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SPINK1 Protein Expression and Prostate Cancer Progression

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Abstract

Purpose: SPINK1 overexpression has been described in prostate cancer and is linked with poor prognosis in many cancers. The objective of this study was to characterize the association between SPINK1 overexpression and prostate cancer–specific survival.

Experimental Design: The study included 879 participants in the U.S. Physicians' Health Study and Health Professionals Follow-Up Study, diagnosed with prostate cancer (1983–2004) and treated by radical prostatectomy. Protein tumor expression of SPINK1 was evaluated by immunohistochemistry on tumor tissue microarrays.

Results: Seventy-four of 879 (8%) prostate cancer tumors were SPINK1 positive. Immunohistochemical data were available for PTEN, p-Akt, pS6, stathmin, androgen receptor (AR), and ERG (as a measure of the TMPRSS2:ERG translocation). Compared with SPINK1-negative tumors, SPINK1-positive tumors showed higher PTEN and stathmin expression, and lower expression of AR ($P < 0.01$). SPINK1 overexpression was seen in 47 of 427 (11%) ERG-negative samples and in 19 of 427 (4%) ERG-positive cases ($P = 0.0003$). We found no significant associations between SPINK1 status and Gleason grade or tumor stage. There was no association between SPINK1 expression and biochemical recurrence ($P = 0.56$). Moreover, there was no association between SPINK1 expression and prostate cancer mortality (there were 75 lethal cases of prostate cancer during a mean of 13.5 years follow-up; HR = 0.71; 95% confidence interval, 0.29–1.76).

Conclusions: Our results suggest that SPINK1 protein expression may not be a predictor of recurrence or lethal prostate cancer amongst men treated by radical prostatectomy. SPINK1 and ERG protein expression do not seem to be entirely mutually exclusive, as some previous studies have suggested. *Clin Cancer Res; 20(18); 4904–11. ©2014 AACR.*

Introduction

SPINK1 encodes for a 56 amino acid peptide which is secreted in the prostate gland and whose function is to inhibit serine proteases such as trypsin (1). Recently, SPINK1 was identified in a meta-analysis as having outlier expression in ETS rearrangement negative prostate cancers, and results indicated that SPINK1 was expressed exclusively in TMPRSS2:ERG-negative prostate cancers (2). These data suggested that SPINK1 overexpression may represent a distinct prostate cancer subtype. Moreover, SPINK1 overexpression has been retrospectively associated with an increased risk of disease progression and biochemical recurrence in hormonally and surgically treated prostate cancer cohorts (2, 3). Ateeq and colleagues demonstrated that SPINK1-positive cancers may potentially be targeted therapeutically through humanized SPINK-1 directed monoclonal antibodies and EGF receptor (EGFR) inhibition (4). No study to date, however, has addressed the association between SPINK1 overexpression and prostate cancer–specific survival in patients treated by radical prostatectomy.

Materials and Methods

This study was based upon the analysis of men treated with radical prostatectomy for prostate cancer who were participants in the Physicians' Health Study and Health Professionals Follow-up Study, and included 879 prostate

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Translational Relevance

SPINK1 overexpression has been described in prostate cancer and is linked with poor prognosis in many cancers. The objective of this study was to characterize the association between SPINK1 overexpression and prostate cancer–specific survival. The study included 879 participants in the U.S. Physicians' Health Study and Health Professionals Follow-Up Study, diagnosed with prostate cancer (1983–2004) and treated by radical prostatectomy. Immunohistochemical data were available for SPINK1, PTEN, p-Akt, pS6, stathmin, androgen receptor, and ERG. Our results suggest that SPINK1 protein expression may not be a predictor of recurrence or lethal prostate cancer amongst men treated by radical prostatectomy. SPINK1 and ERG protein expression do not seem to be entirely mutually exclusive, as some previous studies have suggested.

cancer cases, diagnosed between 1983 and 2004, on whom archival formalin-fixed, paraffin-embedded tumor tissue specimens were available (5, 6). Tumor tissue from radical prostatectomies was reviewed by our pathology team to provide uniform evaluation of Gleason score and to identify areas of high-density tumor for construction of tumor tissue microarrays (TMA). At least three tumor cores (0.6 mm) were sampled from each case (three cores were taken at a minimum from the same dominant tumor nodule with the highest Gleason score).

