross-platform normalization. Bioinformatics 2008;24:1154–60) (**Fig. 1C**). Biological differences between tumors and benign tissues were also maintained while minimizing batch effects (**Fig. 1D**).

[0156] As validation datasets, we assembled another collection of 12 independent cohorts consisting of 2,728 tumors from primary and CRPC/Met samples (**Table 3**). From this collection, 3 datasets, the Swedish watchful waiting cohort (SWD), the Emory cohort (EMORY), and the Health Study Prostate Tumor cohort (HSPT), were obtained from GEO. The gene expression profiles and clinical annotations of The Cancer Gnome Atlas (TCGA) cohort of 333 prostate cancer and SU2C/PCF Dream Team cohort (SU2C) of 118 CRPC/Mets were obtained from cBioPortal (http://www.cbioportal.org/). Seven additional cohorts were obtained from the Decipher GRID database (GRID). The expression datasets from the GRID were generated using a single platform, the Affymetrix Human Exon 1.0 ST Array, using primary tumors for the purpose of developing outcomes and treatment response signatures. We used these 7 cohorts to investigate associations of clinical outcomes with subtype assignment in this study.

[0157] Table 3: List of independent cohorts for validation of the subtypes.

Cohort name	Number of samples	Disease status	Available clinical outcomes	Data from GRID	Abbreviation	PubMed ID
Swedish Watchful-Wainting Cohort	281	Localized	OS	No	SWD	20233430
The Cancer Genome Anatomy	333	Localized	N.A.	No	TCGA	26000489
Emory University	106	Localized	N.A.	No	EMORY	24713434
Health Professionals Follow-up Study and Physicians' Health Study Prostate Tumor Cohort	264	Localized	N.A.	No	HSPT	25371445
Stand Up To Cancer/Prostate Cancer Foundation Dream Team Cohort	118	CRPC/Met	N.A.	No	SU2C	26000489
Mayo Clinic Cohort 1	545	Localized	PMS, TMP, PCSM	Yes	MAYO1	23826159
Mayo Clinic Cohort 2	235	Localized	PMS, TMP, PCSM	Yes	MAYO2	23770138
Thomas Jefferson University cohort	130	Localized	PMS, TMP, PCSM	Yes	TJU	25035207

Cohort name	Number of samples	Disease status	Available clinical outcomes	Data from GRID	Abbreviation	PubMed ID
Cleveland Clinic Foundation Cohort	182	Localized	PMS, TMP, PCSM	Yes	CCF	25466945
Memorial Sloan Kettering Cancer Center cohort	131	Localized	PMS, PCSM	Yes	MSKCC	20579941
Erasmus Medical Centre Cohort	48	Localized	PMS, PCSM	Yes	EMC	23319146
Johns Hopkins Medicine Cohort	355	Localized	PMS, TMP, PCSM	Yes	JHM	25466945

Abbreviations: N.A., not available; OS, overall survival; PMS, progression to metastatic state; PCSM, PC-specific mortality; TMP, time-to-metastatic progression.

Pathway activations describing prostate cancer biology

[0158] Recent studies have demonstrated the advantage of pathway-based analysis in clinical stratification for prostate and other cancer types (Markert EK, Mizuno H, Vazquez A, Levine AJ. Molecular classification of prostate cancer using curated expression signatures. Proc Natl Acad Sci U S A 2011;108:21276–81; Gatza ML, Silva GO, Parker JS, Fan C, Perou CM. An integrated genomics approach identifies drivers of proliferation in luminal-subtype human breast cancer. Nat Genet 2014;46:1051–9; Drier Y, Sheffer M, Domany E. Pathway-based personalized analysis of cancer. Proc Natl Acad Sci USA 2013;110:6388–93), However, to date, there has been no study of prostate cancer using pathway activation profiles in which thousands of patient specimens were used. In addition, the utility of recently characterized molecular lesions such as *AR* amplification/overexpression, *AR-V* expression, transcriptional activation of *EZH2* and forkhead box A1 (*FOXA1*), and *SPOP* mutation have not been fully exploited for classification. Therefore, we employed 22 pathway activation gene expression signatures encompassing prostate cancer–relevant signaling and genomic alterations (**Tables 4 and 5**) in the DISC cohort (n = 1,321). These were ultimately collapsed into 14 pathway signatures that were grouped into 3 categories: (i) prostate cancer–relevant signaling pathways, including activation of AR, AR-V, EZH2, FOXA1, and rat sarcoma viral oncogene homolog (RAS) and inactivation by polycomb repression complex 2 (PRC); (ii) genetic and genomic alterations, including mutation of *SPOP*, *TMPRSS2–ERG* fusion (ERG), and deletion of PTEN; and (iii) biological features related to aggressive prostate cancer progression, including stemness (ES), cell proliferation (PRF), epithelial–mesenchy-mal

transition (MES), proneural (PN), and aggressive prostate cancer with neuroendocrine differentiation (AV). Pathway activation scores were computed in each specimen in the DISC cohort using the *Z*-score method (Levine DM, Haynor DR, Castle JC, Stepaniants SB, Pellegrini M, Mao M, et al. Pathway and gene-set activation measurement from mRNA expression data: the tissue distribution of human pathways. Genome Biol 2006; 7:R93). The conversion of gene expression to pathway activation showed a further reduction of batch effects, while preserving biological differences that are particularly evident in the clustering of metastatic and non-metastatic samples (**Fig. 1E**).

[0159] Table 4: Publications from which the pathway activation gene sets were obtained

Pathway Name	Description	# of genes	PubMed ID.
Androgen receptor (AR)	Three sets of up-regulated genes by AR in human patient tissues and prostate cancer cells	1367	23260764
		253	9289629
		100	12185249
AR-Variant (AR-V)	Two sets of up-regulated genes by presence or high expression of AR-variant in bone metastasis tissues or prostate cancer cells.	114	21552559
		24	22710436
Deletion of phosphatase and tensin homolog (PTEN)	Genes up-regulated by loss of PTEN.	113	17452630
TMPRSS2–ERG fusion (ERG)	Gene expression signature up-regulated by TMPRSS-ERG fusion.	140	18283340, 18505969, 17079440
Forkhead box A1 (FOXA1)	Two gene sets up-regulated by FOXA1 with chromatin binding of FOXA1 in their regulatory regions of DNA.	447	23539448
		175	24292680
Mutation of speckle-type POZ protein (SPOP)	Genes significantly up-regulated in all LNCaP-abl cell with three different SPOP mutations and down-regulated in cells with wildtype SPOP compared to cells with control vector treatment (FDR<0.05).	35	25274033
Enhancer of zeste 2 (EZH2)	EZH2-stimulated genes bound by EZH2 solo peaks	84	23239736
Inactivation by polycomb repression complex 2 (PRC)	Two sets of genes repressed by polycomb repression complex from human embryonic stem cell study and prostate cancer patients.	654	16630818
		87	18006806
Rat sarcoma viral oncogene homolog (RAS)	Genes up-regulated by oncogenic RAS activation.	179	16273092
Stemness (ES)	Genes highly expressed in human embryonic stem cells according to 5 or more out of 20 profiling studies	380	17204602
Aggressive PC with neuroendocrine differentiation (AV)	Genes up-regulated in metastatic neuroendocrine (NE) prostate cancer compared to primary prostate cancer without NE phenotype.	464	22389870

Pathway Name	Description	# of genes	PubMed ID.
Pro-neural (PN)	Genes reflecting neuronal differentiation (Pro-neural) activity.	242	16530701
Epithelial-mesenchymal transition (MES)	Genes represent activation of mesenchymal transition.	141	16530701
Poliferation (PRF)	Genes represent active proliferation.	183	16530701

[0160] Table 5: The genes in the collection of pathway signatures used in this study

Pathway	Reference	Genes (Entrez Gene ID)
AR	Sharma et al., Cancer Cell (2013)	152940, 151258, 399948, 126432, 153129, 442117, 57600, 80820, 79143, 126075, 130355, 152485, 162073, 253012, 285636, 389072, 400451, 401152, 402117, 493869, 646603, 162333, 10162, 2122, 389336, 169166, 4803, 78815, 57185, 9182, 5122, 5128, 5218, 55331, 5339, 54704, 5828, 9743, 51246, 6434, 84900, 121601, 100124539, 780776, 728416, 677841, 677823, 677802, 654463, 646962, 643836, 619279, 613212, 504189, 494551, 445815, 445347, 404220, 404093, 403274, 403273, 401546, 401138, 390437, 390174, 389941, 389337, 388697, 387104, 376940, 375449, 375056, 374882, 373156, 344901, 344838, 344758, 343035, 341032, 340359, 340252, 339512, 339403, 337974, 337968, 286676, 286183, 286151, 286122, 286053, 285704, 285590, 285527, 285386, 284756, 284618, 284613, 284612, 284266, 284186, 284185, 284083, 284076, 284001, 283991, 283554, 283450, 283349, 280636, 261729, 259286, 257313, 257068, 257019, 256987, 256435, 256364, 256281, 255631, 254827, 254158, 254048, 252969, 245972, 222962, 222389, 222255, 222194, 222183, 222166, 221981, 221937, 221935, 221895, 221527, 221481, 221294, 221178, 221143, 221037, 221035, 220965, 219988, 219902, 219899, 219621, 206358, 203286, 203260, 203228, 203197, 203068, 202915, 202151, 201625, 201266, 200162, 200150, 199920, 197370, 197358, 192134, 191585, 170850, 170690, 170506, 168667, 166979, 164045, 163882, 163702, 163486, 162282, 159195, 157680, 155435, 155368, 154810, 154091, 153443, 153241, 153201, 152330, 152006, 150864, 150684, 148641, 147912, 147798, 147463, 146862, 146691, 145482, 145376, 145282, 145226, 143458, 143162, 140460, 138046, 137682, 136227, 135932, 134957, 133686, 132660, 131616, 131566, 131405, 130733, 130617, 130162, 129642, 129531, 129285, 128178, 127670, 127018, 127002, 126868, 126364, 124817, 124540, 124152, 123041, 121504, 120534, 119504, 118426, 117531, 116512, 116285, 116225, 116154, 116113, 115825, 114907, 114899, 114884, 114876, 114825, 114804, 114784, 113829, 113174, 112936, 112858, 112616, 96459, 94241, 94240, 94234, 93129, 92714, 92565, 92400, 92399, 92105, 91869, 91748, 91584, 91526, 91120, 90993, 90576, 90529, 90268, 90102, 89796, 89778, 87178, 85865, 85479, 85476, 85462, 85457, 85444, 85439, 85415, 85377, 85026, 84976, 84955, 84952, 84923, 84919, 84918, 84904, 84902, 84869, 84830, 84679, 84668, 84645, 84623, 84614, 84569, 84532, 84524, 84293, 84263, 84262, 84191, 84135, 84074, 84072, 84068, 84002, 83998, 83988, 83940, 83939, 83938, 83930, 83786, 83648, 83641, 83593, 83544, 83538, 83451, 83449, 81839, 81796, 81789, 81788, 81693, 81671, 81627, 81617, 81606, 81567, 81563, 81553, 81545, 81537, 81037, 81031, 80829, 80824, 80745, 80736, 80727, 80723, 80279, 80176, 80153, 80149, 80036, 80017, 79977, 79974, 79949, 79944, 79915,

Pathway	Reference	Genes (Entrez Gene ID)
		79906, 79905, 79875, 79867, 79846, 79840, 79838, 79831, 79813, 79809, 79794, 79789, 79783, 79772, 79740, 79736, 79712, 79705, 79698, 79695, 79689, 79674, 79668, 79658, 79642, 79582, 79411, 79363, 79170, 79165, 79158, 79135, 79098, 79065, 79038, 79031, 65979, 65266, 65083, 65008, 64921, 64919, 64852, 64849, 64816, 64778, 64769, 64756, 64754, 64748, 64743, 64710, 64420, 64374, 64328, 64207, 64167, 64087, 64084, 64083, 64072, 64067, 64061, 63892, 60678, 60676, 60468, 59352, 59351, 59084, 58517, 58511, 58490, 58480, 57862, 57822, 57763, 57713, 57709, 57706, 57704, 57685, 57664, 57657, 57630, 57623, 57597, 57580, 57560, 57552, 57544, 57533, 57528, 57509, 57507, 57496, 57463, 57458, 57452, 57415, 57337, 57223, 57221, 57188, 57181, 57122, 57118, 57117, 57107, 57097, 57018, 56992, 56980, 56975, 56950, 56943, 56934, 56925, 56922, 56914, 56892, 56302, 56288, 56262, 56243, 56204, 56172, 56164, 55970, 55966, 55917, 55869, 55824, 55812, 55803, 55799, 55785, 55766, 55760, 55700, 55698, 55691, 55689, 55672, 55667, 55650, 55638, 55610, 55554, 55553, 55512, 55503, 55502, 55432, 55422, 55366, 55297, 55291, 55226, 55223, 55220, 55214, 55209, 55204, 55198, 55190, 55187, 55186, 55180, 55164, 55163, 55157, 55156, 55139, 55093, 55062, 55061, 55041, 55039, 55017, 54954, 54948, 54897, 54892, 54882, 54879, 54858, 54848, 54828, 54823, 54820, 54815, 54806, 54805, 54788, 54752, 54742, 54677, 54663, 54622, 54620, 54566, 54545, 54541, 54539, 54532, 54514, 54499, 54491, 54475, 54464, 54463, 54455, 54437, 54328, 54187, 53371, 53343, 51752, 51741, 51735, 51729, 51727, 51704, 51703, 51666, 51633, 51631, 51608, 51601, 51585, 51559, 51555, 51454, 51441, 51426, 51385, 51366, 51350, 51263, 51204, 51199, 51196, 51187, 51174, 51171, 51138, 51130, 51112, 51109, 51092, 51075, 51029, 51019, 50939, 50807, 50640, 50512, 50484, 43847, 29998, 29995, 29927, 29843, 29842, 29087, 29028, 28999, 28998, 28958, 28957, 28951, 27347, 27339, 27314, 27303, 27293, 27250, 27241, 27240, 27230, 27185, 27156, 27151, 27132, 27109, 27086, 27085, 27075, 27074, 27042, 26959, 26747, 26526, 26524, 26468, 26298, 26272, 26240, 26235, 26229, 26227, 26166, 26137, 26130, 26098, 26090, 26085, 26084, 26074, 26053, 26047, 26038, 26018, 26011, 26005, 25996, 25962, 25937, 25932, 25917, 25902, 25885, 25841, 25833, 25831, 25825, 25816, 23760, 23732, 23731, 23705, 23642, 23623, 23576, 23566, 23549, 23545, 23531, 23522, 23514, 23499, 23463, 23403, 23384, 23383, 23368, 23365, 23353, 23350, 23327, 23321, 23316, 23310, 23287, 23286, 23270, 23250, 23247, 23230, 23200, 23189, 23171, 23150, 23143, 23133, 23120, 23107, 23105, 23101, 23097, 23094, 23092, 23085, 23059, 23043, 23029, 23026, 23024, 23012, 23007, 23002, 22989, 22985, 22947, 22941, 22933, 22920, 22917, 22901, 22890, 22889, 22887, 22882, 22877, 22875, 22874, 22873, 22871, 22843, 22820, 15116, 11335, 11331, 11277, 11270, 11243, 11238, 11236, 11167, 11148, 11144, 11141, 11138, 11107, 11103, 11079, 11077, 11057, 11016, 11010, 11005, 10954, 10947, 10919, 10910, 10752, 10742, 10735, 10718, 10712, 10667, 10656, 10648, 10647, 10643, 10637, 10611, 10579, 10578, 10563, 10560, 10551, 10538, 10529, 10521, 10512, 10497, 10490, 10488, 10458, 10455, 10451, 10436, 10418, 10417, 10404, 10402, 10397, 10370, 10329, 10307, 10298, 10276, 10257, 10250, 10242, 10229, 10221, 10217, 10211, 10208, 10207, 10200, 10179, 10160, 10142, 10129, 10124, 10087, 10082, 10067, 10058, 10057, 10036, 10026, 10011, 10008, 9967, 9901, 9886, 9863, 9830, 9827, 9804, 9788, 9781, 9766, 9739, 9734, 9732, 9725, 9723, 9722, 9699, 9698, 9687, 9686, 9679, 9678, 9673, 9657, 9650, 9649, 9645, 9622, 9612, 9609, 9607, 9603, 9586, 9580, 9578, 9577, 9550, 9545, 9518, 9516, 9515, 9501, 9493, 9472, 9455, 9439, 9404, 9382, 9378, 9369,

Pathway	Reference	Genes (Entrez Gene ID)
		9318, 9223, 9219, 9209, 9202, 9197, 9181, 9169, 9166, 9146, 9120, 9117, 9110, 9071, 9066, 8994, 8992, 8915, 8893, 8874, 8864, 8840, 8833, 8814, 8749, 8743, 8738, 8678, 8671, 8667, 8629, 8622, 8621, 8620, 8607, 8600, 8563, 8556, 8555, 8553, 8538, 8496, 8495, 8439, 8434, 8419, 8412, 8379, 8289, 8241, 8226, 8091, 8036, 8000, 7994, 7993, 7982, 7978, 7975, 7881, 7879, 7850, 7837, 7832, 7786, 7704, 7681, 7597, 7534, 7517, 7498, 7494, 7482, 7474, 7464, 7462, 7458, 7433, 7419, 7373, 7369, 7357, 7323, 7294, 7216, 7204, 7169, 7163, 7113, 7104, 7094, 7090, 7088, 7084, 7068, 7052, 7050, 7047, 7038, 7029, 7015, 7014, 7007, 7006, 6938, 6926, 6907, 6897, 6895, 6876, 6873, 6811, 6809, 6788, 6742, 6733, 6726, 6711, 6695, 6687, 6668, 6655, 6642, 6641, 6629, 6622, 6586, 6575, 6533, 6520, 6506, 6491, 6482, 6480, 6455, 6453, 6403, 6400, 6338, 6317, 6303, 6282, 6207, 6198, 6194, 6182, 6176, 6146, 6137, 6119, 6118, 6094, 6093, 6091, 6046, 6016, 6001, 5982, 5935, 5918, 5890, 5873, 5867, 5865, 5834, 5799, 5797, 5795, 5788, 5787, 5784, 5747, 5740, 5641, 5602, 5597, 5596, 5587, 5581, 5580, 5578, 5577, 5550, 5540, 5530, 5500, 5495, 5468, 5435, 5414, 5337, 5327, 5317, 5314, 5313, 5311, 5286, 5244, 5208, 5207, 5201, 5195, 5192, 5169, 5156, 5152, 5149, 5144, 5142, 5139, 5101, 5098, 5090, 5087, 5073, 5049, 5045, 5028, 5019, 5007, 5001, 4953, 4931, 4926, 4925, 4921, 4919, 4915, 4869, 4856, 4849, 4824, 4799, 4783, 4782, 4781, 4774, 4773, 4758, 4718, 4715, 4690, 4681, 4660, 4653, 4651, 4638, 4604, 4430, 4292, 4286, 4254, 4246, 4245, 4224, 4215, 4171, 4154, 4149, 4128, 4121, 4088, 4071, 4026, 4012, 3987, 3977, 3964, 3960, 3930, 3909, 3899, 3851, 3850, 3849, 3848, 3817, 3816, 3782, 3781, 3778, 3768, 3751, 3747, 3732, 3725, 3714, 3709, 3708, 3680, 3664, 3642, 3638, 3632, 3613, 3612, 3592, 3570, 3480, 3475, 3295, 3290, 3191, 3181, 3169, 3158, 3156, 3109, 3108, 3098, 2982, 2975, 2969, 2936, 2932, 2919, 2917, 2909, 2878, 2824, 2823, 2813, 2804, 2781, 2768, 2752, 2737, 2692, 2651, 2632, 2629, 2587, 2585, 2568, 2549, 2515, 2494, 2331, 2329, 2317, 2309, 2242, 2224, 2222, 2201, 2200, 2194, 2169, 2158, 2153, 2138, 2132, 2131, 2120, 2118, 2115, 2104, 2052, 2051, 2029, 2009, 1998, 1982, 1956, 1937, 1896, 1891, 1879, 1857, 1839, 1836, 1805, 1803, 1769, 1767, 1756, 1740, 1719, 1718, 1716, 1674, 1659, 1657, 1630, 1622, 1612, 1611, 1607, 1600, 1591, 1523, 1512, 1501, 1500, 1496, 1489, 1488, 1468, 1452, 1408, 1389, 1365, 1364, 1356, 1345, 1305, 1280, 1198, 1196, 1180, 1131, 1124, 1119, 1112, 1053, 1052, 1050, 1047, 999, 987, 983, 950, 944, 928, 904, 883, 859, 845, 835, 832, 831, 820, 776, 768, 759, 753, 747, 687, 678, 654, 640, 636, 605, 604, 587, 574, 549, 545, 517, 495, 463, 419, 395, 364, 360, 354, 330, 311, 288, 284, 247, 242, 220, 216, 182, 164, 157, 154, 132, 120, 107, 55, 47, 40
AR	Mendiratta et al., JCO (2009)	3817, 3817, 7113, 65986, 27347, 4824, 10257, 55839, 8611, 1047, 56937, 57556, 9687, 2289, 7704, 2181, 7855, 10198, 3781, 10892, 79098, 354, 55839, 133, 10198, 29028, 10512, 5001, 9240, 51347, 354, 5192, 2181, 990, 6675, 22936, 7366, 10788, 8867, 5004, 3156, 445347, 11057, 55892, 220, 8495, 55081, 25816, 5865, 7057, 11057, 10892, 5001, 51514, 114882, 25816, 10638, 7113, 10892, 9935, 27232, 60481, 23052, 2181, 9455, 8611, 51465, 445347, 26046, 10198, 3156, 10645, 400451, 64780, 23099, 990, 56995, 23099, 8560, 5983, 3557, 5583, 79098, 51312, 10560, 2235, 23099, 5395, 22837, 2887, 55840, 1718, 1052, 10725, 5152, 9044, 57178, 5867, 3949, 54491, 55627, 4174, 114882, 7163, 6385, 54861, 10628, 23299, 25803, 481, 3177, 3613, 8756, 27244, 400451, 654342, 10954, 2237, 10059, 5366, 11167, 6936, 25840, 6659, 5062, 100506658, 6303, 1487, 9, 3638, 7088, 3915, 6482, 694, 54733, 90355, 10725, 6659, 4086, 8660, 5558, 55852, 55656, 23310, 993, 6652, 56829, 7976, 694, 2222, 8165,

Pathway	Reference	Genes (Entrez Gene ID)
		6303, 3422, 100132565, 8165, 23327, 11057, 3108, 23086, 9686, 2235, 51002, 23001, 3422, 80232, 5867, 55623, 4173, 10096, 9619, 4172, 23216, 1487, 6652, 9518, 4792, 1487, 10397, 2542, 3817, 384, 79073, 23077, 23598, 50628, 10645, 4790, 55168, 5500, 6239, 10765, 23047, 6764, 5520, 8353, 8555, 4363, 4678, 8507, 10765, 23286, 31, 1119, 3417, 55718, 23112, 6309, 9686, 79170, 8349, 80003, 56985, 7795, 55604, 1831, 3915, 64087, 2800, 3229, 5797, 5293, 23327, 3983, 50814, 8473, 9261, 6867, 51317, 60481, 6774, 4047, 5373, 2222, 5813, 55041, 8050, 22998, 5962, 7703, 10903, 10096, 84187, 4121, 55144, 5074, 8879, 5813, 10276, 2734, 64710, 9894, 643854, 5927, 10715, 4077, 9019, 81671, 9813, 8237, 2063, 2582, 30850, 55252, 5687, 54676, 4953, 7763, 9750, 5908, 23355, 10475, 83440, 6310, 79726, 6809, 25800, 6655, 22845, 22905, 55347, 93487, 357, 3720, 8795, 9175, 26152, 23598, 1534, 3183, 11171, 9775, 8648, 3654, 7150, 10521, 2805, 80111, 8289
AR	Nelson et al., PNAS (2002)	3248, 8611, 6319, 6652, 1718, 6611, 51171, 220, 6303, 3157, 1622, 2683, 5264, 3422, 60481, 7358, 2181, 1644, 10788, 5238, 9455, 9590, 7163, 10611, 10645, 11099, 2982, 8821, 10461, 11217, 590, 5587, 6385, 5178, 56995, 8503, 57007, 6414, 2936, 6446, 27347, 4189, 3817, 354, 9622, 1362, 5274, 11047, 87, 3685, 3880, 3005, 11258, 10627, 4325, 2335, 56937, 567, 81563, 8916, 79689, 9510, 11057, 8241, 6675, 7982, 1801, 4094, 10397, 22936, 25816, 8555, 3398, 3398, 55502, 1487, 1024, 2289, 10497, 10257, 56172, 54407, 6616, 10513, 563, 3998, 5867, 6728, 1836, 9871, 9218, 6337, 2030, 4824, 25803, 65986, 8554, 8848, 84159, 9314, 4609
AR-V	Hornberg et al., PLoS One (2011)	72, 120, 140, 213, 216, 367, 699, 890, 983, 991, 1058, 1063, 1123, 1164, 1525, 1870, 1875, 1917, 2150, 2171, 2261, 2935, 2938, 3123, 3127, 3181, 3248, 3308, 3315, 3485, 3775, 3895, 4126, 4172, 4176, 4192, 4824, 5111, 5166, 5264, 5307, 5360, 5597, 5603, 5792, 5985, 6234, 6281, 6337, 6950, 7020, 7272, 7280, 7298, 7364, 7366, 7367, 7525, 7913, 8140, 8318, 8407, 8644, 8801, 8836, 9055, 9061, 9133, 9168, 9212, 9401, 10024, 10112, 10370, 10457, 10551, 10635, 11004, 11065, 11339, 22974, 23671, 25827, 25923, 25932, 26063, 26271, 29128, 51050, 51203, 51337, 51703, 55502, 55872, 57415, 57556, 57819, 64151, 79019, 81539, 81610, 81620, 81831, 83461, 83596, 83690, 83879, 84034, 84678, 84706, 93100, 116844, 127845, 139886, 140462, 140710, 145837, 146456, 151126, 154043, 201562, 203068, 221935, 253558, 259266, 388468, 388621, 391267, 399942, 400710, 401466, 402644, 440482, 440915, 642460, 645138, 645656, 646163, 647000, 647169, 653377, 653658, 100129028, 100131161
AR-V	Hu et al., Cancer Res (2012)	113130, 332, 699, 3838, 9735, 701, 994, 10459, 1062, 9700, 11004, 4751, 11113, 3835, 890, 11130, 22974, 995, 56992, 4085, 9088, 11065, 5347, 51203
PTEN	Saal et al., PNAS (2007)	330, 699, 891, 1010, 1062, 1164, 1207, 2618, 2999, 3066, 3608, 3833, 3838, 3925, 4172, 4175, 4259, 4291, 4751, 5052, 5290, 5359, 5612, 5718, 5870, 5873, 5889, 5984, 6612, 6619, 6632, 6732, 6772, 6941, 7159, 7307, 7372, 7444, 8208, 8317, 8532, 8833, 9133, 9232, 9392, 9493, 9711, 9833, 9928, 10440, 10541, 10589, 10606, 10951, 10963, 11004, 11073, 11169, 11222, 23279, 24137, 25852, 26973, 27238, 27316, 29028, 29127, 29979, 51642, 54503, 54534, 54625, 54845, 55248, 79694, 79894, 84056, 87178, 151188, 151246, 151636, 157313, 205564, 219988, 64844, 54906, 92342, 126731, 81610, 387103, 219285, 84955, 1503, 64789, 9787, 29105, 51773, 3192, 55827, 3796, 79132, 57380, 7936, 6426, 6434, 6596, 60313, 6790, 7280, 54014
ERG	Tomlins et	183, 272, 347, 397, 658, 776, 950, 999, 1280, 1298, 1485, 1627, 1644,

