Published in final edited form as:

Am J Surg Pathol. 2014 May ; 38(5): 615–626. doi:10.1097/PAS.0000000000000090.

HOXB13 G84E-related Familial Prostate Cancers: A Clinical, Histologic, and Molecular Survey

Steven C. Smith, M.D., Ph.D^{1,%}, Nallasivam Palanisamy, Ph.D^{1,2,4,%}, Kimberly A. Zuhlke, B.A³, Anna M. Johnson, M.S³, Javed Siddiqui, M.S^{1,2}, Arul M. Chinnaiyan, M.D., Ph.D^{1,2,4,5,6}, Lakshmi P Kunju, Ph.D. M.D^{1,2,4}, Kathleen A. Cooney, M.D^{3,4}, and Scott A. Tomlins, M.D., Ph.D^{1,2,4,6,*}

¹Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

²Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

³Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA

⁴Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI USA

⁵Howard Hughes Medical Institute

⁶Department of Urology, University of Michigan Medical School, Ann Arbor, MI USA

Abstract

Recent genetic epidemiologic studies identified a germline mutation in the homeobox transcription factor, *HOXB13* G84E, which is associated with markedly increased risk of prostate cancer, particularly early onset hereditary prostate cancer. The histomorphologic and molecular features of cancers arising in such carriers have not been studied. Here, we reviewed prostatectomy specimens from 23 *HOXB13* G84E mutation carriers, mapping the total cancer burden by anatomically distinct cancer focus and evaluating morphologic features. We also assessed basic molecular subtypes for all cancer foci (ERG/SPINK1 status) by dual immunohistochemistry staining on full sections. The cohort showed median age 58 years, median serum PSA 5.7 ng/ml, and a median of 6 cancer foci (range 1–14) per case. Of evaluable cases, dominant foci were Gleason score 6 in 23%, 3+4=7 in 41%, 4+3=7 in 23%, and 8 in 14%; biochemical recurrence was observed in one case over a median of 36 months follow-up. Histologic review found a high

*Corresponding Author: Scott A. Tomlins, M.D., Ph.D., Departments of Pathology and Urology, University of Michigan Medical School, 1524 Basic Sciences Research Building, 109 Zina Pitcher Place SPC 2200, Ann Arbor, Michigan 48109-2200. Tel. 734-764-1549. Fax. 734-647-7950. tomlinss@umich.edu.

%These authors contributed equally

DISCLOSURES

The University of Michigan has applied for a patent related to the identification of *HOXB13* mutations in men with prostate cancer, on which K.A.C. is listed as an inventor. The University of Michigan has been issued a patent on the detection of ETS gene fusions in prostate cancer, on which S.A.T. and A.M.C. are listed as co-inventors. The University of Michigan licensed the diagnostic field of use to Gen-Probe, Inc., who has sublicensed some rights to Ventana Medical Systems-Roche. S.A.T. serves as a consultant to, and has received honoraria from, Ventana Medical Systems-Roche. A.M.C. has served as a consultant for Gen-Probe, Inc. and Ventana Medical Systems-Roche. N.P. receives research funding from Ventana Medical Systems-Roche. Gen-Probe and Ventana Medical Systems-Roche did not play a role in the design and conduct of this study, in the collection, analysis, or interpretation of the data or in the preparation, review or approval of the article.

Note: Preliminary findings for this work was presented at the Annual Meeting of the United States and Canadian Academy of Pathology Meeting, Baltimore, M.D., in March 2013.

prevalence of cases showing cancers with a spectrum of features previously described with pseudohyperplastic carcinomas, with 45% of cases showing a dominant focus with such features. Molecular subtyping revealed a strikingly low prevalence of ERG⁺ cancer with increased prevalence of SPINK1⁺ cancer (dominant focus ERG⁺ 17%, SPINK1⁺ 26%, ERG⁻/SPINK1⁻ 52%, single ERG⁺/SPINK1⁺ focus 4%). One ERG⁻/SPINK1⁻ dominant focus showed aberrant p63⁺ immunophenotype. In summary, *HOXB13* G84E variant-related prostate cancers show frequent pseudohyperplastic-type features and markedly low prevalence of ERG⁺ cancers relative to unselected cases, and, especially, to early onset cohorts. These findings suggest that novel molecular pathways may drive disease in *HOXB13* G84E carriers.

INTRODUCTION

Germline variations in >70 genes have been associated with the inherited predisposition to prostate cancer, including common variations in multiple genes that together may account for ~30% of familial prostate cancer risk, although these variants individually have small odds ratios (1.1–1.5)^{1–6}. Recently, through linkage analysis and next-generation sequencing, investigators at the University of Michigan and Johns Hopkins University identified a rare germline variation in *HOXB13* (G84E substitution) in families with hereditary prostate cancer⁷. This rare variant (carrier frequency estimates of 0.1–1.5%) has been shown in multiple European-American cohorts to confer an ~3–10× odds ratio for the development of prostate cancer, particularly early onset and hereditary prostate cancer^{8–19}. *HOXB13*, a homeobox domain-containing gene, is strongly expressed in the prostate from embryonic stages to adulthood and has been shown to directly interact with the androgen receptor (AR), functioning to activate or repress distinct AR target genes^{20–25}. The molecular mechanism by which *HOXB13* G84E variants promote prostate cancer development, however, is unknown.

Prostate cancer shows a variety of histologic patterns, most commonly acinar carcinoma, with variant histology observed in 5–10% of cases^{26, 27}. Numerous histologic variants of prostate cancer have been described, including ductal carcinoma, foamy gland carcinoma, pseudohyperplastic carcinoma, mucinous carcinoma and neuroendocrine/small cell carcinoma. Similarly, a variety of robust prostate cancer molecular subtypes have been identified, based primarily on the presence or absence of recurrent gene fusions involving 5' regions of androgen regulated genes (most commonly *TMPRSS2*) fused to nearly full length members of the ETS transcription factor family (most commonly *ERG*)^{28–30}. In PSA-screened, Caucasian cohorts, approximately 50% of prostate cancer foci harbor ETS gene fusions (ETS⁺)³¹. The development of sensitive and specific antibodies against ERG, the 3' partner in ~90% of all ETS fusions, has enabled rapid characterization of ETS fusion status across large cohorts by immunohistochemistry (IHC)^{32, 33}. Additionally, over-expression of *SPINK1* was identified in ~5–10% of prostate cancers, which are nearly exclusively ETS fusion negative (ETS⁻)³⁴. ETS^{+/-}/SPINK1^{+/-} based subtyping of prostate cancer has been supported by recent reports of dual ERG/SPINK1 IHC protocols employed by independent groups, which have confirmed the mutually exclusive nature of these subtypes^{35, 36}. When present, *ERG* rearrangements and corresponding IHC staining are clonal in a given cancer focus, while *SPINK1* often shows heterogeneous staining in a given focus. Given the

multifocal nature of prostate cancer, men can harbor genetically distinct ERG⁺/SPINK1⁻, ERG⁻/SPINK1⁺ and ERG⁻/SPINK1⁻ foci, and ERG/SPINK1 status can be used to evaluate whether two foci of prostate cancer are genetically distinct (multiclonal).