Immunohistochemistry was performed on 4 to 5 μm sections of the TMAs to assess protein expression of SPINK1 (mouse monoclonal, 1:100 dilution; H00006690-M01, Abnova), ERG (rabbit monoclonal, 1:200 dilution; EPR3864, Epitomics Inc.), PTEN (rabbit polyclonal, 1:200 dilution; PN37, Zymed Laboratories), p-AKT (rabbit monoclonal, 1:50 dilution, D9E; Cell Signaling Technology), pS6 (rabbit monoclonal, 1:50 dilution, Ser240/Ser244; Cell Signaling Technology), stathmin (rabbit polyclonal, 1:50 dilution, Cell Signaling Technology), Androgen receptor (AR, rabbit polyclonal, 1:50 dilution; PG-21, Upstate Cell Signaling), and cell proliferation marker Ki-67 (rabbit polyclonal, 1:2000 dilution; Vector Laboratories). To validate concomitant ERG/SPINK1 staining, a dual stain for ERG (rabbit monoclonal, 1:1000 dilution, EPR3864, Abcam) and SPINK1 (1:50 dilution) was performed on three whole tumor sections and the percentage of prostate tumor glands that coexpress both proteins semiquantified. Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay was performed on 5 μm sections of the TMAs to identify the percentage of tumor cells undergoing apoptosis using the Apoptag Peroxidase In-Situ kit (Chemicon International; ref. 7).

SPINK1 and ERG expressions were classified as positive or negative by study pathologists as previously described (refs. 2, 8; Fig. 1). Cases with SPINK1 staining in any cancerous epithelial cells were deemed SPINK1 positive (2). A case was called ERG positive if at least one core from an individual case had positive ERG staining observed within prostate cancer epithelial cells. For all cases, the presence of ERG staining in the vasculature endothelium

