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SPINK1 Defines a Molecular Subtype of Prostate Cancer in Men with More Rapid Progression in an at Risk, Natural History Radical Prostatectomy Cohort

Michael H. Johnson,

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Ashley E. Ross^{*},

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Mohammed Alshalalfa^{*},

GenomeDx Biosciences, Vancouver, British Columbia, Canada

Nicholas Erho^{*},

GenomeDx Biosciences, Vancouver, British Columbia, Canada

Kasra Yousefi^{*},

GenomeDx Biosciences, Vancouver, British Columbia, Canada

Stephanie Glavaris,

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Helen Fedor^{*},

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Misop Han^{*},

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Sheila F. Faraj^{*},

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Stephania M. Bezerra^{*},

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

George Netto^{*},

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Alan W. Partin,

James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Bruce J. Trock^{*},

^{*}Financial interest and/or other relationship with GenomeDx Biosciences.

[†]Correspondence: Tarry Building Room 16-703, 300 E. Superior, Chicago, Illinois 60611 (telephone: 312-908-8145; eschaeffer@jhmi.edu).

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James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, Maryland

Elai Davicioni, and

GenomeDx Biosciences, Vancouver, British Columbia, Canada

Edward M. Schaeffert[†]

Northwestern University Feinberg School of Medicine, Chicago, Illinois

Abstract

Purpose: Prostate cancer is clinically and molecularly heterogeneous. We determined the prognosis of men with ERG-ETS fusions and SPINK1 over expression.

Materials and Methods: Men were identified with intermediate or high risk localized prostate cancer treated with radical prostatectomy and no therapy before metastasis. A case-cohort design sampled a cohort (262) enriched with metastasis from the entire cohort and a cohort (213) enriched with metastasis from patients with biochemical recurrence. We analyzed transcriptomic profiles and subtyped tumors as m-ERG⁺, m-ETS⁺, m-SPINK1⁺ or Triple Negative (m-ERG⁻/m-ETS⁻/m-SPINK1⁻), and multivariable logistic regression analyses, Kaplan-Meier and multivariable Cox models were used to evaluate subtypes as predictors of clinical outcomes.

Results: Overall 36%, 13%, 11% and 40% of prostate cancer was classified as m-ERG⁺, m-ETS⁺, m-SPINK1⁺ and Triple Negative, respectively. Univariable analysis demonstrated that m-SPINK1⁺ tumors were more common in African-American men (OR 5, 95% CI 1.6–16) but less commonly associated with positive surgical margins (OR 0.16, 95% CI 0.03–0.69) compared to the m-ERG⁺ group. Compared to the Triple Negative group, m-SPINK1⁺ showed similar associations with race and surgical margins in univariable and multivariable analyses across the entire cohort. Survival analyses did not show significant differences among m-ERG⁺, m-ETS⁺ and Triple Negative cases. m-SPINK1⁺ independently predicted prostate cancer specific mortality after metastasis (HR 2.48, 95% CI 0.96–6.4) and biochemical recurrence (HR 3, 95% CI 1.1–8).

Conclusions: SPINK1 over expression is associated with prostate cancer specific mortality in at risk men with biochemical and clinical recurrence after prostatectomy. ERG-ETS alterations are not prognostic for outcome.

Keywords

prostatic neoplasms; genomics; prognosis; neoplasm metastasis; transcriptome

PROSTATE cancer is the most common noncutaneous malignancy in males, accounting for 220,800 incident cases in the United States in 2015.¹ Currently, disease diagnosis and treatment decision making rely heavily on Gleason score and surgical pathology.² More recently, genomic technologies have offered unprecedented insight into the molecular biology of PCa, defining its aggressiveness in terms of molecular signatures.^{3–6} These tools have demonstrated the ability to predict patients at risk for post-radiotherapy biochemical failure, post-prostatectomy metastasis and cancer specific mortality.^{7,8}

Transcriptome characterization has also enabled the subtyping of cases of PCa on the basis of mutually exclusive molecular events, namely ERG fusions, ETS fusions, SPINK1 over

expression or none of these (TripleNeg), which may aid in the assessment of tumor lineage and clinical outcomes.⁹⁻¹² However, to date, studies using these molecular events to evaluate clinical outcomes have generated conflicting results and have been limited by small sample sizes, short followup or confounding by multiple treatment modalities. As a result the influence of molecular subtypes on clinical outcome heterogeneity has been inadequately studied.

