Am J Cancer Res 2022;12(4):1660-1670
www.ajcr.us /ISSN:2156-6976/ajcr0139929

Original Article

Identification of novel biomarkers differentially expressed between African-American and Caucasian-American prostate cancer patients

Fei Ye[1], Xiaoxia Han[2], Yonzhao Shao[2], Jingzhi Lo[9], Fengxia Zhang[1], Jinhua Wang[3], Jonathan Melamed[3], Fang-Ming Deng[3], Karen S Sfanos[6], Angelo De Marzo[6], Guoping Ren[7], Dongwen Wang[8], David Zhang[4], Peng Lee[3,4,5]

[1]Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, USA; Departments of [2]Biostatstics, [3]Pathology, [4]Urology, [5]New York Harbor Healthcare System, New York University School of Medicine, New York, USA; [6]Department of Pathology, Johns Hopkins University, Baltimore, MD, USA; [7]Department of Pathology, First Hospital of Zhejiang University, Zhejiang, China; [8]Department of Urology, Cancer Hospital Chinese Academy of Medical Sciences, Shenzhen Center, Guangdong, China; [9]Department of Genomic Medicine Unit, Sanofi, Waltham, MA, USA

Received October 19, 2021; Accepted February 10, 2022; Epub April 15, 2022; Published April 30, 2022

Abstract: Prostate cancer (PCa) incidence and mortality rate vary among racial and ethnic groups with the highest occurrence in African American (AA) men who have mortality rates twice that of Caucasians (CA). In this study, we focused on differential expression of proteins in AA prostate cancer compared to CA using Protein Pathway Array Analysis (PPAA), in order to identify protein biomarkers associated with PCa racial disparity. Fresh frozen prostate samples (n=90) obtained from radical prostatectomy specimens with PCa, including 25 AA tumor, 21 AA benign, 23 CA tumor, 21 CA benign samples were analyzed. A total of 286 proteins and phosphoproteins were assessed using PPAA. By PPAA analysis, 33 proteins were found to be significantly differentially expressed in tumor tissue (n=48, including both CA and AA) in comparison to benign tissue (n=42). We further compared protein expression levels between AA and CA tumor groups and found that 3 proteins were differentially expressed ($P<0.05$ and $q<5\%$). Aurora was found to be significantly increased in AA tumors, while Cyclin D1 and HNF-3a proteins were downregulated in AA tumors. Predicted risk score was significantly different between AA and CA ethnic groups using logistic regression analysis. In conclusion, we identified Aurora, Cyclin D1 and HNF-3a proteins as being differentially expressed between AA and CA in PCa tissue. Our study suggests that these proteins might be involved in different pathways that lead to aggressive PCa behavior in AA patients, potentially serving as biomarkers for the PCa racial disparity.

Keywords: Prostate cancer, racial disparity, African American, Caucasian, molecular markers

Introduction

Prostate cancer (PCa) places an exceptional burden on the African American (AA) population. There have been intensive studies that show there is a disparity of PCa between AA and Caucasian (CA) men; namely, AA men have a notably higher prevalence than CA men and present with PCa at a younger age [1-3]. Contributions to outcome disparities are multifactorial, including socioeconomic differences that may affect access to quality health care [4-8] as well as tumor biology. In order to understand the molecular mechanisms contributing to these disparities, it is crucial to identify biological factors that may contribute to the increased PCa mortality observed among AA men and characterize biomarkers predicting cancer behavior.

The poor prognosis of AA men with PCa may be attributed to biological factors such as tumor biology, genetics and molecular changes [4-10]. Several epidemiologic and genetic investigations have focused on AA men to decipher the molecular basis for such disparities; however, the molecular causes of the more aggressive PCa in AA patients remain to be further elucidated. Androgens and the androgen receptor-mediated signaling pathways that contribute to

the racial disparity of PCa have been investigated at both the protein and genetic level. These studies suggest that androgens along with other hormones and their receptors may have an important role in racial disparity in PCa [11-16]. Recent genome-wide association studies in PCa have shown genetic heterogeneity in PCa association in AA men versus CA such as differences at 8q24 [17, 18] and 17q21 chromosomes [19]. Different levels of methylation for functionally relevant genes were also reported as possible factors in PCa racial disparities [20-22]. Growth factors have been studied regarding the racial disparity in PCa and a well-documented example is the finding that, epidermal growth factor receptor (EGFR) mutations are more common in AA patients than in CA patients [23]. The differences in apoptotic genes related to PCa racial disparity have also been studied and showed a significant association between Bcl-2 positivity and proliferation in AA men [24]. There are several reported single-nucleotide polymorphisms (SNPs) [25] and copy number variations [26] associated with racial diversity, which may influence non-coding RNA expression, epigenetic regulation, and/or post-translational modifications. However, their biological and clinical significance in most cases is unknown. All of these findings suggest that there are distinct molecular and genetic differences contributing to racial differences of PCa development and disease progression, and subsequently, a subset of these factors may contribute to a more aggressive PCa biology in AA patients. Despite all these efforts, studies on the difference between AA and CA PCa are limited at the proteomic landscape level.

In this study, we hypothesize that the gene expression profiles of PCa in AA and CA patients can reveal biological differences between the two populations that may explain the more aggressive cancer phenotype in AA paitents. We used PPAA analysis: a high-throughput protein assay with a capability to characterize hundreds of proteins in tissue samples, to identify and evaluate the alterations of functionally important proteins and phosphoproteins in samples from AA and CA PCa patients and identify a robust set of proteins that are associated with PCa racial disparity. The changes of these proteins were further correlated with an important outcome factor Gleason score and showed distinct protein expressions between AA and CA cohorts.

Materials and methods

Tissue specimens and patient characteristics

For the Protein Pathway Array (PPAA) analysis, a total of 90 fresh frozen tissue specimens with adequate protein including tumor and adjacent normal prostate were obtained during surgical resection on patients with primary PCa from the hospitals of Mount Sinai and the Prostate Cancer Biorepository Network (PCBN) (25 AA tumors, 21 AA benign, 23 CA tumors, 21 CA benign samples). All samples were snap frozen tissue collected before year between 2003 to 2013 with a minimum 10 year follow up at the time of study. All cancer cases were examined by frozen section with at least 80% cancer volume. The clinicopathologic data of the patients are summarized in **Table 1**. This study was reviewed and approved by the institutional ethical review board.