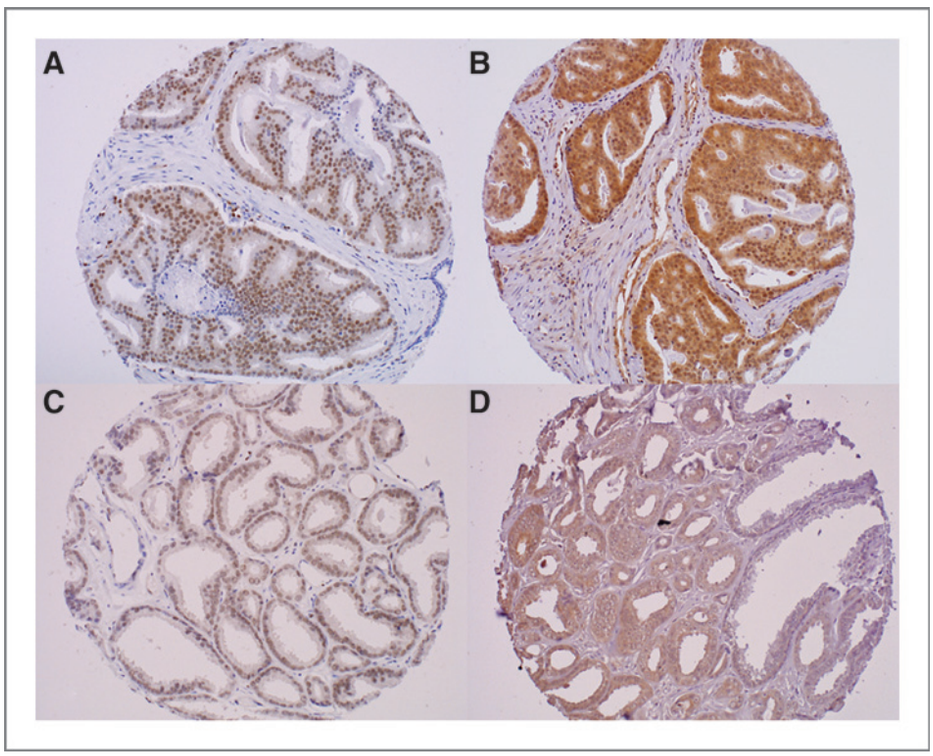

Figure 1. Immunohistochemistry for nuclear marker ERG (left) and cytoplasmic marker SPINK1 (right) showing diffuse positive staining in the exact same tumor cores from a case of prostate adenocarcinoma Gleason score 4 + 4 (A and B; ×20) and from a case of prostate adenocarcinoma Gleason score 3 + 3 (C and D; ×20).

served as a positive internal control, and assessment of ERG expression was restricted to cores in which the positive internal control was observed (8). In addition, each TMA contained internal controls including duplicate cores and normal prostate. All cases were double-read by a study pathologist to validate initial scores and noninformative cases were eliminated from the downstream analysis. Prior studies have shown that ERG overexpression is highly concordant with ERG rearrangement status as assessed by FISH (9, 10) and quantitative PCR (11). Expression of PTEN, p-AKT, pS6, stathmin, AR, and Ki-67 was quantified using the Ariol instrument SL-50 image analysis software (Applied Imaging) and results validated by manual quantification of scores in an estimated 5% of all tissue cores. Semiautomated assessment of staining intensity (scale: 0–255) and percent staining (scale: 0%–100%) was performed using the MultiStain assay. The mean percent staining across cores was used as a measure of PTEN, pAkt, pS6, and stathmin expression. The mean nuclear staining intensity across cores was used as a measure of AR expression. Ki-67 proliferation index was defined as the number of stained nuclei over the total number of tumor nuclei. For TUNEL, the Apoptag sum was calculated as the number of positive cells out of the total number of tumor cells. The whole area of each tumor TMA core was evaluated for the sum. Areas of tumor were manually identified with masking of the stroma and normal/benign glands from image analysis as previously described (12).

Information on tumor stage, prostate-specific antigen (PSA) level at diagnosis, and treatments were abstracted from medical records and pathology reports. Since 2000, newly diagnosed patients with prostate cancer have been followed for biochemical recurrence and development of metastatic disease via mailed questionnaires. For men with prostate cancer in the Health Professionals Follow-up Study, their treating physicians were contacted to collect information about their clinical course and to confirm development of metastases. For men with prostate cancer in the Physicians' Health Study, self-report of metastases by these physician participants was virtually always confirmed when records were available (among 80% of Physicians' Health Study cases), so all metastatic cases were included as outcomes. Biochemical recurrence was participant reported, reported by the treating physician, or abstracted from medical records; defined as PSA above 0.2 ng/mL postsurgery sustained over two measures when abstracted from medical records. Cause of death is assigned following a centralized review of medical records and death certificates by study physicians. Follow-up for mortality is more than 95% in both cohorts (in the Physicians' Health Study mortality follow-up is more than 99%).

We included men who had undergone radical prostatectomy and on whom we had SPINK1 status available ($N = 879$; 364 men from the Physicians' Health Study and 515 men from the Health Professionals Follow-up Study). We investigated whether age at diagnosis and follow-up time differed by SPINK1 status using *t* tests. To test associations with Gleason score and pathologic tumor stage, we used χ^2 tests or Cochrane–Armitage trend tests. The association between SPINK1 status and PSA level at diagnosis, and between SPINK1 status and expression of Ki-67, TUNEL, PTEN, pAKT, pS6, stathmin, and AR, was tested using Wilcoxon rank-sum tests.