Therefore, we performed genome-wide transcriptome analysis using data generated while developing a clinically available prognostic assay (Decipher®) on a natural history cohort of men who underwent RP without further treatment until the time of metastasis. We also evaluated alternative computational tools for molecular subtyping, and determined clinicopathological and clinical outcomes associations. Finally, we evaluated whether molecular subtyping changes the predictive ability of the genomic assay.

MATERIALS AND METHODS

Patient Cohorts

A total of 358 patient expression profiles from 2 cohorts were analyzed from the Johns Hopkins Medical Institutions' prostate cancer biorepository for patients who underwent RP between 1992 and 2010. The first cohort (post-RP, 262) consists of men with localized NCCN intermediate or high risk disease, and at least 5 years of followup after RP, an undetectable PSA after surgery and no additional therapy before metastatic progression.¹³ Patients were excluded from study if they had nodal or metastatic disease at diagnosis, received neoadjuvant therapy, or received radiation or hormonal therapy before clinical evidence of metastasis. The second cohort (post-BCR, 213) included men who had an undetectable PSA after RP and subsequently experienced BCR, defined as a PSA of 0.2 ng/ml or greater with a subsequent confirmatory value. There were 117 patients in the post-BCR cohort who were also sampled in the post-RP cohort. Exclusion criteria for the post-BCR cohort were the same as for the post-RP cohort.

Sample Preparation

All radical prostatectomy cases used in the study were rereviewed and regraded following 2005 International Society of Urological Pathology criteria. For each case the sampled lesion was the specimen containing the highest pathological Gleason grade disease. Two 0.6 mm punch biopsies were used for RNA extraction from formalin fixed paraffin embedded tumor blocks. RNA extraction and quantification were performed using the Decipher assay pipeline. cDNA was amplified using the Ovation® WTA FFPE System, labeled and hybridized to Human Exon 1.0 ST microarrays (Affymetrix, Santa Clara, California). Processing of microarray data and quality control was conducted as previously described.³ All data were normalized using the SCAN algorithm.¹⁴ A more detailed description of the samples is provided in our previous work.¹³

Molecular Subtyping

Patients in this study were grouped in 4 subtypes using microarray classifiers that have been previously described.⁹ A supervised random forest model was trained to predict ERG

rearrangement (m-ERG) and benchmarked to fluorescence in situ hybridization (supplementary fig. 1, A; <http://jurology.com/>). Rearrangements in other genes such as ETV1, ETV4, ETV5 and FLI were predicted using the outlier analysis method implemented in the “extremevalues” R package (m-ETS classifier) (supplementary fig. 1, B; <http://jurology.com/>). Similarly, SPINK1 cases with outlier expression were detected using the outlier analysis method (m-SPINK classifier). Four groups were then defined based on the results of the 3 microarray based classifiers (see supplementary material).

Statistical Analysis

Statistical analyses were performed in R v3.0 and all statistical tests were 2-sided using a $p < 0.05$ significance level. Univariable and multivariable logistic regression analyses were performed to evaluate the associations between subtypes and clinical variables including race, age, preoperative PSA, SMS, EPE, SVI, LNI and GS. Prognosis of risk factors was assessed using multivariable Cox models using the Lin-Ying method for case-cohort design. Receiver operating characteristics were used to measure the predictive power of the subtypes and KM analysis was used to predict the prognostic impact of the subtypes.

RESULTS

Clinical Characteristics of the Study Cohort

Patient pathological and clinical characteristics across the molecular subtypes are summarized in table 1. Approximately 88% were of European-American ancestry and more than 98% of patients had Gleason 7 or greater disease on final pathology (supplementary table 1, <http://jurology.com/>). Based on PSA 31.3% and 9.8% of patients had intermediate and high risk disease, respectively, and 46.4% and 15.6% of patients had intermediate and high risk disease based on biopsy GS. Metastasis developed in 35% of the patients with a median followup of 4 years (IQR 2–7) and PCSM developed in 15% with a median followup of 6 years (IQR 5–9.5).