Pathway	Reference	Genes (Entrez Gene ID)
	al., Neoplasia (2008); Setlur et al., JNCI (2008); Iljin et al., Cancer Res (2006)	1824, 1889, 1983, 2078, 2152, 2153, 2528, 2690, 2705, 2812, 2867, 2982, 3065, 3109, 3249, 3549, 3710, 3781, 3783, 3790, 3800, 3918, 4035, 4217, 4646, 4725, 4883, 4905, 5074, 5140, 5152, 5192, 5226, 5575, 5585, 5597, 5607, 5719, 5754, 5796, 5832, 5989, 6001, 6294, 6602, 6629, 6675, 6833, 6899, 6908, 7027, 7088, 7174, 7291, 7326, 7358, 7520, 7551, 7941, 8030, 8505, 8507, 8618, 8648, 8672, 8766, 9053, 9073, 9112, 9411, 9529, 9766, 9892, 10202, 10269, 10321, 10477, 10551, 10557, 10656, 10801, 11052, 11079, 22877, 22881, 23250, 26037, 26751, 27199, 27314, 27347, 30848, 51365, 54880, 54997, 55384, 55623, 55753, 55884, 56099, 57630, 65108, 79570, 81557, 83988, 147741, 221395, 246100, 266977, 339260, 349160, 389432, 400710, 728239, 100133941, 100506658, 221061, 55614, 90625, 948, 8853, 3831, 5218, 23613, 5891, 4072, 23598
FOXA1	Jin et al., Cancer Res (2013)	1644, 384, 6013, 2353, 3248, 7365, 6820, 150519, 1058, 8501, 84722, 2354, 222, 283651, 24137, 55771, 55388, 8034, 2030, 9609, 6038, 283651, 677765, 26793, 81035, 2731, 374393, 162681, 645121, 283349, 5406, 81624, 8825, 18, 1031, 642569, 100132920, 283130, 643904, 5167, 2187, 388161, 2005, 2521, 1763, 100132106, 57198, 83463, 606551, 23252, 83849, 403, 10384, 730268, 83903, 647718, 653387, 2870, 647250, 441957, 2177, 5558, 648200, 132864, 1846, 282969, 149830, 6019, 643265, 127700, 84904, 729003, 83540, 157313, 57124, 650061, 84750, 387921, 729667, 2981, 7439, 59341, 7035, 11052, 100134006, 387761, 729384, 5651, 11086, 100131871, 653665, 4594, 5783, 4603, 100128295, 55425, 26747, 9687, 641, 409, 124976, 50614, 30820, 5228, 51435, 4135, 730809, 642153, 148103, 100134550, 4477, 653111, 84296, 649984, 646236, 503542, 91431, 654222, 7940, 647748, 729383, 145837, 25, 649067, 100302254, 54784, 590, 29893, 83992, 286207, 4595, 728340, 390507, 729012, 57245, 79187, 10160, 1734, 387775, 653468, 650995, 642130, 27077, 728217, 2030, 143503, 8350, 51102, 652102, 391427, 100134248, 574508, 100131392, 643035, 90381, 650003, 55711, 100128781, 85414, 51313, 388946, 388242, 338692, 23074, 51776, 407046, 649676, 440311, 26809, 84224, 79643, 375449, 645164, 27324, 80742, 8796, 643233, 118738, 100132029, 100131768, 84532, 22836, 389690, 51430, 647336, 6038, 729392, 3158, 440072, 651952, 727722, 388394, 641958, 10868, 135114, 650852, 84140, 386724, 399939, 2587, 65061, 728114, 1553, 3955, 652185, 100128862, 375347, 643326, 100128374, 158326, 643150, 728352, 57144, 5950, 100128653, 340990, 339476, 100132317, 63976, 100128191, 144678, 647534, 11221, 727833, 221150, 440040, 7170, 653620, 51340, 54143, 1587, 100129986, 652610, 284083, 100128765, 572, 483, 255812, 650036, 84767, 100131243, 1804, 642167, 84068, 643085, 728531, 100134365, 728686, 724027, 622, 127002, 4543, 130540, 348021, 10814, 730394, 5178, 644305, 445347, 2267, 55867, 9768, 340970, 339977, 392232, 728780, 168455, 84553, 404785, 100302116, 5239, 652251, 203430, 100133213, 652490, 652046, 642411, 644041, 10659, 646791, 100131454, 56953, 643943, 55120, 57402, 5947, 5741, 641714, 729198, 161635, 645113, 2260, 100126693, 100132649, 2155, 644990, 390705, 22999, 79370, 653527, 388507, 124, 341230, 128506, 92106, 50515, 651381, 644943, 645485, 55329, 201299, 100133599, 54490, 54821, 79097, 23510, 100134651, 643623, 643246, 91614, 645425, 729240, 285755, 10168, 340024, 158471, 64087, 100129200, 140432, 100134539, 643722, 649184, 406911, 55166, 9700, 643906, 256309, 100127952, 645726, 590, 646976, 650749, 650274, 80312, 995, 222, 3164, 388666, 80741, 146481, 100134050, 653333, 23273, 80204, 133609, 85462, 9793, 284260, 26018, 23178, 7480, 158471,

Pathway	Reference	Genes (Entrez Gene ID)
		10720, 126, 648979, 653689, 142827, 121214, 730024, 217, 55199, 644785, 639, 100302203, 254428, 5004, 100128908, 100133311, 645875, 4951, 100129463, 41, 57464, 649214, 7035, 57824, 729051, 219, 730861, 56959, 89944, 57222, 440153, 100073347, 2650, 7033, 8821, 286527, 5831, 55504, 642362, 1404, 9882, 4751, 154091, 11200, 94104, 55247, 6080, 1010, 389206, 5557, 100133898, 5557, 26018, 84058, 57221, 112611, 51073, 4173, 100132464, 64946, 23306, 55504, 594837, 25953, 645691, 89876, 100132964, 259217, 53834, 441484, 2146, 594838, 91057, 53834, 5427, 65062, 55038, 55658, 23286, 151246, 3169, 5631, 4494, 10714, 26272
FOXA1	Robinson et al., Oncogene (2013)	3336, 1981, 644634, 140901, 9415, 115948, 64061, 728643, 4627, 85456, 57805, 2975, 79977, 23420, 103, 220686, 10523, 3609, 388692, 29128, 445347, 9020, 5934, 9993, 25832, 338707, 22974, 221035, 23318, 11051, 11198, 5585, 124565, 8301, 63901, 8916, 10298, 949, 1399, 440270, 390916, 701, 56882, 55683, 116064, 55502, 26039, 644745, 2064, 3714, 10648, 6720, 22998, 10628, 3187, 10193, 728857, 22911, 286075, 7259, 7155, 7913, 10963, 84895, 23310, 2624, 9589, 9191, 3304, 65123, 7317, 6837, 7536, 353131, 6744, 652713, 23244, 56882, 54443, 132671, 3609, 399664, 2870, 4172, 441205, 7475, 653199, 126133, 6522, 9267, 4128, 339287, 1981, 440915, 11167, 4820, 1717, 3091, 392288, 9918, 3158, 27328, 25929, 25957, 326, 1982, 1108, 1949, 50628, 23052, 2289, 329, 7082, 3232, 1639, 652160, 221981, 389322, 729154, 83606, 2101, 5422, 200030, 51701, 55706, 9922, 51402, 26054, 11338, 5213, 9688, 23517, 645879, 8473, 23270, 64207, 647500, 3985, 56829, 56853, 653321, 4302, 649702, 64061, 647983, 54545, 201255, 4605, 55905, 729234, 8539, 23451, 6574, 6238, 100129543, 9013, 7544, 22985, 653419, 23306, 54093, 644322, 647000, 8897, 27333, 5192, 649908, 10985, 4627, 5925, 646665, 5584, 9854, 64848, 10594
SPOP	Geng et al., Cancer Res (2014)	730996, 6446, 3936, 58480, 354, 79054, 6446, 10397, 6446, 646723, 25803, 57801, 54490, 6590, 4070, 5225, 1811, 79054, 54206, 2235, 4316, 585, 55897, 148327, 649970, 4316, 4285, 85012, 8611, 10257, 246, 8611, 23623, 390557, 3710
EZH2	Xu et al., Science (2013)	5036, 9401, 5315, 51069, 55052, 5886, 29082, 91057, 5707, 199699, 64426, 2289, 7398, 5810, 7417, 10155, 8550, 29028, 9768, 11004, 790, 4801, 55299, 122769, 6426, 132, 3029, 10592, 81620, 56834, 57696, 64222, 256126, 26517, 90480, 84262, 128239, 1111, 65003, 64105, 10921, 26528, 51081, 1164, 4796, 6883, 6883, 57819, 79902, 4176, 5514, 4173, 55631, 23204, 5889, 56683, 790955, 7329, 5434, 79171, 51154, 10535, 55146, 1869, 10640, 6520, 4860, 1981, 2091, 1537, 5757, 5718, 23548, 5216, 79959, 1841, 55706, 80179, 4893, 79447, 10426, 1810, 54069, 10614
PRC	Lee et al., Cell (2006)	6833, 25841, 170689, 170692, 105, 196883, 114, 116, 148, 150, 153, 155, 246, 257, 60529, 138649, 84210, 389002, 84079, 362, 57569, 132946, 429, 430, 460, 23245, 463, 467, 474, 84913, 579, 56751, 343472, 56033, 8538, 596, 79365, 128408, 27319, 353500, 646, 7832, 283078, 25789, 375061, 387597, 148753, 149499, 24141, 59271, 25927, 89876, 56934, 774, 776, 777, 8913, 796, 55450, 54897, 869, 140689, 874, 57332, 947, 925, 64072, 1005, 8941, 1031, 1045, 55803, 94027, 94115, 51673, 9023, 140578, 8646, 25884, 64377, 338917, 1149, 4435, 146225, 1184, 161198, 64084, 26507, 1271, 255631, 84570, 85301, 1280, 1287, 1288, 1298, 81035, 1311, 84940, 1394, 9244, 55118, 1412, 64478, 114788, 1501, 57369, 9547, 58191, 1591, 1592, 56603, 340665, 1594, 1602, 117154, 166614, 1630, 54798, 23576, 1608, 9162, 50846, 1735, 25849, 22943, 27123, 54567, 1745, 1746, 1747, 1748, 1761, 10655, 58524, 220164, 8110, 283417, 1816, 1825, 57453,

Pathway	Reference	Genes (Entrez Gene ID)
		53905, 50506, 1846, 9427, 1942, 1944, 25975, 1960, 1961, 55531, 2019, 2020, 8320, 2034, 64097, 2044, 2047, 2049, 2066, 90952, 83715, 80712, 2149, 151647, 163933, 57795, 339479, 284716, 151354, 2201, 2203, 55336, 26273, 54738, 9638, 26281, 2248, 2250, 2254, 344018, 2313, 54508, 79962, 54790, 150538, 151278, 283212, 91607, 400765, 253650, 349152, 129804, 203111, 400591, 388336, 399717, 389064, 23768, 3170, 27023, 2306, 27022, 349334, 200350, 286380, 387054, 2304, 2294, 2290, 2302, 2300, 668, 257019, 2526, 11211, 2535, 2555, 2557, 2572, 2583, 124872, 374378, 8811, 2624, 2625, 2626, 2627, 2637, 392255, 151449, 2668, 2690, 2693, 55340, 2706, 23127, 9630, 2262, 2824, 2834, 83550, 2835, 338557, 54112, 2891, 2894, 2897, 2899, 116443, 2917, 145258, 2928, 219409, 170825, 2982, 3000, 9464, 3039, 3040, 54626, 84667, 23462, 55733, 3087, 64399, 3142, 3110, 3167, 340784, 3211, 10481, 3212, 3213, 3216, 3217, 3218, 3227, 3228, 3221, 3222, 3223, 3224, 3231, 3238, 3239, 3232, 3233, 3234, 3235, 51440, 60495, 8739, 9953, 388605, 266722, 3299, 3310, 3350, 3358, 3363, 7087, 51214, 84966, 26280, 3574, 9118, 84684, 3645, 3651, 79191, 50805, 10265, 3670, 64843, 3676, 3706, 3725, 81621, 3736, 3738, 7881, 3747, 3749, 3752, 3756, 23416, 56660, 56659, 3776, 50801, 3778, 3786, 27012, 22846, 57214, 57535, 85376, 84623, 9365, 9314, 339855, 10660, 3958, 8549, 9355, 89884, 64211, 26468, 431707, 4010, 124842, 127003, 143903, 148898, 150221, 153684, 200030, 340529, 388394, 388407, 389289, 400120, 405753, 440804, 441413, 441425, 441426, 441430, 441459, 56901, 92162, 23284, 4023, 57631, 145581, 4036, 347730, 4053, 4058, 256586, 4081, 10586, 9935, 4118, 5596, 4137, 55283, 55897, 284207, 84803, 145773, 440482, 340419, 403312, 4300, 9242, 4487, 4489, 4490, 4496, 4499, 326343, 4618, 4645, 4654, 162417, 89797, 4684, 4741, 4747, 4745, 4760, 4761, 4762, 63973, 50674, 4784, 90527, 4821, 159296, 26257, 4824, 4825, 84504, 145741, 8715, 4861, 255743, 4883, 4884, 4886, 7026, 8013, 3084, 9542, 220323, 84618, 9423, 84628, 4914, 4915, 266743, 4948, 25903, 10215, 3175, 9480, 4985, 84709, 130497, 133060, 92736, 347741, 23440, 5013, 5015, 64064, 5069, 5075, 5076, 5077, 5080, 5081, 7849, 5083, 27253, 5100, 9659, 5156, 9758, 23037, 5179, 5239, 5241, 401, 8929, 8395, 8544, 5307, 5308, 5309, 63876, 5317, 5339, 5362, 5376, 127435, 5426, 5453, 5456, 5457, 5458, 5459, 22843, 84366, 59335, 54886, 5581, 5592, 60675, 256297, 5729, 5732, 5733, 5734, 5737, 5744, 11122, 10076, 5827, 5697, 84084, 399694, 5923, 83593, 30062, 5950, 9185, 28984, 6001, 8601, 388531, 11035, 79836, 79589, 64221, 27330, 10633, 284654, 349667, 6263, 79966, 6330, 6340, 6344, 80031, 6422, 6425, 130367, 6469, 6473, 6474, 54847, 6493, 6495, 10736, 6496, 4990, 201780, 6506, 6509, 123041, 5172, 11001, 7780, 7781, 7782, 140679, 148641, 6529, 6531, 9152, 6549, 6550, 6578, 81796, 6585, 9353, 114798, 22865, 80235, 55509, 114815, 22986, 8403, 64321, 83595, 9576, 50859, 10418, 6716, 6751, 6752, 8128, 55351, 11075, 29091, 55061, 9899, 91683, 6886, 10716, 6899, 6909, 30009, 6926, 6910, 6920, 6928, 339488, 7056, 113091, 7080, 7092, 3195, 3196, 23671, 57393, 161291, 29767, 970, 7161, 8717, 7200, 55521, 440730, 114088, 7224, 85480, 57348, 7349, 7350, 8633, 389658, 124590, 11023, 25806, 7421, 30813, 49856, 51352, 7471, 80326, 7480, 7481, 51384, 7472, 89780, 7475, 7476, 7490, 284273, 7704, 340595, 9839, 57732, 7545, 84107, 84225, 55079, 80818, 84858, 22806
PRC	Yu et al., Cancer Res (2007)	26, 70, 627, 744, 783, 958, 1075, 1116, 1511, 1571, 1803, 1909, 2051, 2258, 2532, 2774, 3071, 3077, 3371, 3683, 3689, 3696, 3872, 4223, 4625, 4629, 4635, 4646, 5179, 5376, 5592, 5733, 6013, 6387, 6505, 6622, 6863,

Pathway	Reference	Genes (Entrez Gene ID)
		6928, 7040, 7227, 7472, 8013, 8406, 8835, 8854, 9172, 9180, 9506, 9508, 10468, 11080, 11279, 23314, 24141, 25802, 25924, 25937, 26167, 27123, 27151, 28984, 30061, 51309, 51384, 51700, 53405, 54738, 55504, 55816, 57094, 57172, 57418, 57569, 57821, 60495, 64399, 66004, 79258, 79365, 81035, 81553, 91607, 114788, 133584, 168667, 221833, 283078
RAS	Bild et al., Nature (2006)	101, 154, 384, 490, 650, 688, 805, 813, 829, 1316, 1453, 1454, 1594, 1604, 1743, 1839, 1843, 1846, 1847, 1848, 1947, 1958, 1969, 1992, 2004, 2069, 2317, 2353, 2683, 2707, 2709, 2710, 2810, 2919, 2920, 2921, 3099, 3265, 3552, 3553, 3576, 3589, 3598, 3628, 3673, 3710, 3726, 3775, 3783, 3949, 3976, 4084, 4170, 4237, 4323, 4615, 4907, 4953, 5055, 5266, 5268, 5292, 5293, 5329, 5362, 5473, 5621, 5743, 5744, 5791, 5806, 5817, 6277, 6303, 6364, 6374, 6382, 6385, 6515, 6525, 6548, 6574, 6675, 6804, 6926, 7039, 7076, 7150, 7262, 7277, 7378, 7422, 7538, 7804, 7851, 7980, 8651, 8795, 8797, 8848, 8870, 8900, 9123, 9136, 9170, 9221, 9227, 9518, 9590, 9592, 9938, 9943, 9982, 10105, 10135, 10140, 10184, 10221, 10397, 10509, 10687, 10855, 10938, 11007, 11332, 22822, 23135, 23227, 23529, 23645, 23767, 26092, 29005, 29126, 50486, 50515, 50640, 51129, 51228, 51312, 51330, 54676, 54910, 55117, 55149, 55384, 55612, 55700, 56938, 64332, 64750, 64866, 65059, 79413, 79686, 79993, 80328, 80853, 81631, 81848, 83667, 84002, 84803, 84951, 84985, 85450, 89795, 94234, 117195, 119548, 120224, 129642, 135398, 144195, 152519, 163259, 201176, 201799, 285672
ES	Assou et al., Stem Cells (2007)	58, 70, 89, 119, 142, 249, 262, 332, 477, 657, 699, 701, 708, 836, 875, 890, 891, 934, 983, 990, 991, 993, 1075, 1111, 1381, 1382, 1400, 1434, 1525, 1592, 1690, 1719, 1730, 1741, 1763, 1788, 1789, 1829, 1894, 2030, 2041, 2058, 2064, 2115, 2118, 2171, 2237, 2239, 2247, 2258, 2260, 2289, 2308, 2558, 2562, 2571, 2618, 2697, 2731, 2824, 2842, 2956, 3038, 3070, 3149, 3159, 3161, 3182, 3308, 3312, 3329, 3609, 3620, 3710, 3720, 3730, 3790, 3800, 3818, 3838, 3856, 3932, 3964, 4072, 4074, 4124, 4141, 4144, 4171, 4172, 4173, 4174, 4175, 4176, 4240, 4257, 4277, 4361, 4436, 4521, 4522, 4678, 4801, 4838, 4869, 4913, 4922, 4947, 4998, 5058, 5097, 5134, 5163, 5198, 5291, 5331, 5366, 5393, 5411, 5420, 5427, 5460, 5495, 5519, 5521, 5557, 5558, 5613, 5631, 5771, 5803, 5865, 5919, 5932, 5983, 5984, 6059, 6091, 6229, 6241, 6297, 6299, 6337, 6422, 6423, 6426, 6432, 6480, 6535, 6566, 6611, 6626, 6638, 6653, 6657, 6741, 6997, 7004, 7013, 7019, 7072, 7112, 7138, 7360, 7374, 7447, 7546, 7547, 7748, 7804, 7855, 7913, 8087, 8239, 8324, 8433, 8519, 8577, 8607, 8611, 8615, 8745, 8805, 8820, 8842, 8880, 8886, 9053, 9074, 9143, 9184, 9188, 9201, 9212, 9221, 9232, 9282, 9350, 9456, 9473, 9573, 9603, 9787, 9908, 9910, 10011, 10036, 10049, 10053, 10146, 10149, 10153, 10157, 10196, 10308, 10346, 10360, 10383, 10434, 10439, 10459, 10528, 10606, 10606, 10622, 10635, 10637, 10643, 10644, 10797, 10874, 11004, 11040, 11051, 11061, 11083, 11143, 11145, 11168, 11169, 11200, 11245, 11339, 22800, 22823, 22929, 23108, 23170, 23178, 23195, 23242, 23246, 23397, 23401, 23411, 23468, 23534, 23683, 24137, 25788, 25926, 25957, 26018, 26047, 26053, 26135, 26207, 26354, 27022, 27231, 29078, 29785, 29920, 51018, 51053, 51083, 51104, 51268, 51385, 51444, 51491, 51574, 51575, 51582, 51599, 51659, 51704, 51816, 54014, 54069, 54478, 54517, 54566, 54596, 54821, 54845, 54892, 54989, 55003, 55010, 55120, 55211, 55237, 55270, 55299, 55320, 55366, 55388, 55660, 55706, 55726, 55749, 55759, 55920, 55975, 56548, 56915, 57122, 57167, 57181, 57380, 57405, 57486, 57502, 57504, 57541, 57556, 57633, 57685, 58516, 63978, 64318, 64782, 64849, 65981, 79007, 79012, 79023, 79071, 79075, 79158, 79647, 79664, 79727, 79923, 79960, 80155, 80179,

Pathway	Reference	Genes (Entrez Gene ID)
		80324, 80775, 81539, 81542, 81554, 81620, 81848, 83439, 83596, 84101, 84296, 84343, 84549, 84889, 84891, 90806, 90990, 91431, 92667, 93099, 112399, 113130, 114569, 115572, 117156, 120071, 157627, 157695, 220042, 221079, 347733, 548596
AV	Beltran et al., Cancer Discov (2011)	268, 292, 317, 610, 613, 656, 699, 701, 745, 782, 835, 890, 899, 990, 991, 997, 1058, 1062, 1063, 1072, 1072, 1122, 1132, 1141, 1351, 1387, 1412, 1455, 1503, 1663, 1759, 1846, 1850, 1869, 1870, 1871, 1917, 1952, 1978, 2026, 2161, 2176, 2237, 2245, 2256, 2297, 2305, 2491, 2529, 2563, 2583, 2584, 2644, 2781, 2786, 2821, 2918, 3026, 3161, 3195, 3225, 3227, 3609, 3619, 3710, 3714, 3714, 3757, 3777, 3796, 3832, 3992, 4001, 4023, 4131, 4171, 4174, 4175, 4176, 4241, 4288, 4521, 4605, 4642, 4661, 4670, 4751, 4803, 4821, 4841, 4879, 4881, 4917, 4985, 4998, 5050, 5260, 5347, 5355, 5424, 5442, 5478, 5557, 5603, 5623, 5630, 5662, 5753, 5985, 6175, 6241, 6294, 6297, 6455, 6502, 6590, 6597, 6620, 6749, 6790, 6804, 6812, 6833, 6839, 6853, 6855, 6860, 6861, 7023, 7036, 7083, 7153, 7175, 7329, 7425, 7516, 7546, 7703, 8021, 8120, 8175, 8193, 8208, 8290, 8317, 8318, 8359, 8475, 8497, 8614, 8655, 8914, 8927, 8941, 9080, 9088, 9127, 9128, 9133, 9148, 9156, 9203, 9212, 9232, 9319, 9355, 9479, 9480, 9493, 9515, 9524, 9578, 9582, 9700, 9735, 9787, 9824, 9837, 9918, 9928, 10036, 10083, 10112, 10287, 10459, 10460, 10501, 10535, 10635, 10733, 10736, 10744, 10814, 10900, 10908, 10921, 10992, 10994, 11000, 11082, 11113, 11130, 11169, 11178, 11182, 11339, 22859, 22974, 22983, 22994, 23025, 23046, 23138, 23299, 23307, 23370, 23373, 23396, 23594, 23649, 24137, 24148, 25789, 25862, 26000, 26000, 26251, 26255, 26528, 27156, 27245, 27324, 27338, 28231, 28511, 29089, 29128, 29843, 29954, 51203, 51291, 51412, 51512, 51514, 51621, 51673, 51690, 53354, 53615, 53637, 53820, 53820, 54332, 54438, 54443, 54503, 54520, 54734, 54825, 55038, 55071, 55122, 55135, 55143, 55165, 55224, 55229, 55247, 55295, 55355, 55388, 55530, 55635, 55658, 55722, 55723, 55753, 55771, 55789, 55964, 56033, 56675, 56896, 56901, 56905, 56938, 56995, 57082, 57125, 57156, 57405, 57418, 57464, 57468, 57473, 57540, 57574, 57657, 57719, 58492, 58509, 60386, 63967, 64105, 64377, 64711, 64858, 65012, 65055, 79002, 79019, 79075, 79140, 79173, 79575, 79605, 79677, 79709, 79728, 79784, 79801, 79829, 79862, 79968, 80178, 80329, 80757, 81539, 81576, 81620, 81831, 81930, 83481, 83546, 83694, 83723, 83786, 83903, 84131, 84140, 84444, 84464, 84530, 84634, 84684, 84687, 84823, 84894, 85356, 85446, 85446, 85446, 85455, 89796, 89839, 89891, 90249, 90378, 90379, 90557, 90580, 90668, 90835, 91039, 92591, 92691, 93323, 94032, 108961, 113130, 114787, 115650, 115827, 115948, 116028, 124222, 126567, 128239, 134266, 138715, 146330, 146909, 147341, 147841, 149175, 150468, 151835, 153478, 158405, 164284, 165918, 169714, 170393, 170463, 171169, 192683, 195828, 196403, 199699, 201161, 201725, 219988, 220042, 220134, 220359, 221150, 222389, 222662, 245812, 253430, 253982, 254099, 254173, 254263, 254295, 254559, 255349, 256472, 259266, 283385, 283431, 283989, 284069, 284338, 284339, 284403, 284716, 284992, 285643, 286151, 286826, 338707, 339674, 339778, 343702, 348738, 349152, 374407, 374946, 386684, 387273, 389792, 391123, 399665, 401491, 401548, 401647, 401827, 404217, 440021, 494143, 494470, 574029, 645191, 653820, 654429, 728116, 100124700, 100130776, 100133941, 101927813, 101929705, 5426, 84642
PN	Phillips et al., Cancer Cell (2006)	108, 163, 230, 348, 403, 429, 534, 547, 650, 1038, 1272, 1410, 1645, 1826, 2037, 2047, 2147, 2257, 2258, 2259, 2550, 2556, 2562, 2571, 2746, 2747, 2774, 2775, 2890, 2891, 2893, 2894, 2900, 3131, 3400, 3745, 3782, 3798,