We used Cox proportional hazards models to calculate HRs and 95% confidence intervals (CI) of the association between SPINK1 status and disease progression. Prostate cancer progression was defined as (i) time to lethal prostate cancer, defined as development of distant metastases or prostate cancer death and (ii) time to biochemical recurrence. Men who did not report a PSA rise but who reported lymph node metastases, distant metastases, or who died of prostate cancer were assigned a biochemical recurrence on the earliest date of any of these events. Men in the cohort were followed from the date of prostate cancer diagnosis until they experienced outcomes, until they were censored at death from other causes, or at end of follow-up, whichever occurred first. Follow-up for death extended through March 2011 for the Physicians' Health Study and December 2011 for the Health Professionals Follow-up Study. In both cohorts, follow-up for prostate cancer recurrence and metastases ended approximately 2 years before follow-up for death due to questionnaire timing. Men with missing information on pathologic tumor stage ($n = 32$) were assigned a missing indicator variable. We also conducted multivariable analyses limited to men with known tumor stage ($n = 847$). The study was approved by the Institutional Review Boards at the Harvard School of Public Health and Partners Healthcare (Boston, MA).

Results

Data on both SPINK1 and ERG expression were available for 854 men, SPINK1 and Ki-67 expression for 778 men, SPINK1 and TUNEL expression for 675 men, SPINK1 and PTEN expression for 761 men, SPINK1 and p-AKT expression for 741 men, SPINK1 and pS6 expression for 746 men, SPINK1 and stathmin expression for 743 men, and SPINK1 and AR expression for 802 men. The mean age at diagnosis was 65.4 years. The mean follow-up time was 13.5 years. In total, 75 men developed lethal prostate cancer, 213 men developed biochemical recurrence, and 260 men died of any cause during follow-up.

Table 1 presents clinical characteristics amongst the men with prostate cancer overall, as well as stratified by SPINK1 status. Eight percent of the men in the cohort had SPINK1-positive tumors. We found no significant associations between SPINK1 status and clinicopathologic features including cell proliferation marker Ki-67 and TUNEL (apoptotic marker). There was no significant association between SPINK1 status and biochemical recurrence or lethal prostate cancer (Table 2). These results did not vary significantly by cohort, and did not materially change when we restricted the multivariate analyses to men with known pathologic stage.

Expression of PTEN, stathmin, and AR differed significantly according to SPINK1 status. Compared with SPINK1-

Table 1. Clinical characteristics for all men and by SPINK1 expression status among 879[a] men treated with radical prostatectomy for prostate cancer, Physicians' Health Study and Health Professionals Follow-up Study cohorts

Characteristic	All men	SPINK1 negative	SPINK1 positive	P^b
Number	879	805	74	—
Mean follow-up time, y (SD)	13.5 (4.6)	13.4 (4.6)	13.6 (4.5)	0.78
Mean age at diagnosis, y (SD)	65.4 (6.0)	65.4 (6.0)	65.0 (6.2)	0.54
Median PSA at diagnosis, ng/mL (IQR)	7.0 (5.6)	7.0 (5.6)	7.0 (7.5)	0.88
Tumor stage, *n* (%)				—
pT2 N0/Nx	599 (71)	547 (71)	52 (71)	—
pT3 N0/Nx	222 (26)	204 (26)	18 (25)	—
pT4/N1/M1	26 (3)	23 (3)	3 (4)	1.00
Gleason score, *n* (%)				
2–6	185 (21)	173 (21)	12 (16)	—
3 + 4	325 (37)	298 (37)	27 (36)	—
4 + 3	214 (24)	194 (24)	20 (27)	—
8–10	155 (18)	140 (17)	15 (20)	0.25
Lethal prostate cancer[c], *n* (%)				
No	804 (91)	735 (91)	69 (93)	—
Yes	75 (9)	70 (9)	5 (7)	0.57
Biochemical recurrence, *n* (%)				
No	666 (76)	612 (76)	54 (73)	—
Yes	213 (24)	193 (24)	20 (27)	0.56
All cause mortality[d], *n* (%)				
No	619 (70)	568 (71)	51 (69)	—
Yes	260 (30)	237 (29)	23 (31)	0.77
Ki-67 expression, median (IQR)[e]	0.12 (0.45)	0.12 (0.45)	0.11 (0.39)	0.70
TUNEL, median (IQR)[f]	0.5 (2.0)	0.5 (2.0)	0.5 (2.0)	0.11
ERG negative, *n*	427	380	47	—
ERG positive, *n*	427	408	19	0.0003