Protein pathway array analysis (PPAA)

As descripted previously [27, 28], total protein was extracted from 90 fresh frozen prostate samples. 1 mL of 1× lysis buffer (Cell Signaling Technology, Danvers, MA) with 1× protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN) and 1× phosphatase inhibitor cocktail (Roche Applied Science, Indianapolis, IN) were added to each tissue sample. Homogenizer (Benchmark Scientific, Edison, NJ) with 1.7 mm acid washed zirconium beads (Ops Diagnostics, Lebanon, NJ) was used to homogenize the samples. The machine operated for 30 seconds at 4,000 rpm and samples were then put on ice for 15 minutes. The lysate was sonicated 3 times for 15 seconds each time on ice water, then centrifuged at 14,000 rpm for 30 min at 4°C to remove incompletely lysed debris. The protein concentration was determined with the BCA Protein Assay kit (PIERCE, Rockford, IL). 315 μg of lysated protein was loaded in one well across the entire width of 10% SDS polyacrylamide and separated by electrophoresis. After electrophoresis, the protein was transferred electrophoretically to a nitrocellulose membrane (BioRad, Hercules, CA). Three nitrocellulose membranes were made from each sample and each membrane was blocked for 1 hour with blocking buf-

Table 1. Clinicopathologic characteristics of 90 patients

Clinicopathologic characteristic	No. of patients (n=90)
Mean age	
Histology	
Tumor size	
Ethnicity	44 CA, 46 AA
Gleason score	
6	10
7	68
8	0
9	10
NA	2
Pathological TNM stage	
IIA	5
IIB	46
III	35
IV	1
Perineural invasion (Y/N)	
Yes	85
No	3
NA	2
Lymhovascular invasion (Y/N)	
Yes	4
No	57
NA	29
Extra-prostatic extension (Y/N)	
Yes	25
No	25
NA	40

fer including 3% BSA in 1× TBST containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 0.1% Tween-20. The membrane was clamped on a Western blotting manifold (Mini-PROTEAN II Multiscreen apparatus, Bio-Rad, Hercules, CA) which had 20 separate channels across the membrane. The multiplex immunoblot was performed using a total of 286 protein specific or phosphorylation site-specific antibodies (shown in **Table 2**). The antibodies were divided into 9 sets, each set contained 26-36 antibodies. For the first set of 36 primary antibodies, a mixture of 2 antibodies in the blocking buffer were added to each channel and then incubated at 4°C overnight. The membrane was washed with 1× TBS and 1× TBST and was then incubated with secondary anti-rabbit (BioRad, Hercules, CA) or anti-mouse (Bio-Rad, Hercules, CA) or anti-goat (Santa Cruz Biotechnology, Santa Cruz, CA) antibody conjugated with horseradish peroxidase for 1 hour at room temperature. The membrane was developed with chemiluminescence substrate (Immun-Star HRP Peroxide Buffer/Immun-Star HRP Luminol Enhancer) (Bio-Rad, Hercules, CA), and chemiluminescent signals were captured using the ChemiDoc XRS System (Bio-Rad, Hercules, CA). The same membrane was then stripped off using stripping buffer (Restore Western blot stripping buffer, Thermo Scientific, Rockford, IL) and then used for blotting with another set of primary antibodies as described above. Each membrane was blotted for three times.

For PPAA, the signal of each protein was determined by densitometric scanning (Quantity One software package, Bio-Rad) and the background was locally subtracted from raw protein signal [28, 29]. The background-subtracted intensity was normalized by "global median subtraction" method to reduce variation among different experiments, that means, the intensity of each protein from each sample divided by total intensities of all proteins from the same sample and then multiplied by average intensity in all samples.

Statistical analysis

The Significance of Microarrays (SAM) tool (http://www-stat.stanford.edu/tibs/SAM) and t-test were used to identify differentially expressed proteins between tumor tissues and non-tumor tissues. The overlap proteins with q<5% from SAM and P<0.05 from t-test were selected for downstream analysis.

Logistic regression analysis used R Software (Version 3.3.1). Exact Wilcoxon rank sum test is used to compare Gleason score between AA and CA groups. Non-negative matrix factorization (NMF) brunet method (R package NMF) was used to select proteins. Logistic regression was used to model ethnic group (AA vs CA) with Gleason score as the only explanatory variable in baseline model. The full logistic model includes Gleason score and NMF selected proteins as explanatory variables. The area under receiver operating characteristic curve (AUC) and 95% confidence interval were calculated to evaluate model performance. Exact Wilcoxon rank sum test is used to compare the predicted risk score between AA and CA based on the fitted logistic regression model. Two-tailed test

Table 2. List of the antibodies included in the protein pathway array analysis

Antibodies specific for Phosphorylation	p-AKT, p-GSK-3a/B, p-p38 MAPK, p-P44/42 MAPK, p-p53, p-PDK1, p-PKC a/BII, p-PKCa, p-PKCD, p-PTEN, p-Smad1/5, p-Stat3
Antibodies for signal transduction proteins	14-3-3 Beta, ADAM-10, ADH, AIM2, Akt, alpha-tubulin, annexin A1, ASC, ASCL1, ATF-1, Aurora, Auxin, Bak, Bax, B-catenin, Bcl-2, Bcl-6, Bcl-xL, BENC1, BID, Calpain 2, Calretinin, CaMKKa, Caspase-1 p10, CathepsinB-25, CathepsinB-37, CD33, cdc25C, cdc42, cdk2, cdk4, cdk6, c-IAP2, cPKCa, CREB, CTGF, cul1, cx43, cyclin B1, cyclin E, Cyclin D1, Cytokeratin 18, Cytokeratin 19, Cytokeratin 5-, DHFR, Dmnt1, DRG1, E2A, E-cadherin, EGFR, ERa, Era, ERCC1, Factor XIIB, FAH, FAS, FEN-1, FGF-8, FKHR, FTa, fusin, Galectin-3, GLP-1R, Glutamine Synthetase, GSTP1, HDAC1, HGF, Hint1, HIP-3a, HMG-1, HNF-3a, H-Ras, HSP27, HSP70, Hsp90, ICAM-1, IGFBP5, IL-11, IL-18, IL-3Ra, IL-6, ITF, Jagged 1, Keratin 10, LKB1, LSD1, L-Selectin, Lyn, Maspin, MATIIbeta, MDM2, Mesothelin, MetAP-2, MetRS, MGr1-Ag, MMP-13, MMP2, MMP-7, THFD1, MTHFR, NALP1, N-cadherin, NFkB52, NFkBp50, NFkBp65, Nkx-3.1, NM23-H1/2/3, Notch4, NQO1, ODC, p14, p27, P2X7, p38B, p44/42, P504S, Pax-2, PC2, PCNA, PDEF, PEDF, Plk, MAPK, MAPK, PTEN, Raf-B, RAGE, RANKL, Rap1, SK3, SOD-1, SPAK, Stat1, stat3, Tau, TCF-1, TFIIHp89, TIMP3, TIRAP, TRAF6, Tyro3, VAP-1, VEGF, Vimentin, VSV-G, Wnt1, WT1, XIAP, B-actin, GADPH

were used in all analyses. A maximum *p* value of 0.05 was chosen for statistical significance.

