8-18-2022

Loss of KLK4::KLKP1 pseudogene expression by RNA chromogenic in-situ hybridization is associated with PTEN loss and increased risk of biochemical recurrence in a cohort of middle eastern men with prostate cancer

Andrea Bakker

Jonathan C. Slack

Nallasivam Palanisamy
Henry Ford Health, NPALANI1@hfhs.org

Shannon Carskadon
Henry Ford Health, scarska1@hfhs.org

Sunita Ghosh

See next page for additional authors

Recommended Citation
Bakker A, Slack JC, Palanisamy N, Carskadon S, Ghosh S, Khalifeh I, and Bismar TA. Loss of KLK4::KLKP1 pseudogene expression by RNA chromogenic in-situ hybridization is associated with PTEN loss and increased risk of biochemical recurrence in a cohort of middle eastern men with prostate cancer. J Cancer Res Clin Oncol 2022; Epub ahead of print. PMID: 35982181. Full Text

Authors

Andrea Bakker, Jonathan C. Slack, Nallasivam Palanisamy, Shannon Carskadon, Sunita Ghosh, Ibrahim Khalifeh, and Tarek A. Bismar

Journal of Cancer Research and Clinical Oncology
https://doi.org/10.1007/s00432-022-04279-5

RESEARCH

Loss of *KLK4*::*KLKP1* pseudogene expression by RNA chromogenic in-situ hybridization is associated with *PTEN* loss and increased risk of biochemical recurrence in a cohort of middle eastern men with prostate cancer

Andrea Bakker[1] · Jonathan C. Slack[1] · Nalla Palanisamy[2] · Shannon Carskadon[2] · Sunita Ghosh[3,4] · Ibrahim Khalifeh[5] · Tarek A. Bismar[1,6,7]

Received: 7 July 2022 / Accepted: 10 August 2022

Abstract

Background *KLK4::KLKP1* fusion is a recently described pseudogene that is enriched in prostate cancer (PCa). This new biomarker has not been characterized in the Middle Eastern population.

Objective To establish the incidence and prognostic value of *KLK4::KLKP1* fusion in a cohort of Middle Eastern men with PCa and explore the relationship of this marker to other relevant biomarkers (PTEN, ERG, SPINK1).

Design, setting, and participants We interrogated a cohort of 340 Middle Eastern men with localized PCa treated by radical prostatectomy between 2005 and 2015. *KLK4::KLKP1* fusion status was assessed by RNA Chromogenic in situ hybridization (CISH) and correlated to pathological and clinical parameters.

Outcome measurements and statistical analysis RNA-CISH expression of *KLK4::KLKP1* was correlated with prognostic factors, ERG, PTEN, and SPINK1 expression, and biochemical recurrence (BCR) following prostatectomy.

Results and limitations 51.7% of patient samples showed positive *KLK4::KLKP1* expression; more commonly in cores of PCa (38%) versus non-cancer (20.6%) ($p < 0.0001$) and in lower Gleason Grade Group tumors (1–3) vs (4–5). *KLK4::KLKP1* expression positively correlated with ERG positivity and inversely associated with PTEN loss. No significant association was found with SPINK1 expression, seminal vesicle invasion, positive surgical margin, pathological stage, or patient age (< 50 or ≥ 50). The association between PTEN loss and BCR increased when combined with *KLK4::KLKP1* negativity (HR 2.31, CI 1.03–5.20, $p = 0.042$).

Conclusions *KLK4::KLKP1* expression is more common in this cohort of Middle Eastern men than has been reported in North American men. It is associated with ERG positivity and inversely correlated with PTEN loss. In isolation, *KLK4::KLKP1* expression was not significantly associated with clinical outcome or pathological parameters. However, its expression is associated with certain molecular subtypes (*ERG*-positive, *PTEN*-intact) and as we demonstrate may help further stratify the risk of recurrence within these groups.

Keywords *KLK4::KLKP1* · Middle eastern · Prostate cancer · Biomarker · Prognosis

✉ Tarek A. Bismar
tabismar@ucalgary.ca

[1] Department of Pathology and Laboratory Medicine, Alberta Precision Laboratories, University of Calgary Cumming School of Medicine, Rockyview General Hospital, Calgary, AB T2V 1P9, Canada

[2] Department of Urology, Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI, USA

[3] Department of Medical Oncology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

[4] Departments of Mathematical and Statistical Sciences, University of Alberta, Edmonton, AB, Canada

[5] Department of Pathology and Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

[6] Departments of Oncology, Biochemistry, and Molecular Biology, Calgary, AB, Canada

[7] Arnie Charbonneau Cancer Institute and Tom Baker Cancer Center, Calgary, AB, Canada

Published online: 18 August 2022

Introduction

Prostate cancer (PCa) remains one of the commonest cancers worldwide and, excluding skin cancers, is the most common cancer diagnosed in North American (NA) men (Siegel et al. 2021). In the Middle East, the incidence is much lower and may be due to a variety of factors, including lifestyle, diet, obesity, androgen levels, socioeconomic status, and genetics (Al-Abdin and Al-Beeshi 2018). PCa is also known to be a heterogeneous disease, which makes the characterization and clinical implementation of biomarkers imperative to help guide treatment. As such, evaluation of molecular profiles that reflect molecular subtypes of PCa in different ethnicities is needed (Kaffenberger and Barbieri 2016).