The histologic characteristics and molecular subtypes of prostate cancer developing in *HOXB13* G84E carriers have not been explored. Here we comprehensively characterized 23 prostate cancers arising in men harboring this variant through complete mapping and morphologic evaluation of all cancer foci at the time of prostatectomy specimens. Importantly, for each patient, we also evaluated the basic molecular subtype of all cancer foci in each case for ERG/SPINK1 status by IHC, rather than the more typical strategy of just evaluating the dominant focus (usually defined by Gleason score and/or size).

MATERIALS AND METHODS

Cohort and Histologic Evaluation

With prior IRB approval, archival tissue blocks or archival blocks and slides from a total of 24 prostatectomy cases of patients previously identified as germline carriers of *HOXB13* G84E mutation^{7, 17} were identified and retrieved from the University of Michigan Department of Pathology slide library. These prostatectomy cases were from either radical retropubic prostatectomies or robotic-assisted laparoscopic prostatectomies, as indicated in Table 1. The University of Michigan prostatectomy tissue submission protocol is a partial submission of the parenchyma with full submission of the capsule, enabling IRB-approved frozen tissue procurement but full microscopic evaluation of the capsule for staging and margins. It has been detailed previously and validated to demonstrate its diagnostic equivalence to full submission of complete submissions³⁷. Briefly, prostatectomy specimens were weighed, measured, and inked. Seminal vesicles, apex, and base were amputated, and the remaining prostate was serially sectioned at 3-mm to 5-mm intervals perpendicular to the long axis of the gland from the base to apex. Two intervening sections were snap-frozen for tissue procurement after removal of the circumferential capsules of the procurement sections. Capsule sections were submitted for fixation in 10% neutral buffered formalin with the unprocured sections before routine tissue processing and sectioning.

Blocks from the 24 *HOXB13* G84E cases were recut to produce H&E slides by standard means if original archival slides were not available for review. The total cancer burden for each case was mapped in three dimensions as we have detailed recently³⁸ and validated previously by others³⁹. Briefly, by careful reconstruction of each quartered serial section of the prostatectomy as if it were a whole mount, anatomically distinct foci were mapped as independent if they were greater than 3mm from adjacent focus within the same (or adjoining) section and 4mm or more from the closest cancer on an adjacent section. Each focus was reviewed and individually graded by 2005 ISUP consensus guidelines⁴⁰ by the study pathologists (S.C.S. and S.A.T.). The dominant focus was designated as the focus with the highest Gleason Score, and the greatest linear dimension was measured. Any focus with equivocal morphology for carcinoma was resolved by use of basal cell cocktail immunostain (detailed below) and diagnostic consensus (S.C.S., L.P.K. and S.A.T.). Clinical data were assembled by review of the institutional electronic medical record.

After mapping the entire cancer burden by focus, all foci were systematically re-reviewed to identify and tabulate the prevalence of each of selected histologic variants of prostatic adenocarcinoma. These included conventional acinar adenocarcinoma, pseudohyperplastic carcinoma, small cell carcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, atrophic carcinoma, oncocytic carcinoma, ductal adenocarcinoma, carcinoma with Paneth cell-like neuroendocrine differentiation, squamous cell carcinoma, and giant cell carcinoma, as described previously^{26, 27}. A focus was considered variant if the variant morphology was the predominant pattern of the focus. Initial review identified a high prevalence of cancer foci showing features previously described within the spectrum of pseudohyperplastic carcinomas. Given the range of pseudohyperplastic-type features exhibited in these cases, each case was classified as to whether it harbored carcinoma demonstrating cytologic and/or architectural features of pseudohyperplastic carcinoma, and whether these features were present in the dominant cancer focus^{26, 41, 42}. The cytologic features of pseudohyperplastic carcinoma (less specific to this variant), included tall/columnar epithelium, pale to granular foamy cytoplasm, and basally oriented nuclei. The architectural features of pseudohyperplastic carcinoma evaluated were presence of an irregular luminal border and cystically dilated glands with papillary infoldings. Presence of luminal pink amorphous secretions and pink crystalloids, features of both pseudohyperplastic and non-pseudohyperplastic carcinoma (and occasional benign glands), were also tabulated for each cancer focus. Finally, each focus was evaluated for features previously associated with ERG⁺ prostate cancer, including prominent cribriform architecture, intraductal growth, and blue mucin⁴³.

Immunohistochemistry

Dual immunohistochemistry (IHC) for ERG and SPINK1 was performed at the Michigan Center for Translational Pathology as detailed recently³⁵. Briefly, primary antibodies used were rabbit monoclonal anti-ERG (EPR3864, 1:100 dilution, Cat no. 790-4576, Ventana Medical Systems-Roche) and a mouse monoclonal anti-SPINK1 (1:100; H00006690-M01, clone 4D4, Abnova). IHC was performed using an automated protocol on the Discovery XT autostainer (Ventana Medical Systems-Roche), with secondary antibodies and detection using brown/DAB chromogen for ERG and Red for SPINK1 (hematoxylin counterstain). Vascular endothelium was used as an internal positive control for ERG; normal pancreas was the positive control for SPINK1. Basal cell markers were assessed using an automated protocol on the Discovery XT autostainer using a cocktail (Ventana Medical Systems-Roche) containing primary antibodies against high molecular weight cytokeratin HMWCK (34βE12) and p63 (clone 4A4) and secondary ultraMap anti-msHRP antibody. Hematoxylin II was used as a counterstain. All immunostains were evaluated by S.C.S., L.P.K. and S.A.T.

Criteria used for assessment of the molecular status of an adenocarcinoma focus were as reported before^{35, 38}. Briefly, given the usual dichotomous ERG staining in prostate cancer, a focus was considered ERG⁺ if diffuse, strong nuclear staining was observed in all cancer cells (weaker staining was noted if present). A focus was considered SPINK1⁺ if >5% of the cancerous cells showed strong cytoplasmic positivity. A focus was considered dual ERG⁺/SPINK1⁺ if cancerous cells demonstrated diffuse positivity for ERG and 5% of these cells showed concomitant SPINK1⁺ cytoplasmic staining. Lack of basal cell markers (p63 and

high molecular weight cytokeratin) was used to support a diagnosis of carcinoma in challenging cases. In cases where the morphologic dominant focus was found by ERG/SPINK1 dual stain to consist of two different clones, the focus with higher Gleason score was designated dominant, while the focus with larger maximum linear dimension was designated dominant among foci showing the same Gleason score.