NMF (non-negative matrix factorization) *analysis for* the data set, which contains samples of AA tumor and benign, CA tumor and benign, used an un-supervised matrix factorization method. NMF method was used to identify local signatures and de-convolute mixed signals, widely used for dimension deduction and feature selection. We created a matrix with normalized positive protein expression values, and clustering samples and extracting genes using NMF implemented in an R package (https://cran.r-project.org/src/contrib/Archive/NMF/). Meta genes associated with AA tumor in contrast to AA benign, and CA tumor versus CA benign were extracted.

Results

Differential expression of proteins between tumor and normal samples

The PPAA analysis includes 90 samples that contain 48 tumors and 42 matched benign tissues. There were 176 of 286 proteins detected in either tumor or non-tumor tissue (**Table 2**). Of these proteins, 33 proteins were found to be significantly differentially expressed when compared between tumor and non-tumor tissues based on t-test and SAM two class unpaired analysis (t-test P<0.05 and SAM q<5%) (Supplementary Table 1). Among these 33 proteins, 13 proteins and phosphoproteins were predominantly overexpressed in tumor tissues and include: p-PKC a/BII, cyclin E, H-Ras, annexin A1, Aurora, Cyclin D1, NFkBp50, NFkBp52, EGFR, HSP70, 14-3-3 Beta, FAS, and Maspin. In contrast, 20 proteins were found to be down-regulated in tumors including: MetRS, MGr1-AG, CathepsinB-37, PDEF, NQO1, PCNA, p38B, FEN-1, P504S, MetAP-2, Dmnt1, ASC, Cytokeratin 18, IL-18, HSP90, IL-6, ADAM-10, MDM2, ERCC1, and HSP27.

Differential expression of proteins between tumor and normal in AA and CA subgroups

In the comparison of proteins in 23 CA tumors vs 21 CA benign, 28 proteins were found significantly differentially expressed (Supplementary Table 2). There were 15 proteins found to be upregulated: Annexin A1, H-Ras, Cyclin D1, WT1, NFkBp50, FAS, Stat1, NFkB52, cyclin E, Vimentin, PTEN, NFkBp65, L-Selectin, p-PKCa, and TFIIHp89. 13 proteins were found to be downregulated: VSV-G, MGr1-Ag, CathepsinB-37, B-actin, MetRS, ASC, ASCL1, TIMP3, NQO1, Tau, PDEF, MetAP-2, and P504S. Moreover, when proteins were compared between 21 AA benign and 25 AA tumor samples, a total of 12 proteins were found significantly differentially expressed (Supplementary Table 3). Five proteins were found to be upregulated: Aurora, p-p38 MAPK, stat3, p-PKC a/BII, and p-PKC A. 7 were found to be downregulated: MetRS, XIAP, MGr1-Ag, PCNA, Hsp90, HNF-3a, FEN-1.

Differential expression of proteins between tumor-tumor and normal-normal in AA and CA samples

The three proteins Aurora, Cyclin D1 and HNF-3a were found to be differentially expressed

Table 3. Results of logistic regression and predictive ability of models

	OR (95% CI)	*p* value	AUC (95% CI)	*p* value of Predicted risk score (CA vs AA)
Based model				
Gleason score	0.390 (0.106, 0.953)	0.073	0.769 (0.592, 0.946)	0.021
Full model				
HNF3A	1.011 (0.718, 1.39)	0.94	0.939 (0.843, 1)	<0.001
NQO1	1.593 (1.109, 3.311)	0.069		
VAP-1	0.907 (0.615, 1.195)	0.511		
MetRS	0.353 (0.054, 0.81)	0.097		
p-p53	2.259 (1.004, 12.099)	0.174		
L-selectin	0.383 (0.027, 1.559)	0.268		
DNMT1	0.745 (0.398, 1.043)	0.15		
FGF8	2.803 (1.06, 24.629)	0.176		
pGSK3AB	0.372 (0.084, 0.779)	0.051		
Fas	0.175 (0.001, 1.063)	0.317		
pPKCaBII	0.002 (0, 0.272)	0.09		
Gleason score	0.004 (0, 0.196)	0.067		

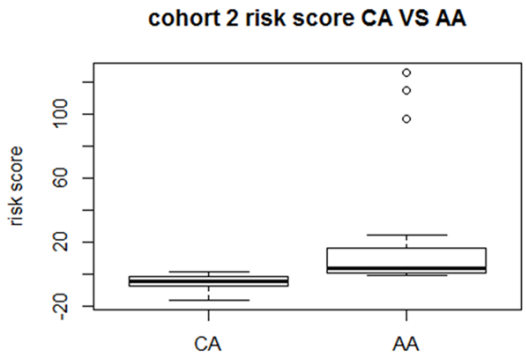

Figure 1. Boxplot of AA and CA risk score distribution. Boxplot of AA and CA patients risk score distribution in cohort 2 showing the CA group has larger median value and quantile range.

between 25 AA and 23 CA tumor samples (Supplementary Table 4). Aurora was upregulated, while Cyclin D1 and HNF-3a were downregulated. In contrast, when comparing 21 AA benign vs 21 CA benign samples, four proteins were found significantly differentially expressed (Supplementary Table 5). FAS and Factor XIIB were up-regulated while SPAK and FGF-8 were downregulated.

Protein expression difference in AA/CA based on Gleason score and selected proteins

To identify a robust set of proteins that could classify the different ethnic groups (AA vs CA) we used predicted risk score analysis. There were 87 non-housekeeping proteins. Proteins with a call rate of <70% for each cohort were removed and 46 proteins were left after quality control. Proteins HNF3A, Tau, NQO1, VAP-1, MetRS, p-p53, L-selectin, DNMT1, FGF8, pGSK-3AB, Fas and pPKCaBII were selected using NMF analysis. **Table 3** summarizes logistic regression results, AUC and *p* values for predicted risk score. For baseline models using only the Gleason score as covariate, the AUC was 0.769. The predicted risk score difference between AA and CA was significant (*P* value: 0.021) (**Figure 1**). Furthermore, when selected proteins were included to the logistic regression analysis in combination with Gleason score, the AUC increased substantially from 0.769 to 0.939 and the predicted risk score between AA and CA groups were significantly different (P<0.001), suggesting that based on the logistic regression model approach HNF3A, Tau, NQO1, VAP-1, MetRS, p-p53, L-selectin, DNMT1, FGF8, pGSK3AB, Fas and pPKCaBII proteins are good markers for distinguishing AA tumors from CA tumors. NMF analysis was performed for sets of samples and revealed distinct protein expression patterns in for both training and validation sets (**Figure 2**).

