Prostate Cancer SubtyPINg BiomarKers and Outcome: Is Clarity EmERGing?

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Molecular prostate cancer subtypes have been proposed on the basis of mutually exclusive SPINK1 and ERG overexpression, with conflicting reports on their prognostic ability. Flavin and colleagues report that SPINK1 is neither prognostic nor absolutely mutually exclusive with ERG, raising important questions about prostate cancer molecular subtyping and prognostic biomarker evaluation. Clin Cancer Res; 20(18); 4733–6. ©2014 AACR.

In this issue of Clinical Cancer Research, Flavin and colleagues evaluated SPINK1 expression by immunohistochemistry (IHC) in prostate cancer resection specimens from participants in the U.S. Physician’s Health Study and Health Professionals Follow-Up Study (1). Flavin and colleagues found that 8% of cases were SPINK1-positive (SPINK1\textsuperscript{+}), with no association with biochemical recurrence or prostate cancer–related mortality. Importantly, this study was performed in a large, well-annotated clinical cohort with prostate cancer–specific mortality as a definitive endpoint. As described below, we have previously proposed prostate cancer molecular subtyping based on mutually exclusive SPINK1 and ERG overexpression (2, 3). Although Flavin and colleagues again report significant exclusivity, SPINK1 expression was observed in 47 of 427 (11%) ERG-negative (ERG\textsuperscript{−}) cases and 19 of 427 (4%) ERG-positive (ERG\textsuperscript{+}) cases, the latter challenging the more absolute degree of exclusivity reported previously. Overall, this study provides helpful independent confirmation of the prevalence of these molecular groups in an exceptionally well-annotated North American cohort with follow-up; it also makes suggestive observations about cellular signaling pathways thought to be downstream of SPINK1 that may be of translational and therapeutic relevance. However, the observations about apparent incomplete mutual exclusivity between SPINK1\textsuperscript{+} and ERG\textsuperscript{+} prostate cancer raise interesting and important issues about prostate cancer subtyping, IHC, and testing prognostic biomarkers.

In 2008, through expression profiling meta-analysis, we reported that approximately 10% of prostate cancer showed marked SPINK1 overexpression (3) and that almost all SPINK1-overexpressing cases lacked ERG rearrangements. Previously, we had shown that ERG overexpression, occurring in approximately 50% of PSA-screened prostate cancer cases, was driven by chromosomal rearrangements bringing the ERG locus under the supra-physiologic transcriptional regulation of a number of genes, most androgen regulated, and most frequently TMPRSS2 (2). By combined IHC/FISH, we found that 9% of prostate cancer cases were SPINK1\textsuperscript{+}, and that these cases were exclusively ERG rearrangement negative (ERG\textsuperscript{−}; Fig. 1A; ref. 3). Hence, we proposed prostate cancer molecular subtypes defined by ERG and SPINK1 status. The subsequent identification of mutations in SPOP (SPOP\textsuperscript{mut}), which also seem to arise only in ERG\textsuperscript{−} prostate cancer, characterizes yet another molecular subtype of ERG\textsuperscript{−} prostate cancer (4), as does CHD1-deleted (CHD1\textsuperscript{del}) prostate cancer (2, 4). Interestingly, overlap of SPINK1\textsuperscript{+}, SPOP\textsuperscript{mut}, and CHD1\textsuperscript{del} has been observed; however, each alteration was discovered as mutually exclusive from ERG\textsuperscript{−} prostate cancer, supporting prostate cancer molecular subclassification and analogous to the expanding repertoire of genetically defined subtypes of other common epithelial tumors, such as lung adenocarcinoma.

Lending to the lack of clarity in the literature for biomarkers, including SPINK1 in prostate cancer, are a number of issues, which may be intrinsic to the experiment (differing techniques, analytes studied, antibodies/protocols used, and criteria and thresholds for “positivity”), the cohort (demography, differences in treatment, follow-up protocols), or even the disease studied (for prostate cancer, the remarkable degree of multifocality as described below). About technical issues, previous studies in contemporary PSA-screened Caucasian cohorts have consistently shown that approximately 10% of prostate cancers show marked SPINK1 transcript overexpression (3, 5). In keeping with these reports, in Fig. 1B, we show ERG and SPINK1.
Table A:

<table>
<thead>
<tr>
<th>Study</th>
<th>Cohort</th>
<th>SPINK1 antibody</th>
<th>SPINK1* (%)</th>
<th>ERG evaluation</th>
<th>ERG*/SPINK1*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavin et al. (1)</td>
<td>RP</td>
<td>4D4</td>
<td>66/854 (8%)</td>
<td>IHC/FISH</td>
<td>19/854 (2%)</td>
</tr>
<tr>
<td>Bhalla et al. (8)</td>
<td>RP, CRPC, XRT</td>
<td>4D4</td>
<td>26/284 (9%)</td>
<td>Dual IHC</td>
<td>1/284 (0.4%)</td>
</tr>
<tr>
<td>Bjarnar et al. (13)</td>
<td>CRPC</td>
<td>4D4</td>
<td>3/51 (6%)</td>
<td>FISH</td>
<td>0/51 (0%)</td>
</tr>
<tr>
<td>Grupp et al. (6)</td>
<td>RP</td>
<td>4D4</td>
<td>2/9, 503 (8%)</td>
<td>IHC/FISH</td>
<td>13/6, 642 (2.2%)</td>
</tr>
<tr>
<td>Smith et al. (12)</td>
<td>RP</td>
<td>4D4</td>
<td>25/145 (17%)</td>
<td>Dual IHC</td>
<td>1/145 (0.7%)</td>
</tr>
<tr>
<td>Tomlins et al. (3)</td>
<td>RP/TURP</td>
<td>4D4</td>
<td>33/387 (9%)</td>
<td>FISH</td>
<td>0/387 (0%)</td>
</tr>
<tr>
<td>Jhavar et al. (5)</td>
<td>RP/TURP</td>
<td>6E7</td>
<td>14/126 (11%)</td>
<td>FISH</td>
<td>3/126 (2%)</td>
</tr>
<tr>
<td>Paju et al. (14)</td>
<td>RP</td>
<td>6E7</td>
<td>114/115 (99%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Leinonen et al. (15)</td>
<td>ADT</td>
<td>6E8</td>
<td>21/186 (11%)</td>
<td>FISH</td>
<td>7/170 (4%)</td>
</tr>
<tr>
<td>Lippolis et al. (7)</td>
<td>RP</td>
<td>6E8</td>
<td>175/3, 385 (5%)</td>
<td>Dual IHC</td>
<td>0/3, 385 (0%)</td>
</tr>
<tr>
<td>Tomlins et al. (3)</td>
<td>RP</td>
<td>6E8</td>
<td>297/817 (36%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Wang et al. (16)</td>
<td>RP</td>
<td>4D4</td>
<td>16/211 (8%)</td>
<td>FISH</td>
<td>0/195 (0%)</td>
</tr>
</tbody>
</table>

Diagram B:

- **n = 587**
- SPINK1 antibody
- ERG
- ERG*/SPINK1*

Diagram C:

- For TMA
- TMA
- ERG/SPINK1 IHC
- ERG*/SPINK1

Diagram D:

- TMA

Diagram E:

- TMA

Diagram F:

- TMA

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expression from 11 prostate cancer datasets in the Oncomine database, including five studies not included in our original analysis. In contrast, much greater variability in SPINK1 positivity (5%–99%) has been observed in IHC studies, with the 8% reported by Flavin and colleagues consistent with other studies using the 4D4 antibody clone (Fig. 1A).

Similarly, conflicting results about SPINK1 and prostate cancer prognosis have been reported (Fig. 1A), which likely pertain to both technical and cohort-dependent differences. Although we first observed SPINK1 outlier expression to be associated with biochemical recurrence after prostatectomy in an mRNA expression profiling study and in two IHC-based studies of referral prostatectomy series (3), the negative results with respect to prognosis reported by Flavin and colleagues among participants in the U.S. Physicians’ Health Study and Health Professionals Follow-Up Study are more consistent with negative results in recent large prostatectomy series (6, 7). Caveats related to biases and even interventions (e.g., vitamins) undertaken in the Physicians cohorts merit consideration. Nonetheless, the cases represented in the Flavin and colleagues cohort add to recent experience finding little to no support for use of SPINK1 as a prognostic biomarker following prostatectomy.

Finally, intriguing are the disease-specific issues about prostate cancer biomarker testing, as were noted in this study. The overall 2% ERG+/SPINK1− rate reported by Flavin and colleagues is the highest rate of dual positivity reported, at least using the 4D4 SPINK1 antibody clone (Fig. 1A). Prostate cancer is commonly multifocal, with most men at resection harboring multiple, clonally independent tumor foci, further complicating subtyping and prognostic studies, particularly those reliant on tissue microarrays (TMA; Fig. 1C–F). During TMA construction, pathologists typically take replicate cores from the tumor focus presumed to drive biologic behavior (the dominant or index focus). Although easier in cases with a readily apparent, highest-grade focus, cases with multiple intermediate-grade foci are challenging to assess with certainty. In addition, through whole-section evaluation, we and others have observed morphologic index foci composed of distinct ERG+ and ERG− areas (8–10). Such foci could be interpreted as a single clone with heterogeneous ERG expression (9), or as a collision of adjacent, morphologically apparent but genetically distinct clones (especially in the peripheral zone, in which carcinoma arises most frequently). Several lines of evidence support the nearly uniform presence or absence of ERG rearrangements in a given clone, suggestive of these rearrangements being early events, driving prostate cancer (2). If ERG rearrangements occurred at any point in prostate cancer development and progression, one would expect to observe with some frequency small pockets of nascent ERG+ cancer arising within large ERG− tumor foci, which has never been reported. In contrast, we and others have published examples of apparent ERG+/ERG−, ERG+/ETV1+ and ERG+/SPINK1−/SPINK1−/SPINK1− cases that represent “collisions” of genetically distinct foci on whole-section evaluation (4, 8, 11, 12).