Statistical Analysis

Descriptive statistics and associations among clinicopathological parameters from different cohorts were assessed using MedCalc 12. Two-tailed non-parametric two sample tests or Fisher exact tests were used for all comparisons and *P* values less than 0.05 were considered statistically significant.

RESULTS

We evaluated archival prostatectomy material for 24 cases of prostatic adenocarcinoma arising in *HOXB13* G84E germline mutation carriers treated by prostatectomy at the University of Michigan. One case showed no carcinoma remaining on deeper sections and was excluded from further study. The 23 case final cohort showed a median age of 58.0 years, median preoperative PSA of 5.7 ng/mL, a median prostatectomy weight of 41.4g; all patients were of European descent. Pathologic staging showed that in 87% (20/23) cases the adenocarcinoma was organ confined (pT2a–b 9% (2/23); pT2c 78% (18/23); pT3a 9% (2/23); pT3b 4% (1/23)). No case showed lymphatic metastasis among the 18/23 cases where lymph nodes were dissected. Surgical margins were positive in 13% (3/23) of cases. Median follow-up time for the cohort was 36.0 months; a single patient experienced biochemical recurrence at 29.2 months and expired from metastatic hormone refractory carcinoma at 56.4 months. One subject had been treated by hormone therapy prior to prostatectomy, and was not Gleason scored. Of the remaining 22 cases, dominant foci were Gleason score 3+3=6 in 23% (5/22), 3+4=7 in 41% (9/22), 4+3=7 in 23% (5/22), and 8 in 14% (3/22). Per case, the median greatest linear dimension of the dominant focus was 1.2 cm. The dominant focus was based in the transition zone in 13% (3/23) of cases. A median of 6 cancer foci (range, 1–14) were identified per case. Table 1 lists clinicopathologic parameters per case for all evaluated patients.

Morphologic evaluation of the entire cancer burden of these cases was performed, identifying typical acinar prostatic adenocarcinoma in all cases, with no foci of ductal adenocarcinoma, urothelial carcinoma, or other neoplasm. Additionally, no foci of neuroendocrine, mucinous, signet ring, giant cell, sarcomatoid, lymphoepithelioma-like, oncocytic, or Paneth cell-like variants of acinar adenocarcinoma were identified. In contrast, morphologic evaluation of the 22 untreated cases showed prevalent pseudohyperplastic-type features (Figure 1 and Table 1)^{27, 41, 42}. Cytologic features of pseudohyperplastic carcinoma (tall/columnar epithelium with pale to finely granular or vacuolated cytoplasm and basally oriented nucleoli, Figure 1A–B) were present in 45% of dominant foci, while the architectural features (dilated glands with papillary infoldings, Figure 1C–D) were present in 41% of dominant foci (Table 1). Across all foci, the cytologic features were more common, with 82% of cases showing at least one such focus, than were architectural features, with

only 55% of cases showing such a focus. Other features, associated with but not specific to pseudohyperplastic carcinoma, prevalent among this cohort were intraluminal pink amorphous secretions (at least one focus in 91% of cases, 20/22) and pink crystalloids (86%, 19/22), see Figure 1A–B. Additionally, three cases showed at least one focus of with features of foamy gland carcinoma (14%, 3/22), one case showed a focus of atrophic carcinoma (5%, 1/22), while another case showed a focus of acinar adenocarcinoma with a number of glands showing a PIN-like morphology, including stratified epithelium reminiscent of tufted PIN (5%, 1/22).

Several morphologic features, previously associated with the ERG⁺ prostatic adenocarcinoma molecular subtype⁴³ and not typical of pseudohyperplastic carcinoma, appeared infrequent during initial mapping studies. These included blue mucin, prominent cribriform growth pattern, and intraductal growth. Systematic re-review of all foci demonstrated that 23% (5/22) of cases showed at least one focus of carcinoma showing luminal blue mucin. Prominent cribriform growth and intraductal growth were also infrequent, with only 18% (4/22) and 5% (1/22) of cases showing such a focus, respectively. The benign prostatic parenchyma was anatomically and histologically unremarkable in these cases.

To assess basic molecular subtypes of prostate carcinoma arising in *HOXB13* G84E carriers, we assessed ERG and SPINK1 status by dual IHC. Dual ERG/SPINK1 IHC was performed on full tissue sections representing the dominant foci and all unique mapped foci from all 23 cases to ascertain the prevalence of these two essentially mutually exclusive prostate cancer molecular subtypes^{34–36}. In *HOXB13* G84E carriers, if positive, ERG showed strong, diffuse expression across all cancer cell nuclei in a cancer focus (Figure 2A–B), while SPINK1 IHC showed heterogeneous expression, particularly at the periphery and invasive front of foci (Figure 2C–D). Both of these staining patterns are similar to previous observations using the same antibodies in unselected prostate cancer cohorts^{33, 35, 38, 44}. Importantly, the ability to assess clonality of a focus through dual ERG/SPINK1 IHC revealed that the morphologic dominant focus was composed of two different clones in 5 of the 23 cases (22%), as shown in Figure 3A–D.

Dual IHC showed that the dominant focus was ERG[−]/SPINK1[−] in 52% (12/23), ERG⁺/SPINK1[−] in 17% (4/23), SPINK1⁺/ERG[−] 26% (6/23), and ERG⁺/SPINK1⁺ in 4% (1/23) of cases. All but one case showed at least one ERG[−]/SPINK[−] focus (96%, 22/23), while over half of cases showed at least one ERG⁺/SPINK[−] or ERG[−]/SPINK1⁺ focus (57%, 13/23 and 52%, 12/23, respectively). Of the 145 independent foci mapped across all cases, 66% (95/145) were ERG[−]/SPINK1[−], 17% (25/145) were ERG⁺/SPINK1[−], 17% (24/145) were ERG[−]/SPINK1⁺, and a single focus, 1% (1/145) was ERG⁺/SPINK1⁺.