Abbreviation: IQR, interquartile range.

[a]Numbers may not add up to 879 because men with missing information for a characteristic are not included in that characteristic.

[b]*P* values are based on the following tests: *t* test for follow-up time and age at diagnosis; Wilcoxon rank-sum test for PSA at diagnosis, Ki-67 expression, and TUNEL; exact Cochran–Armitage trend test for tumor stage, Cochran–Armitage trend test for Gleason sum; χ^2 test for lethal prostate cancer, biochemical recurrence, all-cause mortality, and ERG status.

[c]Lethal prostate cancer includes metastases to distant organs, and prostate cancer death.

[d]All-cause mortality includes prostate cancer death and death due to any other cause.

[e]Ki-67 expression was available for 778 men with known SPINK1 status.

[f]TUNEL was available for 675 men with known SPINK1 status.

negative tumors, SPINK1-positive tumors showed higher PTEN and stathmin expression ($P < 0.01$), and lower expression of AR ($P < 0.01$; Table 3). There was no significant association between SPINK1 status and p-AKT expression ($P = 0.22$) or pS6 expression ($P = 0.23$). SPINK1 expression was seen in 47 of 427 (11%) ERG-negative samples and in 19 of 427 (4%) ERG-positive cases ($P = 0.0003$; via χ^2 test). In 12 of the 19 (63%) cases staining positive for both SPINK1 and ERG, dual SPINK1/ERG staining was seen in at least two of three same tumor cores (Table 4). Concomitant ERG/SPINK1 staining was validated on three whole tumor sections using a dual stain for ERG and SPINK1 (Fig. 2). In two of these three cases, the percentage of prostate tumor glands that coexpressed both proteins was less than 5% of the overall tumor volume in that nodule; in the third case, it was less than 10%.

Discussion

Overexpression of SPINK1 has been associated with prognosis in many cancers. Initial work in ETS rearrangement negative prostate cancers indicated that SPINK1-positive prostate cancers were a distinct cancer subtype with an aggressive phenotype (2). In this study, we used the same criteria and antibody procedures as Tomlins and colleagues (2) to define SPINK1 positivity, but we found no positive association between SPINK1 status and clinicopathologic factors or prostate cancer–specific survival, despite the frequency of SPINK1-positive tumors in our cohort being

Table 2. HRs and 95% CI for prostate cancer recurrence and death by SPINK1 expression status among 879 men treated with radical prostatectomy for prostate cancer, Physicians' Health Study and Health Professionals Follow-up Study cohorts

	Reduced model[a]		Full model[b]	
Characteristic	**HR**	**95% CI**	**HR**	**95% CI**
Lethal prostate cancer ($n = 75$)[c]				
SPINK1−	1.00	—	1.00	—
SPINK1+	0.71	(0.29–1.76)	0.60	(0.24–1.50)
Biochemical recurrence ($n = 213$)				
SPINK1−	1.00	—	1.00	—
SPINK1+	1.01	(0.63–1.60)	1.03	(0.65–1.65)

[a]Adjusted for age at diagnosis (<60, 60–64, 65–69, 70+), and cohort (Physicians' Health Study and Health Professionals Follow-up Study).