Discussion

In this study, we proposed to identify novel biomarkers associated with racial disparity in PCa. We examined protein alterations in AA and CA samples using PPAA. Of 286 proteins tested, 176 proteins were detected out of 286 proteins tested in either tumor or non-tumor tissue. From these 176 identified proteins, 33 were dif-

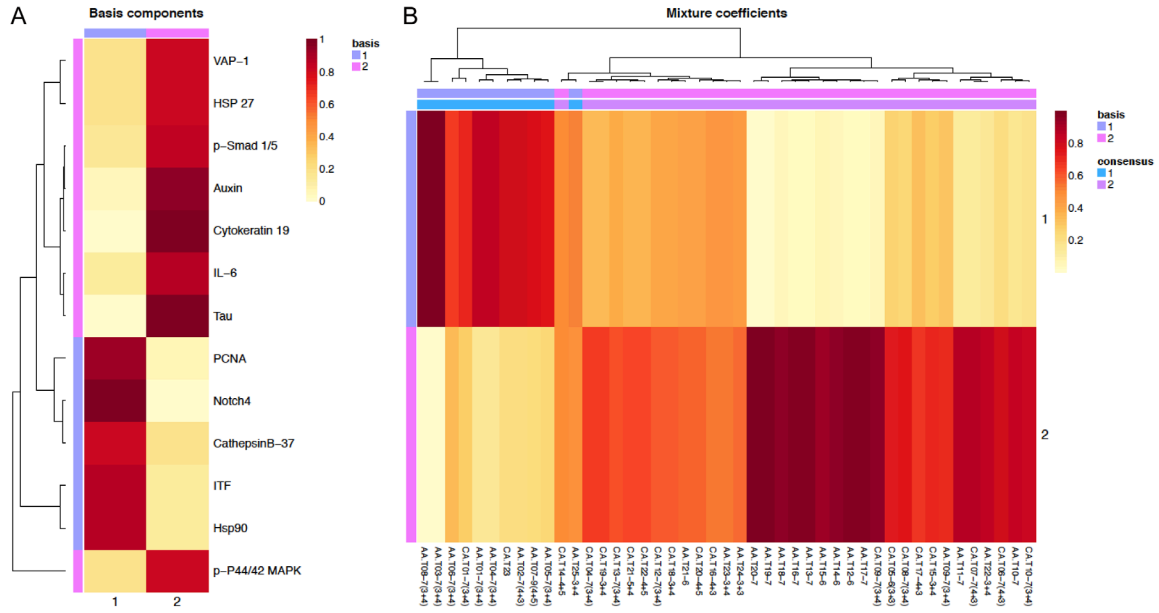

Figure 2. Non-negative Matrix factorization analysis of gene features separating AA and CA group (A) and AA/CA sample clustering using the feature gene sets (B). (A) 13 genes have significant differential expression between AA and CA; (B) AA and CA samples segregate into subgroups using the 13 gene features.

ferentially expressed between non-tumor tissues and tumors, suggesting that there is significant dysregulation of signaling proteins in tumors. These are primarily signaling proteins and are important in diverse cellular processes including the cell cycle, signal transduction, cell-cell interactions, cell adhesion, DNA damage repair and inflammation. Some of the dysregulated proteins were reported to be involved in PCa in previous studies, which is consistent with our findings. For example, Aurora kinase A (AURKA) is significantly overexpressed in models of AR-positive castration-resistant PCa samples [30] and its levels were significantly elevated in both primary human and mouse PCas and cell lines [31]. Two recent studies reported that immunoregulatory protein Annexin 1 increased the tumor stem cell dynamics and induced EMT changes, which ultimately resulted invasion and progression in PCa cells [32, 33]. PDEF was down-regulated in both metastatic PCa cell lines and clinical patient samples [34]. The expression of some of the proteins in our study differed from that described in previous publications. For example, Maspin expression was increased in our study however, decreased levels of Maspin expression have been previously reported by other investigators [35, 36]. When we evaluate the down regulated genes, our study found FEN1, p504S and MetAP-2 down-regulation in PCa samples compared to normal samples, in contrast, increased levels of those proteins have been showed in previous studies [37-39]. ADAM10 protein was also found to be down-regulated in our study while increased nuclear localization of ADAM10 was previously demonstrated [40, 41]. The differences may be explained by the technique that the authors used such as immunohistochemistry which measures only the staining intensity and localization while PPAA has the advantage of measuring total protein level. Other factors might be related to these discrepancies may also be biological factors including antibodies used, methodology (immunoblot versus immunohistochemistry), tumor origin and differences in tumor biology. Interestingly, only few proteins from these 33 altered proteins have been previously studied in PCa racial disparity. Previous work from our group showed high expression of Mdm protein in CA patients compared to AA patient samples, which is consistent with our current findings [42]. Shuch et al. also studied the association of EGFR expression and race in 202 patients and showed a high EGFR expression in AA patients [23]. It's important to note that, some of the dysregulated proteins found in our study including MetRS, MGr1-ag, and 14-3-3-beta proteins have not been reported

previously in PCa demonstrating the utility of the PPAA method to identify new markers in PCa. This finding also suggests the role of multiple pathways in carcinogenesis and emphasizes the importance of combinational therapies in the management of treatment.

When we evaluated differential expression of specific proteins among AA and CA tumor subgroups, three individual proteins showed statistically significant differential expression; Aurora kinase A was up-regulated, while, cyclin D1 and HNF3A were down-regulated in AA versus CA tumors. Interestingly, Zhang et al. [47] recently reported that the genes with different expression levels between AA and EA patients with prostate cancers in the same grade category are very limited in numbers, which is similar finding with our data showing only three significant proteins changed in the tumors with similar Gleason scores between AA and CA. However, Zhang et al. [47] concluded that the contribution of the Gleason patterns to the variability of gene expression level is much more significant than patient races, which is contradictory to other reported literatures that there are distinct molecular and genetic differences contributing to racial differences of PCa [4-26]. High expression of Aurora has been shown in Castration resistant PCa [43] and correlated with poor prognosis in PCA. The role of Aurora in racial disparity has been studied in other cancers including breast cancer but not in PCa [44]. This study represents first time identification of Aurora as an ethnic marker for AA group. Cyclin D1 in contrast was decreased in our AA tumors samples compared to CA tumors. This is a novel finding because most of the prior molecular genetic studies investigating the role of cyclin D1 in PCa primarily focused on CA PCa patients, in whom cyclin D1 expression was higher in PCa overall [45]. For example, Pereira et al. studied cyclin D1 in PCa samples and the patient population used was composed of 79% CA men [45]. The transcription factor FoxA1 (FOXA1), also named HNF-3a, was also downregulated in our AA tumor samples. Reports of FoxA1 function in prostate cancer have been controversial; for example, some studies have shown that high FoxA1 expression is associated with worse prognosis probably by enhancing AR activity [27, 28], while other studies have shown an association of decreased FoxA1 expression with castration-resistant, poor prognosis PCas [29]. In a recent study, Jin et al. focused the dual role of FOXA1 in PCa carcinogenesis and showed an AR-independent function of FOXA1 as a metastasis inhibitor in *in vivo* and *in vitro* samples [46]. Overall, these findings suggest Cyclin D1 and HNF3A regulate oncogenic process via different molecular mechanisms, including apoptosis and cell proliferation pathways, in AA compared to CA PCa patients, which is consistent to most recent study reported by Rayford et al.[48].