Alteration in ETS-related gene (*ERG*) and phosphatase and tensin homolog (*PTEN*) are among the most common genomic alterations in prostate cancer. Rearrangements between the androgen-regulated gene *ERG* and fusion partner *TMPRSS2* are identified in approximately half of PCa's in North America (Tomlins et al. 2008; Taylor et al. 2010) and often co-exist with *PTEN* deletions (Taylor et al. 2010; Bismar et al. 2018). Although *ERG* rearrangements have been associated with poor prognosis in some studies (Kaffenberger and Barbieri 2016), others have noted an association with better outcomes in the setting of androgen deprivation therapy (Bismar et al. 2012). These seemingly conflicting relationships between molecular subtype and outcome likely reflect differences between specific patient populations and cohorts being investigated, as well as clinical endpoints assessed (Tomlins et al. 2008; Abou-Ouf et al. 2016). For example, *ERG* rearrangements were of lower incidence in a mixed Jordanian-Arab cohort with transurethral and peripheral tumor localization (33.2%) (Aldaoud et al. 2017) as well as in a study of a broader Middle Eastern (ME) cohort with tumors that manifested clinically (42.7%) (Abdelsalam et al. 2020).

PTEN deletions, as assessed by a surrogate of reduced immunohistochemistry (IHC) expression, have been associated with worse clinical outcomes in PCa (Bismar et al. 2018; Guedes et al. 2017). In some studies, this adverse clinical outcome appears to be more notable among men with ERG-negative tumors compared to those with ERG-positive tumors (Bismar et al. 2018; Ahearn et al. 2015).

Serine Protease Inhibitor, Kazal Type 1 (*SPINK1*), a trypsin inhibitor, has been documented to be overexpressed in a subset of ERG-negative tumors (Tomlins et al. 2008). In a study by Flavin et. al, SPINK1 expression by IHC was detected in about 11% of ERG-negative samples and 4% of ERG-positive cases. They found no association between SPINK1 expression by IHC and Gleason grade grouping, tumor stage, biochemical recurrence (BCR), or PCa-specific mortality (Flavin et al. 2014). This contrasts with other studies documenting an association between SPINK1 expression and BCR (Terry et al. 2015).

Recently, we have started investigating molecular differences between ME and North American (NA) cohorts, in order to identify and characterize any molecular differences that could play a significant role in explaining the difference in incidence and PCa progression between these two groups and ultimately provide prognostic or therapeutic data.

Previously, we reported that *ERG*, *PTEN*, and *SPINK1* genomic alterations occur less frequently in Middle Eastern men and that the association between of ERG positivity and PTEN loss noted in North American men was not observed in this population (Abdelsalam et al. 2020). As such, ethnically relevant molecular classification schema, and ultimately subsequent biomarker implementation, are vitally needed—what is of clinical relevance to one ethnic background may be of limited utility to another.

However, unlike other common cancers, such as has been seen with lung or breast cancer, there has been limited clinical implementation of molecular biomarkers. Although PTEN loss has recently been proposed as a potentially useful biomarker by the International Society of Urological Pathology (ISUP), systematic use of prognostic biomarkers in prostate cancer is currently not recommended by urological societies (Lotan et al. 2020), and the search for effective clinical biomarkers continues.

Recently a new fusion has been identified that is enriched in PCa. This involves the fusion of the androgen-regulated gene *KLK4* (Kallikrein Related Peptidase 4) and the adjacent pseudogene *KLKP1* (Kallikrein Pseudogene 1). Both *KLK4* and *KLKP1* belong to the kallikrein family of serine proteases, and their genes are located adjacent to each other in a cluster of 15 genes on chromosome 19 (q13.33–q13.4), also containing the well-known *KLK3* (Prostate-Specific Antigen) (Clements et al. 2001). The resulting chimeric sequence fuses the first two exons of *KLK4* with the last two exons of *KLKP1* and retains an open reading frame, incorporating 54 amino acids encoded by the *KLKP1* pseudogene in the putative chimeric protein (Kalyana-Sundaram et al. 2012). The initial study documented this fusion to be highly expressed in 30–50% of prostate cancer tissues (Kalyana-Sundaram et al. 2012). In contrast, the fusion, if present at all, was only expressed in very low relative levels in benign prostate tissue controls (Kalyana-Sundaram et al. 2012). Interestingly, this readthrough was recently described in the PCa cell line LNCaP as a cis sense-antisense chimeric transcript (Lai et al. 2010). A group in Sweden has identified another androgen-regulated transcript from the same region (KLK4T2), which appears to be a splice variant of *KLK4* with exons of *KLKP1*. In their study, they observed decreasing expression of both