Pathway	Reference	Genes (Entrez Gene ID)
		3821, 3823, 3983, 4093, 4094, 4137, 4168, 4330, 4675, 4684, 4915, 4926, 4974, 4978, 5017, 5027, 5046, 5067, 5138, 5164, 5166, 5590, 5662, 5730, 5881, 5911, 6125, 6137, 6167, 6252, 6319, 6328, 6445, 6456, 6505, 6509, 6571, 6585, 6751, 6752, 6886, 7067, 7079, 7168, 7477, 7915, 8502, 8549, 8787, 8812, 9026, 9185, 9229, 9241, 9568, 9699, 9844, 9846, 9892, 9951, 10014, 10083, 10129, 10203, 10215, 10276, 10580, 10633, 10683, 10690, 10718, 10882, 10900, 11074, 22885, 22986, 23017, 23046, 23220, 23236, 23373, 23492, 23493, 23542, 23544, 23769, 25789, 25817, 25956, 26032, 26033, 26050, 26052, 26232, 26999, 27087, 27254, 27344, 27439, 28514, 29106, 29767, 30812, 30845, 51560, 51704, 53342, 53616, 53826, 53829, 53844, 54988, 55022, 55217, 55273, 55553, 55612, 55966, 56288, 56475, 56479, 56521, 56884, 56899, 56961, 57338, 57348, 57406, 57447, 57453, 57512, 57628, 58473, 58504, 59277, 63827, 63876, 64093, 64101, 64376, 65258, 78986, 79176, 79187, 79754, 80309, 80351, 83698, 83937, 84440, 84457, 84502, 84631, 89874, 90362, 91752, 114788, 114805, 116154, 116173, 116448, 118738, 128414, 129049, 129807, 140767, 153811, 219654, 219736, 219931, 220164, 255426, 256987, 259217, 259232, 283455, 283576, 284244, 286499, 338645, 340554, 349136, 386618, 728215, 5414, 10777, 222389, 738, 196500, 28984, 55857, 134701, 4325, 375704, 286097, 220965, 2681, 143381, 202451, 254559, 650392, 8123, 387590, 54886
MES	Phillips et al., Cancer Cell (2006)	59, 87, 285, 290, 602, 715, 771, 861, 871, 976, 977, 1116, 1200, 1282, 1284, 1889, 1902, 1948, 2012, 2014, 2034, 2200, 2242, 2266, 2316, 2321, 2355, 2358, 2615, 2683, 3101, 3269, 3371, 3383, 3675, 3678, 3679, 3726, 3976, 4323, 4627, 4642, 5002, 5054, 5069, 5154, 5157, 5265, 5266, 5322, 5328, 5329, 5371, 5606, 5756, 5819, 6237, 6238, 6263, 6282, 6448, 6464, 6876, 7056, 7076, 7791, 8572, 8693, 8793, 8828, 8829, 9021, 9103, 9123, 9180, 9235, 9260, 9454, 9961, 10395, 10398, 10410, 10581, 10630, 11082, 11178, 22904, 25825, 26031, 26231, 27443, 29126, 30008, 30846, 50619, 51129, 51279, 51312, 53834, 53918, 55020, 55240, 56926, 56937, 56975, 56996, 57124, 57167, 57381, 57619, 63892, 64116, 79156, 80270, 80305, 81622, 81844, 83855, 83871, 84875, 90853, 91107, 114897, 126133, 140825, 196410, 199720, 221395, 284119, 284207, 388115, 399473, 647115, 8553, 55267, 9780, 100132244, 151300, 727901
PRF	Phillips et al., Cancer Cell (2006)	97, 580, 672, 699, 890, 891, 990, 995, 1017, 1029, 1031, 1058, 1062, 1063, 1111, 1164, 1719, 1869, 1894, 1964, 2013, 2146, 2177, 2491, 2730, 3070, 3148, 3161, 3598, 3673, 4001, 4085, 4116, 4171, 4175, 4291, 4678, 4751, 4867, 4925, 5074, 5111, 5163, 5303, 5393, 5480, 5558, 5634, 5888, 5932, 5965, 5984, 6119, 6240, 6241, 6474, 6491, 6790, 6941, 7112, 7153, 7272, 7298, 7398, 7465, 7518, 7913, 7979, 8089, 8208, 8833, 8836, 8914, 9134, 9140, 9360, 9493, 9735, 9768, 9787, 9833, 9837, 9928, 10036, 10040, 10051, 10052, 10403, 10592, 10605, 10635, 10733, 10926, 11130, 11169, 22823, 22995, 23089, 23366, 23397, 23421, 23461, 23658, 24137, 26271, 27101, 29957, 29969, 29980, 51203, 51514, 51605, 51659, 51668, 54821, 54970, 55055, 55110, 55151, 55215, 55329, 55355, 55521, 55732, 55839, 55871, 56938, 57001, 57415, 58487, 64105, 64149, 64151, 79022, 79733, 79980, 79989, 80173, 80204, 81853, 81930, 83540, 83879, 84057, 84250, 84283, 84288, 89891, 91057, 91687, 92092, 92610, 121227, 132430, 132884, 139886, 144455, 147841, 151246, 165055, 171586, 195828, 221662, 259266, 374618, 441054, 63926, 55010, 574036, 84791, 89876, 84984, 387103, 115106, 983, 54801, 643911, 79682, 23594, 122769, 8487

Identification and validation of molecular subgroups

[0161] We performed unsupervised clustering based on consensus NMF clustering (Brunet JP, Tamayo P, Golub TR, Mesirov JP. Metagenes and molecular pattern discovery using matrix factorization. Proc Natl Acad Sci U S A 2004;101:4164–9) using the 14 pathway activation profiles in the DISC cohort. A consensus map of the NMF clustering results shows clear separation of the samples into three clusters (**Fig. 2A**). To identify the optimal number of clusters and to assess robustness of the clustering result, we computed the cophenetic coefficient and silhouette score using different numbers of clusters (Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007;448:595–9; Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, et al. Gene expression correlates of clinical prostate cancer behavior. Cancer Cell 2002;1:203–9; Lapointe J, Li C, Higgins JP, van de RijnM, Bair E, Montgomery K, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A 2004;101:811–6; Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22; Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–43). These results indicate that 3 clusters is a statistically optimal representation of the data (**Fig. 2B**). A heatmap of 3 sample clusters demonstrates highly consistent pathway activation patterns within each group (**Fig. 2C**). These analyses suggest that the clusters correspond to three prostate cancer subtypes. We compared the magnitude of activation of each pathway across the 3 clusters evident in **Fig. 2C** using the Wilcoxon rank-sum test for pairwise comparisons (**Fig. 2I**). The PCS1 subtype exhibits high activation scores for EZH2, PTEN, PRF, ES, AV, and AR-V pathways. In contrast, ERG pathway activation predominates in PCS2, which is also characterized by high activation ofAR, FOXA1, and SPOP. PCS3 exhibits high activation of RAS, PN, MES, while AR and AR-V activation are low.

[0162] High enrichment of PRC and low AR within PCS3 raises the question of whether this subtype is an artifact of contaminating nontumor tissues. However, PCA demonstrates that samples in PCS3 are as distinct from benign tissues as samples in the other subtypes (**Fig. 2D**). To further confirm the difference from benign tissue, we made use of a gene signature shown to discriminate benign prostate tissue from cancer in a previous study (Stuart RO, Wachsman W, Berry CC, Wang-Rodriguez J, Wasserman L, Klacansky I, et al. In silico

dissection of cell-type-associated patterns of gene expression in prostate cancer. Proc Natl Acad Sci U S A 2004;101:615–20) and found a significant difference ($P < 0.001$) in all the tumors in the subtypes compared with benign tissues (**Fig. 2J**). These results demonstrate that prostate cancers retain distinct gene expression profiles between subtypes, which are not related to the amount of normal tissue contamination.

[0163] To validate the PCS classification scheme, a 14-pathway classifier was developed using a naïve Bayes machine learning algorithm (see details in Materials and Methods). This classifier was applied to 9 independent cohorts of localized tumors (i.e., SWD, TCGA, EMORY, HSPT, MAYO1/2, CCF, TJU, and JHM) and the SU2C cohort of CRPC/Met tumors. Out of these 10 independent cohorts, 5 cohorts (i.e., MAYO1/2, TJU, CCF, and JHM) were from the GRID (**Fig. 2E**; **Table 1**; Tomlins SA, Alshalalfa M, Davicioni E, Erho N, Yousefi K, Zhao S, et al. Characterization of 1577 primary prostate cancers reveals novel biological and clinicopathologic insights into molecular subtypes. Eur Urol 2015; 68:555–67). The 14-pathway classifier reliably categorized tumors in the DISC cohort into 3 subtypes, with an average classification performance = 0.89 ($P < 0.001$). The 3 subtypes were identified in all cohorts. Their proportions were similar across the localized disease cohorts, demonstrating the consistency of the classification algorithm across multiple practice settings (**Fig. 2E**). The 2 cohorts consisting of CRPC/Met tumors (DISC and SU2C) showed some differences in the frequency of PCS1 and PCS3; the most frequent subtype in the DISC CRPC/Met cohort was PCS1 (66%), while the most frequent subtype in SU2C was PCS3 (45%; **Fig. 2F**). PCS2 was the minor subtype in both CRPC/Met cohorts.

[0164] To determine whether the PCS classification is relevant to laboratory models of prostate cancer, we analyzed 8 human prostate cancer cell lines from The Cancer Cell Line Encyclopedia (CCLE; GSE36133; Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483:603–7 and 11 prostate cancer mouse models (Aytes A, Mitrofanova A, Lefebvre C, Alvarez MJ, Castillo-Martin M, Zheng T, et al. Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. Cancer Cell 2014;25:638–51; Mulholland DJ, Kobayashi N, Ruscetti M, Zhi A, Tran LM, Huang J, et al. Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. Cancer Res 2012;72:1878–89). There are two datasets for mouse models. The first dataset (GSE53202) contains transcriptome profiles of 13 genetically

engineered mouse models, including normal epithelium (i.e., wild-type), low-grade PIN (i.e., Nkx3.1 and APT), high-grade PIN, and adenocarcinoma (i.e., APT-P, APC, Myc, NP, Erg-P, and NP53), CRPC (i.e., NP-Ai), and metastatic prostate cancer (i.e., NPB, NPK, and TRAMP). Because of no available data for samples without drug treatment, the Nkx3.1 and APC models were excluded from this analysis. The second dataset (GSE34839) contains transcriptome profiles from mice with *PTEN*-null/*KRAS* activation mutation-driven high-grade, invasive prostate cancer and mice with only the *PTEN*-null background. This analysis revealed that all 3 prostate cancer subtypes were represented in the 8 human prostate cancer cell lines (**Fig. 2G**), while only 2 subtypes (PCS1 and PCS2) were represented in the mouse models (**Fig. 2H**). This result provides evidence that the subtypes are recapitulated in genetically engineered mouse models and persist in human cancer cells in cell culture.

Evaluation of PCS subtypes in comparison with other subtypes

[0165] Several categorization schemes of prostate cancer have been described, based mostly on tumor-specific genomic alterations and in some cases with integration of transcriptomic and other profiling data (Markert EK, Mizuno H, Vazquez A, Levine AJ. Molecular classification of prostate cancer using curated expression signatures. Proc Natl Acad Sci U S A 2011;108:21276–81; Tomlins SA, Alshalalfa M, Davicioni E, Erho N, Yousefi K, Zhao S, et al. Characterization of 1577 primary prostate cancers reveals novel biological and clinicopathologic insights into molecular subtypes. Eur Urol 2015; 68:555–67; Erho N, Crisan A, Vergara IA, Mitra AP, Ghadessi M, Buerki C, et al. Discovery and validation of a prostate cancer genomic classifier that predicts early metastasis following radical prostatectomy. PLoS One 2013;8:e66855). This prompted us to compare the PCS classification scheme with the genomic subtypes derived by TCGA (Cancer Genome Atlas Research Network. Electronic address scmo, Cancer Genome Atlas Research N. The Molecular Taxonomy of Primary Prostate Cancer. Cell 2015;163:1011–25) , because comprehensive genomic categorization was recently made available (Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161:1215–28). We also compared the PCS classification with the subtypes recently defined by Tomlins and colleagues from RNA expression data (Tomlins SA, Alshalalfa M, Davicioni E, Erho N, Yousefi K, Zhao S, et al. Characterization of 1577 primary prostate cancers reveals novel biological and clinicopathologic insights into molecular subtypes. Eur Urol 2015; 68:555–67). The Tomlins subtyping scheme is defined

using the 7 GRID cohorts (i.e., MAYO1/2, TJU, CCF, MSKCC, EMC, and JHM) that we used for validating the PCS system. The large number of cases in the 7 GRID cohorts (n = 1,626) is comparable with our DISC cohort in terms of heterogeneity and complexity. TCGA identified several genomic subtypes, named ERG, ETV1, ETV4, FLI1, SPOP, FOXA1, IDH1, and "other." Tomlins and colleagues described 4 subtypes based on microarray gene expression patterns that are related to several genomic aberrations [i.e., $ERG^{\pm}$, $ETS^{\pm}$, $SPINK1^{\pm}$, and triple negative ($ERG^{-}/ETS^{-}/SPINK1^{-}$)].

[0166] A comparison of the PCS categories with the TCGA genomic subtypes showed that the tumors classified as ERG, ETV1/4, SPOP, FOXA1, and "other" were present across all the PCS categories in the TCGA dataset (n = 333; **Fig. 3A**). SPOP cancers were enriched in PCS1 (OR: 3.53), while PCS2 tumors were overrepresented in TCGA/ERG cancers (OR: 1.82) and TCGA/"other" cancers were enriched in PCS3 (OR: 1.79; **Fig. 3B**). In the GRID cohorts, we observed all PCS categories in all classification groups as defined by Tomlins and colleagues (**Fig. 3C and Fig. 3D**). We found a high frequency of the Tomlins/$ERG^{\pm}$ subtype in PCS2, but not in PCS1. PCS1 was enriched for Tomlins/$ETS^{\pm}$ and Tomlins/$SPINK1^{\pm}$ subtypes, while PCS3 was enriched for the triple-negative subtype but not the $ERG^{\pm}$ or $ETS^{\pm}$ subgroups. Finally, we compared the Tomlins classification method with the PCS classification using 5 of 7 GRID cohorts. PCS1 demonstrated significantly shorter metastasis-free survival compared with PCS2 and PCS3 ($P < 0.001$; **Fig. 3E**). In contrast, no difference in metastatic progression was seen among the Tomlins categories (**Fig. 3F**).

[0167] PCS1 contained the largest number of prostate cancers with GS > 8 (**Fig. 2C**). Given the overall poorer outcomes seen in PCS1 tumors, we tested whether this result was simply a reflection of the enrichment of high-grade disease in this group (i.e., GS > 8). For this analysis, we merged 5 GRID cohorts (i.e., MAYO1/2, TJU, CCF, and JHM) into a single dataset and separately analyzed low and high-grade disease. We observed a similarly significant ($P < 0.001$) association between subtypes and metastasis-free survival in GS < 7 and in GS > 8 (**Fig. 3G**). Thus, tumors in the PCS1 group exhibit the poorest prognosis, including in tumors with low Gleason sum score. Finally, in the DISC cohort, although CRPC/Met tumors were present in all PCS categories, PCS1 predominated (66%), followed by PCS3 (27%) and PCS2 (7%) tumors. To confirm whether this clinical correlation is replicated in individual cohorts, we also assessed association with time to metastatic progression, prostate cancer–specific mortality (PCSM), and overall survival (OS) in 5 individual cohorts in the GRID (i.e., MAYO1/2, CCF, TJU, and JHM) and in the SWD

cohorts. PCS1 was seen to be the most aggressive subtype, consistent with the above results (**Fig. 3H(i-x)**).

PCS categories possess characteristics of basal and luminal prostate epithelial cells

[0168] Prostate cancer may arise from oncogenic transformation of different cell types in glandular prostate epithelium (Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. Identification of a cell of origin for human prostate cancer. Science 2010;329:568–71; Wang ZA, Mitrofanova A, Bergren SK, Abate-Shen C, Cardiff RD, Califano A, et al. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. Nat Cell Biol 2013;15:274–83; Baird AA, Muir TC. Membrane hyperpolarization, cyclic nucleotide levels and relaxation in the guinea-pig internal anal sphincter. Br J Pharmacol 1990;100:329–35). Breast cancers can be categorized into luminal and basal subtypes, which are associated with different patient outcomes (Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. Genes Dev 2009;23:2563–77) . It is unknown whether this concept applies to human prostate cancer. To examine whether the 3 PCS categories are a reflection of different cell types, we identified 428 SEGs (SEG1–3; 86 for PCS1, 123 for PCS2, and 219 for PCS3; **Table 6**) in each subtype. As expected, these genes are involved in pathways that are enriched in each subtype (**Fig. 4A**) and that define the perturbed cellular processes of the subtype. We then identified the cellular processes that are associated with the SEGs. Proliferation and lipid/steroid metabolism are characteristic of SEG1 and SEG2, while extracellular matrix organization, inflammation, and cell migration are characteristic of SEG3 (**Fig. 4B**). This result suggests that distinct biological functions are associated with the PCS categories.

[0169] Table 6: List of 428 SEGs.

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
1	699	BUB1	1	0.733	-0.29	-0.359
2	24137	KIF4A	1	0.797	-0.36	-0.354
3	890	CCNA2	1	0.705	-0.23	-0.389
4	1062	CENPE	1	0.607	-0.25	-0.29
5	1164	CKS2	1	1.037	-0.26	-0.649
6	9787	DLGAP5	1	0.832	-0.31	-0.423
7	11004	KIF2C	1	0.737	-0.37	-0.289
8	701	BUB1B	1	0.742	-0.23	-0.428

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
9	983	CDK1	1	0.965	-0.3	-0.547
10	990	CDC6	1	0.617	-0.17	-0.374
11	1058	CENPA	1	0.704	-0.34	-0.291
12	9493	KIF23	1	0.61	-0.32	-0.227
13	891	CCNB1	1	0.796	-0.16	-0.539
14	991	CDC20	1	0.918	-0.46	-0.365
15	1063	CENPF	1	1.176	-0.45	-0.593
16	3161	HMMR	1	0.917	-0.29	-0.519
17	6241	RRM2	1	0.963	-0.26	-0.582
18	6790	AURKA	1	0.789	-0.26	-0.435
19	9133	CCNB2	1	0.869	-0.2	-0.561
20	9232	PTTG1	1	1.163	-0.55	-0.492
21	9735	KNTC1	1	0.611	-0.26	-0.287
22	9928	KIF14	1	0.58	-0.32	-0.203
23	11130	ZWINT	1	0.904	-0.19	-0.602
24	51203	NUSAP1	1	1.089	-0.33	-0.632
25	113130	CDCA5	1	0.688	-0.3	-0.311
26	259266	ASPM	1	0.913	-0.38	-0.434
27	4173	MCM4	1	0.662	-0.25	-0.341
28	9768	KIAA0101	1	1.068	-0.27	-0.668
29	22974	TPX2	1	1.099	-0.39	-0.579
30	29128	UHRF1	1	0.748	-0.35	-0.316
31	51514	DTL	1	0.687	-0.36	-0.262
32	332	BIRC5	1	0.927	-0.4	-0.423
33	1894	ECT2	1	0.654	0.15	-0.698
34	2171	FABP5	1	0.59	-0.08	-0.428
35	4001	LMNB1	1	0.691	-0.26	-0.357
36	7153	TOP2A	1	1.213	-0.33	-0.733
37	7272	TTK	1	0.785	-0.2	-0.493
38	7298	TYMS	1	0.717	-0.34	-0.303
39	8318	CDC45	1	0.602	-0.25	-0.286
40	9088	PKMYT1	1	0.608	-0.37	-0.182
41	9833	MELK	1	1.008	-0.35	-0.538
42	10112	KIF20A	1	0.878	-0.38	-0.406
43	11113	CIT	1	0.587	-0.35	-0.181
44	54845	ESRP1	1	0.61	0.232	-0.736
45	55355	HJURP	1	0.656	-0.23	-0.347
46	64151	NCAPG	1	0.872	-0.35	-0.429
47	79019	CENPM	1	0.59	-0.31	-0.221
48	81831	NETO2	1	0.61	0.162	-0.672
49	55502	HES6	1	0.604	-0.27	-0.273
50	2146	EZH2	1	1.007	-0.2	-0.676
51	7366	UGT2B15	1	0.609	-0.43	-0.122
52	54443	ANLN	1	0.696	-0.32	-0.3
53	54892	NCAPG2	1	0.611	-0.12	-0.416
54	56992	KIF15	1	0.699	-0.31	-0.312
55	83540	NUF2	1	0.753	-0.31	-0.358
56	213	ALB	1	0.631	-0.32	-0.249

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
57	367	AR	1	0.739	-0.09	-0.555
58	2305	FOXM1	1	0.693	-0.34	-0.279
59	3148	HMGB2	1	0.594	-0.18	-0.346
60	3832	KIF11	1	0.603	-0.21	-0.326
61	3925	STMN1	1	0.756	-0.2	-0.465
62	4288	MKI67	1	0.634	-0.18	-0.382
63	7083	TK1	1	0.835	-0.49	-0.267
64	9055	PRC1	1	0.881	-0.29	-0.487
65	9134	CCNE2	1	0.6	-0.18	-0.353
66	9156	EXO1	1	0.604	-0.31	-0.235
67	10024	TROAP	1	0.723	-0.39	-0.26
68	10460	TACC3	1	0.619	-0.38	-0.185
69	11065	UBE2C	1	1.164	-0.47	-0.566
70	29089	UBE2T	1	0.894	-0.39	-0.411
71	29127	RACGAP1	1	0.749	-0.24	-0.42
72	55143	CDCA8	1	0.619	-0.26	-0.287
73	55165	CEP55	1	0.698	-0.28	-0.336
74	55872	PBK	1	0.895	-0.34	-0.458
75	79682	MLF1IP	1	0.8	-0.17	-0.531
76	374393	FAM111B	1	0.581	-0.19	-0.326
77	3223	HOXC6	1	0.633	0.21	-0.735
78	1033	CDKN3	1	0.868	-0.29	-0.481
79	1951	CELSR3	1	0.659	-0.39	-0.202
80	6472	SHMT2	1	0.599	-0.03	-0.485
81	6696	SPP1	1	0.841	-0.37	-0.383
82	8438	RAD54L	1	0.618	-0.32	-0.234
83	10615	SPAG5	1	0.785	-0.31	-0.387
84	10721	POLQ	1	0.581	-0.28	-0.238
85	29923	HILPDA	1	0.796	-0.31	-0.4
86	51155	HN1	1	0.631	-0.13	-0.419
87	8611	PPAP2A	2	-0.23	0.73	-0.472
88	10551	AGR2	2	-0.58	0.974	-0.395
89	4824	NKX3-1	2	-0.31	0.585	-0.276
90	4072	EPCAM	2	0.349	0.63	-0.879
91	5865	RAB3B	2	-0.18	0.895	-0.672
92	6480	ST6GAL1	2	-0.56	0.691	-0.159
93	23671	TMEFF2	2	0.147	0.789	-0.852
94	262	AMD1	2	-0.32	0.657	-0.326
95	10040	TOM1L1	2	-0.03	0.611	-0.537
96	384	ARG2	2	-0.45	0.625	-0.192
97	776	CACNA1D	2	0.129	0.628	-0.688
98	2982	GUCY1A3	2	-0.09	0.655	-0.527
99	6675	UAP1	2	-0	0.682	-0.624
100	354	KLK3	2	-0.56	0.738	-0.196
101	2153	F5	2	0.265	0.774	-0.939
102	3109	HLA-DMB	2	-0.43	0.833	-0.399
103	3781	KCNN2	2	-0.02	0.834	-0.751
104	10257	ABCC4	2	-0.04	0.841	-0.741

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
105	27347	STK39	2	-0.13	0.623	-0.458
106	57630	SH3RF1	2	0.047	0.602	-0.594
107	445347	TARP	2	-0.14	0.94	-0.743
108	1298	COL9A2	2	-0.19	0.674	-0.453
109	1803	DPP4	2	-0.86	0.714	0.082
110	2690	GHR	2	-0.43	0.657	-0.24
111	4646	MYO6	2	0.077	0.905	-0.898
112	81035	COLEC12	2	-0.09	0.589	-0.468
113	55	ACPP	2	-1.24	0.798	0.326
114	220	ALDH1A3	2	-0.75	0.875	-0.16
115	288	ANK3	2	-0.18	0.585	-0.386
116	1718	DHCR24	2	-0.1	0.661	-0.519
117	1824	DSC2	2	-0.17	0.732	-0.528
118	2078	ERG	2	-0.48	1.143	-0.643
119	2152	F3	2	-0.77	0.7	0.014
120	2181	ACSL3	2	-0.16	0.777	-0.579
121	2331	FMOD	2	-0.97	0.848	0.049
122	2650	GCNT1	2	-0.1	0.819	-0.671
123	2705	GJB1	2	-0.16	0.678	-0.484
124	3249	HPN	2	0.233	0.714	-0.856
125	3817	KLK2	2	-0.52	0.619	-0.124
126	3936	LCP1	2	-0.58	0.625	-0.081
127	4070	TACSTD2	2	-0.68	0.711	-0.069
128	4477	MSMB	2	-1.67	0.865	0.635
129	4604	MYBPC1	2	-0.68	0.713	-0.071
130	5238	PGM3	2	-0.12	0.676	-0.522
131	5530	PPP3CA	2	-0.01	0.613	-0.555
132	6652	SORD	2	-0.42	0.644	-0.236
133	6695	SPOCK1	2	-0.43	0.959	-0.512
134	7113	TMPRSS2	2	-0.35	0.626	-0.278
135	7941	PLA2G7	2	-0.27	1.198	-0.872
136	8671	SLC4A4	2	-0.37	0.704	-0.328
137	9073	CLDN8	2	-0.17	0.826	-0.617
138	10269	ZMPSTE24	2	-0.05	0.611	-0.521
139	10321	CRISP3	2	-0.16	1.018	-0.802
140	10611	PDLIM5	2	0.137	0.592	-0.661
141	10788	IQGAP2	2	-0.32	0.907	-0.565
142	10954	PDIA5	2	-0.09	0.582	-0.46
143	23316	CUX2	2	-0.43	0.605	-0.185
144	23327	NEDD4L	2	-0.06	0.646	-0.541
145	25800	SLC39A6	2	-0.06	0.629	-0.524
146	51109	RDH11	2	-0.38	0.588	-0.212
147	51313	FAM198B	2	-0.17	0.591	-0.399
148	51365	PLA1A	2	-0.13	0.826	-0.652
149	57600	FNIP2	2	-0.12	0.742	-0.578
150	58511	DNASE2B	2	-0.07	0.682	-0.568
151	59084	ENPP5	2	-0.27	0.585	-0.304
152	60481	ELOVL5	2	-0.12	0.621	-0.47