There are several proteins differentially expressed in benign tissue of African and Caucasian patients but not in prostate cancer including SPAK, FGF-8, FAS, and Factor XIIIB. SPAK is WNK (with no lysin kinase) signal kinase [49]. It has lower level of expression in benign AA prostate tissue compared with CA tissue. There is no significant difference in cancer between AA and CA tissue. The results indicate that, relatively, there is an increase in the level of SPAK expression in cancerous compared to benign AA prostate tissue. SPAK is involved in cellular homeostasis, angiogenesis [49, 50] and promotion of cell invasion and tumor growth [50]. Our data correlated these publications and indicates SPAK may be related with AA and CA prostate cancer disparity. The FGF-8 provides instructions for making a protein called fibroblast growth factor 8 (FGF-8) [51]. Its expression in benign AA is lower than CA, but no difference in cancer between AA and CA, which indicates that FGF-8 expression is relative higher in cancer compared with benign AA prostate. FGF-8 is involved in many processes, such as promoting cell division, regulation of cell growth and maturation. Therefore, our results indicate that FGF-8 may be suggested to be involved in the progression of prostate cancer and correlated with AA and CA prostate cancer disparity [51]. Fatty acid synthase (FAS), a key enzyme of the fatty acid biosynthetic pathway, functions as a central regulator of lipid metabolism and plays a critical role in the growth and survival of tumors with lipogenic phenotypes [52]. It has been shown to be overexpressed in various types of human cancer, including the prostate, breast, ovarian, endometrial, lung, colorectal, stomach and skin cancer [52]. Factor XIIIB, also known by the name fibrin stabilizing factor, is a key clotting factor in the coagulation cascade known for

stabilizing the formation of a blood clot, and abnormal FXIII expression levels are found in some cancer patients [53]. Since both FAS and Factor XIIIB are known to be important in tumor growth, tumor stroma formation and metastasis [52, 53], it has been suggested that FAS and FXIIIB expression levels may also be important in tumor development and metastasis. Factor XIIIB has higher level of expression in benign AA prostate tissue, the significance is uncertain.

We used logistic regression based model to identify predictive biomarkers which are related to PCa in men of African descent versus the men of Caucasians. We first used a base model using Gleason score to classify the proteins and found that there was an association between Gleason score and different ethnic groups. However, when we used the full model using the selected proteins including HNF3A, Tau, NQO1, VAP-1, MetRS, p-p53, L-selectin, DNMT1, FGF8, pGSK3AB, Fas and pPKCaBII in combination with Gleason score accurately predicted risk in an ethnicity dependent manner. Predicted risk score between AA and CA was significantly different. Those eleven differentially expressed genes were associated with various molecular pathways including endocrine signaling, adhesion, DNA methylation, cell division, and programmed cell death. Thus, these data suggest AA PCa patients have differentially expressed genes that could contribute to the aggressiveness of PCa. Although Gleason score is one of the best predictors of PCa aggressiveness, there are limited number of studies and potential biomarkers which can effectively identify the aggressive disease in PCa patients from African descent. Identification of new molecular targets may significantly help predicting aggressive disease and assist in the development of new targeted therapies especially for AA men with PCa.

While we have identified novel protein biomarkers associated with PCa racial disparity, our study has minor limitations. Because it is difficult to collect tissue of both tumoral and non-tumoral tissue from AA and CA patients, our sample size is limited. In addition, the significantly altered markers especially in AA versus CA group patient samples were not yet assessed by other means such as immunohistochemistry, which would be a validation to our findings. Future clinical studies will be needed to further validate our findings and evaluate the associations of these potential markers with PCa tumor characteristics in the racial disparity of PCa using larger-sized patient cohorts.

In conclusion, our data show a multiple dysregulated signaling proteins contributing to racial disparity in PCa patients. Importantly, the results suggest that these differentially expressed proteins may in part explain the difference in clinical outcomes between AA and Ca PCa patients.

Acknowledgements

This work is supported by NIH U01CA149556 (PL and DZ), The Department of Defense Prostate Cancer Research Program, Award No W81XWH-18-1-0272 (PL), W81XWH-10-2-0056 and W81XWH-10-2-0046 to Prostate Cancer Biorepository Network (PCBN).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Peng Lee, Department of Pathology, New York University School of Medicine, New York, USA. E-mail: Peng.lee@nyumc.org; Dr. David Zhang, Department of Urology, New York University School of Medicine, New York, USA. E-mail: Dyzhang01@gmail.com

References

[1] Hoffman RM, Gilliland FD, Eley JW, Harlan LC, Stephenson RA, Stanford JL, Albertson PC, Hamilton AS, Hunt WC and Potosky AL. Racial and ethnic differences in advanced-stage prostate cancer: the prostate cancer outcomes study. J Natl Cancer Inst 2001; 93: 388-395.

[2] Thompson I, Tangen C, Tolcher A, Crawford E, Eisenberger M and Moinpour C. Association of African-American ethnic background with survival in men with metastatic prostate cancer. J Natl Cancer Inst 2001; 93: 219-225.

[3] Gokce MI, Sundi D, Schaeffer E and Pettaway C. Is active surveillance a suitable option for African American men with prostate cancer? A systemic literature review. Prostate Cancer Prostatic Dis 2017; 20: 127-136.

[4] McGinley KF, Tay KJ and Moul JW. Prostate cancer in men of African origin. Nat Rev Urol 2016; 13: 99-107.

[5] Powell IJ and Bollig-Fischer A. Minireview: the molecular and genomic basis for prostate cancer health disparities. Mol Endocrinol 2013; 27: 879-891.