[b]Adjusted for age at diagnosis (<60, 60–64, 65–69, 70+), cohort (Physicians' Health Study and Health Professionals Follow-up Study), tumor stage (pT2 N0/NX, pT3 N0/NX, pT4/N1/M1, unknown), and Gleason score ($\leq$6, 3 + 4, 4 + 3, $\geq$8).

[c]Lethal prostate cancer includes metastases to distant organs, and prostate cancer death.

similar to what has been previously published (8%; refs. 2, 3). Leinonen and colleagues investigated the association between SPINK1 status and clinicopathologic factors and progression-free survival among 186 men primarily treated hormonally for prostate cancer (3). They observed no statistically significant associations between SPINK1 status and Gleason score, clinical stage, or Ki-67 expression, but observed a significantly shorter progression-free survival amongst men with SPINK1 positive compared with SPINK1-negative tumors (RR 2.3; 95% CI, 1.1–4.6). In the Lippolis and colleagues' study, including 3,385 men treated with radical prostatectomy for prostate cancer, SPINK1 was weakly associated with pathologic tumor stage, but otherwise the findings were consistent with the current report with no association between SPINK1 positivity and biochemical recurrence or development of metastatic disease (13). Grupp and colleagues found no association between SPINK1 status and clinicopathologic factors or biochemical recurrence among more than 8,000 (presumably including the 3385 men in the Lippolis study) surgically treated prostate cancer patients. Taken together, these data suggest that SPINK1 protein expression is not a strong predictor of biochemical recurrence or lethal prostate cancer amongst men treated by radical prostatectomy.

Recent work by Ateeq and colleagues suggests that the phosphoinositide 3-kinase (PI3K) pathway is one of the few key signaling pathways downstream of the SPINK1-EGFR axis. As such we looked at the associations between SPINK1 status and pAKT, pS6, stathmin, and PTEN expression (4). We found higher expression of wild-type PTEN ($P < 0.01$), stathmin ($P < 0.01$), and pAKT ($P = 0.22$) in SPINK1 positive compared with negative tumors, though the latter association was not statistically significant. Higher levels of pAKT and stathmin may indicate that there is activation of the PI3K pathway in SPINK1-overexpressing tumors. PTEN deletion is associated with subsequent activation of the PI3K pathway, which promotes cell proliferation, survival, and other cellular pathways (14, 15). These data suggest that any interaction of SPINK1 with the PI3K pathway seems to be downstream of PTEN. Indeed, a recent small study of 59

Table 3. Tumor protein expression of Ki-67, TUNEL, PTEN, pAKT, pS6, stathmin, and AR by SPINK1 protein expression status among men treated with radical prostatectomy for prostate cancer, Physicians' Health Study and Health Professionals Follow-up Study cohorts

Marker	**No.**	**Range**	**SPINK1 − Median (IQR)**	**SPINK1 + Median (IQR)**	***r*[a]**	***P*[b]**
PTEN	761	0–0.91	0.14 (0.22)	0.27 (0.35)	+0.17	<0.01
pAKT	741	0–0.73	0.02 (0.12)	0.03 (0.14)	+0.04	0.22
pS6	746	0–0.97	0.08 (0.24)	0.04 (0.16)	−0.06	0.09
Stathmin	743	0–0.82	0.01 (0.02)	0.02 (0.04)	+0.21	<0.01
AR	802	91–152	118 (11)	114 (11)	−0.10	<0.01

Abbreviation: IQR, interquartile range.

[a]Spearman correlation coefficient.

[b]*P* values from the Wilcoxon rank-sum test.