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
153	79054	TRPM8	2	-0.52	0.886	-0.372
154	79689	STEAP4	2	-0.26	0.78	-0.493
155	116285	ACSM1	2	0.164	0.723	-0.806
156	130733	TMEM178A	2	-0.69	0.848	-0.19
157	143503	OR51E1	2	-0.12	0.641	-0.483
158	148327	CREB3L4	2	-0.19	0.621	-0.412
159	151258	SLC38A11	2	-0.19	0.589	-0.378
160	9185	REPS2	2	-0.05	0.647	-0.549
161	2203	FBP1	2	-0.37	0.713	-0.34
162	7782	SLC30A4	2	-0.49	0.678	-0.201
163	10481	HOXB13	2	-0.04	0.611	-0.531
164	11001	SLC27A2	2	0.078	0.581	-0.602
165	57535	KIAA1324	2	-0.6	0.837	-0.258
166	120224	TMEM45B	2	0.173	0.677	-0.772
167	306	ANXA3	2	-0.91	0.918	-0.061
168	957	ENTPD5	2	-0.15	0.696	-0.509
169	2346	FOLH1	2	0.03	0.926	-0.877
170	3081	HGD	2	-0.57	0.717	-0.175
171	4744	NEFH	2	-1.38	0.58	0.646
172	4852	NPY	2	-1.12	1.599	-0.513
173	5320	PLA2G2A	2	-0.88	0.833	-0.012
174	5874	RAB27B	2	-0.4	0.595	-0.206
175	6296	ACSM3	2	2E-04	0.653	-0.601
176	6558	SLC12A2	2	-0.41	0.74	-0.326
177	6646	SOAT1	2	-0.13	0.602	-0.445
178	7103	TSPAN8	2	-0.43	0.63	-0.214
179	9375	TM9SF2	2	-0.25	0.587	-0.328
180	9413	FAM189A2	2	-0.52	0.58	-0.089
181	10103	TSPAN1	2	-0.42	0.716	-0.302
182	11013	TMSB15A	2	-0.04	0.851	-0.753
183	23600	AMACR	2	0.188	1.177	-1.244
184	25874	MPC2	2	0.115	0.594	-0.645
185	26503	SLC17A5	2	-0.08	0.591	-0.475
186	26872	STEAP1	2	0.065	0.6	-0.608
187	26996	GPR160	2	0.169	0.821	-0.9
188	27249	MMADHC	2	-0.31	0.662	-0.343
189	51084	CRYL1	2	-0.32	0.619	-0.298
190	51170	HSD17B11	2	-0.06	0.601	-0.506
191	51280	GOLM1	2	-0.31	0.914	-0.574
192	51302	CYP39A1	2	-0.29	0.624	-0.323
193	51635	DHRS7	2	-0.37	0.742	-0.364
194	51809	GALNT7	2	-0.11	0.78	-0.623
195	54431	DNAJC10	2	-0.14	0.767	-0.59
196	54502	RBM47	2	-0.21	0.585	-0.359
197	55790	CSGALNACT1	2	-0.58	0.877	-0.313
198	56165	TDRD1	2	-0.4	1.094	-0.661
199	64094	SMOC2	2	-0.5	0.621	-0.147

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
200	80110	ZNF614	2	-0.05	0.607	-0.517
201	80157	CWH43	2	-0.35	0.614	-0.261
202	81285	OR51E2	2	-0.51	1.197	-0.661
203	84419	C15orf48	2	-0.46	0.607	-0.166
204	84899	TMTC4	2	-0.08	0.66	-0.54
205	90701	SEC11C	2	-0.29	0.742	-0.437
206	92292	GLYATL1	2	-0.06	0.704	-0.595
207	131034	CPNE4	2	-0.29	0.788	-0.477
208	219595	FOLH1B	2	0.156	0.635	-0.718
209	284370	ZNF615	2	-0.09	0.586	-0.464
210	70	ACTC1	3	-1.02	-0.15	1.011
211	72	ACTG2	3	-1.77	0.32	1.218
212	477	ATP1A2	3	-0.87	-0.17	0.899
213	5919	RARRES2	3	-0.66	-0.29	0.839
214	2919	CXCL1	3	-0.46	-0.24	0.612
215	5239	PGM5	3	-1.25	-0.01	1.08
216	6876	TAGLN	3	-0.95	-0.05	0.856
217	7881	KCNAB1	3	-0.51	-0.17	0.591
218	10418	SPON1	3	-0.55	-0.21	0.662
219	284	ANGPT1	3	-0.69	-0.17	0.75
220	1674	DES	3	-1.32	-0.07	1.193
221	1805	DPT	3	-0.62	-0.27	0.779
222	2354	FOSB	3	-1.03	0.277	0.629
223	2568	GABRP	3	-0.39	-0.28	0.595
224	4638	MYLK	3	-1.44	0.28	0.973
225	4660	PPP1R12B	3	-0.76	0.013	0.637
226	4681	NBL1	3	-0.58	-0.19	0.667
227	4921	DDR2	3	-0.62	-0.06	0.581
228	5918	RARRES1	3	-0.67	-0.18	0.738
229	5947	RBP1	3	-0.28	-0.37	0.581
230	7047	TGM4	3	-0.71	-0.12	0.719
231	7169	TPM2	3	-1.14	-0.15	1.114
232	9510	ADAMTS1	3	-0.57	-0.17	0.651
233	10563	CXCL13	3	-0.22	-0.52	0.66
234	3371	TNC	3	-0.58	-0.12	0.606
235	4684	NCAM1	3	-0.27	-0.42	0.619
236	59	ACTA2	3	-1.07	0.044	0.877
237	290	ANPEP	3	-0.86	0.065	0.678
238	467	ATF3	3	-0.81	0.106	0.6
239	1288	COL4A6	3	-0.68	-0.23	0.791
240	1410	CRYAB	3	-0.72	-0.39	0.983
241	2294	FOXF1	3	-0.64	-0.19	0.722
242	2316	FLNA	3	-0.8	-0.06	0.739
243	2920	CXCL2	3	-0.46	-0.24	0.611
244	3678	ITGA5	3	-0.51	-0.28	0.695
245	3679	ITGA7	3	-0.58	-0.18	0.655
246	3872	KRT17	3	-0.59	-0.22	0.71
247	4118	MAL	3	-0.3	-0.4	0.63

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
248	4629	MYH11	3	-1.55	0.135	1.203
249	5179	PENK	3	-0.42	-0.41	0.73
250	5268	SERPINB5	3	-0.5	-0.19	0.597
251	5376	PMP22	3	-0.58	-0.23	0.712
252	5730	PTGDS	3	-1.01	-0.03	0.89
253	6277	S100A6	3	-0.63	-0.22	0.746
254	6387	CXCL12	3	-0.46	-0.21	0.587
255	6525	SMTN	3	-0.73	-0.21	0.818
256	6716	SRD5A2	3	-1.02	0.009	0.864
257	7168	TPM1	3	-0.88	0.135	0.631
258	7538	ZFP36	3	-1.11	0.393	0.592
259	8013	NR4A3	3	-0.65	-0.03	0.586
260	8406	SRPX	3	-0.57	-0.14	0.621
261	8854	ALDH1A2	3	-0.78	-0.03	0.696
262	8870	IER3	3	-0.53	-0.24	0.668
263	9021	SOCS3	3	-0.77	-0.02	0.672
264	9260	PDLIM7	3	-0.49	-0.25	0.645
265	9506	PAGE4	3	-1.39	0.087	1.109
266	10398	MYL9	3	-1.13	-0.16	1.117
267	10580	SORBS1	3	-0.98	0.011	0.831
268	22943	DKK1	3	-0.37	-0.3	0.592
269	25802	LMOD1	3	-1.04	-0.13	1.011
270	30008	EFEMP2	3	-0.36	-0.32	0.609
271	50859	SPOCK3	3	-0.86	-0.06	0.789
272	53826	FXYD6	3	-0.55	-0.32	0.764
273	64093	SMOC1	3	-0.45	-0.22	0.589
274	284119	PTRF	3	-0.8	-0.08	0.754
275	316	AOX1	3	-0.74	-0.12	0.747
276	390	RND3	3	-0.8	-0.05	0.735
277	443	ASPA	3	-0.45	-0.26	0.618
278	493	ATP2B4	3	-0.56	-0.14	0.607
279	629	CFB	3	-0.64	-0.05	0.593
280	653	BMP5	3	-0.29	-0.36	0.583
281	710	SERPING1	3	-0.68	-0.18	0.75
282	716	C1S	3	-0.81	-0.03	0.723
283	857	CAV1	3	-0.93	-0.08	0.872
284	858	CAV2	3	-0.52	-0.16	0.595
285	894	CCND2	3	-0.51	-0.16	0.583
286	1066	CES1	3	-0.71	-0.19	0.788
287	1191	CLU	3	-0.7	-0.31	0.891
288	1264	CNN1	3	-1.54	0.019	1.302
289	1291	COL6A1	3	-0.4	-0.41	0.719
290	1292	COL6A2	3	-0.53	-0.24	0.677
291	1307	COL16A1	3	-0.51	-0.29	0.708
292	1346	COX7A1	3	-0.8	-0.23	0.904
293	1465	CSRP1	3	-1.1	0.122	0.832
294	1577	CYP3A5	3	-0.58	-0.23	0.711
295	1580	CYP4B1	3	-0.4	-0.27	0.591

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
296	1593	CYP27A1	3	-0.57	-0.21	0.682
297	1672	DEFB1	3	-0.4	-0.29	0.612
298	1675	CFD	3	-0.58	-0.31	0.777
299	1809	DPYSL3	3	-0.7	-0.07	0.665
300	2192	FBLN1	3	-1.13	0.033	0.934
301	2202	EFEMP1	3	-0.54	-0.2	0.647
302	2263	FGFR2	3	-0.67	-0.09	0.655
303	2273	FHL1	3	-1.11	-0.01	0.962
304	2274	FHL2	3	-0.84	-0.03	0.745
305	2318	FLNC	3	-0.75	-0.29	0.911
306	2564	GABRE	3	-0.72	-0.18	0.776
307	2619	GAS1	3	-0.72	-0.11	0.716
308	2934	GSN	3	-0.82	-0.02	0.725
309	2944	GSTM1	3	-0.57	-0.23	0.696
310	2946	GSTM2	3	-0.7	-0.25	0.828
311	2949	GSTM5	3	-0.61	-0.2	0.708
312	2950	GSTP1	3	-0.81	-0.31	0.979
313	3397	ID1	3	-0.75	-0.15	0.779
314	3399	ID3	3	-0.55	-0.16	0.622
315	3489	IGFBP6	3	-0.75	-0.27	0.891
316	3491	CYR61	3	-1.01	0.247	0.635
317	3569	IL6	3	-0.39	-0.33	0.64
318	3764	KCNJ8	3	-0.37	-0.3	0.585
319	3779	KCNMB1	3	-0.95	-0.25	1.044
320	3852	KRT5	3	-0.95	-0.18	0.987
321	3860	KRT13	3	-0.61	-0.19	0.701
322	3866	KRT15	3	-1.1	-0.08	1.022
323	3910	LAMA4	3	-0.37	-0.33	0.623
324	3914	LAMB3	3	-0.59	-0.23	0.719
325	3934	LCN2	3	-0.71	-0.19	0.781
326	3956	LGALS1	3	-0.64	-0.23	0.762
327	4057	LTF	3	-1.1	0.124	0.828
328	4129	MAOB	3	-0.94	0.026	0.783
329	4147	MATN2	3	-0.74	0.051	0.583
330	4211	MEIS1	3	-0.71	-0.05	0.651
331	4212	MEIS2	3	-0.83	-0.03	0.732
332	4239	MFAP4	3	-0.7	-0.19	0.775
333	4920	ROR2	3	-0.49	-0.18	0.589
334	4969	OGN	3	-0.86	0.074	0.667
335	5099	PCDH7	3	-0.52	-0.17	0.601
336	5121	PCP4	3	-1.57	0.231	1.133
337	5176	SERPINF1	3	-0.64	-0.26	0.785
338	5348	FXYD1	3	-0.53	-0.32	0.75
339	5350	PLN	3	-0.85	0.008	0.721
340	5579	PRKCB	3	-0.39	-0.3	0.606
341	5648	MASP1	3	-0.44	-0.22	0.586
342	5764	PTN	3	-0.98	0.065	0.779
343	5837	PYGM	3	-0.52	-0.16	0.591

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
344	6273	S100A2	3	-0.54	-0.14	0.599
345	6275	S100A4	3	-0.42	-0.39	0.726
346	6347	CCL2	3	-0.78	0.006	0.663
347	6376	CX3CL1	3	-0.68	-0.21	0.78
348	6401	SELE	3	-0.8	0.056	0.635
349	6442	SGCA	3	-0.41	-0.26	0.59
350	6518	SLC2A5	3	-0.51	-0.22	0.638
351	6563	SLC14A1	3	-0.79	-0.06	0.739
352	6604	SMARCD3	3	-0.36	-0.32	0.607
353	6769	STAC	3	-0.47	-0.21	0.596
354	6840	SVIL	3	-0.67	-0.03	0.595
355	7041	TGFB1I1	3	-0.52	-0.25	0.667
356	7043	TGFB3	3	-0.57	-0.29	0.759
357	7077	TIMP2	3	-0.44	-0.26	0.614
358	7123	CLEC3B	3	-0.34	-0.36	0.618
359	7145	TNS1	3	-0.85	-0.09	0.809
360	7205	TRIP6	3	-0.47	-0.24	0.62
361	7356	SCGB1A1	3	-0.46	-0.33	0.693
362	7414	VCL	3	-0.6	-0.11	0.619
363	7732	RNF112	3	-0.37	-0.28	0.582
364	8309	ACOX2	3	-0.51	-0.21	0.631
365	8404	SPARCL1	3	-1.2	0.169	0.874
366	8425	LTBP4	3	-0.53	-0.15	0.596
367	8613	PPAP2B	3	-0.67	-0.04	0.612
368	8626	TP63	3	-1.07	0.025	0.896
369	8639	AOC3	3	-0.72	-0.14	0.74
370	8654	PDE5A	3	-0.88	0.092	0.67
371	9843	HEPH	3	-0.45	-0.27	0.638
372	10231	RCAN2	3	-0.64	-0.22	0.749
373	10278	EFS	3	-0.5	-0.23	0.636
374	10290	SPEG	3	-0.54	-0.24	0.685
375	10335	MRVI1	3	-0.66	-0.16	0.709
376	10406	WFDC2	3	-0.64	-0.23	0.76
377	10562	OLFM4	3	-1.1	0.132	0.823
378	10826	FAXDC2	3	-0.48	-0.23	0.623
379	10974	ADIRF	3	-1.01	0.115	0.758
380	11030	RBPMS	3	-0.63	-0.17	0.701
381	11117	EMILIN1	3	-0.41	-0.27	0.601
382	11155	LDB3	3	-0.53	-0.22	0.656
383	11170	FAM107A	3	-0.87	-0.13	0.867
384	11259	FILIP1L	3	-0.6	-0.18	0.685
385	11341	SCRG1	3	-0.48	-0.35	0.731
386	23022	PALLD	3	-0.75	-0.03	0.674
387	23336	SYNM	3	-1.45	0.191	1.067
388	23584	VSIG2	3	-0.6	-0.14	0.642
389	23650	TRIM29	3	-0.82	-0.18	0.871
390	25959	KANK2	3	-0.56	-0.14	0.61
391	25984	KRT23	3	-0.76	-0.14	0.778

Figure 4A Order	Entrez Gene ID	Symbol	Subtype ID	Fold change in PCS1	Fold change in PCS2	Fold change in PCS3
392	25999	CLIP3	3	-0.39	-0.41	0.71
393	26353	HSPB8	3	-0.91	-0.17	0.933
394	26577	PCOLCE2	3	-0.73	-0.11	0.728
395	27122	DKK3	3	-0.7	-0.09	0.684
396	27129	HSPB7	3	-0.36	-0.32	0.598
397	29951	PDZRN4	3	-0.83	-0.01	0.714
398	51285	RASL12	3	-0.57	-0.31	0.769
399	51676	ASB2	3	-0.56	-0.16	0.632
400	55679	LIMS2	3	-0.54	-0.26	0.703
401	58189	WFDC1	3	-0.86	-0.28	0.996
402	59353	TMEM35	3	-0.73	-0.05	0.676
403	64091	POPDC2	3	-0.59	-0.13	0.627
404	79625	NDNF	3	-0.49	-0.23	0.634
405	79630	C1orf54	3	-0.42	-0.26	0.597
406	80206	FHOD3	3	-0.5	-0.22	0.635
407	83643	CCDC3	3	-0.34	-0.31	0.583
408	83716	CRISPLD2	3	-0.7	-0.02	0.621
409	84417	C2orf40	3	-0.7	-0.25	0.823
410	84617	TUBB6	3	-0.57	-0.19	0.667
411	89927	C16orf45	3	-0.46	-0.23	0.604
412	91624	NEXN	3	-0.89	-0.06	0.815
413	91851	CHRDL1	3	-0.99	-0.05	0.896
414	93649	MYOCD	3	-0.61	-0.13	0.64
415	94274	PPP1R14A	3	-0.46	-0.32	0.688
416	112464	PRKCDBP	3	-0.49	-0.26	0.655
417	113146	AHNAK2	3	-0.49	-0.31	0.709
418	116535	MRGPRF	3	-0.64	-0.13	0.67
419	118425	PCAT4	3	-0.84	0.126	0.604
420	126393	HSPB6	3	-0.51	-0.29	0.704
421	140597	TCEAL2	3	-0.82	-0.13	0.83
422	146713	RBFOX3	3	-0.6	-0.1	0.611
423	147906	DACT3	3	-0.52	-0.16	0.591
424	148741	ANKRD35	3	-0.57	-0.2	0.676
425	171024	SYNPO2	3	-1.27	0.266	0.842
426	253827	MSRB3	3	-0.64	-0.08	0.625
427	387763	C11orf96	3	-0.48	-0.27	0.661
428	728264	MIR143HG	3	-0.67	-0.1	0.673

[0170] To determine whether the PCS categories reflect luminal or basal cell types of the prostatic epithelium, we analyzed the mean expression of genes known to be characteristic of luminal (EZH2, AR, MKI67, NKX3-1, KLK2/3, and ERG) or basal (ACTA2, GSTP1, IL6, KRT5, and TP63) prostatic cells (**Fig. 4C**). We observed a strong association (FDR < 0.001; fold change > 1.5) between luminal genes and PCS1 and PCS2, and basal genes and PCS3. To verify this observation, we used two independent datasets derived from luminal and basal

cells from human (Liu H, Cadaneanu RM, Lai K, Zhang B, Huo L, An DS, et al. Differential gene expression profiling of functionally and developmentally distinct human prostate epithelial populations. Prostate 2015;75:764–76) and mouse (GSE39509; Wang ZA, Mitrofanova A, Bergren SK, Abate-Shen C, Cardiff RD, Califano A, et al. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. Nat Cell Biol 2013;15:274–83 prostates. The assignment of a basal designation to PCS3 is further supported by the highly significant enrichment in PCS3, in comparison with the other two subtypes, of a recently described prostate basal cell signature derived from CD49f-Hi versus CD49f-Lo benign and malignant prostate epithelial cells (**Fig. 4D**; Smith BA, Sokolov A, Uzunangelov V, Baertsch R, Newton Y, Graim K, et al. A basal stem cell signature identifies aggressive prostate cancer phenotypes. Proc Natl Acad Sci U S A 2015;112:E6544–52). In addition, using the 14-pathway classifier, mouse basal tumors and human basal cells from benign tissues were classified as PCS3, while mouse luminal tumors and benign prostate human luminal cells were classified into PCS2 (**Fig. 4E**). These results are consistent with the conclusion that the PCS categories can be divided into luminal and basal subtypes.

A gene expression classifier for assignment to subtypes

[0171] Given the potential advantages of the PCS system to classify tumor specimens, we constructed a classifier that can be applied to an individual patient specimen in a clinical setting (**Fig. 5C**). First, of 428 SEGs, 93 genes were selected on the basis of highly consistent expression patterns in 10 cohorts (i.e., SWD, TCGA, EMORY, HSPT, SU2C, MAYO1/2, CCF, TJU, and JHM). Second, using a random forest machine learning algorithm, we selected 37 genes with feature importance scores >0.5, showing a comparable level of error with the full model based on 428 SEGs (**Fig. 5D**). Performance of the classifier was assessed in the GRID cohort (AUC $^{1}/_{4}$ 0.97). The 37-gene panel displays significantly different expression patterns between the three subtypes in the DISC cohort (**Fig. 5A**).

[0172] The robust performance of the gene panel led us to determine whether it could be used to profile circulating tumor cells (CTC) from patients with CRPC. We analyzed single-cell RNA-seq data from 77 intact CTCs isolated from 13 patients (Miyamoto DT, Zheng Y, Wittner BS, Lee RJ, Zhu H, Broderick KT, et al. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science 2015;349:1351–6). Prior to the clustering analysis to investigate the expression patterns of these CTC data, the

normalized read counts as read-per-million (RPM) mapped reads were transformed on a log2 scale for each gene. The 77 CTCs were largely clustered into two groups using median-centered expression profiles corresponding to the 37-gene PCS panel by the hierarchical method (**Fig. 5B**). One group (GROUP I), consisting of 67 CTCs displays low expression of PCS1-enriched genes, while the other group (GROUP II) consisting of 10 CTCs has high expression of PCS1-enriched genes. In addition, we observed that PCS3-enriched genes in the panel were not detected or have very low expression changes across all CTCs as shown in the heatmap of **Fig. 5B**. The results suggest that CTCs can be divided into two groups with the 37-gene PCS panel. Given this result, we hypothesized that the 37-gene classifier might assign CTCs to PCS1 or PCS2, consistent with the clustering result. The bar graph below the heatmap illustrates the probability of likelihood of PCS assignment, with the result that all the CTCs were assigned to PCS1 (n = 12) or PCS2 (n = 65), while no PCS3 CTCs were assigned on the basis of the largest probability score. By comparing with the CTC group assignment, 7 (70%) of 10 CTCs in the GROUP II were assigned to PCS1 by the 37-gene classifier and 62 (95%) of 65 CTCs in the GROUP I were assigned to PCS2 by the classifier. We then tested whether GROUP I and II exhibit any difference in terms of therapeutic responses. Of note, 5 of the 7 CTCs in GROUP II (OR: 1.74; 95% confidence interval: 0.49–6.06) were from patients whose cancer exhibited radiographic and/or PSA progression during enzalutamide therapy, suggesting that the 37-gene PCS panel can potentially identify patients with resistance to enzalutamide therapy.

[0173] Collectively, the results demonstrate that the 37-gene classifier has a potential to assign individual prostate cancers to PCS1 using both prostate tissues and blood CTCs, suggesting that the classifier can be applied to subtype individual prostate cancers using clinically relevant technology platforms (Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 2008;26:317–25; Morrison T, Hurley J, Garcia J, Yoder K, Katz A, Roberts D, et al. Nanoliter high throughput quantitative PCR. Nucleic Acids Res 2006;34:e123), including by noninvasive methods.

[0174] Herein, the inventors describe a novel classification system for prostate cancer, based on an analysis of over 4,600 prostate cancer specimens, which consists of only 3 distinct subtypes, designated PCS1, PCS2, and PCS3. PCS1 exhibits the highest risk of progression to advanced disease, even for low Gleason grade tumors. Although sampling methods across the cohorts we studied were different, classification into the 3 subtypes was reproducible. For

example, the SWD cohort consists of specimens that were obtained by transurethral resection of the prostate rather than radical prostatectomy; however, subtype assignment and prognostic differences between the subtypes were similar to the other cohorts we examined (**Fig. 3H(x)**). Genes that are significantly enriched in the PCS1 category were highly expressed in the subset of CTCs (58%, 7 CTCs out of 12) from patients with enzalutamide-resistant tumors. This proportion of resistant cases in PCS1 CTCs is very high compared with PCS2 CTCs (8%, 5 CTCs out of 65). The characteristics of the PCS categories are summarized in **Table** 7.

[0175] Table 7: Summary of PCS characteristics

Sample Type	**Features**	**PCS1**	**PCS2**	**PCS3**
Patient Tumors	Proportion	6%	47%	47%
	Pathology	Enriched GS ≥8	Enriched GS ≤ 7	Enriched GS ≤ 7
	Prognosis	Poor	Variable	Variable
	Subtypes – TCGA	SPOP	ERG	'Other'
	Subtypes - Tomlins	ETS+, SPINK+	ERG+	Triple Negative
	Pathway signatures	AR-V, ES, PTEN, PRF, EZH2, NE	AR, FOXA1, SPOP, ERG	PRC, RAS, PN, MES
	Cell Lineage	Luminal-like	Luminal-like	Basal-like
Patient CTCs	Proportion	16%	84%	0%
	Enzalutamide resistance	Yes (58%)	No (8%)	Unknown

[0176] Previously published prostate cancer classifications have defined subtypes largely based on the presence or absence of genomic alterations (e.g., *TMPRSS2-ERG* translocations). Tumors with ERG rearrangement (ERG^b) are overrepresented in PCS2; however, it is not the presence or absence of an ERG rearrangement that defines the PCS2 subtype, but rather ERG pathway activation features based on coordinate expression levels of genes in the pathway. Our findings provide evidence for biologically distinct forms of prostate cancer that are independent of Gleason grade, currently the gold standard for clinical decision-making. In addition, by comparing prognostic profiles between the PCS categories and the Tomlins and colleagues categories, prognostic information was evident only from the PCS classification scheme in the same cohort. Taken together, this indicates that the PCS classification is unique.