Critically, collision tumors complicate the interpretation of replicate index focus TMA cores that show discordant ERG status. In our opinion, they indicate the presence of multiple genetically distinct foci, and such cases should be considered independently or resolved through whole-section evaluation. Hence, the 7 of 19 ERG+/SPINK1− cases described by Flavin and colleagues with discordant ERG+/SPINK1− status in TMA cores are challenging to interpret without whole-section evaluation. Interestingly, in the 3 ERG+/SPINK1+ cases evaluated on whole sections by Flavin and colleagues and in the one observed in our recent series (12), only small areas of dual positivity were observed in otherwise ERG+/SPINK1− foci. Such observations underscore the value of whole-section–based studies, and, given the lack of homogeneous ERG+/SPINK1+ tumor foci, these exceptions seem to prove the rule observed at the transcript level. Finally, and importantly with respect to criteria for positivity, in whole-section–based studies, we commonly...

Figure 1. Molecular subtyping of prostate cancer through ERG/SPINK1 expression. Prostate cancer molecular subtypes have been proposed on the basis mutually exclusive overexpression of ERG (due to chromosomal rearrangements) and SPINK1 (unknown mechanism). A, summary of SPINK1 IHC studies in prostate cancer. Results from prostatectomy (RP), castration-resistant prostate cancer (CRPC), radiation treated (XRT), and hormonally treated (ADT) cohorts are indicated, along with the SPINK1 antibody clone, number of SPINK1+ cases (and frequency), concurrent evaluation of ERG status (and method), and number of ERG+/SPINK1+ cases (and frequency). Studies with or without significant associations between SPINK1+ and clinical outcome (biochemical/clinical recurrence or prostate cancer–related mortality) are indicated in blue and red type, respectively. All cases evaluated by Lippolis et al. (7) were presumably included in the study by Grupp et al. (6). B, normalized gene expression (Z-score units) for ERG and SPINK1 in 587 localized prostate cancers is plotted from 11 microarray studies in the Oncomine database. The lack of ERG/SPINK1 coexpression supports marked overexpression of ERG (ERG++, blue) or SPINK1 (SPINK1++, yellow) as defining unique prostate cancer molecular subtypes (approximate frequencies in PSA-screened Caucasian cohorts shown in the inset). C–F, prostate cancer multifocality confounds molecular subtyping and biomarker evaluation. At prostatectomy, most men with prostate cancer harbor multiple genetically distinct cancer clones. C, the largest/highest-grade focus (index focus, gray) is used to assign pathology and smaller/lower-grade foci (white) are not specifically described. To facilitate IHC or molecular assays across large cohorts, replicate cores (black circles) are often taken from the index focus to generate. D–F, multiple groups have evaluated ERG and/ or SPINK1 by IHC using whole sections or TMAs. D, in our experience, ERG is nearly always homogeneously present or absent in a given focus and replicate TMA cores (green, an ERG+/SPINK1+ focus is indicated in blue). E, in a minority of cases, ERG+HC staining on whole sections supports collision of two independent foci (ERG+/SPINK1− and ERG−/SPINK1−, yellow shown), which can demonstrate heterogeneous ERG/SPINK1 status among replicate TMA cores. F, very rarely, we and Flavin et al. (1) have observed a small area of ERG+/SPINK1− (green) tumor inside a larger ERG+/SPINK1+ focus. "Denotes our recent whole-section–based analysis of a cohort of hereditary prostate cancers defined genetically by harboring the HOXB13 G84E susceptibility allele (12)."
observe heterogeneous SPINK1 expression, both in intensity and percentage, with particularly strong staining in the leading edge of tumors (8, 12). Given the lack of prognostic ability of SPINK1 in prostatectomy series, the major near-term utility of this biomarker will likely be in subtyping. Hence, careful whole-section evaluation of genetic, transcript, and protein expression will likely be required to conclusively evaluate subtyping based on ERG/SPINK1/SPOP status and other biomarkers. Similar studies will also be required to advance prognostic biomarkers into routine practice.

Disclosure of Potential Conflicts of Interest
S.A. Tomlins reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Ventana Medical Systems/Roche, and has ownership interest (including patents) in Ventana Medical Systems/Roche and Hologic/GenProbe. No potential conflicts of interest were disclosed by the other authors.

References

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.C. Smith
Writing, review, and/or revision of the manuscript: S.C. Smith, S.A. Tomlins

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