Table 4. Dual ERG/SPINK1-positive tumor cases among men treated with radical prostatectomy for prostate cancer, Physicians' Health Study and Health Professionals Follow-up Study cohorts

One core[a] +	Two cores[a] +	Three cores[a] +	Different replicate cores +	Total number of cases
7 (37)	6 (31.5)	6 (31.5)	0 (0)	19 (100)

[a]Exact same tumor cores.

patients with castration-resistant prostate cancer found an association between PTEN deletion and SPINK1 overexpression (16). Previous studies have shown reduced PTEN expression in tumors overexpressing ERG (17–20). If SPINK1 protein is mutually exclusive from ERG, then SPINK1 in theory could be associated with higher wild-type PTEN; further work is needed to explore this potential relationship.

SPINK1 protein is expressed in the androgen independent but androgen-responsive 22RV1 xenograft prostate cancer cell line (1), and seems to be regulated by androgens. As such we looked at the association between SPINK1 status and AR protein expression in prostate tumors. Herein, we found lower expression of AR in SPINK1-positive tumors compared with SPINK1-negative cases ($P < 0.01$). In contrast, Bismar and colleagues found no AR amplification in SPINK1-overexpressing tumors (16). A possible explanation for this discrepancy in results is that decreased levels of AR in tumor overexpressing SPINK1 reflects a compensatory AR downregulation as a result of decreased levels of one or more AR regulated genes. ERG-overexpressing tumors seem to have higher levels of AR versus tumors not overexpressing ERG (21).

Some studies suggest that SPINK1 is expressed exclusively in TMPRSS2:ERG-negative prostate cancers (2, 13, 16, 22, 23). However, additional studies have demonstrated the presence of TMPRSS2:ERG in a small but significant percentage of SPINK1-positive prostate cancers (3, 23, 24). Our results corroborate these latter studies, with SPINK1 expression being more frequent in ERG-negative (11%) than in ERG-positive cancers (4%; $P = 0.0003$). The discrepancy in study results may reflect differences in methodology used to elucidate the presence of both SPINK1 (at the protein or mRNA level) and the TMPRSS2:ERG fusion, intratumoral heterogeneity or may be related to the power of the studies in question. However, it seems that coexpression of both SPINK and ERG is a focal event in prostate tumors, and this most likely explains why previously coexpression of both markers was felt to be mutually exclusive. Interestingly, Jhavar and colleagues found that higher expression of SPINK1 mRNA was restricted to cancers that lack ERG rearrangement, but