[0177] Although the current report has provided evidence that PCS classification can assign subtypes within groups of "indolent" as well as aggressive tumors, and in a wide range of

preclinical models, it remains to be determined whether the PCS categories might be stable during tumor evolution in an individual patient. An interesting alternative possibility is that disease progression results in phenotypic diversification with respect to the PCS assignment. We have shown that preclinical model systems, including genetically engineered mouse models (GEMM), can be assigned with high statistical confidence to the PCS categories. We believe the simplest explanation for this finding is that these subtypes reflect distinct epigenetic features of chromatin that are potentially stable, even in the setting of genomic instability associated with advanced disease. This possibility needs to be formally tested. The human prostate cancer cell lines we evaluated could be assigned to all 3 subtypes; however, the GEMMs we tested could only be assigned to PCS1 and PCS2. This finding suggests that approximately 1 of 3 of human prostate cancers are not being modeled in widely used GEMMs. It should be feasible to generate mouse models for PCS3 through targeted genetic manipulation of pathways that are deregulated in PCS3 and through changing chromatin structure, such as by altering the activity of the PRC2 complex.

[0178] A major clinical challenge remains the early recognition of aggressive disease, in particular, due to the multifocal nature of prostate cancer (Martin NE, Mucci LA, Loda M, Depinho RA. Prognostic determinants in prostate cancer. Cancer J 2011;17:429–37). The classification scheme we describe predicts the risk of progression to lethal prostate cancer in patients with a diagnosis of low-grade localized disease (**Fig. 3G**). It is possible that in these cancers, pathway activation profiles are independent of Gleason grade and that pathways indicating high risk of progression are manifested early in the disease process and throughout multiple cancer clones in the prostate. In addition to predicting the risk of disease progression, PCS subtyping might also assist with the selection of drug treatment in advanced cancer by profiling CTCs in patient blood. With the 37-gene classifier we present here, it will be possible to assign individual tumors to PCS categories in a clinical setting. This new classification method may provide novel opportunities for therapy and clinical management of prostate cancer.

[0179] The various methods and techniques described above provide a number of ways to carry out the application. Of course, it is to be understood that not necessarily all objectives or advantages described can be achieved in accordance with any particular embodiment described herein. Thus, for example, those skilled in the art will recognize that the methods can be performed in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objectives or advantages as

taught or suggested herein. A variety of alternatives are mentioned herein. It is to be understood that some preferred embodiments specifically include one, another, or several features, while others specifically exclude one, another, or several features, while still others mitigate a particular feature by inclusion of one, another, or several advantageous features.

[0180] Furthermore, the skilled artisan will recognize the applicability of various features from different embodiments. Similarly, the various elements, features and steps discussed above, as well as other known equivalents for each such element, feature or step, can be employed in various combinations by one of ordinary skill in this art to perform methods in accordance with the principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in diverse embodiments.

[0181] Although the application has been disclosed in the context of certain embodiments and examples, it will be understood by those skilled in the art that the embodiments of the application extend beyond the specifically disclosed embodiments to other alternative embodiments and/or uses and modifications and equivalents thereof.

[0182] Preferred embodiments of this application are described herein, including the best mode known to the inventors for carrying out the application. Variations on those preferred embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. It is contemplated that skilled artisans can employ such variations as appropriate, and the application can be practiced otherwise than specifically described herein. Accordingly, many embodiments of this application include all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the application unless otherwise indicated herein or otherwise clearly contradicted by context.

[0183] All patents, patent applications, publications of patent applications, and other material, such as articles, books, specifications, publications, documents, things, and/or the like, referenced herein are hereby incorporated herein by this reference in their entirety for all purposes, excepting any prosecution file history associated with same, any of same that is inconsistent with or in conflict with the present document, or any of same that may have a limiting affect as to the broadest scope of the claims now or later associated with the present document. By way of example, should there be any inconsistency or conflict between the

description, definition, and/or the use of a term associated with any of the incorporated material and that associated with the present document, the description, definition, and/or the use of the term in the present document shall prevail.

[0184] It is to be understood that the embodiments of the application disclosed herein are illustrative of the principles of the embodiments of the application. Other modifications that can be employed can be within the scope of the application. Thus, by way of example, but not of limitation, alternative configurations of the embodiments of the application can be utilized in accordance with the teachings herein. Accordingly, embodiments of the present application are not limited to that precisely as shown and described.

[0185] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[0186] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

[0187] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention.

CLAIMS

1. A method for classifying prostate cancer into subtypes, comprising:
   a) obtaining a sample from a subject;
   b) assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values;
   c) determining the presence of an expression pattern of the one or more genes associated with the subtype in the sample based on the detected changes; and
   d) classifying the cancer in the subject into the subtype if the expression pattern of the one or more genes associated with the subtype is detected in the sample.

2. The method of claim 1, wherein the subtype is PCS1, PCS2, or PCS3.

3. The method of claim 1, wherein the one or more genes comprise one, two, three, four, five, six, or more, or all of the genes listed in Table 1.

4. The method of claim 1, wherein the genes are STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

5. The method of claim 1, wherein the one or more genes comprise one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

6. The method of claim 1, wherein the sample is a tissue sample or blood.

7. The method of claim 1, wherein the sample is a prostate tissue or blood circulating tumor cells.

8. The method of claim 7, wherein the blood circulating tumor cells are classified into the PCS1 subtype.

9. The method of claim 2, wherein the PCS1 subtype is resistant to enzalutamide.

10. The method of claim 2, wherein the PCS1 subtype is characterized in that it has an increased probability of progressing to metastatic disease or prostate cancer specific mortality when compared to the PCS2 subtype or PCS3 subtype.

11. The method of claim 2, wherein the PCS1 subtype has increased expression levels in STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, and KNTC1 genes; and decreased expression levels in RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45 genes.

12. The method of claim 2, wherein the PCS2 subtype has increased expression levels in RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2 genes; and decreased expression levels in STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45 genes.

13. The method of claim 2, wherein the PCS3 subtype has increased expression levels in CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45 genes; and decreased expression levels in STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2 genes.

14. The method of claim 11, wherein the subtype is PCS1, and the method further comprises administering to the subject a therapeutically effective amount of one or more DNA damaging agents selected from cisplatin, PARP inhibitors, or combinations thereof.

15. The method of claim 12, wherein the subtype is PCS2, and the method further comprises administering to the subject a therapeutically effective amount of an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, an androgen synthesis inhibitor, enzalutamide, a mitotic inhibitor, or docetaxel, or combinations thereof.

16. The method of claim 13, wherein the subtype is PCS3, and the method further comprises administering to the subject a therapeutically effective amount of dasatinib or docetaxel, or combinations thereof.

17. A method for prognosing a cancer in a subject, comprising:
    a) obtaining a sample from the subject;
    b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values;
    c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; and
    d) prognosing the cancer in the subject.

18. The method of claim 17, wherein the subtype is PCS1, and the cancer is prognosed with a poor clinical outcome.

19. The method of claim 18, wherein the poor clinical outcome comprises lower metastasis-free survival, higher risk of metastatic progression, higher rate of cancer specific mortality, lower overall survival, or more aggressive form of cancer, or a combination thereof.

20. A method for treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of a cancer in a subject, comprising:
    a) obtaining a sample from the subject;
    b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values;
    c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; and
    d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of the cancer.

21. The method of claim 20, wherein the subtype is PCS1, and the administered therapeutic agent is one or more DNA damaging agents selected from cisplatin, PARP inhibitors, or combinations thereof.

22. The method of claim 20, wherein the subtype is PCS1, and the administered therapeutic agent is a mitotic inhibitor.

23. The method of claim 20, wherein the subtype is PCS1, and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

24. The method of claim 20, wherein the subtype is PCS2, and the administered therapeutic agent is an antiandrogen, an androgen receptor (AR) antagonist, a selective AR modulator, or an androgen synthesis inhibitor, or a combination thereof.

25. The method of claim 20, wherein the subtype is PCS2, and the administered therapeutic agent is enzalutamide, or a functional equivalent, analog, derivative or salt of enzalutamide, or a combination thereof.

26. The method of claim 20, wherein the subtype is PCS2, and the administered therapeutic agent is a mitotic inhibitor.

27. The method of claim 20, wherein the subtype is PCS2, and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

28. The method of claim 20, wherein the subtype is PCS3, and the administered therapeutic agent is a Src signaling inhibitor, a Src family tyrosine kinase inhibitor, or a Bcr-Abl tyrosine kinase inhibitor, or a combination thereof.

29. The method of claim 20, wherein the subtype is PCS3, and the administered therapeutic agent is dasatinib, or a functional equivalent, analog, derivative or salt of dasatinib, or a combination thereof.

30. The method of claim 20, wherein the subtype is PCS3 and the administered therapeutic agent is docetaxel, or a functional equivalent, analog, derivative or salt of docetaxel, or a combination thereof.

31. A method for treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of a cancer in a subject, comprising:
    a) obtaining a sample from the subject;
    b) assaying the sample to detect a marker for a subtype of the cancer;
    c) detecting the marker for the subtype in the sample; and

d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of the cancer.

32. The method of claim 31, where the marker for the subtype comprises:
a) an increased expression level in one, two, three, four, five, six, or more, or all of the PCS1 SEGs (SubtypeID = 1) listed in Table 1; or
b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS1 SEGs (SubtypeID ≠ 1) listed in Table 1.

33. The method of claim 31, where the marker for the subtype comprises:
a) an increased expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, and KNTC1; or
b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, SLC12A2, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

34. The method of claim 31, where the marker for the subtype comprises:
a) an increased expression level in one, two, three, four, five, six, or more, or all of the PCS2 SEGs (SubtypeID=2) listed in Table 1; or
b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS2 SEGs (SubtypeID ≠ 2) listed in Table 1.

35. The method of claim 31, where the marker for the subtype comprises:
a) an increased expression level in one, two, three, four, five, six, or more, or all of RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2; or
b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45.

36. The method of claim 31, where the marker for the subtype comprises:

a) an increased expression level in one, two, three, four, five, six, or more, or all of the PCS3 SEGs (SubtypeID=3) listed in Table 1: or

b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of the non-PCS3 SEGs (SubtypeID $\neq$ 3) listed in Table 1.

37. The method of claim 31, where the marker for the subtype comprises:

a) an increased expression level in one, two, three, four, five, six, or more, or all of CFD, COL6A1, PTGDS, LTBP4, SOCS3, SPEG, GABRP, PENK, SMARCD3, CLIP3, ACTC1, ASPA, COL4A6, CYP4B1, ROR2, SGCA, SLC2A5, PAGE4, ACOX2, and C16orf45; or

b) a decreased or insignificantly changed expression level in one, two, three, four, five, six, or more, or all of STMN1, MCM4, CCNB1, CDC6, CDKN3, EZH2, TPX2, FOXM1, KIF11, HMMR, MKI67, KNTC1, RAB3B, SLC4A4, ANK3, GJB1, and SLC12A2.

38. A method for classifying a prostate cancer into a prostate cancer subtype, comprising:

a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens;

b) converting the pathway activation gene expression signatures into pathway activation profiles;

c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to the prostate cancer subtype; and

d) classifying the prostate cancer into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the prostate cancer.

39. The method of claim 38, wherein the pathway activation profiles are selected from PTEN, ES, AR-V, PRF, EZH2, AV, AR, SPOP, FOXA1, ERG, RAS, MES, PRC, and PN.

40. The method of claim 38, wherein the prostate cancer subtype is PCS1, PCS2, or PCS3.

41. The method of claim 40, wherein the PCS1 subtype comprises pathway activation profiles PTEN, ES, AR-V, PRF, EZH2, or AV, or combinations thereof; the PCS2 subtype comprises pathway activation profiles AR, SPOP, FOXA1, or ERG, or combinations thereof; and the PCS3 subtype comprises pathway activation profiles RAS, MES, PRC, or PN, or combinations thereof.

42. The method of claim 38, wherein determining pathway activation gene expression signatures in the prostate cancer specimens comprises:

a) obtaining a first prostate cancer dataset, wherein the first prostate cancer dataset comprises gene expression profiles;

b) selecting a second prostate cancer dataset from the first prostate dataset, wherein the second prostate cancer dataset is numerically smaller than the first prostate cancer dataset;

c) normalizing the second prostate cancer dataset;

d) removing gene expression profiles for benign prostate tissues; and

e) normalizing the gene expression profiles to obtain a merged dataset comprising the pathway activation gene expression signatures.

43. The method of claim 42, wherein the gene expression profiles comprise gene expression profiles for benign prostate tissues and gene expression profiles for malignant prostate tissues.

44. The method of claim 43, wherein the malignant prostate tissues are primary tumors, metastatic prostate cancers, or castration resistant prostate cancers, or combinations thereof.

45. The method of claim 42, wherein normalizing the second prostate cancer dataset is performed using a quantile method.

46. The method of claim 42, wherein normalizing the gene expression profiles is performed using median centering and quantile scaling.

47. The method of claim 38, wherein converting the pathway activation gene expression signatures into pathway activation profiles comprises:

a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization;

b) calculating Z-scores for the pathway activation gene expression signatures; and

c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures.

48. The method of claim 38, wherein grouping the pathway activation profiles into independent clusters comprises, determining a number of independent clusters by applying a consensus non-negative matrix factorization clustering method.

49. A method for classifying a prostate cancer in a subject, comprising:

a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens;

b) converting the pathway activation gene expression signatures into pathway activation profiles;

c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to a prostate cancer subtype;

d) obtaining a sample from the subject;

e) determining a pathway activation profile in the sample; and

f) classifying the prostate cancer in the subject into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the sample.

50. The method of claim 49, wherein the pathway activation profiles are selected from PTEN, ES, AR-V, PRF, EZH2, AV, AR, SPOP, FOXA1, ERG, RAS, MES, PRC, and PN.

51. The method of claim 49, wherein the prostate cancer subtype is PCS1, PCS2, or PCS3.

52. The method of claim 51, wherein the PCS1 subtype comprises pathway activation profiles PTEN, ES, AR-V, PRF, EZH2, or AV, or combinations thereof; the PCS2 subtype comprises pathway activation profiles AR, SPOP, FOXA1, or ERG, or

combinations thereof; and the PCS3 subtype comprises pathway activation profiles RAS, MES, PRC, or PN, or combinations thereof.

53. The method of claim 51, wherein the PCS1 subtype is characterized in that it has an increased probability of progressing to metastatic disease or prostate cancer specific mortality when compared to the PCS2 subtype or PCS3 subtype.

54. The method of claim 49, wherein determining pathway activation gene expression signatures in the prostate cancer specimens comprises:

a) obtaining a first prostate cancer dataset, wherein the first prostate cancer dataset comprises gene expression profiles;

b) selecting a second prostate cancer dataset from the first prostate dataset, wherein the second prostate cancer dataset is numerically smaller than the first prostate cancer dataset;

c) normalizing the second prostate cancer dataset;

d) removing gene expression profiles for benign prostate tissues; and

e) normalizing the gene expression profiles to obtain a merged dataset comprising the pathway activation gene expression signatures.

55. The method of claim 54, wherein the gene expression profiles comprise gene expression profiles for benign prostate tissues and gene expression profiles for malignant prostate tissues.

56. The method of claim 55, wherein the malignant prostate tissues are primary tumors, metastatic prostate cancers, or castration resistant prostate cancers, or combinations thereof.

57. The method of claim 54, wherein normalizing the second prostate cancer dataset is performed using a quantile method.

58. The method of claim 54, wherein normalizing the gene expression profiles is performed using median centering and quantile scaling.

59. The method of claim 49, wherein converting the pathway activation gene expression signatures into pathway activation profiles comprises:

a) calculating the difference between (i) an error-weighted mean of expression values of the genes in the pathway activation gene expression signatures and (ii) an error-weighted mean of all genes after normalization;

b) calculating Z-scores for the pathway activation gene expression signatures; and

c) preparing a matrix of pathway activation scores from the pathway activation gene expression signatures.

60. The method of claim 49, wherein grouping the pathway activation profiles into independent clusters comprises, determining a number of independent clusters by applying a consensus non-negative matrix factorization clustering method.

61. The method of claim 49, wherein the sample is a tissue sample or blood.

62. The method of claim 49, wherein the sample is a prostate tissue or blood circulating tumor cells.

63. The method of claim 62, wherein the blood circulating tumor cells are classified into the PCS1 subtype.

64. The method of claim 49, further comprising identifying the cancer as having resistance to enzalutamide.

FIG. 1A

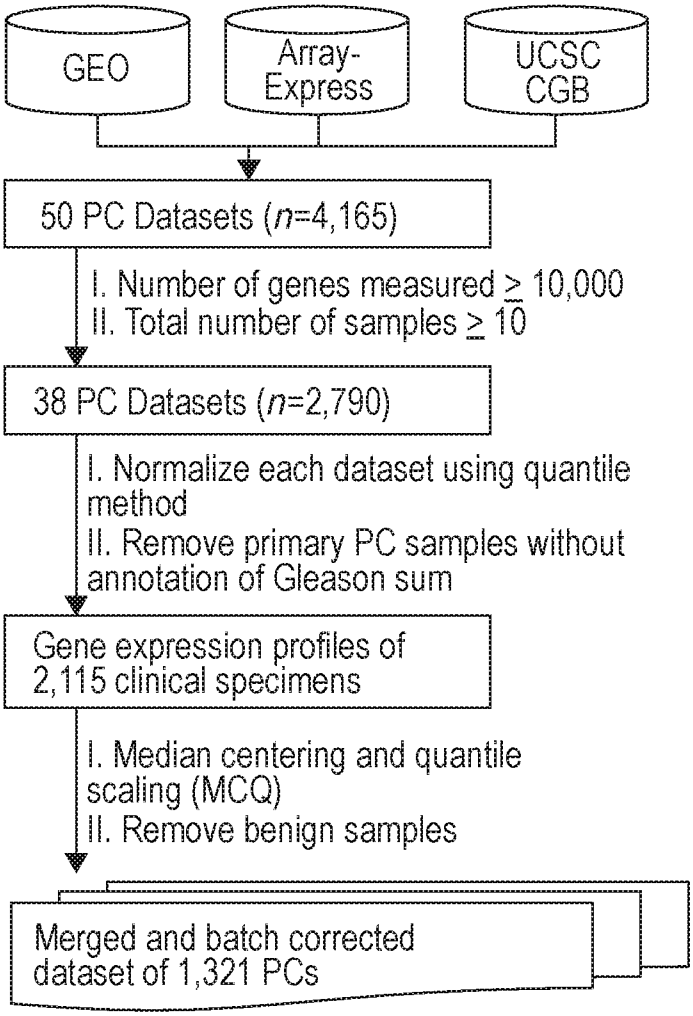
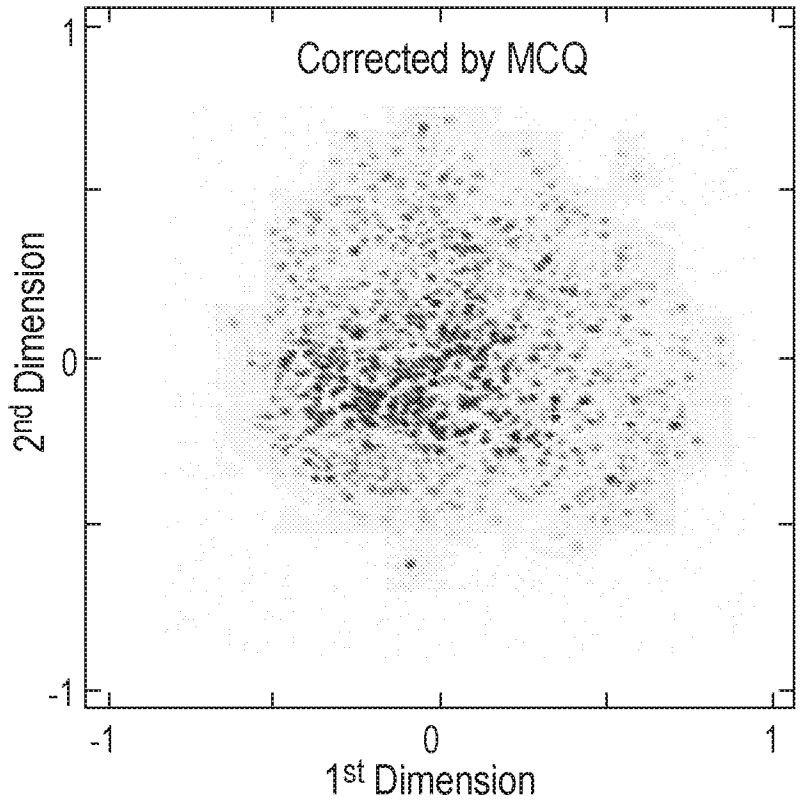


FIG. 1B

Disease status	Number of samples	Sample composition
Benign	794	Benign prostate tissue
GS < 7	328	Primary PC with Gleason sum < 7
GS = 7	530	Primary PC with Gleason sum = 7
GS > 7	203	Primary PC with Gleason sum > 7
CRPC/Met	260	metastatic or castration-resistant PC

FIG. 1C


Corrected by MCQ
1
0
-1
2nd Dimension
-1
0
1
1st Dimension

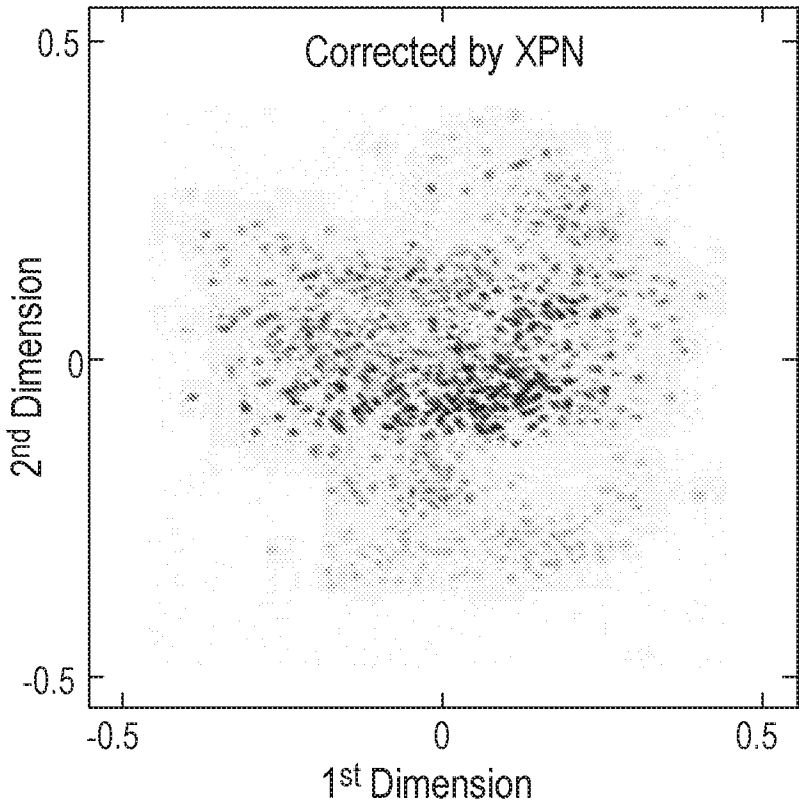

Corrected by XPN
0.5
0
-0.5
2nd Dimension
-0.5
0
0.5
1st Dimension

FIG. 1D

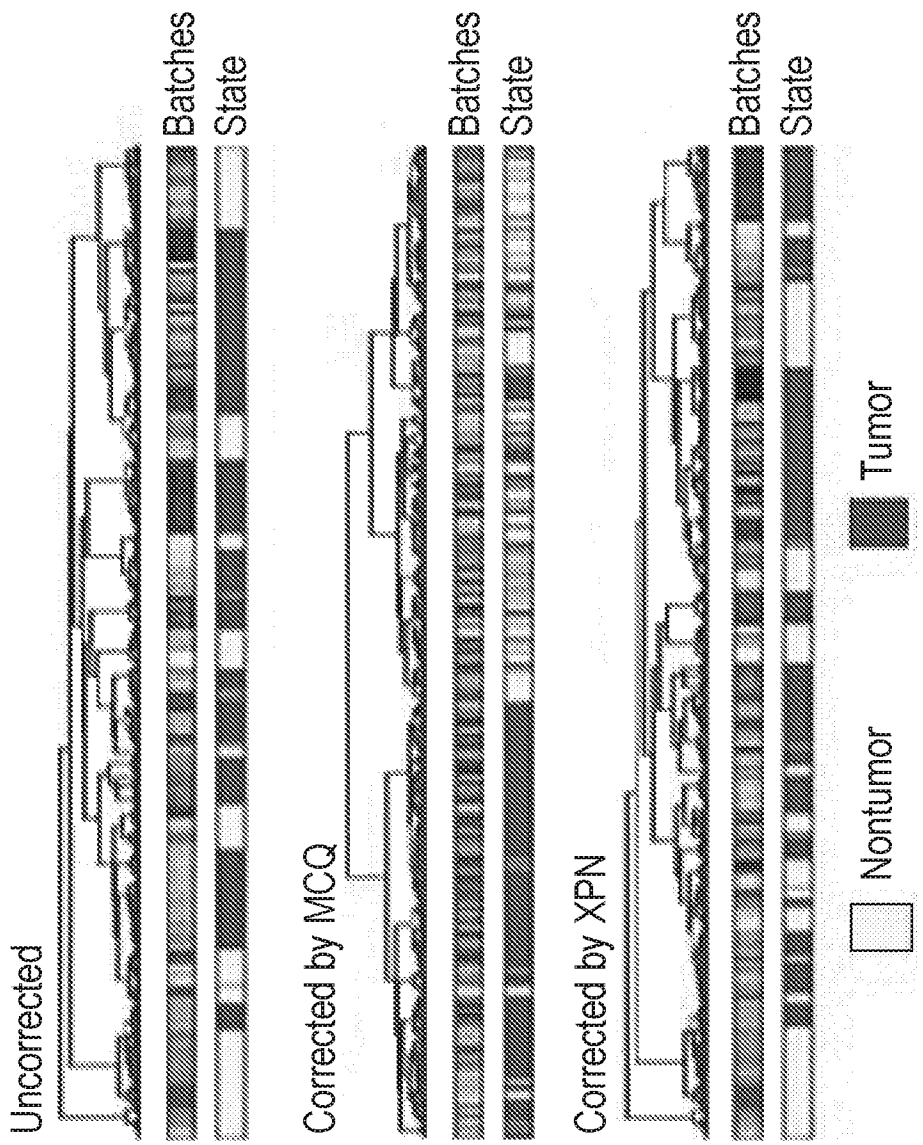
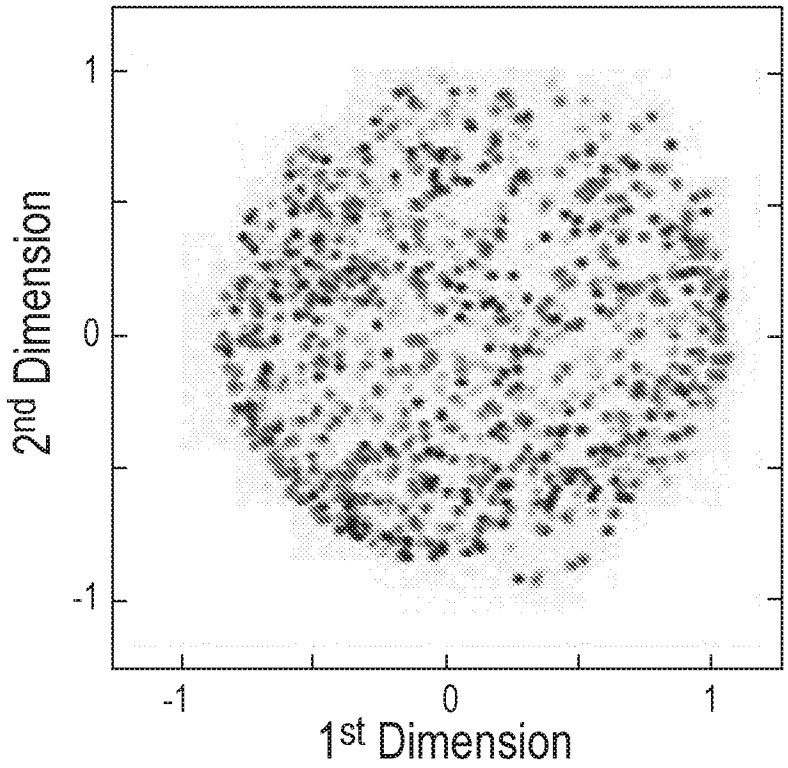