We noted several unique observations in these *HOXB13* G84E-carrier cases. In addition to the single focus with a small area of ERG⁺/SPINK1⁺ prostate cancer embedded in a larger ERG⁺ focus (Figure 4A–B), we also observed a case with a collision between ERG⁺/SPINK1[−] and ERG[−]/SPINK1⁺ foci. In this case, distinct ERG⁺/SPINK1[−] and ERG[−]/SPINK1⁺ cells were present in the same gland (Figure 4C–D), which we interpret as glandular fusion between separate clones. Also of note, one case demonstrated aberrant

diffuse expression of p63 among the carcinoma cells at the time of diagnostic needle biopsy workup, a rare aberrant immunophenotype reported previously^{45, 46}. To assess for the prevalence of this feature among *HOXB13* G84E carriers, IHC for basal cell markers (HMWCK/p63) was performed on full sections containing the dominant focus for all cases (as well as on any additional foci equivocal for carcinoma, see **Materials and Methods**, 47 total sections stained). Review of the stained sections redemonstrated only this single case with dominant focus showing aberrant p63⁺ immunophenotype carcinoma (ERG⁻/SPINK1⁻) (Figure 4E–F), which showed an atrophic to basaloid morphology consistent with Gleason score 4+5=9, though grading remains controversial in these cases^{46, 47}. Intriguingly, this patient also harbored a distinct focus of acinar adenocarcinoma, with a number of glands showing a PIN-like morphology, which was ERG⁺/SPINK1⁻ (Figure 4G–I).

DISCUSSION

Recently, Ewing *et al.* determined through epidemiologic studies and targeted next generation sequencing of over 90 multicase families that a germline variant in the homeobox gene, *HOXB13* (G84E), was associated with predisposition to prostate cancer⁷. Subsequent studies have confirmed that the *HOXB13* G84E variant is rare (carrier frequencies of 0.1–1.5%), most common among men of Western European descent, and, particularly amongst young men with a positive family history, associated with increased prostate cancer risk (odds ratios ~3–10)^{8–19}. However, the pathologic features of such cases have not been studied in detail. Thus, we performed a systematic clinicopathologic, histomorphologic, and molecular review of the University of Michigan subset of the Ewing *et al.* *HOXB13* G84E cases.

In terms of morphology, we observed a prevalent and characteristic appearance of the carcinomas arising in a cohort of 23 *HOXB13* G84E germline mutation carriers: a substantial majority of these cases showed foci of carcinoma with a bland appearance, reminiscent of benign hyperplasia. Cancers demonstrating this constellation of features have been previously described by Humphrey *et al.* as the pseudohyperplastic variant of prostate carcinoma, an infrequent variant^{27, 41, 42}. Review of all cancer foci in these *HOXB13* G84E cases demonstrated frequent pseudohyperplastic-type features among the cancers, involving the dominant focus in 45% and at least one cancer focus in 82% of these cases. As has been described for pseudohyperplastic cancers, there was very frequent admixture of conventional acinar adenocarcinoma. Such nonspecific features should not serve as a screening tool to identify *HOXB13* G84E-related cases (genotyping is required). However, awareness of this prevalent, “pseudobenign”^{41, 42} morphology may be of use in biopsy evaluation of individual cases from known *HOXB13* G84E kindreds, especially as experts contemplate increased surveillance of genetically affected individuals^{9, 11}.

In this *HOXB13* G84E-related cohort, we observed a median age of 58 years and a median serum PSA at diagnosis of 5.7 ng/mL; all patients were of European descent. A median of 6 cancer foci per case were identified and the dominant focus showed Gleason score 6, 3+4=7, 4+3=7 and 8 in 23%, 41%, 23% and 14% of cases, respectively. We have recently studied two additional cohorts suitable for comparison. We employed this same focus mapping and

ERG molecular subtyping strategy in a genetically *unselected* prostatectomy cohort (N=41, also of predominantly European descent, 86%), referred hereafter as “Cohort 2”, with median age of 59 years and PSA of 5ng/mL³⁸. Cohort 2 showed a median of 3 cancer foci, and dominant foci of Gleason Score 6, 3+4=7, 4+3=7 and 8 in 17%, 61%, 15% and 7% of cases, respectively. We also recently applied our dual ERG/SPINK1 IHC in a tissue microarray study of dominant cancer foci of 228 genetically *unselected* men undergoing prostatectomy, referred hereafter as “Cohort 3”. Cohort 3 demonstrated a median age of 61 years, median serum PSA of 6.2ng/mL frequent European descent (94%)³⁵. Dominant foci showed Gleason Score 6, 3+4=7, 4+3=7 and 8 in 19%, 49%, 20% and 11% of cases, respectively. Compared to Cohorts 2 and 3, our current cohort of *HOXB13* G84E carriers was significantly younger than Cohort 3 (Mann-Whitney U-test P=0.002); however, they were otherwise quite similar, suggesting valid comparisons may be made with respects to distributions of molecular subtypes of carcinoma.

Thus, in addition to evaluating histologic features of *HOXB13* G84E-related carcinomas, we also assessed basic molecular subtypes by IHC. We and others have shown that approximately 50% of PSA-screened prostate cancers harbor recurrent gene rearrangements that fuse regulatory regions of androgen regulated genes to ETS transcription factor family members (most commonly *TMPRSS2* fused to *ERG*)^{28, 30, 31}. ERG IHC has been extensively validated as a diagnostic biomarker for carcinoma^{30, 33, 44}; thus, determination of the prevalence of this molecular subtype in *HOXB13* G84E cases would be of use for evaluation of future biopsies for such patients. Assessing all foci of cancer arising in *HOXB13* G84E carriers for ERG/SPINK1 status by IHC, we observed a remarkably low prevalence of ERG⁺/SPINK1⁻ foci. Amongst dominant foci, only 22% were ERG⁺, with 17% of all foci ERG⁺ (including the ERG⁺/SPINK1⁺ focus). For comparison, in the similarly mapped and stained Cohort 2³⁸, we observed significantly higher ERG⁺ prevalence (59% of dominant foci and 49% of all foci, P=0.008 and P<0.0001, respectively vs. *HOXB13* Cohort). The same trend was apparent in Cohort 3³⁵, where we found that 43% of dominant foci used for TMA construction were ERG⁺ (P=0.07 vs. *HOXB13* Cohort). For clarity, Figure 5A plots these key differences in ERG prevalence graphically. Further contrasting the markedly low prevalence of ERG⁺ cancer in the *HOXB13* G84E cases is comparison to a recent meta-analysis of >10,000 cases by Stephensen *et al.* who reported that 47% of prostate cancers were *TMPRSS2:ERG*⁺³¹.