KLK4 and KLK4T2 from benign prostate to primary tumor, to bone metastases, respectively (Lundwall et al. 2021).

Utilizing cell culture and a chicken chorioallantoic membrane (CAM) assay, expression of the *KLK4::KLKP1* fusion transcript was shown to affect cell proliferation, cell invasion, tumor formation, and lymphovascular spread (Chakravarthi et al. 2019). *KLK4::KLKP1* expression was studied via RNA in situ hybridization in a cohort of radical prostatectomy specimens from a racially diverse cohort that included 38% African Americans, which noted positivity in 32% of PCa samples vs. 17% in benign prostate tissue; with no association between KLK expression and Gleason Grade Groups or race (African American vs Caucasian) (Chakravarthi et al. 2019). However, there was an increased expression in the younger age group (< 50) as well as an association between *KLK4::KLKP1* expression with positive ERG expression ($p < 0.001$) and lack of PTEN loss ($p = 0.032$) by IHC. No association was noticed with SPINK1 expression by IHC or ETV1, ETV4, or ETV5 by dual RNA-CISH (Chakravarthi et al. 2019).

Since the study was reported in 2019, there has yet to be further characterization of this fusion in terms of its expression in other ethnicities. This is the first paper to our knowledge to further explore this recurrent gene fusion in PCa within the ME population.

Methods

Tissue microarray construction

Study population and tissue microarray construction

The study cohort consisted of Middle Eastern men diagnosed with localized PCa ($n = 340$). The cohort samples were collected between 2005 and 2015, with a median follow-up of 6 years. The study was approved by the University of Calgary, Cumming School of Medicine Ethics Review Board. The cohort's samples were assembled on five tissue microarrays (TMAs) with an average of two to five cores per patient, including PCa, and adjacent benign tissue when available, using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD, USA).

KLK4::KLKP1 RNA-CISH and IHC

RNA Chromogen in situ hybridization (CISH) was performed as described previously using RNAscope2.5 HD Reagent Kit (ACDBio, catalog #322,350) according to the manufacturer's instructions. In brief, after baking, deparaffinization, and target retrieval per manufacturer's instructions, TMA slides were incubated with target probes for *KLK4::KLKP1* for 2 h at 40 °C in a humidity chamber. After detection and color development, slides were washed twice in deionized water and then counterstained in hematoxylin (AgilentDAKO, catalog #K800821-2) for 5 min. Slides were washed several times in tap water, then dried, dipped in xylene, and mounted in EcoMount (Fisher, catalog #50-828-32). Next, the slides were scanned using a digital imaging system (Aperio Scanner, Leica). The images were reviewed, and the RNA-CISH signal on the TMAs was scored. Distinct punctate cytoplasmic dots were regarded as positive *KLK4::KLKP1*(Fig. 1A–E) (Chakravarthi et al. 2019). Of the original cohort ($n = 340$), 331 patients had analyzable results for *KLK4::KLKP1* expression.

PTEN and ERG protein expression were assessed using an ERG-PTEN dual-color IHC staining protocol and SPINK1 as single-color IHC as described by Huang et al. (2016). Benign prostatic glands and stromal tissue acted as internal positive controls (Bismar et al. 2018). PTEN IHC expression was assessed using a four-tiered system (0, negative; 1, weak; 2, moderate; and 3, high expression). SPINK1 and ERG IHC were assessed as a two-tiered system (0, negative; 1, positive) (Fig. 1F–H).

Pathological analysis

Histological diagnoses of individual TMA cores were confirmed by one study pathologist (*T.A.B.*) on the initial slides. Gleason score grouping was assessed according to the 2014 World Health Organization/ International Society of Urological Pathology Grade Groups (GGs). In each patient, the two predominant patterns of PCa were sampled and included on the TMAs for analysis.