[6] Castro P, Creighton CJ, Ozen M, Berel D, Mims MP and Ittmann M. Genomic profiling of prostate cancers from African American men. Neoplasia 2009; 11: 305-312.
[7] Singh S, Plaga A and Shukla GC. Racial disparities: disruptive genes in prostate carcinogenesis. Front Biosci (Schol Ed) 2017; 9: 244-253.
[8] Farrell J, Petrovics G, McLeod DG and Srivastava S. Genetic and molecular differences in prostate carcinogenesis between African American and Caucasian American men. Int J Mol Sci 2013; 14: 15510-15531.
[9] Berger AD, Satagopan J, Lee P, Taneja SS and Osman I. Differences in clinicopathologic features of prostate cancer between black and white patients treated in the 1990s and 2000s. Urology 2006; 67: 120-124.
[10] Cullen J, Brassell SA, Chen Y, Porter C, L'Esperance J, Brand T and McLeod DG. Racial/Ethnic patterns in prostate cancer outcomes in an active surveillance cohort. Prostate Cancer 2011; 2011: 234519.
[11] Ross RK, Bernstein L, Lobo RA, Shimizu H, Stanczyk FZ, Pike MC and Henderson BE. 5-alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. Lancet 1992; 339: 887-889.
[12] Hatcher D, Daniels G, Osman I and Lee P. Molecular mechanisms involving prostate cancer racial disparity. Am J Transl Res 2009; 1: 235-248.
[13] Powell IJ, Zhou J, Sun Y, Sakr WA, Patel NP, Heilbrun LK and Everson RB. CYP3A4 genetic variant and disease-free survival among white and black men after radical prostatectomy. J Urol 2004; 172: 1848-1852.
[14] Zeigler-Johnson C, Friebel T, Walker AH, Wang Y, Spangler E, Panossian S, Patacsil M, Aplenc R, Wein AJ, Malkowicz SB and Rebbeck TR. CYP3A4, CYP3A5, and CYP3A43 genotypes and haplotypes in the etiology and severity of prostate cancer. Cancer Res 2004; 64: 8461-8467.
[15] Bangsi D, Zhou J, Sun Y, Patel NP, Darga LL, Heilbrun LK, Powell IJ, Severson RK and Everson RB. Impact of a genetic variant in CYP3A4 on risk and clinical presentation of prostate cancer among white and African-American men. Urol Oncol 2006; 24: 21-27.
[16] Abd Elmageed ZY, Moroz K, Srivastav SK, Fang Z, Crawford BE, Moparty K, Thomas R and Abdel-Mageed AB. High circulating estrogens and selective expression of ERbeta in prostate tumors of Americans: implications for racial disparity of prostate cancer. Carcinogenesis 2013; 34: 2017-2023.
[17] Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Cazier JB, Sainz J, Jakobsdottir M, Kostic J, Magnusdottir DN, Ghosh S, Agnarsson K, Birgisdottir B, Le Roux L, Olafsdottir A, Blondal T, Andresdottir M, Gretarsdottir OS, Bergthorsson JT, Gudbjartsson D, Gylfason A, Thorleifsson G, Manolescu A, Kristjansson K, Geirsson G, Isaksson H, Douglas J, Johansson JE, Balter K, Wiklund F, Montie JE, Yu X, Suarez BK, Ober C, Cooney KA, Gronberg H, Catalona WJ, Einarsson GV, Barkardottir RB, Gulcher JR, Kong A, Thorsteinsdottir U and Stefansson K. A common variant associated with prostate cancer in European and African populations. Nat Genet 2006; 38: 652-658.
[18] Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ, Greenway SC, Stram DO, Le Marchand L, Kolonel LN, Frasco M, Wong D, Pooler LC, Ardlie K, Oakley-Girvan I, Whittemore AS, Cooney KA, John EM, Ingles SA, Altshuler D, Henderson BE and Reich D. Multiple regions within 8q24 independently affect risk for prostate cancer. Nat Genet 2007; 39: 638-644.
[19] Haiman CA, Chen GK, Blot WJ, Strom SS, Berndt SI, Kittles RA, Rybicki BA, Isaacs WB, Ingles SA, Stanford JL, Diver WR, Witte JS, Hsing AW, Nemesure B, Rebbeck TR, Cooney KA, Xu J, Kibel AS, Hu JJ, John EM, Gueye SM, Watya S, Signorello LB, Hayes RB, Wang Z, Yeboah E, Tettey Y, Cai Q, Kolb S, Ostrander EA, Zeigler-Johnson C, Yamamura Y, Neslund-Dudas C, Haslag-Minoff J, Wu W, Thomas V, Allen GO, Murphy A, Chang BL, Zheng SL, Leske MC, Wu SY, Ray AM, Hennis AJ, Thun MJ, Carpten J, Casey G, Carter EN, Duarte ER, Xia LY, Sheng X, Wan P, Pooler LC, Cheng I, Monroe KR, Schumacher F, Le Marchand L, Kolonel LN, Chanock SJ, Van Den Berg D, Stram DO and Henderson BE. Genome-wide association study of prostate cancer in men of African ancestry identifies a susceptibility locus at 17q21. Nat Genet 2011; 43: 570-573.
[20] Woodson K, Hayes R, Wideroff L, Villaruz L and Tangrea J. Hypermethylation of GSTP1, CD44, and E-cadherin genes in prostate cancer among US blacks and whites. Prostate 2003; 55: 199-205.
[21] Woodson K, Hanson J and Tangrea J. A survey of gene-specific methylation in human prostate cancer among black and white men. Cancer Lett 2004; 205: 181-188.
[22] Rubicz R, Zhao S, Geybels M, Wright JL, Kolb S, Klotzle B, Bibikova M, Troyer D, Lance R, Ostrander EA, Feng Z, Fan JB and Stanford JL. DNA methylation profiles in African American prostate cancer patients in relation to disease progression. Genomics 2019; 111: 10-16.