Molecular Subtypes of Patients

All patients underwent molecular subtyping using genome-wide expression profiling. The 4 molecular subtypes have a unique genomic fingerprint based on the expression of 105 genes associated with the subtypes (fig. 1, A). m-ETS⁺ and m-ERG⁺ were found to present a similar yet distinct molecular profile, unlike SPINK1⁺ and TripleNeg, which had a similar molecular profile. The androgen receptor activity score, as calculated in Kumar et al,¹⁵ was distinct in the TripleNeg subtype (fig. 1, B). However, the proliferation index measured by *mki67* expression was similar across subtypes (fig. 1, C).

The accuracy of the m-ERG model was evaluated using IHC data available for 248 patients. Of 102 IHC positive cases m-ERG correctly called 92 (data not shown). The ERG model had 90% sensitivity and 98% specificity. An additional 13% of cases were m-ETS⁺ (m-ETV1–8.4%, m-ETV4–2.8%, m-ETV5–1.7%, m-FLI1–0.1%) and 11% were m-SPINK1⁺ (fig. 2, A and supplementary fig. 1, B; <http://jurology.com/>). The m-SPINK1⁺ model achieved a sensitivity of 96% and a specificity of 66% when validated against IHC for these samples. These results are in line with previously reported percentages for m-ETS⁺ and m-

SPINK1⁺ in prostate tumors using fluorescence in situ hybridization or IHC. The remaining 40% were classified as TripleNeg.

We next determined the relative percentages of m-ERG⁺, m-ETS⁺, m-SPINK1⁺ and TripleNeg cases based on race and clinicopathological features. Of the Caucasian cases 38% were m-ERG⁺ and of the African-American cases 18% were m-ERG⁺ ($p=0.02$, fig. 2, B). African-American cases men were more likely to be m-SPINK1⁺ or TripleNeg (tables 2 and 3). In regard to pathological features, as Gleason score increased there were no statistically significant trends in the relative percentages of the subtypes (fig. 2, C). m-ETS⁺ was associated with SVI compared to TripleNeg cases (OR 2.4, 95% CI 1.17–5.05), and m-SPINK1⁺ cases were least likely to have a positive surgical margin (OR 0.16, 95% CI 0.03–0.68) compared to m-ERG⁺ (tables 2 and 3). Multinomial MVA showed that the 4 subtypes are clinically distinct (supplementary table 2, <http://jurology.com/>).

Clinical Associations of Molecular Subtypes

We next characterized the impact of molecular subtypes on prognosis through several analyses. We assessed the discriminative ability of the subtypes for outcome. ROC analysis shows that the 4 subtypes are poor predictors of clinical metastasis and PCSM rates in the post-RP and post-BCR settings (supplementary fig. 2, <http://jurology.com/>). In addition, the evaluation of KM curves for the aforementioned outcomes in the post-RP setting demonstrated that m-SPINK1⁺ had a slightly faster time to PCSM but not metastasis or BCR. All of these outcomes failed to demonstrate significant differences among the molecular subtypes (supplementary fig. 3, <http://jurology.com/>).

We then evaluated KM curves and performed multivariable Cox modeling on the subset of men in whom metastasis developed and their rate of PCSM. PCSM tends to develop faster in m-SPINK1⁺ cases than in other subtypes ($p=0.075$; fig. 3, A). This significance was more pronounced when limiting the analysis to a comparison of m-SPINK1⁺ to TripleNeg ($p=0.025$; fig. 3, D). Using Cox MVA m-SPINK1⁺ had a hazard ratio of 2.48 for independent prognostic information on PCSM after metastasis without achieving statistical significance (HR 2.48, $p=0.06$) when adjusting for clinical variables and other subtypes (fig. 3, G). m-SPINK1⁺ remained the only prognostic biomarker of PCSM after adjusting for time to metastasis (HR 2.6, $p=0.04$).