Although ERG status has been evaluated in several variants of prostate cancer finding similar rates of ERG⁺ as typical acinar carcinoma^{48–50}, we are unaware of published reports of ERG status in the pseudohyperplastic variant. Thus, it is unclear whether the observed lower prevalence of ERG⁺ cancers is a cause, effect, or only associated with the morphology observed. Moreover, histologic features previously associated with the ERG⁺ subtype⁴³ were uncommon in our current cohort. Lower rates of ERG⁺ prostate cancer have been reported in non-Caucasian populations (~25–40%) and cancers arising in the transition zone^{31, 51–56}, neither of which were prominent in our cohort. The disparity of ERG⁺ prevalence in this cohort is even more remarkable compared to higher rates of ERG⁺ cancer reported among early onset cancers and/or those with low serum PSA⁵⁷.

Similarly, approximately 5–10% of prostate cancers, which are almost exclusively ETS fusion negative, show marked overexpression of SPINK1^{34, 58–60}. Thus, SPINK1 expression defines a second molecular class of ETS fusion negative carcinomas. While the clinical significance of SPINK1 expression remains unknown, preclinical studies in animal models have proven the principle that it is an attractive therapeutic target³⁴. Opposite the observation of low ERG⁺ prevalence in this *HOXB13* G84E-related cohort, we found that the SPINK1⁺ molecular subtype of prostate cancer was over-represented in *HOXB13* G84E carriers, with 30% of men harboring SPINK1⁺ dominant foci and 17% of all foci SPINK1⁺ (including the ERG⁺/SPINK1⁺ focus). Although we are unaware of studies assessing SPINK1 status across all foci in multifocal prostate cancer, in our initial characterization of unselected cases, we found that ~10% of prostate cancers were SPINK1⁺³⁴, while recent tissue microarray studies on >12,000 cases suggests that the SPINK1⁺ prevalence is ~5–6%^{36, 61}. In Cohort 3, we found that amongst 228 unselected localized prostate cancers only 5% were SPINK1⁺ (P=0.003 vs. *HOXB13* cohort, see Figure 5B)³⁵. Our findings of a case with an ERG⁺ dominant focus showing patchy SPINK1 expression and a case with a dominant focus (ERG⁻/SPINK1⁻) with aberrant expression of p63⁴⁵ again underscore the uniqueness of these cases.

Of more general relevance to the diagnosis and study of prostate cancer are observations regarding clonality of carcinoma. Given that ETS gene fusions are highly clonal in an individual focus^{29, 30}, IHC for ERG, either alone or in combination with SPINK1, can be used to aid in identifying genetically distinct foci in multifocal prostate cancer. Importantly, in five cases, we identified a dominant focus that was in fact a “collision tumor” of genetically distinct clones that would otherwise have been considered a single focus by histology. Importantly, our observation of frequent dominant foci composed of “collision tumors” that can be appreciated on whole sections may explain a recent report of frequent intrafocal ERG heterogeneity observed in large tumors sampled by multiple tissue microarray cores⁶². These observations call for rigorous whole-section-based studies of dominant focus clonality and whether molecular assessment could assist in dominant focus assignment and Gleason scoring.

In summary, in this first histologic and molecular survey of cancers arising in *HOXB13* G84E carriers, we identified aberrant morphologic and molecular features. Such findings need to be confirmed in additional cohorts of cancers arising in *HOXB13* G84E carriers but may be relevant to future diagnostic practice. Preliminarily, however, these findings imply that novel molecular pathways may drive carcinogenesis in these cases and support comprehensive assessment of molecular alterations in prostate cancers arising in *HOXB13* G84E carriers.

Acknowledgments

Support

The authors thank Ventana Medical Systems-Roche for providing immunohistochemical reagents. This work was supported in part by the Prostate Cancer Foundation, the University of Michigan Comprehensive Cancer Center Prostate SPORE (P50CA 069568), the National Institutes of Health (R01 CA 132874 to A.M.C. and R01 CA 79596 to K.A.C.), and the Early Detection Research Network (U01 CA111275 and U01 CA113913). A.M.C. and S.A.T. are supported by a Stand Up To Cancer - Prostate Cancer Foundation Prostate Dream Team Translational Cancer

Research Grant. Stand Up To Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research (SU2C-AACR-DT0712). A.M.C. is supported by the Prostate Cancer Foundation, Alfred A. Taubman Institute, the Howard Hughes Medical Institute, and a Doris Duke Charitable Foundation Clinical Scientist Award. N.P. and S.A.T. are supported by University of Michigan Prostate SPORC Career Development Awards.

REFERENCES

1. Pomerantz M, Freedman ML. Clinical uncertainty of prostate cancer genetic risk panels. *Sci Transl Med*. 2013; 5:182ed186.
2. Goh CL, Schumacher FR, Easton D, et al. Genetic variants associated with predisposition to prostate cancer and potential clinical implications. *J Intern Med*. 2012; 271:353–365. [PubMed: 22308973]
3. Akamatsu S, Takata R, Haiman CA, et al. Common variants at 11q12, 10q26 and 3p11.2 are associated with prostate cancer susceptibility in Japanese. *Nat Genet*. 2012; 44:426–429. S421. [PubMed: 22366784]
4. Eeles RA, Olama AA, Benlloch S, et al. Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. *Nat Genet*. 2013; 45:385–391. [PubMed: 23535732]
5. Gudmundsson J, Sulem P, Gudbjartsson DF, et al. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet*. 2009; 41:1122–1126. [PubMed: 19767754]
6. Collins CC, Volik SV, Lapuk A, et al. Next Generation Sequencing of Prostate Cancer from a Patient Identifies a Deficiency of Methylthioadenine Phosphorylase (MTAP), an Exploitable Tumor Target. *Mol Cancer Ther*. 2012
7. Ewing CM, Ray AM, Lange EM, et al. Germline mutations in HOXB13 and prostate-cancer risk. *The New England journal of medicine*. 2012; 366:141–149. [PubMed: 22236224]
8. Akbari MR, Trachtenberg J, Lee J, et al. Association between germline HOXB13 G84E mutation and risk of prostate cancer. *J Natl Cancer Inst*. 2012; 104:1260–1262. [PubMed: 22781434]
9. Breyer JP, Avritt TG, McReynolds KM, et al. Confirmation of the HOXB13 G84E germline mutation in familial prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2012; 21:1348–1353. [PubMed: 22714738]
10. Gudmundsson J, Sulem P, Gudbjartsson DF, et al. A study based on whole-genome sequencing yields a rare variant at 8q24 associated with prostate cancer. *Nat Genet*. 2012; 44:1326–1329. [PubMed: 23104005]
11. Karlsson R, Aly M, Clements M, et al. A Population-based Assessment of Germline HOXB13 G84E Mutation and Prostate Cancer Risk. *Eur Urol*. 2012
12. Stott-Miller M, Karyadi DM, Smith T, et al. HOXB13 mutations in a population-based, case-control study of prostate cancer. *Prostate*. 2012
13. Chen Z, Greenwood C, Isaacs WB, et al. The G84E mutation of HOXB13 is associated with increased risk for prostate cancer: results from the REDUCE trial. *Carcinogenesis*. 2013
14. Kluzniak W, Wokolorczyk D, Kashyap A, et al. The G84E mutation in the HOXB13 gene is associated with an increased risk of prostate cancer in Poland. *Prostate*. 2013
15. Laitinen VH, Wahlfors T, Saaristo L, et al. HOXB13 G84E Mutation in Finland: Population-Based Analysis of Prostate, Breast, and Colorectal Cancer Risk. *Cancer Epidemiol Biomarkers Prev*. 2013; 22:452–460. [PubMed: 23292082]
16. Macinnis RJ, Severi G, Baglietto L, et al. Population-Based Estimate of Prostate Cancer Risk for Carriers of the HOXB13 Missense Mutation G84E. *PLoS One*. 2013; 8:e54727. [PubMed: 23457453]
17. Schroeck FR, Zuhlke KA, Siddiqui J, et al. Testing for the Recurrent HOXB13 G84E Germline Mutation in Men with Clinical Indications for Prostate Biopsy. *J Urol*. 2013; 189:849–853. [PubMed: 23036981]
18. Xu J, Lange EM, Lu L, et al. HOXB13 is a susceptibility gene for prostate cancer: results from the International Consortium for Prostate Cancer Genetics (ICPCG). *Hum Genet*. 2013; 132:5–14. [PubMed: 23064873]