[23] Shuch B, Mikhail M, Satagopan J, Lee P, Yee H, Chang C, Cordon-Cardo C, Taneja SS and Osman I. Racial disparity of epidermal growth factor receptor expression in prostate cancer. J Clin Oncol 2004; 22: 4725-4729.
[24] Guo Y, Sigman DB, Borkowski A and Kyprianou N. Racial differences in prostate cancer growth: apoptosis and cell proliferation in Caucasian and African-American patients. Prostate 2000; 42: 130-136.
[25] Rose AE, Satagopan JM, Oddoux C, Zhou Q, Xu R, Olshen AB, Yu JZ, Dash A, Jean-Gilles J, Reuter V, Gerald WL, Lee P and Osman I. Copy number and gene expression differences between African American and Caucasian American prostate cancer. J Transl Med 2010; 8: 70.
[26] Ledet EM, Hu X, Sartor O, Rayford W, Li M and Mandal D. Characterization of germline copy number variation in high-risk African American families with prostate cancer. Prostate 2013; 73: 614-623.
[27] Sahu B, Laakso M, Ovaska K, Mirtti T, Lundin J, Rannikko A, Sankila A, Turunen JP, Lundin M, Konsti J, Vesterinen T, Nordling S, Kallioniemi O, Hautaniemi S and Janne OA. Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. EMBO J 2011; 30: 3962-3976.
[28] Gerhardt J, Montani M, Wild P, Beer M, Huber F, Hermanns T, Muntener M and Kristiansen G. FOXA1 promotes tumor progression in prostate cancer and represents a novel hallmark of castration-resistant prostate cancer. Am J Pathol 2012; 180: 848-861.
[29] Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, Glass CK, Rosenfeld MG and Fu XD. Reprogramming transcription by distinct classes of enhancers functionally defined by eRNA. Nature 2011; 474: 390-394.
[30] Jones D, Noble M, Wedge SR, Robson CN and Gaughan L. Aurora a regulates expression of AR-V7 in models of castrate resistant prostate cancer. Sci Rep 2017; 7: 40957.
[31] Lee EC, Frolov A, Li R, Ayala G and Greenberg NM. Targeting Aurora kinases for the treatment of prostate cancer. Cancer Res 2006; 66: 4996-5002.
[32] Bizzarro V, Belvedere R, Milone MR, Pucci B, Lombardi R, Bruzzese F, Popolo A, Parente L, Budillon A and Petrella A. Annexin A1 is involved in the acquisition and maintenance of a stem cell-like/aggressive phenotype in prostate cancer cells with acquired resistance to zoledronic acid. Oncotarget 2015; 6: 25076-25092.
[33] Geary LA, Nash KA, Adisetiyo H, Liang M, Liao CP, Jeong JH, Zandi E and Roy-Burman P. CAF-secreted annexin A1 induces prostate cancer cells to gain stem cell-like features. Mol Cancer Res 2014; 12: 607-621.
[34] Johnson TR, Koul S, Kumar B, Khandrika L, Venezia S, Maroni PD, Meacham RB and Koul HK. Loss of PDEF, a prostate-derived Ets factor is associated with aggressive phenotype of prostate cancer: regulation of MMP 9 by PDEF. Mol Cancer 2010; 9: 148.
[35] Hall DC, Johnson-Pais TL, Grubbs B, Bernal R, Leach RJ and Padalecki SS. Maspin reduces prostate cancer metastasis to bone. Urol Oncol 2008; 26: 652-658.
[36] Cher ML, Biliran HR Jr, Bhagat S, Meng Y, Che M, Lockett J, Abrams J, Fridman R, Zachareas M and Sheng S. Maspin expression inhibits osteolysis, tumor growth, and angiogenesis in a model of prostate cancer bone metastasis. Proc Natl Acad Sci U S A 2003; 100: 7847-7852.
[37] Lam JS, Seligson DB, Yu H, Li A, Eeva M, Pantuck AJ, Zeng G, Horvath S and Belldegrun AS. Flap endonuclease 1 is overexpressed in prostate cancer and is associated with a high Gleason score. BJU Int 2006; 98: 445-451.
[38] Jiang Z, Woda BA, Rock KL, Xu Y, Savas L, Khan A, Pihan G, Cai F, Babcook JS, Rathanaswami P, Reed SG, Xu J and Fanger GR. P504S: a new molecular marker for the detection of prostate carcinoma. Am J Surg Pathol 2001; 25: 1397-1404.
[39] Tucker LA, Zhang Q, Sheppard GS, Lou P, Jiang F, McKeegan E, Lesniewski R, Davidsen SK, Bell RL and Wang J. Ectopic expression of methionine aminopeptidase-2 causes cell transformation and stimulates proliferation. Oncogene 2008; 27: 3967-3976.
[40] Arima T, Enokida H, Kubo H, Kagara I, Matsuda R, Toki K, Nishimura H, Chiyomaru T, Tatarano S, Idesako T, Nishiyama K and Nakagawa M. Nuclear translocation of ADAM-10 contributes to the pathogenesis and progression of human prostate cancer. Cancer Sci 2007; 98: 1720-1726.
[41] McCulloch DR, Akl P, Samaratunga H, Herington AC and Odorico DM. Expression of the disintegrin metalloprotease, ADAM-10, in prostate cancer and its regulation by dihydrotestosterone, insulin-like growth factor I, and epidermal growth factor in the prostate cancer cell model LNCaP. Clin Cancer Res 2004; 10: 314-323.
[42] Wang G, Firoz EF, Rose A, Blochin E, Christos P, Pollens D, Mazumdar M, Gerald W, Oddoux C, Lee P and Osman I. MDM2 expression and regulation in prostate cancer racial disparity. Int J Clin Exp Pathol 2009; 2: 353-360.
[43] Kivinummi K, Urbanucci A, Leinonen K, Tammela TLJ, Annala M, Isaacs WB, Bova GS, Nykter M and Visakorpi T. The expression of AUR-

KA is androgen regulated in castration-resistant prostate cancer. Sci Rep 2017; 7: 17978.
[44] Albain KS, Barlow WE, Shak S, Hortobagyi GN and Hayes DF. Potential biologic causes of the racial survival disparity in adjuvant trials of ER-positive breast cancer. J Clin Oncol 2010; 28: 511.
[45] Pereira RA, Ravinal RC, Costa RS, Lima MS, Tucci S, Muglia VF, Reis RB and Silva GE. Cyclin D1 expression in prostate carcinoma. Braz J Med Biol Res 2014; 47: 515-521.
[46] Jin HJ, Zhao JC, Ogden I, Bergan RC and Yu J. Androgen receptor-independent function of FoxA1 in prostate cancer metastasis. Cancer Res 2013; 73: 3725-3736.
[47] Zhang W, Dong Y, Sartor O, Flemington EK and Zhang K. SEER and gene expression data analysis deciphers racial disparity patterns in prostate cancer mortality and the public health implication. Sci Rep 2020; 10: 6820.
[48] Rayford W, Beksac AT, Alger J, Alshalalfa M, Ahmed M, Khan I, Falagario UG, Liu Y, Davicioni E, Spratt DE, Schaeffer EM, Feng FY, Mahal B, Nguyen PL, Den RB, Greenberger MD, Bradley R, Watson JM, Beamer M, Stamatakis L, Carmen DJ, Awasthi S, Hwang J, Weil R, Merisaari H, Mohamed N, Deane LA, Chakravarty D, Yadav KK, Yamoah K, Nair SS and Tewari AK. Comparative analysis of 1152 African-American and European-American men with prostate cancer identifies distinct genomic and immunological differences. Commun Biol 2021; 4: 670.
[49] Gallolu Kankanamalage S, Karra AS and Cobb MH. WNK pathways in cancer signaling network. Cell Commun Signal 2018; 16: 72.
[50] Chiu MH, Liu HS, Wu YH, Shen MR and Chou CY. SPAK mediates KCC3-enhanced cervical cancer tumorigenesis. FEBS J 2014; 281: 2353-2365.
[51] Valve EM, Nevalainen MT, Nurmi MJ, Laato MK, Martikainen PM and Härkönen PL. Increased expression of FGF-8 isoforms and FGF receptors in human premalignant prostatic intraepithelial neoplasia lesions and prostate cancer. Lab Invest 2001; 81: 815-826.
[52] Bandyopadhyay S, Pai SK, Watabe M, Gross SC, Hirota S, Hosobe S, Tsukada T, Miura K, Saito K, Markwell SJ, Wang Y, Huggenvik J, Pauza ME, Iiizumi M and Watabe K. FAS expression inversely correlates with PTEN level in prostate cancer and a PI 3-kinase inhibitor synergizes with FAS siRNA to induce apoptosis. Oncogene 2005; 24: 5389-5395.
[53] Sawai Y, Yamanaka Y and Nomura S. Clinical significance of factor XIII activity and monocyte-derived microparticles in cancer patients. Vasc Health Risk Manag 2020; 16: 103-110.