Uncorrected
Batches
State
Corrected by MCQ
Batches
State
Corrected by XPN
Batches
State
Nontumor
Tumor

FIG. 1E


1
0
-1
2nd Dimension
-1
0
1
1st Dimension

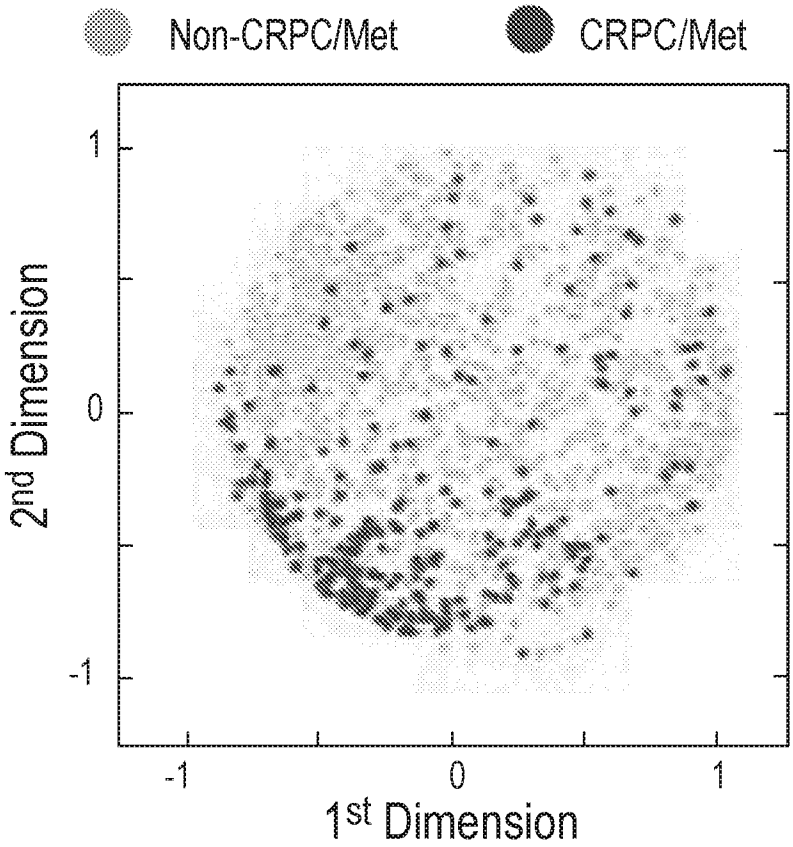

Non-CRPC/Met
CRPC/Met
1
0
-1
2nd Dimension
-1
0
1
1st Dimension

FIG. 2A

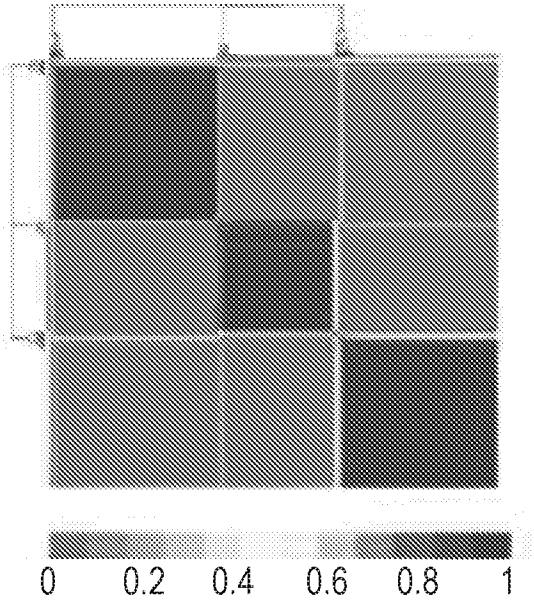
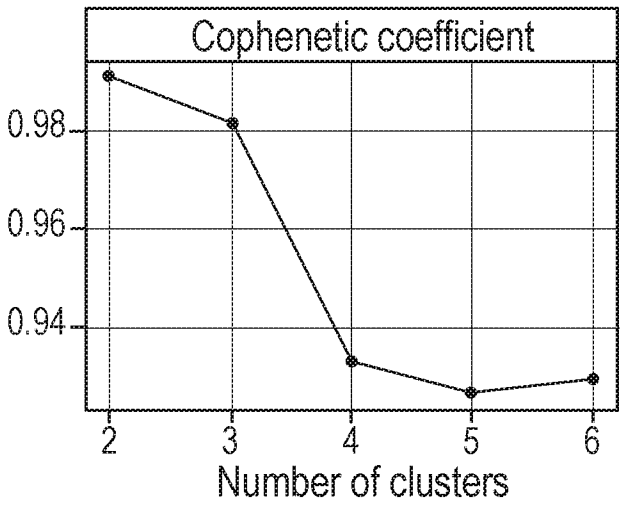

0
0.2
0.4
0.6
0.8
1

FIG. 2B


Cophenetic coefficient
0.98
0.96
0.94
2
3
4
5
6
Number of clusters

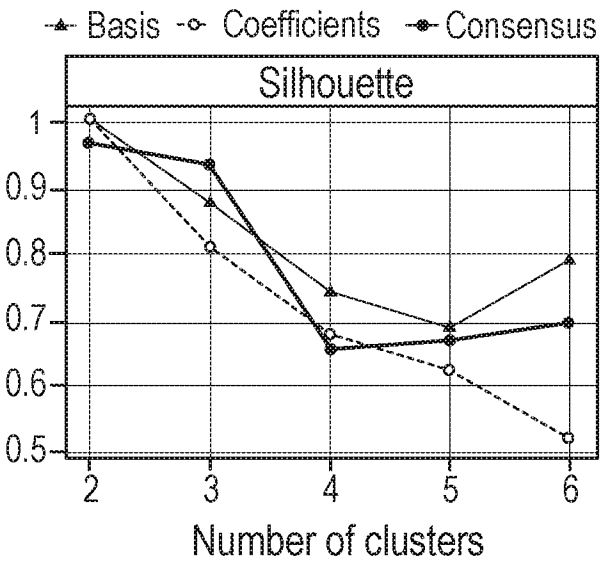

Basis
Coefficients
Consensus
Silhouette
1
0.9
0.8
0.7
0.6
0.5
2
3
4
5
6
Number of clusters

FIG. 2C

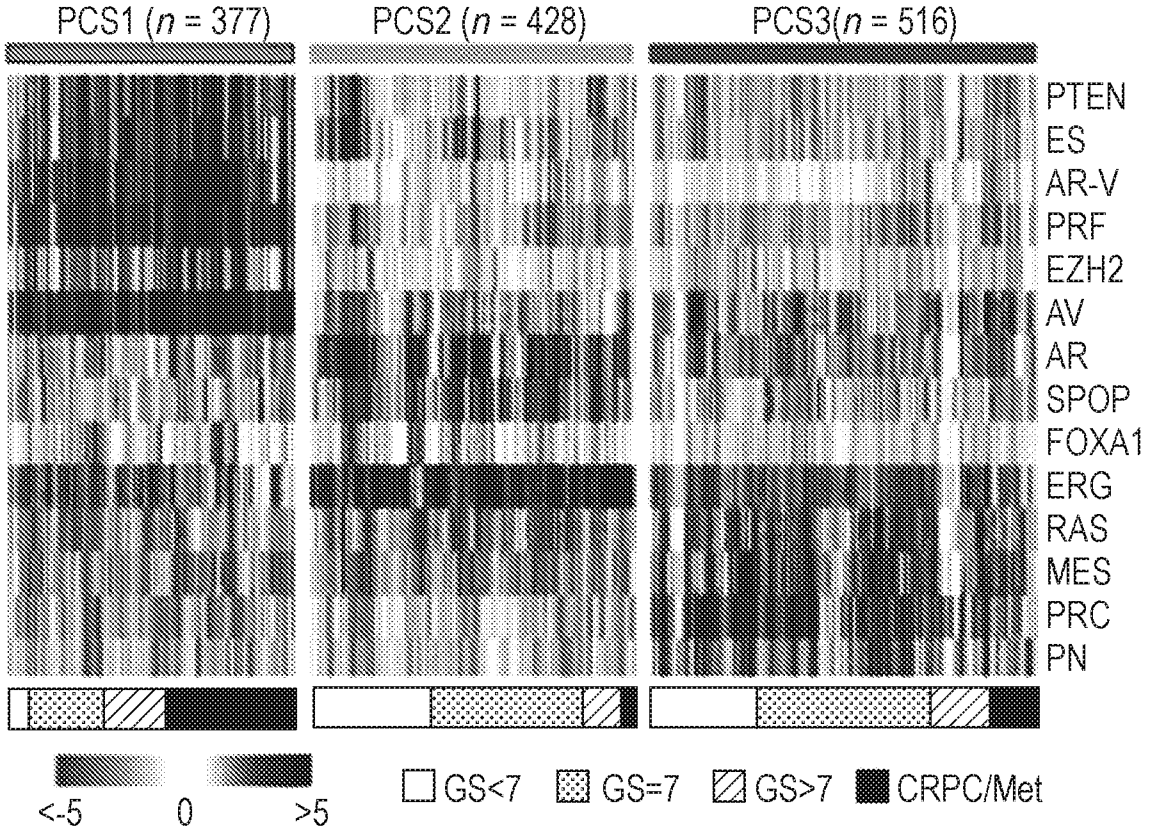

PCS1 (n = 377)
PCS2 (n = 428)
PCS3(n = 516)
PTEN
ES
AR-V
PRF
EZH2
AV
AR
SPOP
FOXA1
ERG
RAS
MES
PRC
PN
<-5
0
>5
GS<7
GS=7
GS>7
CRPC/Met

FIG. 2D

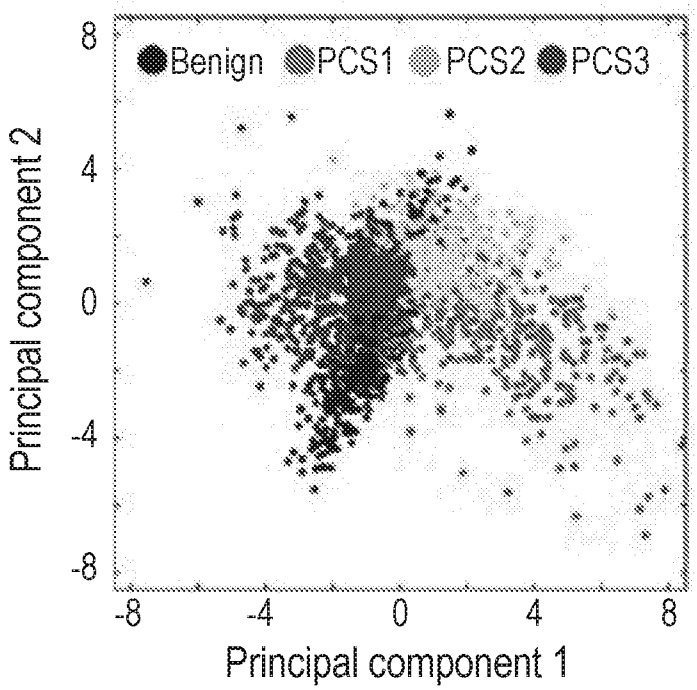

Benign
PCS1
PCS2
PCS3
Principal component 2
Principal component 1
8
4
0
-4
-8
-8
-4
0
4
8

FIG. 2E

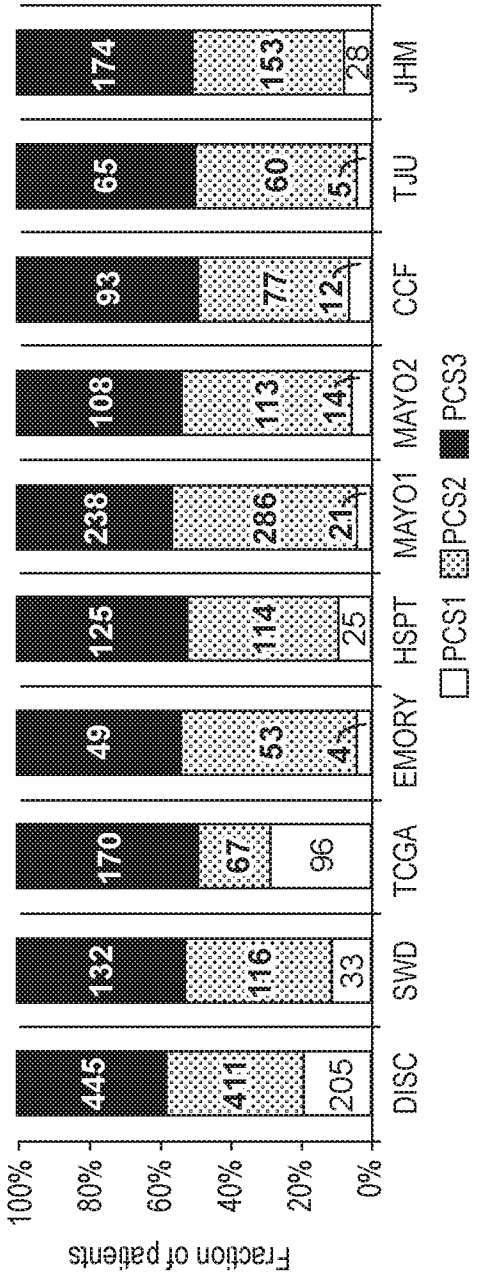


FIG. 2F

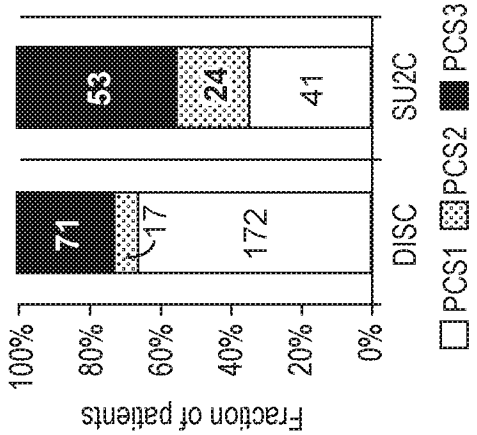


FIG. 2G

FIG. 2H

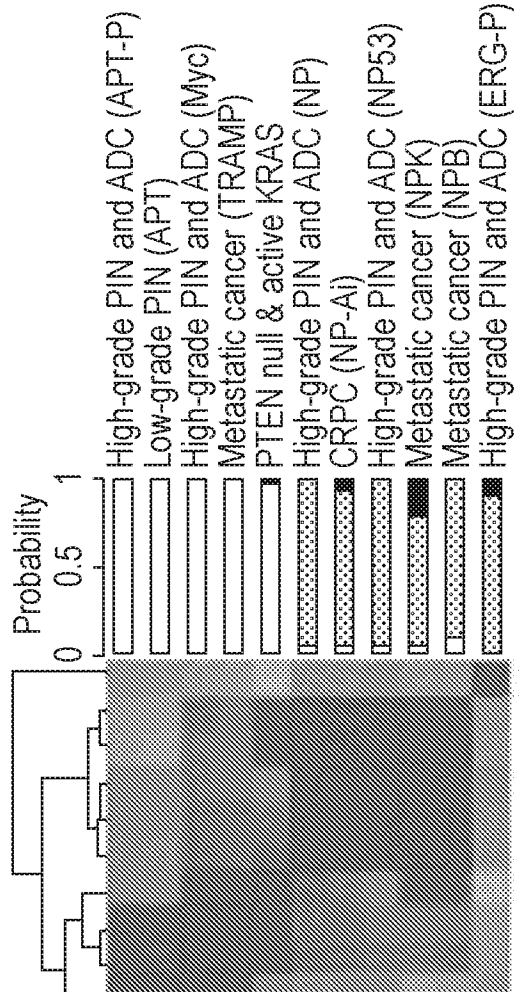
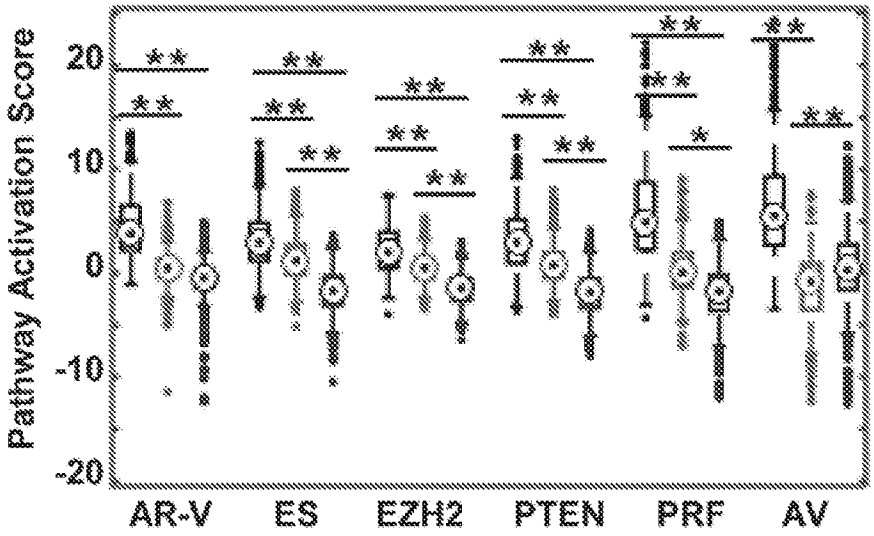
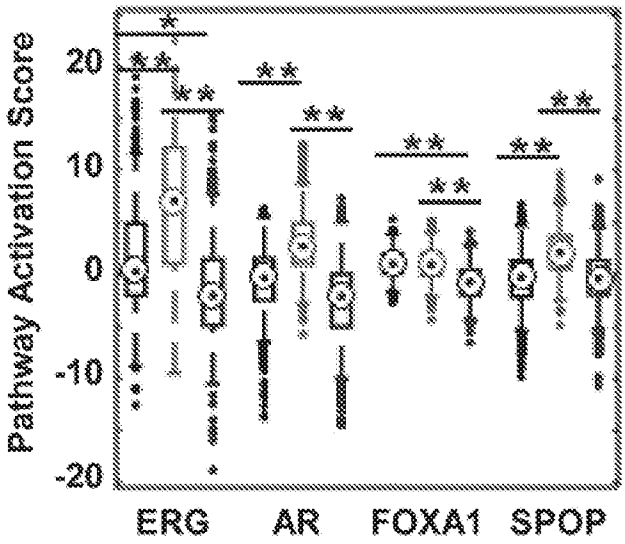


FIG. 2I


Pathway Activation Score
20
10
0
-10
-20
AR-V
ES
EZH2
PTEN
PRF
AV


Pathway Activation Score
20
10
0
-10
-20
ERG
AR
FOXA1
SPOP

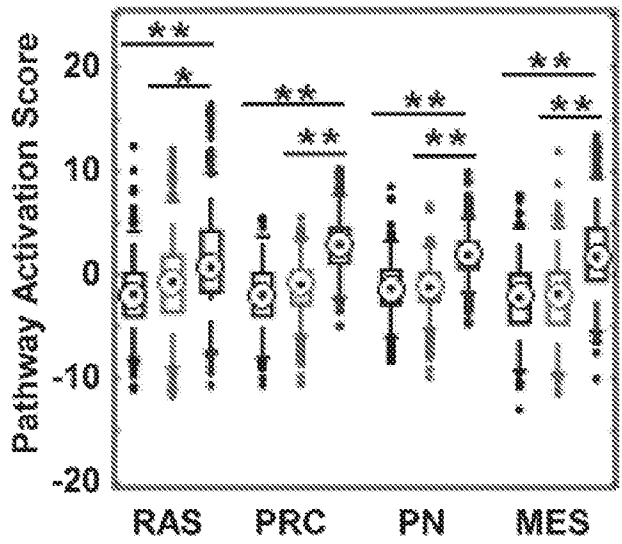

Pathway Activation Score
20
10
0
-10
-20
RAS
PRC
PN
MES

FIG. 2J

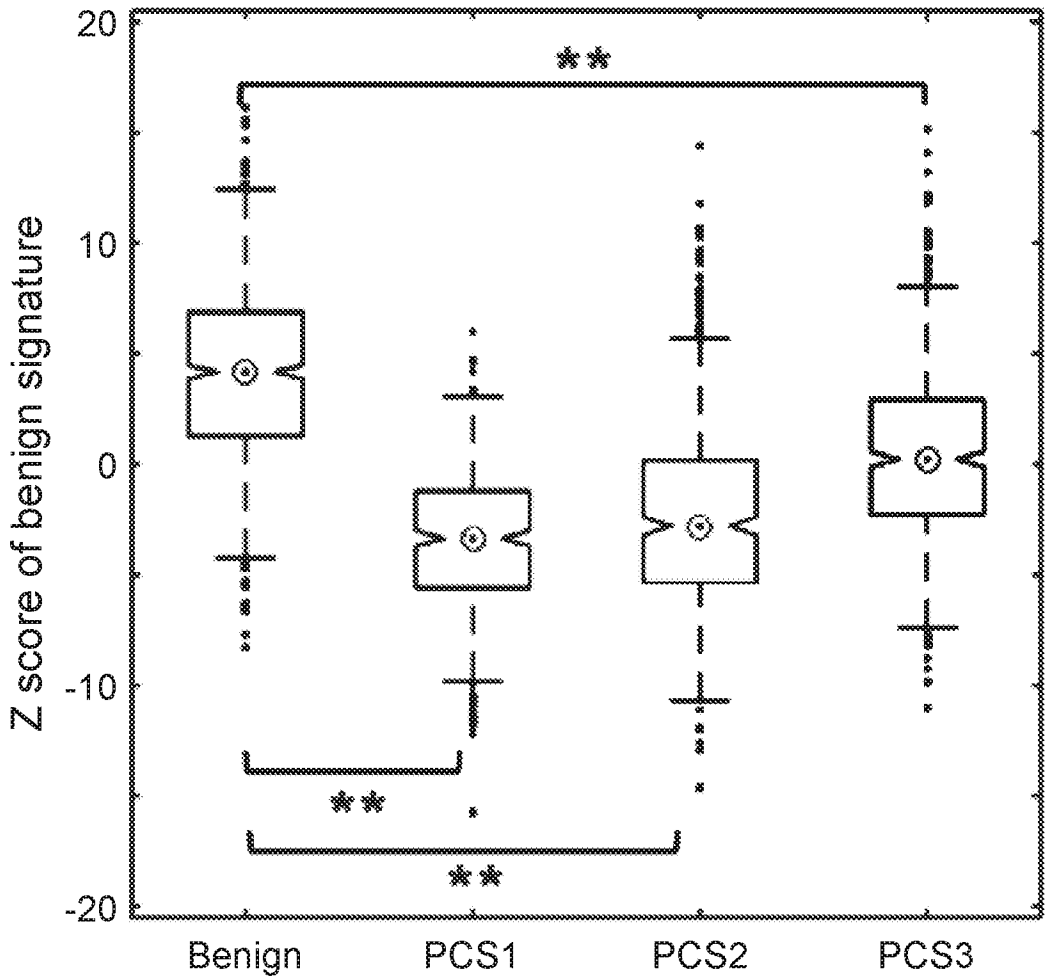

20
10
0
-10
-20
Z score of benign signature
**
**
**
Benign
PCS1
PCS2
PCS3

FIG. 3A

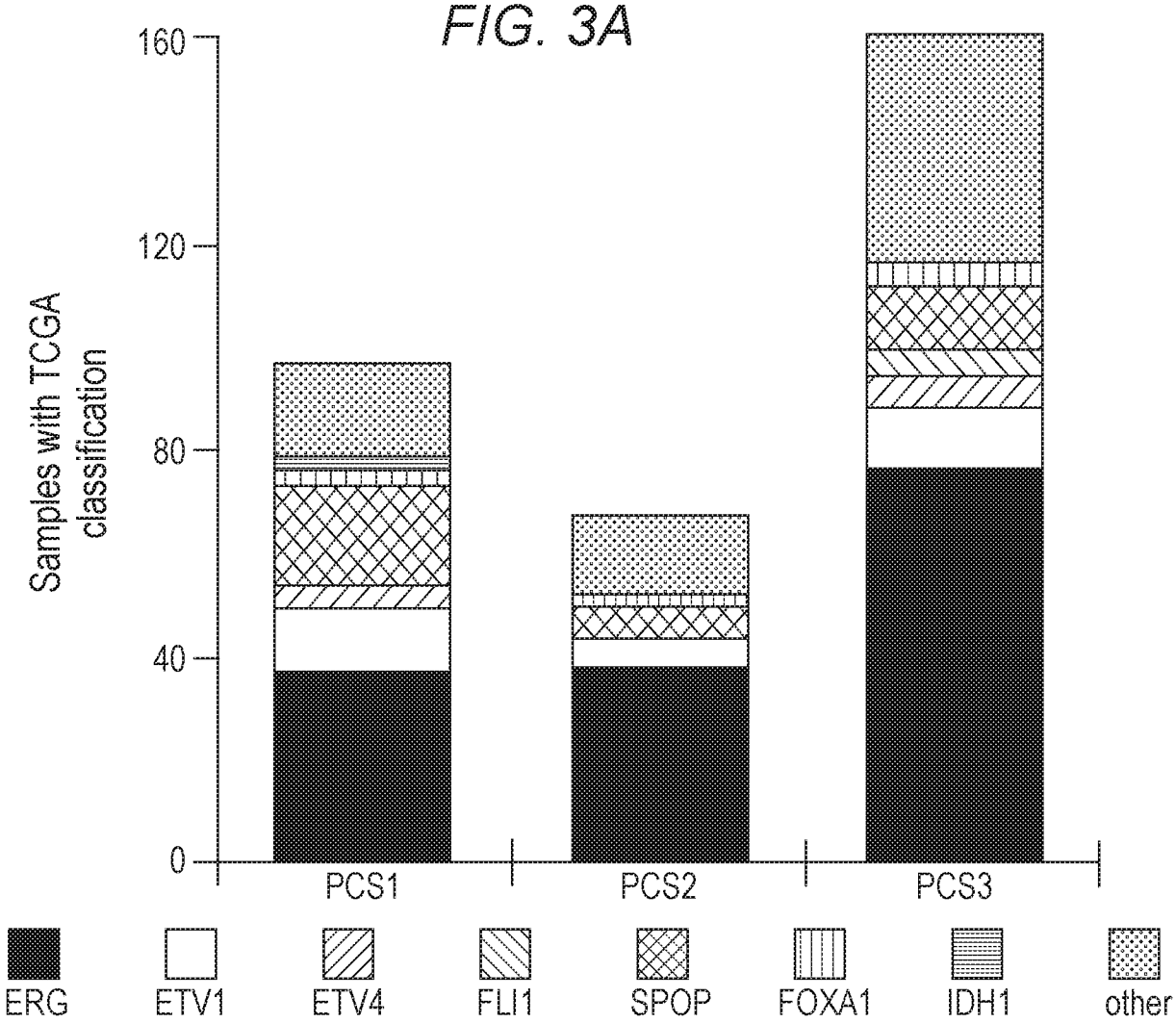
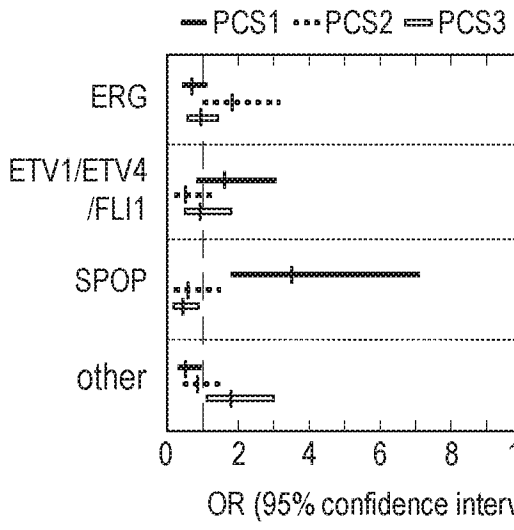