19. Witte JS, Mefford J, Plummer S, et al. HOXB13 Mutation and Prostate Cancer: Studies of Siblings and Aggressive Disease. *Cancer Epidemiol Biomarkers Prev.* 2013
20. Economides KD, Capecchi MR. Hoxb13 is required for normal differentiation and secretory function of the ventral prostate. *Development.* 2003; 130:2061–2069. [PubMed: 12668621]
21. Huang L, Pu Y, Hepps D, et al. Posterior Hox gene expression and differential androgen regulation in the developing and adult rat prostate lobes. *Endocrinology.* 2007; 148:1235–1245. [PubMed: 17138648]
22. Jung C, Kim RS, Lee SJ, et al. HOXB13 homeodomain protein suppresses the growth of prostate cancer cells by the negative regulation of T-cell factor 4. *Cancer Res.* 2004; 64:3046–3051. [PubMed: 15126340]
23. Jung C, Kim RS, Zhang HJ, et al. HOXB13 induces growth suppression of prostate cancer cells as a repressor of hormone-activated androgen receptor signaling. *Cancer Res.* 2004; 64:9185–9192. [PubMed: 15604291]
24. Norris JD, Chang CY, Wittmann BM, et al. The homeodomain protein HOXB13 regulates the cellular response to androgens. *Mol Cell.* 2009; 36:405–416. [PubMed: 19917249]
25. Sreenath T, Orosz A, Fujita K, et al. Androgen-independent expression of hoxb-13 in the mouse prostate. *Prostate.* 1999; 41:203–207. [PubMed: 10517879]
26. Epstein JI, Netto GJ, et al. Biopsy interpretation of the prostate. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.
27. Humphrey PA. Histological variants of prostatic carcinoma and their significance. *Histopathology.* 2012; 60:59–74. [PubMed: 22212078]
28. Beltran H, Rubin MA. New strategies in prostate cancer: translating genomics into the clinic. *Clin Cancer Res.* 2013; 19:517–523. [PubMed: 23248095]
29. Rubin MA, Maher CA, Chinnaiyan AM. Common gene rearrangements in prostate cancer. *J Clin Oncol.* 2011; 29:3659–3668. [PubMed: 21859993]
30. Tomlins SA, Bjartell A, Chinnaiyan AM, et al. ETS Gene Fusions in Prostate Cancer: From Discovery to Daily Clinical Practice. *Eur Urol.* 2009; 56:275–286. [PubMed: 19409690]
31. Pettersson A, Graff RE, Bauer SR, et al. The TMPRSS2:ERG Rearrangement, ERG Expression, and Prostate Cancer Outcomes: a Cohort Study and Meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2012
32. Furusato B, Tan SH, Young D, et al. ERG oncoprotein expression in prostate cancer: clonal progression of ERG-positive tumor cells and potential for ERG-based stratification. *Prostate Cancer Prostatic Dis.* 2010; 13:228–237. [PubMed: 20585344]
33. Park K, Tomlins SA, Mudaliar KM, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. *Neoplasia.* 2010; 12:590–598. [PubMed: 20651988]
34. Tomlins SA, Rhodes DR, Yu J, et al. The role of SPINK1 in ETS rearrangement-negative prostate cancers. *Cancer cell.* 2008; 13:519–528. [PubMed: 18538735]
35. Bhalla R, Kunju LP, Tomlins SA, et al. Novel dual-color immunohistochemical methods for detecting ERG-PTEN and ERG-SPINK1 status in prostate carcinoma. *Mod Pathol.* 2013
36. Lippolis G, Edsjo A, Stenman UH, et al. A high-density tissue microarray from patients with clinically localized prostate cancer reveals ERG and TATI exclusivity in tumor cells. *Prostate Cancer Prostatic Dis.* 2013
37. Hollenbeck BK, Bassily N, Wei JT, et al. Whole mounted radical prostatectomy specimens do not increase detection of adverse pathological features. *J Urol.* 2000; 164:1583–1586. [PubMed: 11025709]
38. Young A, Palanisamy N, Siddiqui J, et al. Correlation of Urine TMPRSS2:ERG and PCA3 to ERG + and Total Prostate Cancer Burden. *Am J Clin Pathol.* 2012; 138:685–696. [PubMed: 23086769]
39. Siu W, Dunn RL, Shah RB, et al. Use of extended pattern technique for initial prostate biopsy. *J Urol.* 2005; 174:505–509. [PubMed: 16006881]
40. Epstein JI, Allsbrook WC Jr, Amin MB, et al. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. *Am J Surg Pathol.* 2005; 29:1228–1242. [PubMed: 16096414]