Evaluating patients in the post-BCR setting, those in the m-SPINK1⁺ group seemed to have an increased incidence of progression to metastatic disease and ultimately PCSM, although these incidence curves failed to show any statistical significance (fig. 3, B and C). However, Cox MVA showed that m-SPINK1⁺ is an independent prognostic marker of metastasis (HR 2.7, $p=0.002$) and PCSM (HR 3, $p=0.03$; fig. 3, H and I). In addition, m-SPINK1⁺ cases were shown to be at higher risk for metastasis ($p=0.04$) and PCSM ($p=0.02$) after BCR compared to TripleNeg (fig. 3, E and F).

Performance of Multigene PCa Prognostic Classifiers across Subtypes

Incorporating molecular subtyping into available prognostic assays ensures the robustness of prognostic biomarkers across subtypes. To address this, we evaluated Decipher,³ Penney⁵ and microarray derived cell cycle progression⁶ multigene signatures to determine if the

molecular subtypes have an impact on the prognostic power of each prostate cancer classifier. The Decipher scores demonstrated similar discrimination for metastasis in all 4 subtypes, suggesting its robustness against molecular heterogeneity (fig. 4). Unlike Decipher, microarray derived cell cycle progression failed to discriminate metastatic cases in m-ERG and TripleNeg subtypes. On the other hand, Penney scores are discriminative of metastasis in all 4 subtypes, but the distribution of the scores is not uniform as the median of scores in m-ERG⁺ is twice the median in TripleNeg (supplementary fig. 4, <http://jurology.com/>).

Delineating the Biology of SPINK1

We further explored the biology underlying SPINK1 over expression, finding the most correlated genes with SPINK1 in the current data and in a set of 2,293 prospective RP samples from patients from the Decipher test. A total of 167 genes were in common among the top 500 correlated genes in each data set. Among them 70 genes were associated with each other either functionally or physically based on the STRING online tool (fig. 5). The top correlated genes were complement component 5 (C5) that is part of the innate immune system that has an important role in inflammation and host homeostasis, and acyl-CoA synthetase long-chain family member 5 (ACSL5). When characterizing the biology underlying the 167 genes, the genes were mainly enriched with metabolic pathways related to retinoid metabolism, drug metabolism, cellular carbohydrate metabolism, FAS signaling, and regulation of cell aging and WNT signaling (supplementary table 3, <http://jurology.com/>).

DISCUSSION

High throughput characterization of the PCa genome and transcriptome has led to the identification of molecular subtypes defined by mutually exclusive genetic/transcriptomic events.¹⁶ For example, approximately 50% of PCa foci from PSA screened Caucasian cohorts harbor rearrangements between the 5' untranslated region of the androgen responsive gene *TMPRSS2* and members of the ETS transcription factor family (most commonly *ERG* and more rarely *ETV1*, *ETV4*, *ETV5* or *FLII*).¹¹ Similarly, approximately 10% of PCa cases, which are nearly exclusively ETS fusion negative (ETS⁻), harbor marked *SPINK1* over expression, consistent with a unique molecular subtype (SPINK1⁺).¹⁰ Although well validated assays have been developed for research and clinical applications, comprehensive subtyping using these assays remains challenging.

The current literature reveals conflicting results about the relationship between SPINK1 and PCa prognosis. Early reports showed that SPINK1 over expression mediates progression and cancer invasion via epidermal growth factor receptor, making it a potential therapeutic target.¹⁷ However, Flavin et al used a tissue microarray from the Physicians' Health Study and Health Professionals Follow-Up Study to show SPINK1 protein expression did not predict lethal PCa after RP.¹⁸ These conclusions were limited by pre-PSA era tissues and were potentially confounded by adjuvant treatments. Indeed, studies linking molecular subtypes with prognosis may be confounded by cohort differences (eg PSA screened vs

unscreened, biopsy vs transurethral resection, and treatment modality) as well as detection methodologies.¹⁹

In our analysis of 358 NCCN intermediate and high risk patients who underwent RP without adjuvant treatment until metastasis, we demonstrate a positive correlation between SPINK1 over expression and the development of metastasis after BCR. In previous work m-SPINK1⁺ and TripleNeg were molecularly similar in a cohort of patients (1,577) who underwent different treatment.⁹ In this study we demonstrate a positive correlation between SPINK1 expression and outcomes when compared to TripleNeg (supplementary fig. 3, <http://urology.com/>). This represents the largest study to our knowledge to date that correlates molecular subtypes of prostate cancer with clinical outcomes in a treatment-free cohort free of the typical selection or treatment biases that plague similar studies.