2.4. Statistical analysis SPSS version 25 was used to conduct all statistical analyses (IBM SPSS Statistics for Windows, version 25.0, released 2017; IBM Corp., Armonk, NY, USA). Frequency and proportions were reported for categorical data. The chi-square test was used to compare two categorical variables and Fisher's exact test was used where the cell frequencies were < 5. A p value of < 0.05 was used for statistical significance, and two-sided tests were utilized.

Results

KLK4::KLKP1 RNA-CISH expression in PCa in ME men and relation to Gleason score grouping, pathological parameters, and other known biomarkers

Overall, *KLK4::KLKP1* RNA-CISH positivity was noted in 171/331 (51.7%) patients. *KLK4::KLKP1* staining was observed in both cancer and adjacent benign tissue but was noted to be more common in PCa, seen in 38% of

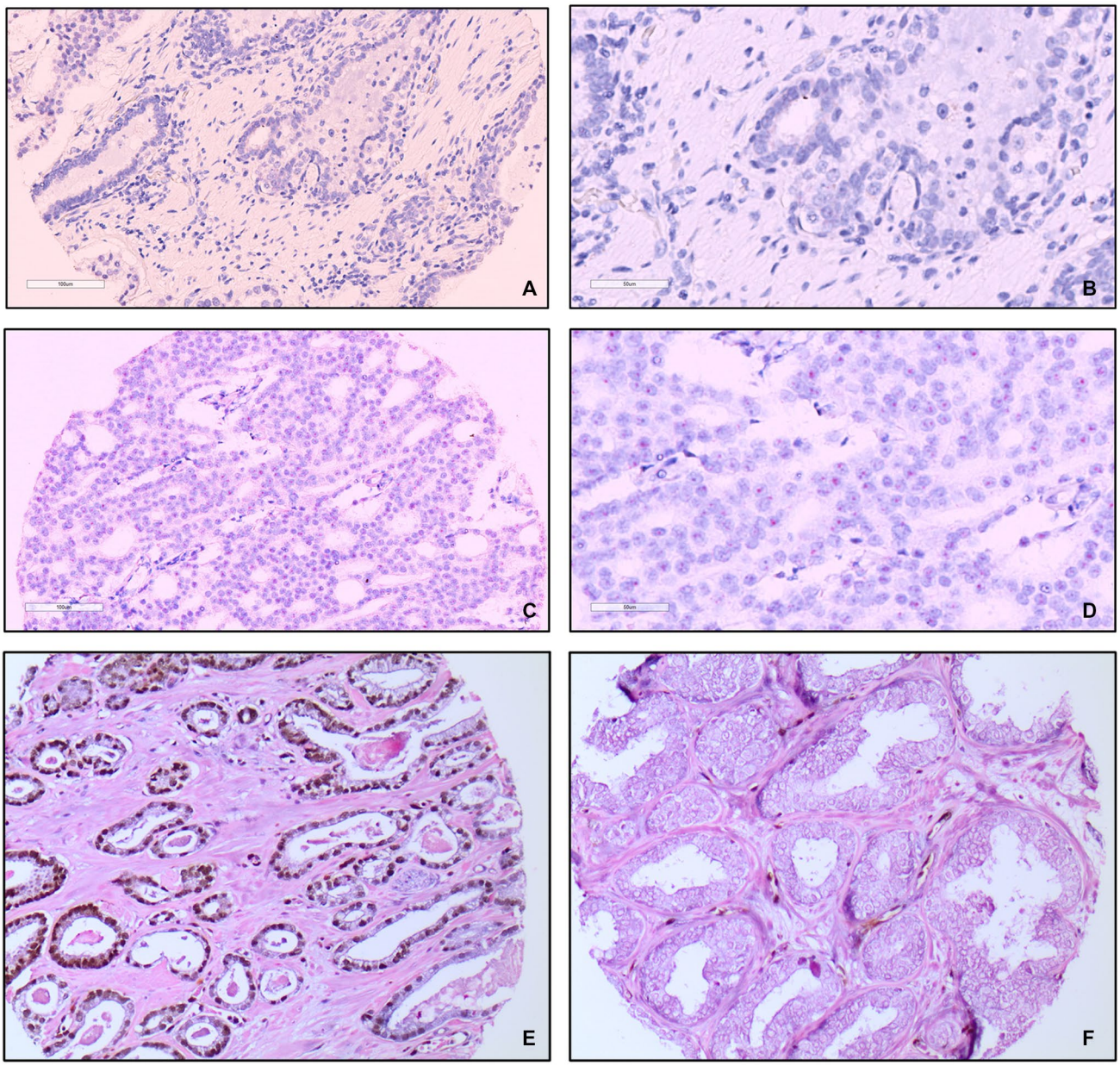

Fig. 1 *KLK4::KLKP1* RNA-CISH expression: negative signal in GG1 PCa (**A** 10x, **B** 20x). Positive signals in GG2 PCa (**C** 10x, **D** 20x). PTEN IHC (purple) and ERG IHC (brown) expression in GG1 PCa: PTEN pos, ERG pos (**E**), PTEN pos, ERG neg (**F**) (Notice the endothelial cells acting as internal positive controls for ERG)

cancer samples versus 20.6% of non-cancer prostate samples ($p < 0.0001$) (Fig. 2).