41. Humphrey PA, Kaleem Z, Swanson PE, et al. Pseudohyperplastic prostatic adenocarcinoma. *Am J Surg Pathol.* 1998; 22:1239–1246. [PubMed: 9777986]
42. Levi AW, Epstein JI. Pseudohyperplastic prostatic adenocarcinoma on needle biopsy and simple prostatectomy. *Am J Surg Pathol.* 2000; 24:1039–1046. [PubMed: 10935644]
43. Mosquera JM, Perner S, Demichelis F, et al. Morphological features of TMPRSS2-ERG gene fusion prostate cancer. *J Pathol.* 2007; 212:91–101. [PubMed: 17385188]
44. Tomlins SA, Palanisamy N, Siddiqui J, et al. Antibody-Based Detection of ERG Rearrangements in Prostate Core Biopsies, Including Diagnostically Challenging Cases: ERG Staining in Prostate Core Biopsies. *Arch Pathol Lab Med.* 2012; 136:935–946. [PubMed: 22849743]
45. Osunkoya AO, Hansel DE, Sun X, et al. Aberrant diffuse expression of p63 in adenocarcinoma of the prostate on needle biopsy and radical prostatectomy: report of 21 cases. *Am J Surg Pathol.* 2008; 32:461–467. [PubMed: 18300803]
46. Giannico G, Ross H, Lotan T, et al. Adenocarcinoma of the prostate with aberrant expression of p63: a radical prostatectomy study. *Mod Pathol.* 2013; 26:212A.
47. Wu AJ, Kunju LP. Prostate Cancer with Aberrant Diffuse p63 Expression: Report of a Case and Review of the Literature and Morphologic Mimics. *Arch Pathol Lab Med.* In Press.
48. Han B, Mehra R, Suleman K, et al. Characterization of ETS gene aberrations in select histologic variants of prostate carcinoma. *Mod Pathol.* 2009; 22:1176–1185. [PubMed: 19465903]
49. Lotan TL, Gupta NS, Wang W, et al. ERG gene rearrangements are common in prostatic small cell carcinomas. *Mod Pathol.* 2011; 24:820–828. [PubMed: 21336263]
50. Scheble VJ, Braun M, Wilbertz T, et al. ERG rearrangement in small cell prostatic and lung cancer. *Histopathology.* 2010; 56:937–943. [PubMed: 20636794]
51. Braun M, Scheble VJ, Menon R, et al. Relevance of cohort design for studying the frequency of the ERG rearrangement in prostate cancer. *Histopathology.* 2011; 58:1028–1036. [PubMed: 21707704]
52. Falzarano SM, Navas M, Simmerman K, et al. ERG rearrangement is present in a subset of transition zone prostatic tumors. *Mod Pathol.* 2010; 23:1499–1506. [PubMed: 20693982]
53. Guo CC, Zuo G, Cao D, et al. Prostate cancer of transition zone origin lacks TMPRSS2-ERG gene fusion. *Mod Pathol.* 2009; 22:866–871. [PubMed: 19396154]
54. Liu S, Yoshimoto M, Trpkov K, et al. Detection of ERG gene rearrangements and PTEN deletions in unsuspected prostate cancer of the transition zone. *Cancer biology & therapy.* 2011; 11:562–566. [PubMed: 21178509]
55. Magi-Galluzzi C, Tsusuki T, Elson P, et al. TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of caucasian, african-american and japanese patients. *Prostate.* 2010
56. Mao X, Yu Y, Boyd LK, et al. Distinct genomic alterations in prostate cancers in Chinese and Western populations suggest alternative pathways of prostate carcinogenesis. *Cancer Res.* 2010; 70:5207–5212. [PubMed: 20516122]
57. Weischenfeldt J, Simon R, Feuerbach L, et al. Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. *Cancer cell.* 2013; 23:159–170. [PubMed: 23410972]
58. Barbieri CE, Baca SC, Lawrence MS, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat Genet.* 2012; 44:685–689. [PubMed: 22610119]
59. Grasso CS, Wu YM, Robinson DR, et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature.* 2012; 487:239–243. [PubMed: 22722839]
60. Baca SC, Prandi D, Lawrence MS, et al. Punctuated evolution of prostate cancer genomes. *Cell.* 2013; 153:666–677. [PubMed: 23622249]
61. Grupp K, Diebel F, Sirma H, et al. SPINK1 expression is tightly linked to 6q15- and 5q21-deleted ERG-fusion negative prostate cancers but unrelated to PSA recurrence. *Prostate.* 2013
62. Minner S, Gartner M, Freudenthaler F, et al. Marked heterogeneity of ERG expression in large primary prostate cancers. *Mod Pathol.* 2013; 26:106–116. [PubMed: 22899295]

Figure 1. Prostate adenocarcinoma with pseudohyperplastic features occurs frequently in cancers arising in *HOXB13* G84E carriers

Morphologic assessment of all cancer foci arising in our cohort of *HOXB13* G84E cases showed prevalent features previously associated with the pseudohyperplastic variant of prostatic adenocarcinoma. A subset of cases showed the (more specific) architectural features of this variant, including irregular/tufted luminal borders and cystically dilated glands with papillary infoldings, which involved the dominant focus in over half of cases (representative focus pictured in **A.**, 4X, H&E, and **B.** 4X, basal cell cocktail). The less specific cytologic features of pseudohyperplastic carcinoma, including tall/columnar epithelium, pale to granular cytoplasm, and basally oriented nucleoli were even more prevalent. Pink amorphous luminal secretions were frequent (**C.**, 40X, H&E), as were pink crystalloids (**D.**, 40X, H&E), each present in a cancer focus in >85% of cases.

Figure 2. ERG and SPINK1 expression patterns are similar in *HOXB13* G84E cases to genetically unselected cases

In in ERG⁺ foci of adenocarcinoma (A., 10x, H&E), the staining pattern was of strong, diffuse, uniform nuclear positivity (B., 10X, ERG immunostain with DAB/brown chromogen), similar to previous reports using this clone/staining protocol^{35, 38, 44}. An asterisk shows an endothelial-lined lymphovascular space, with staining of internal positive control endothelial cells. In SPINK1⁺ foci (C., 10X, H&E), the staining pattern was of cytoplasmic positivity, frequently decorating glands at the periphery or invasive front, and heterogeneous (D., 10X, SPINK1 immunostain, red chromogen).

Figure 3. Dual ERG/SPINK1 immunostaining on whole sections identifies clonally independent cancer foci that are morphologically indistinguishable

Several dominant foci of *HOXB13* G84E cases were identified as “collision tumors” of clonally independent adenocarcinomas using ERG/SPINK1 dual immunostain for molecular subtyping (5 of 23 cases, 22%). The polyclonal nature of these foci was not otherwise distinguishable on morphologic grounds (representative examples: **A.** & **C.**, 4X, H&E). As is evident on dual ERG/SPINK1 immunostain, such cases included collisions of ERG⁺/SPINK1⁻ and ERG⁻/SPINK1⁺ clones (**B.**, 4X) and collisions of ERG⁺/SPINK1⁻ and ERG⁻/SPINK1⁻ clones (**D.**, 4X). ERG immunostain with DAB/brown chromogen; SPINK1 immunostain with red chromogen.