Unlike SPINK1, we did not identify a correlation between PCSM and over expression of ERG. It is likely that ERG⁺ status has been consistently associated with high pathological stage at prostatectomy. In a recent study Berg et al reported that ERG⁺ status was a strong predictor of progression on active surveillance.²⁰ Thus, as ERG⁺ tumors may grow faster or be more locally aggressive than ERG⁻ tumors, assessment of ERG status or other molecular subtypes may be relevant in patients considering active surveillance. In addition, ERG⁺ was shown to have a role beyond regulating gene expression and functions outside the nucleus to cooperate with tubulin toward taxane insensitivity in castration resistant prostate cancer.²¹ Determining ERG rearrangement status may aid in patient selection for docetaxel or cabazitaxel therapy and/or influence co-targeting approaches.

The ETS transcription factor family also failed to show an association with PCSM. Palanisamy et al recently profiled biopsy specimens of patients from the REDEEM study who were ERG⁻ and SPINK1⁻ for ETS rearrangements.²² They concluded that there was no evidence that ETS rearrangements caused disease progression. Our cohort supported these findings and, interestingly, we found that ETS⁺ cases were at increased risk for SVI but not PCSM. The recent study by Tomlins et al using the same molecular subtyping methodology conducted on a pooled set of patients (1,577) from multiple institutions showed similar clinical associations between subtypes and clinical variables, including ETS and SVI, SPINK and African-American men, SPINK and surgical margins.⁹ However, they failed to find a link between these subtypes and outcome, possibly due to the variety of treatments after RP.

Despite the lack of evidence demonstrating SPINK1 as a prognostic indicator for all patients undergoing prostatectomy, the ability to evaluate cancer biology on a transcriptomic level is hypothesis generating, will lead to further refinement of markers for molecular subtyping and will direct future research efforts. Since these subtypes are run as part of the Decipher test, determining the true prognosis of SPINK1 in a large cohort (more than 10,000 patients from the Decipher test) will be feasible in the near future.

This study includes the inherent limitations of a retrospective study. While the median followup was 4 years, data from Pound et al suggest that this may not capture all of the metastatic events.²³ Longer followup may better differentiate patient subtypes and

outcomes. Further prospective clinical trials and biomolecular studies will be essential in confirming our findings.

Incorporating molecular subtyping into a clinically available prognostic assay has several areas of potential near term clinical use. The uptake of subtyping in prognostic assays will make the expression profile and molecular subtypes available for thousands of patients in the near future to further study the association with outcome and systematic therapy. Likewise, it may be useful in evaluating multifocality/clonality indistinguishable by routine histology. Lastly, molecular subtyping will allow for enrichment for clinical trials.

CONCLUSIONS

We evaluated the use of data generated from a clinically available prognostic assay for determining ERG, ETS and SPINK genomic aberrations. Although clinical associations between subtypes and outcomes have been adequately described, little is known about these associations in treatment-free cohorts. In a retrospective case-cohort study of NCCN intermediate and high risk patients who did not undergo adjuvant treatment after RP until the time of metastasis, SPINK1 expression positively correlates with PCSM after BCR and with metastatic progression. Further molecular subtyping of PCa and disease stratification will be important to better identify subsets of post-RP patients who will benefit from adjuvant therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations and Acronyms

BCR	biochemical recurrence
EPE	extraprostatic extension
GS	Gleason score
IHC	immunohistochemistry
KM	Kaplan-Meier
LNI	lymph node involvement
MVA	multivariable analysis
NCCN®	National Comprehensive Cancer Network®
PCa	prostate cancer
PCSM	prostate cancer specific mortality
PSA	prostate specific antigen
RP	radical prostatectomy

SMS	surgical margin status
SVI	seminal vesicle invasion
TripleNeg	triple negative

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Figure 1.