Positive *KLK4::KLKP1* RNA-CISH signal occurred more frequently in patients with moderate/high PTEN staining, compared to negative/weak PTEN staining (49/77; 63.6% vs. 122/250; 48.8% of cases ($p = 0.023$)). *KLK4::KLKP1* RNA positivity was higher in patients with ERG positivity (86/132; 65.2% of cases compared to 83/189; 43.9% of ERG negative ($p < 0.0001$)). No significant difference was noted between *KLK4::KLKP1* expression and SPINK1 expression. In this cohort, *KLK4::KLKP1* positivity occurred in 73/134 (54.5%) of SPINK1 negative patients vs 17/35 (48.6%) SPINK1 positive ($p = 0.53$).

Next, we analyzed *KLK4::KLKP1* RNA-CISH expression in association with other biomarkers on a core-by-core basis. In this cohort, any PTEN positivity (score 1, 2, or 3) was associated with a higher incidence of *KLK4::KLKP1*

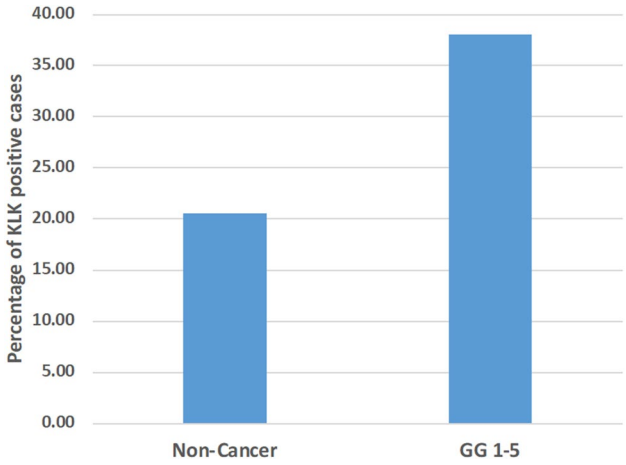

Fig. 2 Incidence of *KLK4::KLKP1* RNA-CISH expression in prostate tissue of ME men. Non-cancer 20.6% and prostatic adenocarcinoma 38%

RNA-CISH positivity (80.4% vs 74.7%), and ERG positivity was associated with a higher incidence of *KLK4::KLKP1* RNA-CISH positivity (34.5% vs 15.8%) ($p = 0.035$ and $p < 0.0001$, respectively) (Table 1).

Investigating *KLK4::KLKP1* RNA-CISH positivity in association with other pathological parameters, there was no association between *KLK4::KLKP1* RNA-CISH signal and Gleason Score ($p = 0.581$), seminal vesicle invasion ($p = 0.775$), positive surgical margin ($p = 0.112$), or pathological stage ($p = 0.812$). Additionally, there was no association between *KLK4::KLKP1* RNA-CISH positivity and patient age < 50 vs ≥ 50 ($p = 0.286$) (Table 2).

In summary, in this cohort of ME men, *KLK4::KLKP1* RNA-CISH expression was inversely associated with PTEN loss and positively associated with ERG expression. There was no association with SPINK1 expression.

Table 1 *KLK4::KLKP1* status in association with other biomarkers (PTEN, ERG and SPINK1) based on core-by-core status

Variables	*KLK4::KLKP1* negative	*KLK4::KLKP1* positive	*p* value
PTEN Score 0	194 (25.3%)	73 (19.6%)	0.035
PTEN Score 1, 2 or 3	574 (74.7%)	299 (80.4%)	
PTEN Score 0 or 1	463 (60.3%)	179 (48.1%)	< 0.0001
PTEN Score 2 or 3	305 (39.7%)	193 (51.9%)	
ERG Score 0	631 (84.2%)	239 (65.5%)	< 0.0001
ERG Score 1	118 (15.8%)	126 (34.5%)	
SPINK1 Score 0	382 (91.4%)	176 (92.1%)	0.407
SPINK1 Score 1	36 (8.6%)	15 (7.8%)	

PTEN; 0 negative, 1 weak, 2 moderate, 3 high intensity

ERG, SPINK1; 0 negative, 1 positive intensity

Table 2 *KLK4::KLKP1* status in association with pathological and clinical parameters