Figure 4. Unique immunomorphologic observations in *HOXB13* G84E cases

One case with conventional acinar adenocarcinoma morphology (A., 10X, H&E) showed a dominant focus that was ERG⁺/SPINK1⁺ (B., 10X, ERG/SPINK1 dual immunostain, ERG in DAB, SPINK1 red chromogen), a phenomenon previously identified in only one of nearly 4500 genetically unselected cases^{35, 36}. In another case, apparent glandular fusion between independent ERG⁺/SPINK1⁻ and ERG⁻/SPINK1⁺ clones was seen in a collision focus (C., 20X, H&E); individual cells within the same gland showed either nuclear positivity for ERG (DAB/brown) or cytoplasmic positivity for SPINK1 (red chromogen), without any dual

positive cells (**D.**, 20X, ERG/SPINK1 dual immunostain). In a single case, the dominant focus (ERG⁻/SPINK1⁻, **E.**, 20X, H&E) showed aberrant diffuse nuclear expression of p63 (**F.**, 20X, basal cell cocktail), a rare immunophenotype reported previously⁴⁵. Within the same section, this case also showed a separate focus of acinar adenocarcinoma with a number of glands showing a PIN-like morphology (**G.**, H&E, **H.**, basal cell cocktail, both 10X). This focus was ERG⁺/SPINK1⁻ (**I.**, 10X, ERG immunostain).

Figure 5. Prevalence of prostate cancer molecular subtypes in *HOXB13* G84E carriers compared to other reported cohorts

A. Among the *HOXB13* G84E-related cohort described in this study (first panel), we found significantly lower prevalence of ERG⁺ dominant foci (outer rings, pie charts) and total ERG⁺ foci among all mapped foci (inner rings, pie charts) than in our recently reported³⁸, unselected Cohort 2 (second panel). Lower prevalence of ERG⁺ dominant foci among *HOXB13* cases was also noted as compared to our recently reported³⁵, unselected Cohort 3, where only the dominant nodule was evaluated (third panel, outer ring of pie chart). **B.** In contrast, among the *HOXB13* G84E-related cohort (first panel), we found a significantly higher prevalence of SPINK1⁺ dominant foci (outer rings, pie charts) as compared to unselected Cohort 3 where only the dominant nodule was evaluated (second panel, outer ring of pie chart). All P-values are for two-tailed Fisher Exact tests comparing the *HOXB13* G84E-related cohort to Cohorts 2 or 3.

Table 1
Clinical, pathological and molecular features of prostate cancers arising in HOXB13 G84E variant carriers

Subject #	Age (years)	PSA (ng/mL)	Surgery ¹	Stage ²	Follow up ³	Gleason Score	Dominant Cancer Focus				All Cancer Foci				
							Size ⁴	Location ⁵	PH cyto ⁶	PH arch ⁷	Molecular Subtype ⁸	Neg (#)	ERG ⁺ (#)	SPINK1 ⁺ (#)	Total (#)
21	59	4.7	RRP	pT2c	52	treated (3+4)	1.1	TZ	treated	treated	Neg	3	0	0	3
12	40	4.9	RRP	pT2c	39	3+3	1.2	PZ	1	1	Neg	8	0	0	8
22	40	3.1	RRP	pT2c	177	3+3	1.5	PZ	1	1	Neg	3	0	0	3
11	60	4.9	RRP	pT2c+	131	3+4	1.1	PZ	1	1	Neg	8	3	2	13
20	68	3.2	RALP	pT2c	46	3+4	2.2	PZ	1	1	Neg	3	3	0	6
13	58	7.1	RRP	pT2c	12	4+3	0.8	PZ	1	1	Neg	12	0	1	13
15	54	7.4	RRP	pT2c	10	4+3	1.2	PZ	1	1	Neg	7	4	3	14
4	51	4.5	RALP	pT2c	38	3+4	1.2	PZ	1	1	SPINK1 ⁺	1	2	3	6
8	58	4.6	RALP	pT2c	0	3+4	0.4	PZ	1	1	SPINK1 ⁺	6	0	5	11
14	61	7.3	RRP	pT2c	137	4+3	2.2	PZ	1	1	SPINK1 ⁺	5	2	2	9
17	57	5.6	RALP	pT2c	2	3+4	3.2	PZ	1	0	Neg	6	1	0	7
1	64	6.4	RALP	pT2a-b	7	3+3	1.4	TZ	0	0	ERG ⁺	0	1	0	1
23	64	8.4	RRP	pT2c	115	3+3	1	PZ	0	0	ERG ⁺	5	1	0	6
9	42	7.4	RRP	pT2c	132	3+4	1.5	PZ	0	0	ERG ⁺	3	3	1	7
10	64	5.2	RALP	pT2c	27	3+4	1.1	PZ	0	0	ERG ⁺	3	2	0	5
2	53	6.5	RRP	pT2c	36	4+3	2.2	PZ	0	0	ERG ⁺ /SPINK1 ⁺	1	0	1	3
7	51	5.7	RRP	pT2c	17	3+3	0.3	PZ	0	0	Neg	7	0	0	7
16	58	18.2	RRP	pT2c	18	3+4	1.1	PZ	0	0	Neg	5	0	0	5
19	43	5.1	RALP	pT3a	37	3+4	1.4	PZ	0	0	Neg	3	1	0	4
5	59	4.6	RALP	pT3a	22	4+5 ⁸	1.4	PZ	0	0	Neg, p63+	2	1	1	4
6	53	14.0	RALP	pT2c+	16	4+3	1.1	PZ	0	0	SPINK1 ⁺	3	1	2	6
3	63	12.6	RALP	pT2a-b	34	4+4	1.9	PZ	0	0	SPINK1 ⁺	1	0	2	3
18	57	30.2	RRP	pT3b+	55 [*]	4+5	1.4	TZ	0	0	SPINK1 ⁺	0	0	1	1

¹ RRP, radical retropubic prostatectomy; RALP, robot-assisted laparoscopic prostatectomy.

² All patients were N0Mx or NxMx.

³ Follow-up in months.

* Patient experienced biochemical recurrence.

⁴ Greatest linear dimension of the dominant focus.

⁵ Primary location of tumor in peripheral zone (PZ) or transition zone (TZ).

⁶ Cytologic features of pseudohyperplastic (PH) carcinoma were present (1) or absent (0).

⁷ Architectural features of pseudohyperplastic (PH) carcinoma were present (1) or absent (0).

⁸ Molecular subtype by immunohistochemistry for ERG and SPINK1. Foci negative (Neg) for both ERG and SPINK1 are indicated.

⁸ Gleason score included for descriptive purposes only, as appropriateness of Gleason score for p63+ cancers remains controversial.