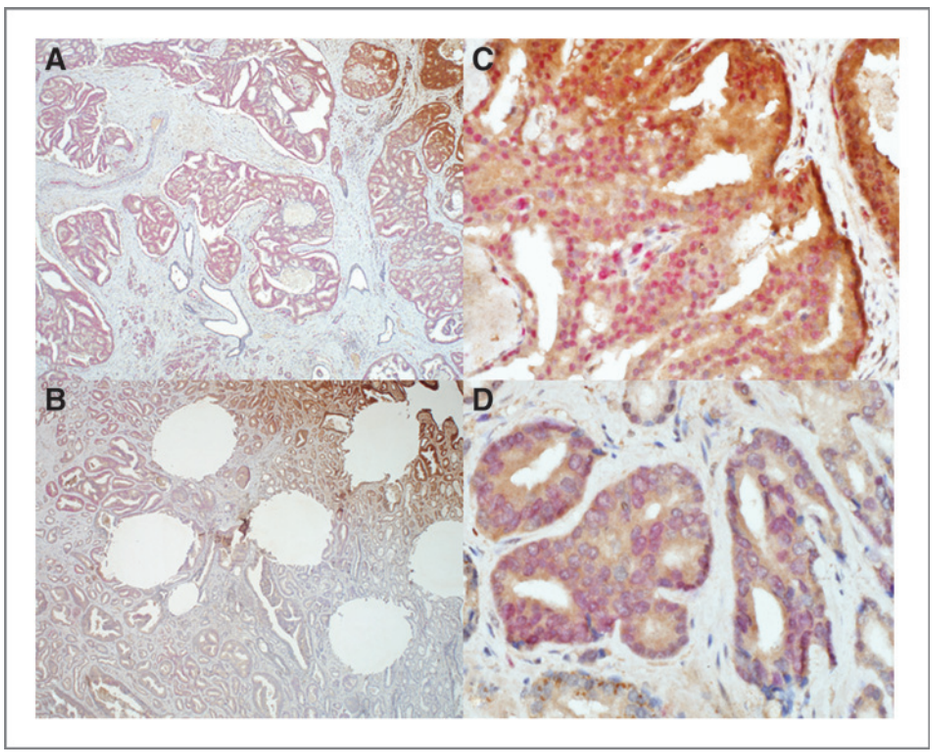

Figure 2. Dual immunohistochemical staining for nuclear marker ERG (red) and cytoplasmic marker SPINK1 (brown) showing positive staining in discrete foci of whole tumor sections from two separate cases (A and C; B and D) of prostate adenocarcinoma [×4 (left column); ×40 (right column). Note in B, there areas of dual positivity for SPINK1 and ERG (top) and areas of tumor negative for both markers (bottom).

a poor correlation was found between SPINK1 mRNA and SPINK1 protein expression, and ERG and SPINK1 were not mutually exclusive when measured at the protein level (23). In this study, we used ERG immunohistochemistry as a surrogate marker for the presence of the fusion in contrast to 2- and 3-color FISH using in several prior studies (2, 3, 23). Leinonen and colleagues found SPINK1 expression (same antibody as current study) in 12 of 110 (11%) of TMPRSS2:ERG-negative (assessed by 3-color FISH) cases and in seven of 60 (12%) TMPRSS2:ERG-positive cases from prostate needle biopsies. In 123 prostatectomy treated patients, they found SPINK1 expression in 11 of 78 (14%) of TMPRSS2:ERG-negative cases and two of 46 (4%) of TMPRSS2:ERG-positive cases. In contrast, in the study by Lippolis and colleagues ($n = 3,385$; ref. 13), ERG and SPINK1 were completely mutually exclusive, however, different scoring methodologies were used in antibody assessment and importantly only one tumor core from each patient was represented on their TMA which may fail to highlight intratumoral heterogeneity which has been observed in SPINK1 staining (22). In the study by Grupp and colleagues ($n = 8,642$), presumably including all cases in Lippolis and colleagues study and an additional 5,257 cases, SPINK1 (same antibody as current study) was almost exclusively expressed in ERG-negative cases; SPINK1 was seen in 506 of 4,861 (10.4%) ERG-negative cases and 13 of 3,781 (0.3%) ERG-positive cases. As in the study by Lippolis and colleagues, different scoring methodologies were used in antibody assessment and only one tumor core from each patient was represented on their TMA. In the Bhalla and colleagues' study, ERG and SPINK1 expressions (same antibody as current study) were essentially mutually exclusive; however, two TMA cores showed dual ERG and SPINK1 staining (2% of ERG-positive cases) albeit only one core showed concomitant expression in the same tumor focus. Interestingly, in our study, a similar but slightly higher percentage of ERG-positive cases showed concomitant SPINK1 staining, that is, 4% (22). The study by Bhalla and colleagues is a smaller cohort ($n = 284$) compared with this present study and has significant differences in terms of sample selection where metastatic tumors and rare morphologic variants were included in their cohort. Further studies are needed to assess the clinical significance of this potentially rare dual SPINK and ERG-positive molecular subtype.

In conclusion, our results suggest that SPINK1 protein expression may not be a predictor of recurrence or lethal prostate cancer amongst men treated by radical prostatectomy. In addition, SPINK1 and ERG protein overexpressions do not seem to be entirely mutually exclusive as some previous studies have suggested.

Disclosure of Potential Conflicts of Interest

L.A. Mucci is a consultant/advisory board member of Metamark genetics. No potential conflicts of interest were disclosed by the other authors.

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