Parameter	Score	*KLK4::KLKP1* RNA-CISH negative n (%)	*KLK4::KLKP1* RNA-CISH positive n (%)	*p* value
Seminal vesicle invasion	Absent	140 (89.2%)	149 (88.2%)	0.775
	Present	17 (10.8%)	20 (11.8%)	
Positive surgical margin	Absent	91 (58.0%)	83 (49.4%)	0.112
	Present	66 (42.0%)	85 (50.6%)	
Pathological stage	Stage 2	114 (72.6%)	120 (71.4%)	0.812
	Stage 3	43 (27.4%)	48 (28.6%)	
Gleason Score	6	56 (35.9%)	54 (32.0%)	0.581
	3 + 4	54 (34.6%)	54 (32.0%)	
	4 + 3	27 (17.3%)	41 (24.3%)	
	8	14 (9.0%)	12 (7.1%)	
	9	5 (3.2%)	8 (4.7%)	
Age (individual data)	Age ≥ 50	155 (98.7%)	163 (96.4%)	0.286
	Age < 50	2 (1.3%)	6 (3.6%)	

KLK4::KLKP1 RNA-ISH expression in relation to BCR post radical prostatectomy

There was no significant association between *KLK4::KLKP1* RNA-CISH expression and biochemical recurrence (BCR). Only PTEN loss was associated with a higher risk for BCR post radical prostatectomy (HR 1.98, CI 1.19–3.30, $p = 0.009$). Combining two biomarkers, PTEN loss/*KLK4::KLKP1* negativity was the only combination showing significant association with BCR (HR 2.18, CI 1.03–4.62, $p = 0.043$), which was higher than PTEN alone. This prognostic association remained significant in multivariate analysis after adjusting for Gleason score, surgical margins, and pathological stage (HR 2.31, CI 1.03–5.20, $p = 0.042$) (Table 3).

Discussion

There is a great need to characterize biomarkers that are reflective of the different molecular subtypes of PCa. Describing these biomarkers in relation to ethnic backgrounds will enable better implementation in specific populations. In this study, we characterized the incidence and significance of *KLK4::KLKP1* expression, a newly described gene fusion found in PCa, in a cohort of ME men, and explored its relationship to known biomarkers including *PTEN*, *ERG*, and *SPINK1* as well as its prognostic parameters in a surgical cohort.

In contrast to Chakravarthi et al.'s study, which described this novel gene fusion in a NA cohort, *KLK4::KLKP1* expression was found at higher rates in both noncancer and

Table 3 *KLK4::KLKP1* and biomarkers status in association with BCR post-radical prostatectomy

Variables	Odds ratio	95% CI	*p* value
KLK4::KLKP1 Negative			
Positive	0.99	0.60–1.62	0.958
ERG (Negative-score 0)			
Positive- score 1	0.93	0.55–1.55	0.769
SPINK1 (Positive-Score 1)			
Negative-score 0	0.90	0.37–2.16	0.812
PTEN (Positive-score 1,2,3)			
Loss-score 0	1.98	1.19–3.30	0.009
KLK4::KLKP1 and ERG combined (*KLK4::KLKP1* Negative and ERG Negative)			
KLK4::KLKP1 Positive and ERG Positive	0.93	0.48–1.80	0.827
KLK4::KLKP1 Negative and ERG Positive	0.91	0.40–2.08	0.827
KLK4::KLKP1 Positive and ERG Negative	0.97	0.50–1.88	0.930
PTEN and ERG combined (PTEN Positive and ERG Negative)			
PTEN Negative and ERG Positive	1.73	0.89–3.38	0.107
PTEN Negative and ERG Negative	1.83	0.86–3.90	0.117
PTEN Positive and ERG Positive	0.84	0.42–1.67	0.616
PTEN and *KLK4::KLKP1* combined (PTEN Positive and *KLK4::KLKP1* Negative)			
PTEN Negative and *KLK4::KLKP1* Positive	2.01	0.94–4.28	0.070
PTEN Negative and *KLK4::KLKP1* Negative	2.18	1.03–4.62	0.043
PTEN Positive and *KLK4::KLKP1* Positive	1.16	0.59–2.28	0.662
KLK4::KLKP1 and ERG combined (*KLK4::KLKP1* negative and ERG Negative)*			
KLK4::KLKP1 Positive and ERG Positive	0.95	0.46–1.93	0.877
KLK4::KLKP1 Negative and ERG Positive	1.05	0.43–2.54	0.917
KLK4::KLKP1 Positive and ERG Negative	1.05	0.52–2.14	0.884
PTEN and ERG combined (PTEN Positive and ERG Negative)*			
PTEN Negative and ERG Positive	1.63	0.80–3.34	0.180
PTEN Negative and ERG Negative	1.47	0.65–3.34	0.354
PTEN Positive and ERG Positive	0.95	0.46–1.97	0.888
PTEN and *KLK4::KLKP1* combined (PTEN gain and *KLK4::KLKP1* negative)*			
PTEN Negative and *KLK4::KLKP1* Positive	1.66	0.73–3.76	0.227
PTEN Negative and *KLK4::KLKP1* Negative	2.31	1.03–5.20	0.042
PTEN Positive and *KLK4::KLKP1* Positive	1.35	0.66–2.80	0.413