FIG. 3B



	PCS1	PCS2	PCS3
ERG	0.69 (0.42, 1.12)	1.82* (1.05, 3.14)	0.92 (0.59, 1.43)
ETV1/ETV4 /FLI1	1.59 (0.83, 3.07)	0.54 (0.22, 1.33)	0.95 (0.51, 1.77)
SPOP	3.53* (1.75, 7.11)	0.57 (0.21, 1.53)	0.42 (0.20, 0.86)
other	0.54 (0.29, 0.97)	0.85 (0.45, 1.58)	1.79* (1.08, 2.97)

*Statistically significant

FIG. 3C

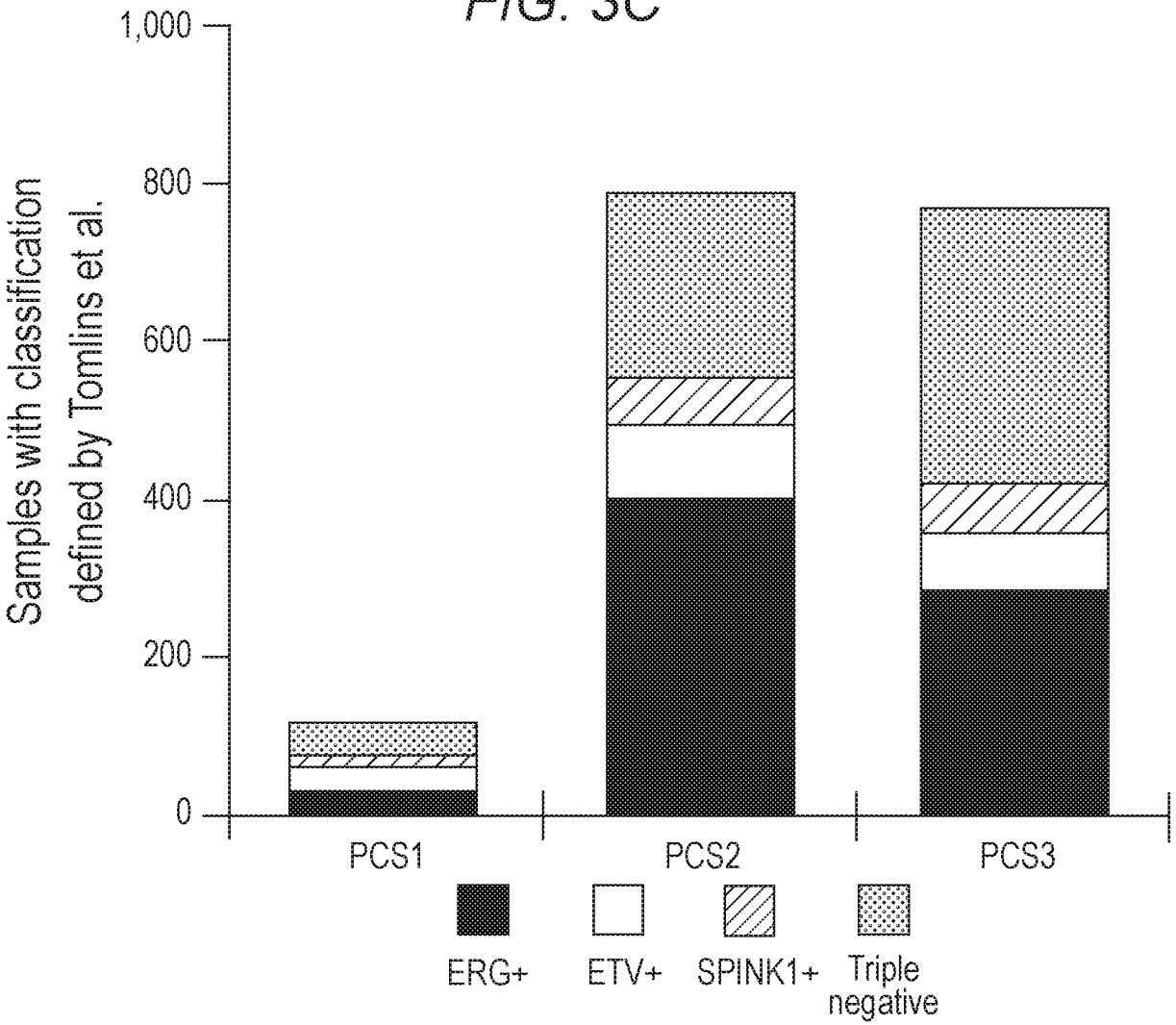
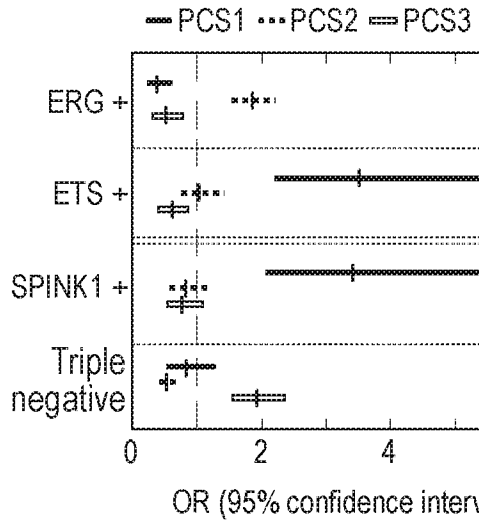


FIG. 3D



	PCS1	PCS2	PCS3
ERG +	0.51 (0.33, 0.80)	1.81* (1.48, 2.21)	0.64 (0.52, 0.78)
ETS +	3.52* (2.22, 5.59)	1.05 (0.78, 1.42)	0.62 (0.45, 0.85)
SPINK1 +	3.41* (2.07, 5.63)	0.857 (0.61, 1.19)	0.77 (0.55, 1.09)
Triple negative	0.84 (0.55, 1.30)	0.54 (0.44, 0.66)	1.92* (1.57, 2.36)

*Statistically significant

FIG. 3E

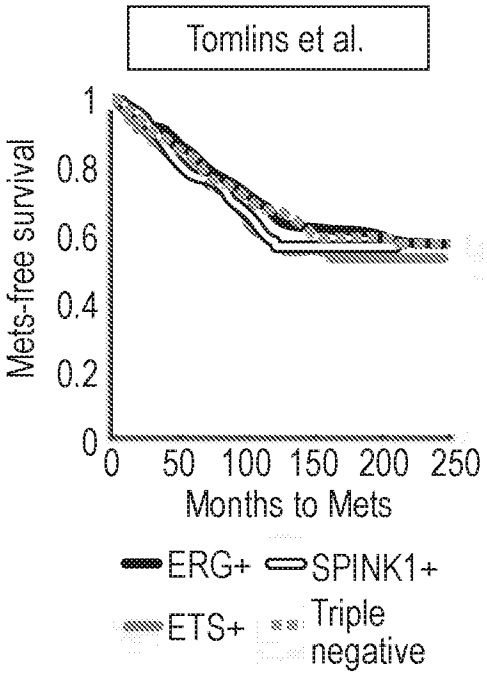


FIG. 3F

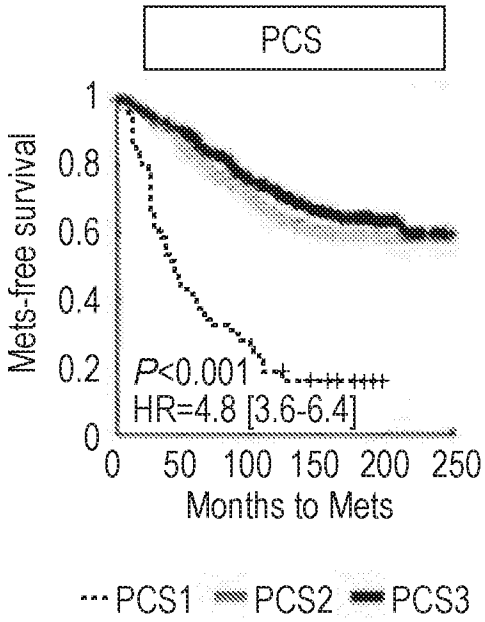
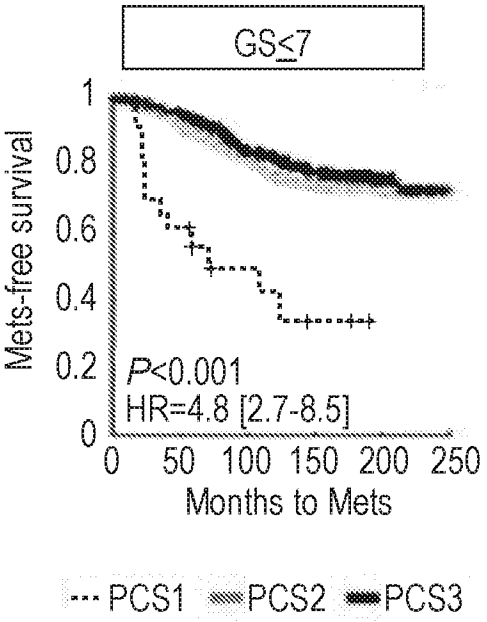


FIG. 3G



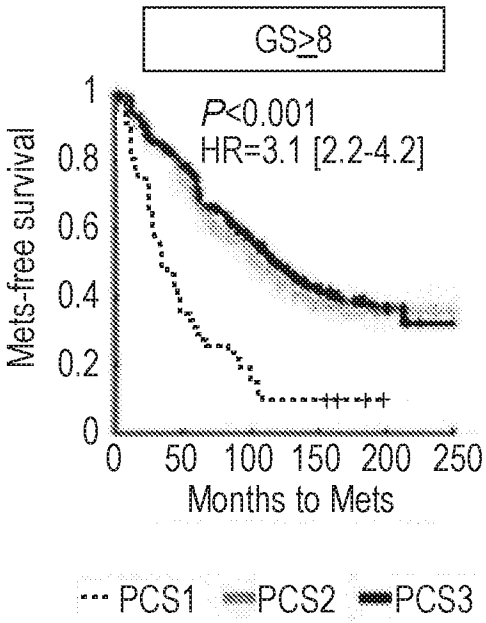
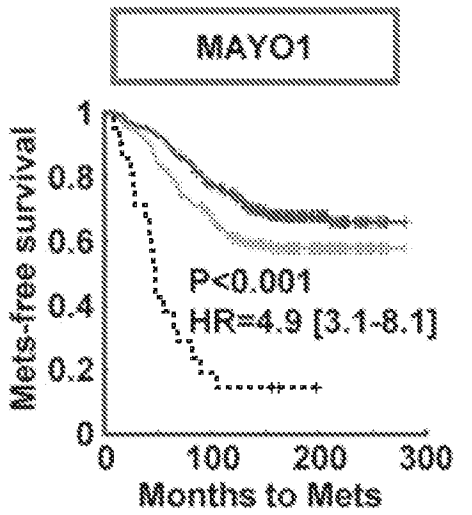


FIG. 3H(i)

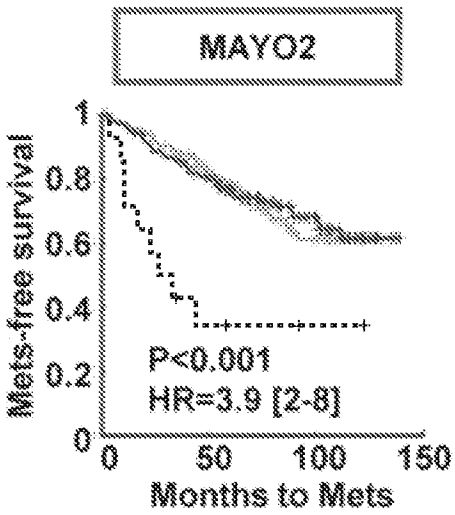


	PCS 1	PCS 2	PCS 3
Mets	18	118	76
No Mets	3	168	162

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 3H(ii)

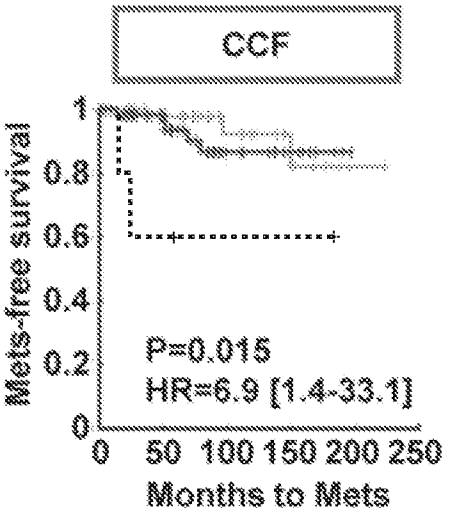


	PCS 1	PCS 2	PCS 3
Mets	9	34	33
No Mets	5	79	75

Chi-square P = 0.0311

PCS1 PCS2 PCS3

FIG. 3H(iii)

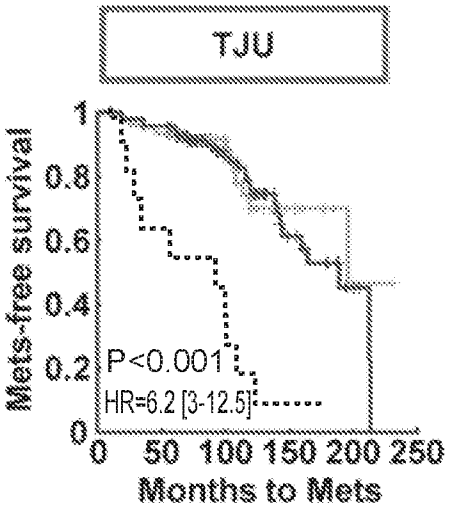


	PCS 1	PCS 2	PCS 3
Mets	2	3	5
No Mets	3	57	60

Chi-square P = 0.0186

PCS1 PCS2 PCS3

FIG. 3H(iv)

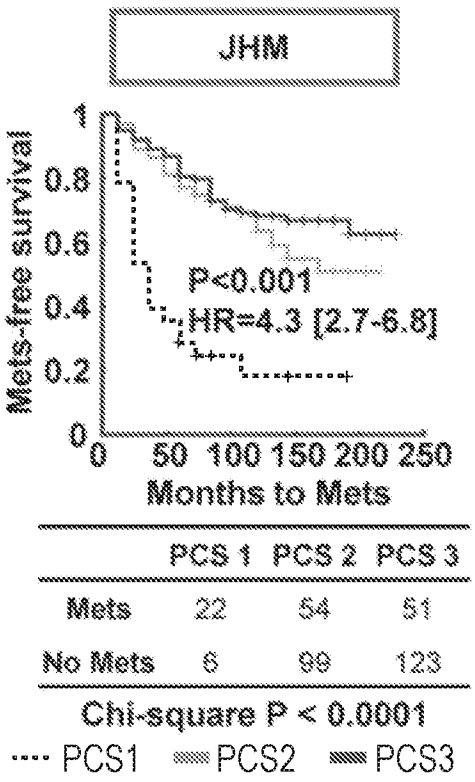


	PCS 1	PCS 2	PCS 3
Mets	10	13	26
No Mets	2	64	67

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 3H(v)

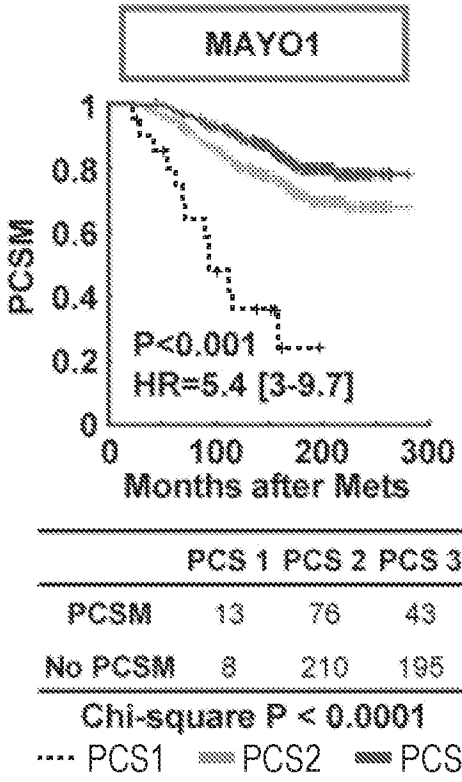


	PCS 1	PCS 2	PCS 3
Mets	22	54	51
No Mets	6	99	123

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 3H(vi)

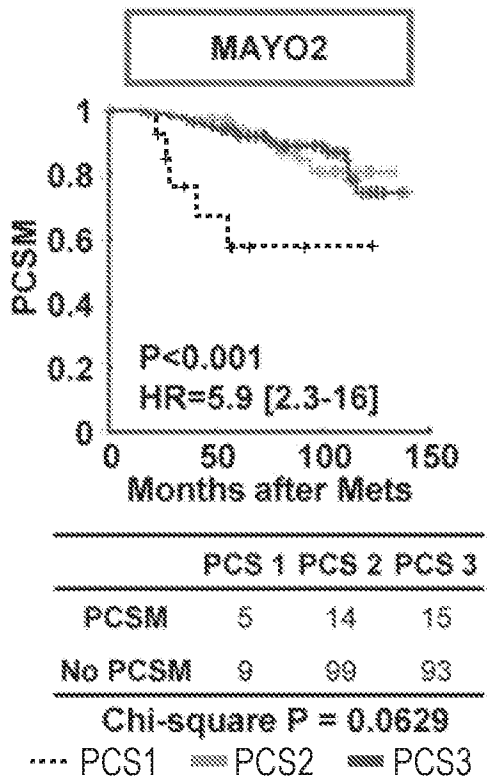


	PCS 1	PCS 2	PCS 3
PCSM	13	76	43
No PCSM	8	210	195

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 3H(vii)

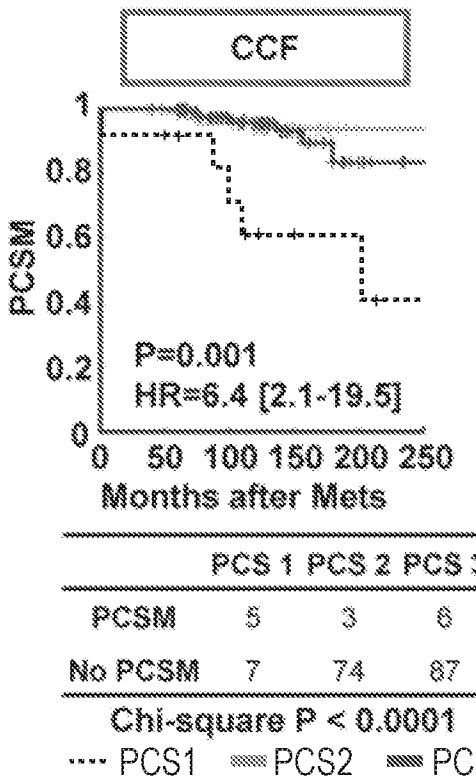


	PCS 1	PCS 2	PCS 3
PCSM	5	14	15
No PCSM	9	99	93

Chi-square P = 0.0629

PCS1 PCS2 PCS3

FIG. 3H(viii)

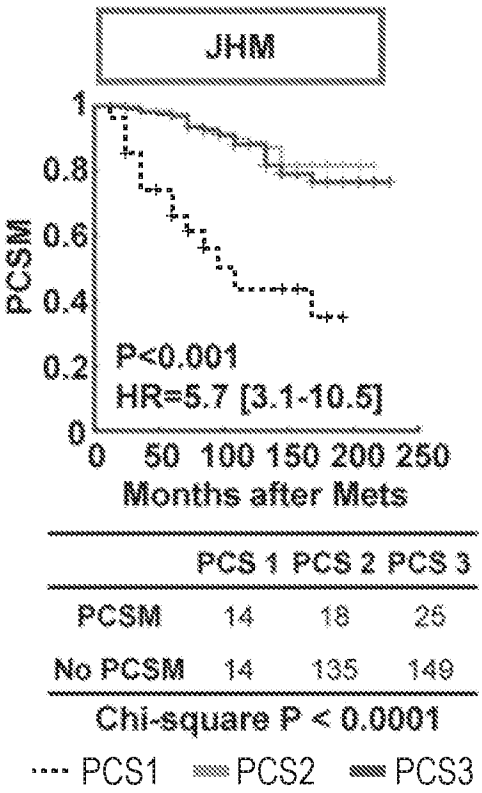


	PCS 1	PCS 2	PCS 3
PCSM	5	3	6
No PCSM	7	74	87

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 3H(ix)

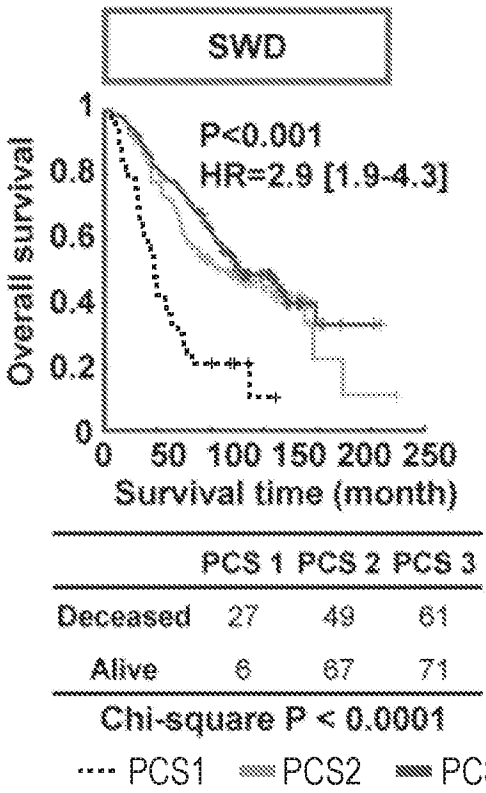


	PCS 1	PCS 2	PCS 3
PCSM	14	18	25
No PCSM	14	135	149

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 3H(x)