Molecular subtype signatures in natural history RP cohort. *A*, 4 molecular subtypes have unique genomic fingerprint based on expression of 105 genes associated with subtypes. ERG+ and ETS+ are genomically more similar than TripleNeg. *Met*, metastasis. *B* and *C*, proliferation index and androgen receptor (*AR*) activity of subtypes are similar.

Figure 2.
Molecular subtypes (A) in spectrum of race (B) and pathological GS (C)

Figure 3.

Prognostic impact of SPINK1 after clinical and biochemical recurrence. *A-C*, KM plots showing SPINK1 is at relatively higher risk for PCSM after metastasis and after BCR compared to other subtypes. *D-F*, SPINK1 prognosis with respect to TripleNeg. SPINK1 is at higher risk for metastasis and PCSM compared to TripleNeg. *G-I*, Cox MVA showing that SPINK1 is independent prognostic variable of metastasis and PCSM after adjusting for clinical variables.

Figure 4.

Performance of Decipher PCa classifier across molecular subtypes. Decipher scores of whole cohort (358) were plotted across 4 subtypes. Color of points indicates metastatic outcome and bars indicate median of Decipher score. AUC for Decipher scores prediction of metastasis in each subtype is given. Based on AUCs Decipher is significant prognostic classifier across subtypes.

Figure 5.
Functional interaction network of genes correlated with SPINK1. Based on STRING online tool, SPINK1 correlated genes are highly interconnected with SRC and AHR as hub genes.

Table 1.

Demographic and clinical characteristics of patients used across molecular subtypes

	m-ERG ⁺	m-ETS ⁺	m-SPINK1 ⁺	TripleNeg	Overall
No. race (%):					
European-American	120 (93.02)	43 (95.56)	28 (73.68)	121 (84.03)	314 (87.71)
African-American	6 (4.65)	2 (4.44)	7 (18.42)	19 (13.19)	34 (9.5)
Other	2 (1.55)	0 (0)	3 (7.89)	1 (0.69)	6 (1.68)
Unknown	1 (0.8)	0 (0)	0 (0)	3 (2)	4 (1)
Median pt age (range)	60 (38, 72)	60 (52, 69)	60 (46, 71)	59 (40, 71)	59 (38, 72)
No. ng/ml preop PSA (%):					
Less than 10	80 (62.02)	26 (57.78)	22 (57.89)	83 (57.64)	211 (58.94)
10–Less than 20	38 (29.46)	17 (37.78)	12 (31.58)	43 (29.86)	112 (31.28)
Greater than 20	11 (8.53)	2 (4.44)	4 (10.53)	18 (12.5)	35 (9.78)
No. biopsy GS (%):					
6 or Less	51 (39.53)	17 (37.78)	17 (44.74)	51 (35.42)	136 (37.99)
7	60 (46.51)	20 (44.44)	15 (39.47)	69 (47.92)	166 (46.37)
8	14 (10.85)	4 (8.89)	2 (5.26)	20 (13.89)	40 (11.17)
9 or Greater	4 (3.1)	4 (8.89)	4 (10.53)	4 (2.78)	16 (4.47)
No. pathological GS (%):					
6 or Less	2 (1.55)	1 (2.22)	0 (0)	4 (2.78)	7 (1.96)
7	81 (62.79)	30 (66.67)	26 (68.42)	80 (55.56)	218 (60.89)
8	16 (12.4)	3 (6.67)	3 (7.89)	13 (9.03)	35 (9.78)
9 or Greater	30 (23.26)	11 (24.44)	9 (23.68)	47 (32.64)	98 (27.37)
No. pos EPE (%)	93 (72.09)	33 (73.33)	22 (57.89)	91 (63.19)	241 (67.32)
No. pos SVI (%)	30 (23.26)	17 (37.78)	7 (18.42)	30 (20.83)	86 (24.02)
No. pos SMS (%)	35 (27.13)	13 (28.89)	2 (5.26)	52 (36.11)	102 (28.49)
No. pos LNI (%)	28 (21.71)	11 (24.44)	5 (13.16)	18 (12.5)	63 (17.6)
No. metastasis pos (%)	51 (39.53)	15 (33.33)	14 (36.84)	46 (31.94)	128 (35.75)
No. BCR pos (%)	84 (65.12)	32 (71.11)	22 (57.89)	88 (61.11)	228 (63.69)
No. PCSM pos (%)	22 (17.05)	7 (15.56)	8 (21.05)	19 (13.19)	57 (15.92)