Supplementary Table 1. Proteins differentially expressed between tumor and non-tumor tissues

	Protein ID	Fold Change	t-test *p*-value	SAM-test q-value (%)
Up	p-PKC a/BII	1.4285	7E-06	0.00
	H-ras	1.7223	0.0031	0.00
	Cyclin E	1.6705	0.0015	0.00
	Annexin A1	1.3307	2E-05	0.00
	Aurora	1.5535	0.0051	0.00
	Cyclin D1	1.5108	0.0063	0.00
	NFkBp50	1.3078	0.0077	2.44
	NFkB52	1.3614	0.0251	4.75
	EGFR	1.4119	0.0444	4.75
	HSP70	1.2065	0.0116	4.75
	14-3-3 Beta	1.1787	0.0069	4.75
	FAS	1.3148	0.0378	4.75
	Maspin	1.2504	0.0381	4.75
Down	METRS	-2.121	8E-08	0.39
	MGr1-Ag	-1.74	5E-06	0.00
	CathepsinB-37	-1.426	0.0002	0.00
	PDEF	-1.433	0.001	0.00
	NQO1	-1.391	0.0004	0.00
	PCNA	-1.613	0.0026	0.00
	P38B	-1.604	0.0048	0.00
	FEN-1	-1.305	0.0009	0.39
	P450S	-1.349	0.0022	0.00
	MetAP-2	-1.342	0.0262	0.00
	Dmnt1	-1.267	0.0093	1.5323
	ASC	-1.291	0.0104	1.5323
	Cytokeratin 18	-1.416	0.0117	1.5323
	IL-18	-1.26	0.0117	1.5323
	Hsp90	-1.321	0.0117	1.5323
	IL-6	-1.283	0.0299	2.7941
	ADAM-10	-1.236	0.0157	2.7941
	MDM2	-1.432	0.0488	3.4756
	ERCC1	-1.182	0.0025	3.4756
	HSP27	-1.239	0.0353	0.00

Supplementary Table 2. Proteins differentially expressed between tumor and normal tissues in CA group

	Protein ID	Fold Change	t-test *p*-value	SAM-test q-value (%)
Up	Annexin A1	1.47683	1.14E-06	0.00
	H-Ras	2.358472	0.00171	0.00
	Cyclin D1	1.991868	0.00171	0.00
	WT1	1.781772	0.003446	0.00
	NFkB52	1.603709	0.005614	0.00
	FAS	1.67749	0.003209	0.00
	Stat1	1.871136	0.016646	0.00
	Cyclin E	1.726876	0.01076	0.00
	Vimentin	1.699801	0.009418	3.095238
	L-Selectin	1.360884	0.07438	3.095238
	PTEN	1.589591	0.014173	3.095238
	NFkBp50	1.409385	0.004232	3.095238
	NFkBp65	1.671406	0.023684	3.095238
	p-KCa/BII	1.355816	0.03862	3.095238
	TFIIHp89	1.604384	0.030086	3.095238
Down	VSV-G	-2.05298	0.00065	0.00
	MGr1-Ag	-1.81574	0.000282	0.00
	B-actin	-1.50146	1.8E-05	0.00
	Cathepsin	-1.57687	0.000289	0.00
	METRS	-1.89054	0.000232	0.00
	ASC	-1.50073	0.00197	2.826087
	ASCL1	-1.49965	0.007427	2.826087
	TIMP3	-1.37636	0.007633	2.826087
	NQO1	-1.47493	0.017944	2.826087
	Tau	-1.54888	0.02315	4.642857
	PDEF	-1.46369	0.01253	4.642857
	MetAP-2	-1.49145	0.034101	4.642857
	P450S	-1.45163	0.008653	4.642857

Supplementary Table 3. Proteins differentially expressed between tumor and normal tissues in AA group

	Protein ID	Fold Change	t-test *p*-value	SAM-test q-value (%)
Up	Aurora	2.302838	3.23E-05	0.00
	p-PKC a/BII	1.480663	0.00061	0.00
	Stat3	1.503772	0.001583	0.00
	p-p38	1.746332	0.00994	0.00
	p-PKCa	1.489588	0.003452	0.00
Down	MetRS	-2.13794	7.89E-05	0.00
	HNF-3a	-1.41654	0.009417	4.6875
	XIAP	-1.61331	0.008217	4.6875
	PCNA	-1.82138	0.007571	4.6875
	MGr1-Ag	-1.58536	0.007764	4.6875
	FEN-1	-1.37112	0.006264	4.6875
	Hsp90	-1.53575	0.012458	4.6875

Supplementary Table 4. Proteins differentially expressed between tumor tissues in AA and CA group

	Protein ID	Fold Change	t-test *p*-value	SAM-test q-value (%)
Up	Aurora	2.13077984	0.000167	0.00
Down	Cyclin D1	-0.52183808	0.05468	0.00
	HNF-3a	-0.67310347	0.021776	0.00

Supplementary Table 5. Proteins differentially expressed between normal tissues in AA and CA group

	Protein ID	Fold Change	t-test *p*-value	SAM-test q-value (%)
Up	FAS	1.737631896	0.009907	0.00
	Factor XIIIB	1.597850001	0.007869	0.00
Down	SPAK	-0.69737255	0.014339	0.021803
	FGF-8	-0.707158336	0.01414	0.001502