*Adjusted for Gleason score, pathology stage and surgical margin

PCa cases in this ME cohort (20.6% and 38% vs 17% and 33%), respectively (Chakravarthi et al. 2019). This adds further data that suggested variable *KLK4::KLKP1* expression in different ethnicities: 51.4% in this cohort of ME men compared to 28% in African Americans and 34% in Caucasian men in their study (Chakravarthi et al. 2019). We did not find an association between *KLK4::KLKP1* positivity and patient age or other pathological parameters, including seminal vesicle invasion, Gleason score, surgical margin status, or pathological stage. These findings mirror those of the NA cohort (Chakravarthi et al. 2019).

As in the NA cohort, *KLK4::KLKP1* expression was more common in ERG-positive cases and cases with increased *PTEN* expression. Interestingly, although no association between *KLK4::KLKP1* expression and BCR was noted, when combined with PTEN status, *KLK4::KLKP1* negativity/*PTEN* loss demonstrated the highest risk of BCR in univariate and multivariate analysis as compared to *PTEN* loss alone. This is in line with literature suggesting that PTEN loss is associated with worse disease (Bismar et al. 2018; Guedes et al. 2017). It also suggests that *KLK4::KLKP1* positivity could potentially be somewhat protective when combined with PTEN loss, as is the case for ERG expression when assessed in combination with PTEN expression.

This latest observation is of particular note since we previously observed that ME men seem to not share the enrichment for *PTEN* deletions seen in ERG-positive tumors seen in North America (Abdelsalam et al. 2020). Our observation that *KLK4::KLKP1* positivity tended to occur more in those with retained PTEN may suggest a unique aspect of molecular biology or tumorigenesis in ME men, and warrants further investigation.

In summary, herein we describe *KLK4::KLKP1* expression patterns in a PCa in a Middle Eastern cohort and outline several similarities, as well as some important differences between the original NA study (Chakravarthi et al. 2019). In this ME cohort, the expression of *KLK4::KLKP1* was noted at increased rates in both benign and malignant prostate samples but retained similar expression patterns in relation to Gleason grade groups and other biomarkers as compared to the NA population. Additionally, the incidence of *KLK4::KLKP1* expression in this ME cohort was higher compared to what was reported in Caucasian and African American populations (51.4% vs 34 and 28%, respectively). Although *KLK4::KLKP1* did not show any prognostic value, it showed a somewhat protective effect when combined with PTEN loss. Its clustering with certain molecular markers suggests a unique molecular profile (i.e., ERG-positive, PTEN-retained) that may be associated with different pathways in PCa. Additional studies are needed to investigate if *KLK4::KLKP1* could be a useful marker to further stratify PCa patients.

Author contributions Andrea Bakker wrote the manuscript and performed evaluation, Jonathan Slack performed evaluation, Nalla Palanisamy and Shannon Carskadon provided and assisted in staining methods and provided input for manuscript, Sunita Ghosh performed statistical analysis, Ibrahim Khalifeh assisted in sample preparation and acquisition and Tarek A. Bismar supervised and provided study concept. All authors reviewed the manuscript and approved this version.

Funding The study was supported in part by the Prostate Cancer Foundation Young Investigator Award (T.A.B) (Grant YI2012). This work is also supported by Prostate cancer Canada and Canada Research Society #400391.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The study was approved by the University of Calgary Cumming School of Medicine ethics review board and in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

References

Abdelsalam RA, Khalifeh I, Box A et al (2020) Molecular characterization of prostate cancer in Middle Eastern population highlights differences with Western populations with prognostic implication. J Cancer Res Clin Oncol 146(7):1701–1709. https://doi.org/10.1007/s00432-020-03221-x

Abou-Ouf H, Zhao L, Bismar TA (2016) ERG expression in prostate cancer: biological relevance and clinical implication. J Cancer Res Clin Oncol 142(8):1781–1793. https://doi.org/10.1007/s00432-015-2096-x

Ahearn TU, Pettersson A, Ebot EM et al (2015) A Prospective investigation of PTEN loss and ERG expression in lethal prostate cancer. JNCIJ. 108(2):djv346. https://doi.org/10.1093/jnci/djv346