	PCS 1	PCS 2	PCS 3
Deceased	27	49	61
Alive	6	67	71

Chi-square P < 0.0001

PCS1 PCS2 PCS3

FIG. 4A

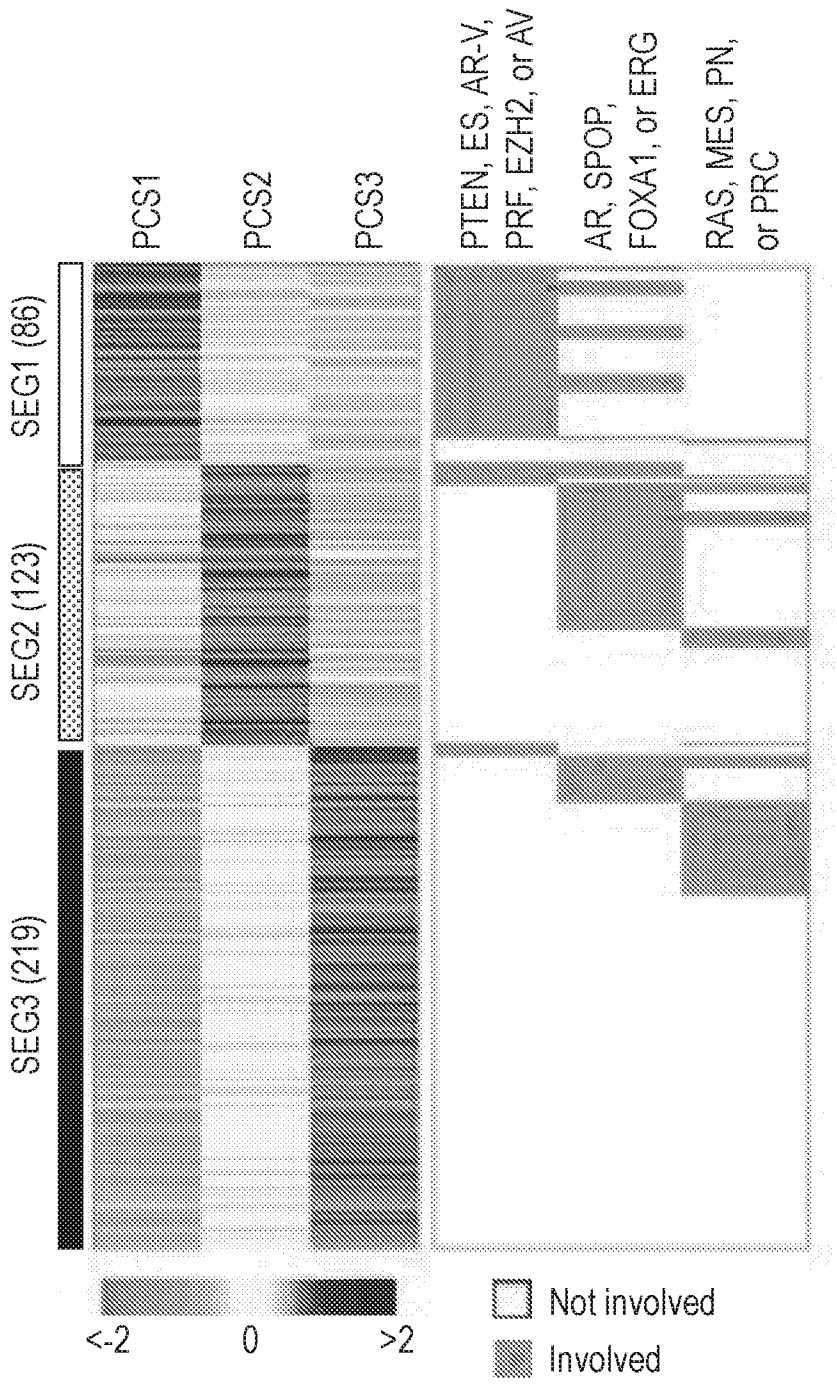

PCS1
PCS2
PCS3
PTEN, ES, AR-V, PRF, EZH2, or AV
AR, SPOP, FOXA1, or ERG
RAS, MES, PN, or PRC
SEG1 (86)
SEG2 (123)
SEG3 (219)
<-2
0
>2
Not involved
Involved

FIG. 4B

	Term	P
SEG1	Chromosome organization	<0.001
	Cell proliferation	<0.001
	DNA repair	0.001
SEG2	Lipid biosynthetic process	0.008
	Steriod biosynthetic process	0.022
SEG3	Extracellular matrix organization	<0.001
	inflammatory response	<0.001
	cell migration	0.003

FIG. 4C

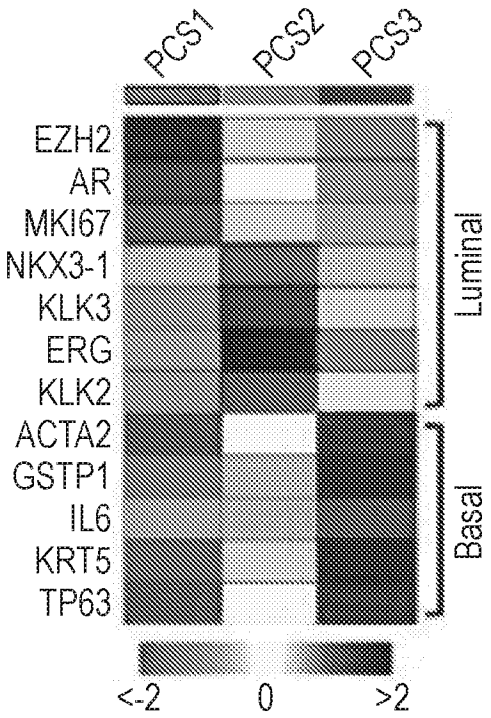


FIG. 4D

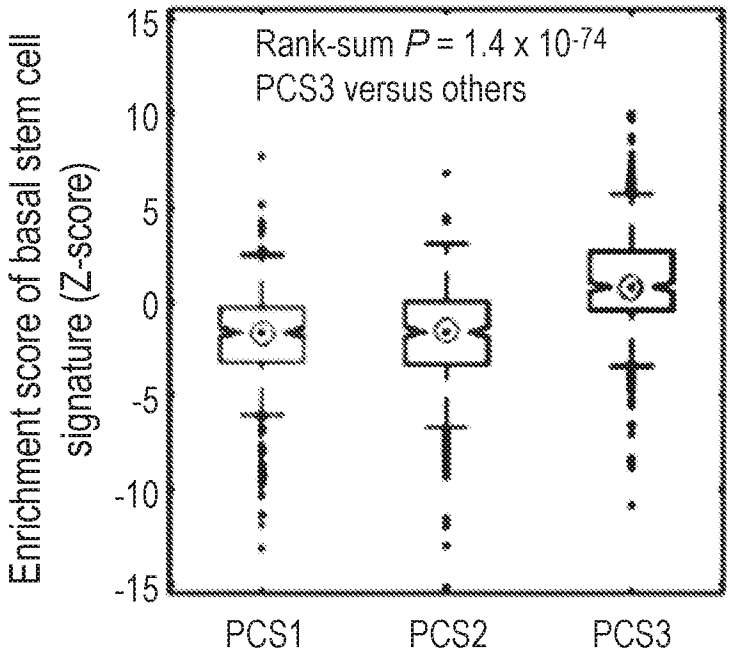

Rank-sum P = 1.4 x 10-74
PCS3 versus others
Enrichment score of basal stem cell signature (Z-score)
15
10
5
0
-5
-10
-15
PCS1
PCS2
PCS3

FIG. 4E

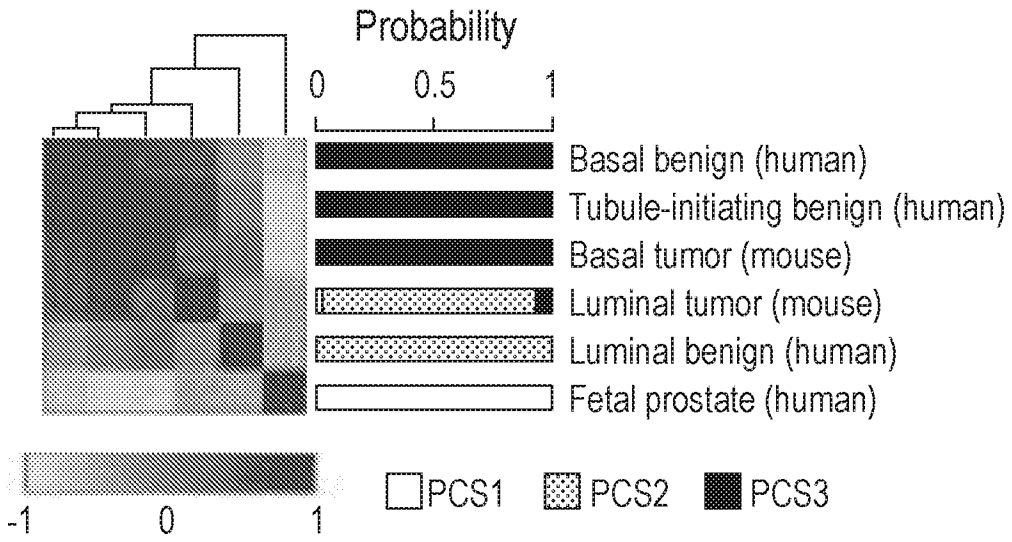

Probability
0
0.5
1
Basal benign (human)
Tubule-initiating benign (human)
Basal tumor (mouse)
Luminal tumor (mouse)
Luminal benign (human)
Fetal prostate (human)
-1
0
1
PCS1
PCS2
PCS3

FIG. 5A

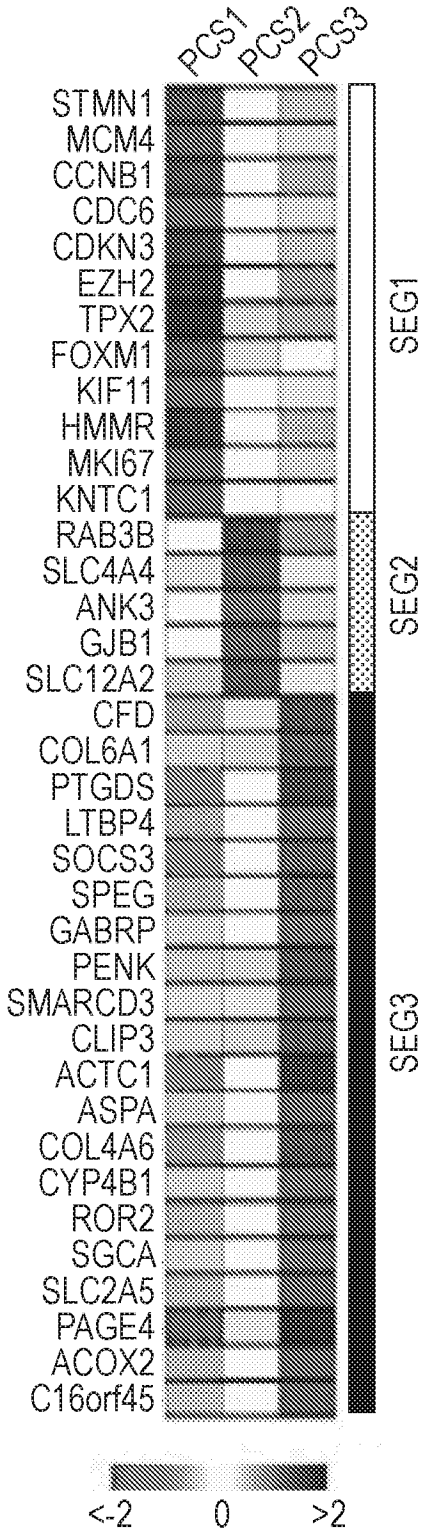

PCS1
PCS2
PCS3
STMN1
MCM4
CCNB1
CDC6
CDKN3
EZH2
TPX2
FOXM1
KIF11
HMMR
MKI67
KNTC1
RAB3B
SLC4A4
ANK3
GJB1
SLC12A2
CFD
COL6A1
PTGDS
LTBP4
SOCS3
SPEG
GABRP
PENK
SMARCD3
CLIP3
ACTC1
ASPA
COL4A6
CYP4B1
ROR2
SGCA
SLC2A5
PAGE4
ACOX2
C16orf45
SEG1
SEG2
SEG3
<-2
0
>2

FIG. 5B

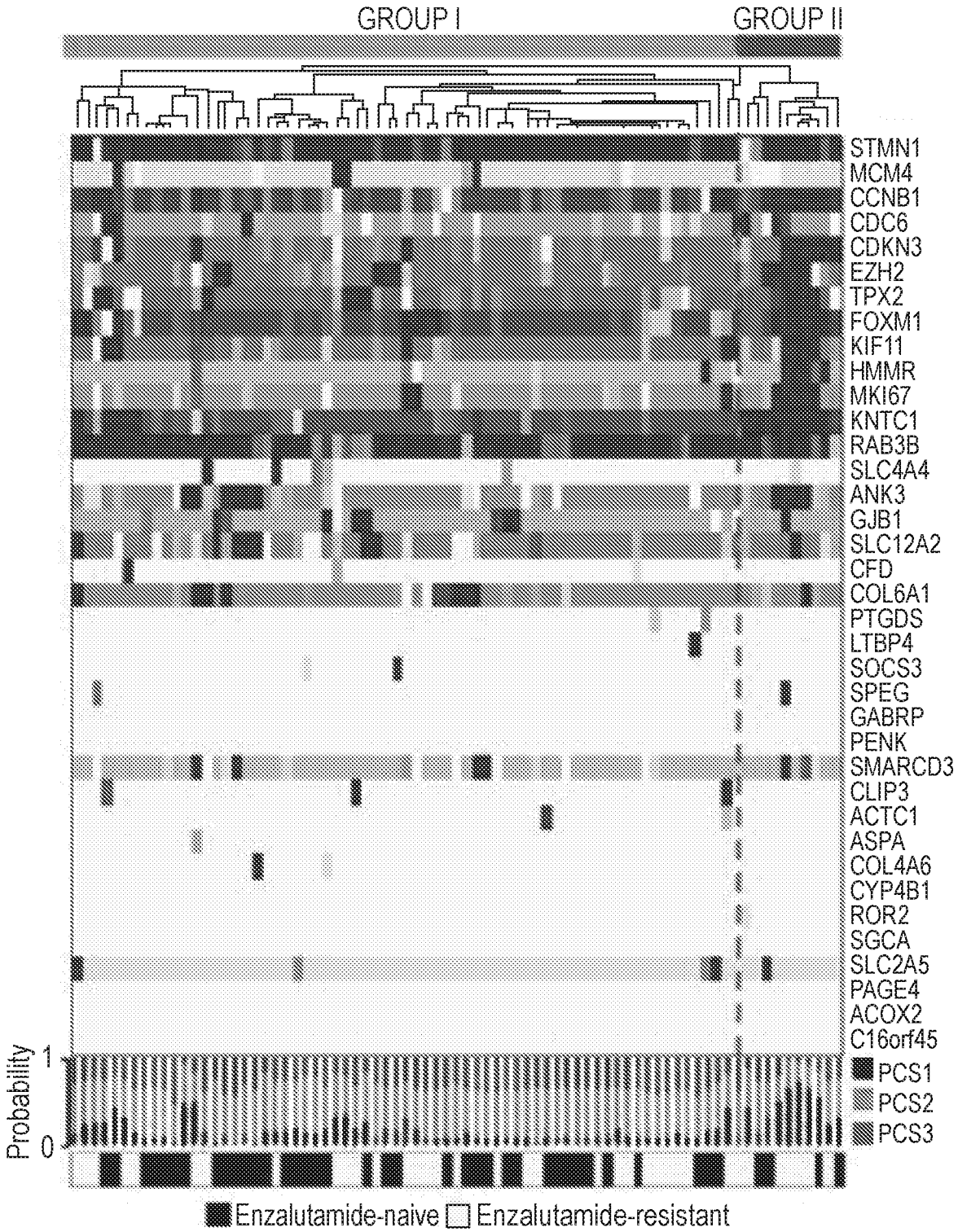

GROUP I
GROUP II
STMN1
MCM4
CCNB1
CDC6
CDKN3
EZH2
TPX2
FOXM1
KIF11
HMMR
MKI67
KNTC1
RAB3B
SLC4A4
ANK3
GJB1
SLC12A2
CFD
COL6A1
PTGDS
LTBP4
SOCS3
SPEG
GABRP
PENK
SMARCD3
CLIP3
ACTC1
ASPA
COL4A6
CYP4B1
ROR2
SGCA
SLC2A5
PAGE4
ACOX2
C16orf45
PCS1
PCS2
PCS3
Probability
1
0
Enzalutamide-naive
Enzalutamide-resistant

FIG. 5C

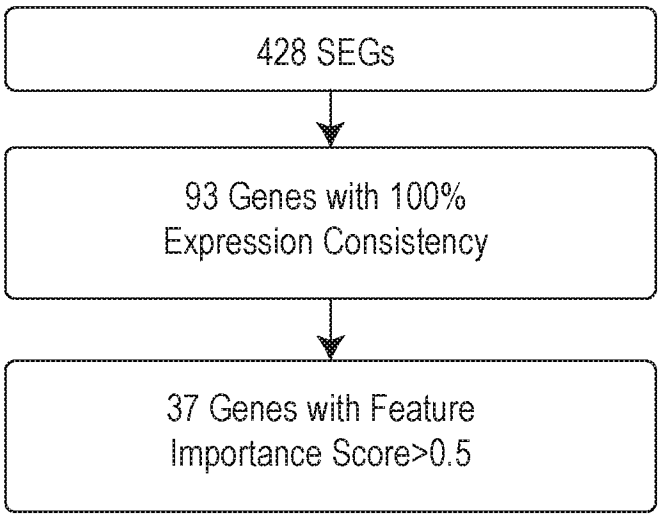
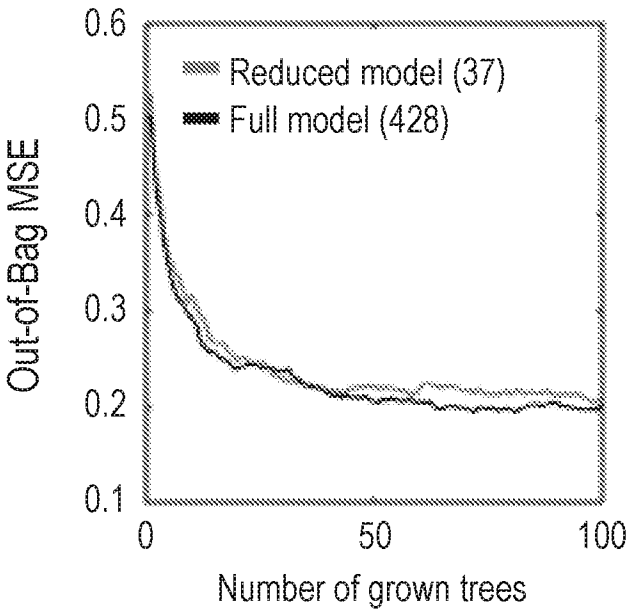

428 SEGs
93 Genes with 100% Expression Consistency
37 Genes with Feature Importance Score>0.5

FIG. 5D


0.6
0.5
0.4
0.3
0.2
0.1
Out-of-Bag MSE
Reduced model (37)
Full model (428)
0
50
100
Number of grown trees

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/55573

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; G06F 19/18 (2017.01)
CPC - C12Q 1/6886, 1/6881; G01N 33/57407, 33/57415; G06F 19/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) Classifications: C12Q 1/68; G06F 19/18; CPC Classifications: C12Q 1/6886, 1/6881; G01N 33/57407, 33/57415; G06F 19/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO); USPTO Web Page; Google Scholar; EBSCO; Entrez Pubmed; Science Direct; Search terms -- 'prostate cancer', classification, subtypes, signature, profile, diagnosis, prognosis, treatment, enzalutamide, PTEN, RAS, 'androgen receptor', BUB1, metastisis, docetaxel, dasatinib, cluster, matrix, normalization

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2014/0243433 A1 (CHUNDSELL MEDICALS AB) August 28, 2014; abstract; paragraphs [0016], [0017], [0020], [0023], [0034], [0036], [0041], [0055], [0058], [0061], [0066], [0067], [0069], [0077], [0081], [0083], [0106], [0119], [0128], [0142], [0135]; figures 5, 7	1, 2, 6, 7, 10, 17-19 ---------- 3, 8, 9, 20-31, 38-47, 49-59, 61-64
Y	US 2011/0165566 A1 (WITTLIFF, JL et al.) July 7, 2011; paragraphs [0040], [0059]; figure 29	3
Y	US 2014/0235479 A1 (DEPINHO, RA et al.) August 21, 2014; abstract; paragraphs [0002], [0005], [0011], [0014]	8, 63
Y	WO 2015/065919 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) May 7, 2015; abstract; paragraphs [0014], [0076], [0089]	9, 24, 25, 41-46, 52, 54-59, 64
Y	WO 2008/086342 A2 (BRISTOL-MYERS SQUIBB COMPANY) July 17, 2008; paragraphs [0013], [0014], [0067], [0075], [0126], [0316]	20-31
Y	US 2014/0342924 A1 (HARKIN, DP et al.) November 20, 2014; paragraphs [0028], [0061], [0065], [0090], [0092], [0128], [0132], [0136], [0137], [0142]	38-47, 49-59, 61-64
Y	US 2013/0281312 A1 (RICHARDSON, AL et al.) October 24, 2013; paragraphs [0006], [0066], [0157]	21
Y	US 2014/0308202 A1 (VANDERBILT UNIVERSITY) October 16, 2014; paragraphs [0008], [0060], [0068], [0217]	22, 26, 39, 41, 50, 52
Y	US 2015/0100244 A1 (SEQUENOM, INC.) April 9, 2015; paragraphs [0140], [0192], [0224], [0352]	47, 59

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2017 (27.01.2017)	27 FEB 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/55573

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

-***-Continued Within the Next Supplemental Box-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6-10, 17-31, 38-64; restricted to BUB1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/55573

-***-Continued from Box III: Observations where unity of invention is lacking-***-

Groups I+, Claims 1-64, and BUB1 are directed toward methods of classifying prostate cancer into subtypes; prognosing cancer in a subject based thereon; and treating cancer in a subject.

The methods will be searched to the extent they encompass a marker gene encompassing BUB1 (first exemplary marker gene). Applicant is invited to elect additional marker gene(s) to be searched. Additional marker gene(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2, 3 (in-part), 6-10, 17-31 and 38-64 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass BUB1 (marker gene). Applicants must specify the claims that encompass any additionally elected marker gene(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a marker gene encompassing STMN1 (first exemplary elected marker gene).

No technical features are shared between the marker genes of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a method for classifying prostate cancer into subtypes, comprising: a) obtaining a sample from a subject; b) assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; c) determining the presence of an expression pattern of the one or more genes associated with the subtype in the sample based on the detected changes; and d) classifying the cancer in the subject into the subtype if the expression pattern of the one or more genes associated with the subtype is detected in the sample; a method for prognosing a cancer in a subject, comprising: a) obtaining a sample from the subject; b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values; c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; and d) prognosing the cancer in the subject; a method for treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of a cancer in a subject, comprising: a) obtaining a sample from the subject; b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values; c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; and d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of the cancer; a method for treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of a cancer in a subject, comprising: a) obtaining a sample from the subject; b) assaying the sample to detect a marker for a subtype of the cancer; c) detecting the marker for the subtype in the sample; and d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating, preventing, reducing the likelihood of having, reducing the severity of or slowing the progression of the cancer; and a method for classifying a prostate cancer in a subject, comprising: a) determining pathway activation gene expression signatures in a plurality of prostate cancer specimens; b) converting the pathway activation gene expression signatures into pathway activation profiles; c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to a prostate cancer subtype; d) obtaining a sample from the subject; e) determining a pathway activation profile in the sample; and f) classifying the prostate cancer in the subject into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the sample.

-***-Continued Within the Next Supplemental Box-***-

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/55573

-***-Continued from Previous Supplemental Box-***-

However, these shared technical features are previously disclosed by US 2014/0342924 A1 to Harkin et al. (hereinafter 'Harkin').

Harkin discloses a method for classifying prostate cancer into subtypes (identification of cancer subtypes, including prostate cancer, and classifying a patient within a subtype (a method for classifying prostate cancer into subtypes); abstract, paragraph [0153]), comprising: a) obtaining a sample from a subject (obtaining a sample from a subject; paragraph [0045]); b) assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values (assaying the sample to detect changes in gene expression of one or more genes relative to reference samples or values; paragraphs [0043], [0045]); c) determining the presence of an expression pattern of the one or more genes associated with the subtype in the sample based on the detected changes (determining the presence of an expression pattern of the one or more genes associated with the subtype in the sample based on the detected changes; paragraphs [0043], [0045]); and d) classifying the cancer in the subject into the subtype if the expression pattern of the one or more genes associated with the subtype is detected in the sample (classifying the cancer in the subject into the subtype if the expression pattern of the one or more genes associated with the subtype is detected in the sample; paragraph [0045]); a method for prognosing a cancer in a subject (a method for determining a prognostic indicator for a subject (a method for prognosing a cancer in a subject); abstract, paragraphs [0021], [0045]), comprising: a) obtaining a sample from the subject (obtaining a sample from a subject; paragraph [0045]); b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values (assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values; paragraphs [0043], [0045]); c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes (determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; paragraphs [0043], [0045]); and d) prognosing the cancer in the subject (prognosing the cancer in the subject; paragraphs [0021], [0045], [0069]); a method for treating a cancer in a subject (a method of guiding the treatment (treating) of cancer in a subject; paragraph [0022]), comprising: a) obtaining a sample from the subject (obtaining a sample from a subject; paragraph [0045]); b) assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values (assaying the sample to detect changes of expression levels of one or more genes relative to reference samples or values; paragraphs [0043], [0045]); c) determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes (determining the presence of a subtype's expression pattern of the one or more genes in the sample based on the detected changes; paragraphs [0043], [0045]); and d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating the cancer (administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating the cancer; paragraphs [0022], [0042]); a method for treating a cancer in a subject (a method of guiding the treatment (treating) of cancer in a subject; paragraph [0022]), comprising: a) obtaining a sample from the subject (obtaining a sample from a subject; paragraph [0045]); b) assaying the sample to detect a marker for a subtype of the cancer (assaying the sample to detect a marker for a subtype of the cancer; paragraphs [0043], [0045]); c) detecting the marker for the subtype in the sample (detecting the marker for the subtype in the sample; paragraphs [0043], [0045]); and d) administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating the cancer (administering a therapeutically effective amount of a therapeutic agent to the subject, thereby treating the cancer; paragraphs [0022], [0042]); and a method for classifying a prostate cancer in a subject (identification of cancer subtypes, including prostate cancer, and classifying a patient within a subtype (a method for classifying prostate cancer into subtypes); abstract, paragraph [0153]), comprising: a) determining pathway activation gene expression signatures (determining pathway activation gene expression signatures; paragraphs [0043], [0045], [0065]) in a plurality of prostate cancer specimens (in a plurality of prostate cancer specimens; paragraphs [0028], [0032], [0153]); b) converting the pathway activation gene expression signatures into pathway activation profiles (converting the pathway activation gene expression signatures into pathway activation profiles; paragraphs [0043], [0065]); c) grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to a prostate cancer subtype (grouping the pathway activation profiles into independent clusters, wherein each independent cluster corresponds to a prostate cancer subtype; paragraphs [0043], [0065], [0153]); d) obtaining a sample from the subject (obtaining a sample from a subject; paragraph [0045]); e) determining a pathway activation profile in the sample (determining a pathway activation profile in the sample; paragraphs [0045], [0065]); and f) classifying the prostate cancer in the subject into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the sample (classifying the prostate cancer in the subject into the prostate cancer subtype if the pathway activation profile corresponding to the prostate cancer subtype is detected in the sample; paragraphs [0045], [0065]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Harkin reference, unity of invention is lacking.