For all variables, distribution across the 4 subtypes for all profiles' samples (358).

Univariable logistic regression analysis between clinicopathological variables and molecular subtypes across the whole cohort using reference TripleNeg

Table 2.

	m-ERG ⁺		m-ETS ⁺		m-SPINK1 ⁺	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Reference TripleNeg						
Preop PSA	0.817 (0.499–1.337)	0.421	1.003 (0.508–1.98)	0.993	0.915 (0.43–1.947)	0.818
Race (Ref European-American)	0.318 (0.123–0.825)	0.018	0.296 (0.066–1.325)	0.111	1.592 (0.61–4.154)	0.342
EPE	1.49 (0.886–2.506)	0.133	1.576 (0.748–3.319)	0.232	0.86 (0.402–1.836)	0.696
SVI	1.25 (0.698–2.239)	0.453	2.429 (1.169–5.046)	0.017	1 (0.396–2.524)	1
Pathological GS less than 7 (Ref 7)	0.494 (0.088–2.774)	0.423	0.642 (0.069–5.977)	0.697	0 (0-Inf)	0.99
Pathological GS greater than 7 (Ref 7)	0.77 (0.468–1.266)	0.303	0.609 (0.297–1.25)	0.177	0.598 (0.271–1.318)	0.202
SMS	0.671 (0.399–1.129)	0.133	0.709 (0.341–1.472)	0.356	0.106 (0.024–0.459)	0.003
Age	1.004 (0.966–1.045)	0.826	1.027 (0.969–1.089)	0.366	1.049 (0.983–1.12)	0.146
LNI	1.848 (0.962–3.55)	0.065	2.193 (0.946–5.084)	0.067	0.875 (0.276–2.77)	0.82

Univariable logistic regression analysis between clinicopathological variables and molecular subtypes across the whole cohort using reference ERG⁺

Table 3.

	m-ETS ⁺		m-SPINK1 ⁺		TripleNeg	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Reference ERG ⁺						
Preop PSA	1.228 (0.614–2.456)	0.561	1.121 (0.521–2.412)	0.771	1.224 (0.748–2.004)	0.421
Race (Ref European-American)	0.93 (0.181–4.785)	0.931	5 (1.559–16.037)	0.007	3.14 (1.212–8.136)	0.018
EPE	1.058 (0.491–2.278)	0.886	0.577 (0.264–1.259)	0.167	0.671 (0.399–1.129)	0.133
SVI	1.943 (0.937–4.027)	0.074	0.8 (0.317–2.016)	0.636	0.8 (0.447–1.433)	0.453
Pathological GS less than 7 (Ref 7)	1.3 (0.114–14.873)	0.833	0 (0-Inf)	0.989	2.026 (0.36–11.386)	0.423
Pathological GS greater than 7 (Ref 7)	0.791 (0.381–1.645)	0.531	0.777 (0.349–1.732)	0.537	1.299 (0.79–2.137)	0.303
SMS	1.056 (0.497–2.244)	0.887	0.158 (0.036–0.692)	0.014	1.49 (0.886–2.506)	0.133
Age	1.021 (0.964–1.082)	0.477	1.042 (0.977–1.111)	0.208	0.996 (0.957–1.035)	0.826
LNI	1.186 (0.532–2.646)	0.676	0.473 (0.154–1.457)	0.192	0.541 (0.282–1.039)	0.065