Al-Abdin OZ, Al-Beeshi IZ (2018) Prostate cancer in the Arab population: an overview. SMJ 39(5):453–458. https://doi.org/10.15537/smj.2018.5.21986

Aldaoud N, Abdo N, Al Bashir S et al (2017) Prostate cancer in Jordanian-Arab population: ERG status and relationship with clinicopathologic characteristics. Virchows Arch 471(6):753–759. https://doi.org/10.1007/s00428-017-2160-9

Bismar TA, Dolph M, Teng LH, Liu S, Donnelly B (2012) ERG protein expression reflects hormonal treatment response and is associated with Gleason score and prostate cancer specific mortality. Eur J Cancer 48(4):538–546. https://doi.org/10.1016/j.ejca.2012.01.001

Bismar TA, Hegazy S, Feng Z et al (2018) Clinical utility of assessing PTEN and ERG protein expression in prostate cancer patients: a proposed method for risk stratification. J Cancer Res Clin Oncol 144(11):2117–2125. https://doi.org/10.1007/s00432-018-2730-5

Chakravarthi BV, Dedigama-Arachchige P, Carskadon S et al (2019) Pseudogene associated recurrent gene fusion in prostate cancer. Neoplasia 21(10):989–1002. https://doi.org/10.1016/j.neo.2019.07.010

Clements J, Hooper J, Dong Y, Harvey T (2001) The expanded human Kallikrein (KLK) gene family: genomic organisation, tissue-specific expression and potential functions. Biol Chem. https://doi.org/10.1515/BC.2001.002

Flavin R, Pettersson A, Hendrickson WK et al (2014) SPINK1 protein expression and prostate cancer progression. Clin Cancer Res 20(18):4904–4911. https://doi.org/10.1158/1078-0432.CCR-13-1341

Guedes LB, Tosoian JJ, Hicks J, Ross AE, Lotan TL (2017) PTEN loss in Gleason score 3 + 4 = 7 prostate biopsies is associated with nonorgan confined disease at radical prostatectomy. J Urol 197(4):1054–1059. https://doi.org/10.1016/j.juro.2016.09.084

Huang KC, Bégin LR, Palanisamy N, Donnelly B, Bismar TA (2016) SPINK1 expression in relation to PTEN and ERG in matched primary and lymph node metastatic prostate cancer: Implications for biomarker development. Urol Oncol 34(5):235.e1-235.e10. https://doi.org/10.1016/j.urolonc.2015.11.015

Kaffenberger SD, Barbieri CE (2016) Molecular subtyping of prostate cancer. Curr Opin Urol 26(3):213–218. https://doi.org/10.1097/MOU.0000000000000285

Kalyana-Sundaram S, Kumar-Sinha C, Shankar S et al (2012) Expressed pseudogenes in the transcriptional landscape of human cancers. Cell 149(7):1622–1634. https://doi.org/10.1016/j.cell.2012.04.041

Lai J, Lehman ML, Dinger ME et al (2010) A variant of the KLK4 gene is expressed as a cis sense-antisense chimeric transcript in prostate cancer cells. RNA 16(6):1156–1166. https://doi.org/10.1261/rna.2019810

Lotan TL, Tomlins SA, Bismar TA et al (2020) Report from the International Society of Urological Pathology (ISUP) consultation conference on molecular pathology of urogenital cancers. I. Molecular biomarkers in prostate cancer. Am J Surg Pathol 44(7):e15–e29. https://doi.org/10.1097/PAS.0000000000001450

Lundwall Å, Ylitalo EB, Wikström P, Brattsand M (2021) KLK4T2 is a hormonally regulated transcript from the KLK4 locus. IJMS 22(23):13023. https://doi.org/10.3390/ijms222313023

Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer statistics, 2021. CA A Cancer J Clin 71(1):7–33. https://doi.org/10.3322/caac.21654

Taylor BS, Schultz N, Hieronymus H et al (2010) Integrative genomic profiling of human prostate cancer. Cancer Cell 18(1):11–22. https://doi.org/10.1016/j.ccr.2010.05.026

Terry S, Nicolaiew N, Basset V et al (2015) Clinical value of ERG, TFF3, and SPINK1 for molecular subtyping of prostate cancer: a Survey of ERG, TFF3 and SPINK1 in PCa. Cancer 121(9):1422–1430. https://doi.org/10.1002/cncr.29233

Tomlins SA, Rhodes DR, Yu J et al (2008) The role of SPINK1 in ETS rearrangement-negative prostate cancers. Cancer Cell 13(6):519–528. https://doi.org/10.1016/j.ccr.2008.04.016