Characterization of TMPRSS2-ERG Fusion High-Grade Prostatic Intraepithelial Neoplasia and Potential Clinical Implications

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Abstract Purpose: More than 1,300,000 prostate needle biopsies are done annually in the United States with up to 16% incidence of isolated high-grade prostatic intraepithelial neoplasia (HGPIN). HGPIN has low predictive value for identifying prostate cancer on subsequent needle biopsies in prostate-specific antigen–screened populations. In contemporary series, prostate cancer is detected in ~20% of repeat biopsies following a diagnosis of HGPIN. Further, discrete histologic subtypes of HGPIN with clinical implication in management have not been characterized. The TMPRSS2-ERG gene fusion that has recently been described in prostate cancer has also been shown to occur in a subset of HGPIN. This may have significant clinical implications given that TMPRSS2-ERG fusion prostate cancer is associated with a more aggressive clinical course.

Experimental Design: In this study, we assessed a series of HGPIN lesions and paired prostate cancer for the presence of TMPRSS2-ERG gene fusion.

Results: Fusion-positive HGPIN was observed in 16% of the 143 number of lesions, and in all instances, the matching cancer shared the same fusion pattern. Sixty percent of TMPRSS2-ERG fusion prostate cancer had fusion-negative HGPIN.

Conclusions: Given the more aggressive nature of TMPRSS2-ERG prostate cancer, the findings of this study raise the possibility that gene fusion-positive HGPIN lesions are harbingers of more aggressive disease. To date, pathologic, molecular, and clinical variables do not help stratify which men with HGPIN are at increased risk for a cancer diagnosis. Our results suggest that the detection of isolated TMPRSS2-ERG fusion HGPIN would improve the positive predictive value of finding TMPRSS2-ERG fusion prostate cancer in subsequent biopsies.

In the United States, approximately 1,300,000 prostate biopsies were done in 2006 with the detection of 234,460 new cases of prostate cancer (American Cancer Society, Cancer facts & figures 2006). The incidence of isolated high-grade prostatic intraepithelial neoplasia (HGPIN) without carcinoma ranges from <1% to 16% (1–5), and the risk of finding carcinoma on subsequent biopsies is 10% to 39% [median risk of 24% (6)] depending on the time of repeat biopsy and number of cores (7–10). A decline in the predictive value of HGPIN for prostate cancer to ~20% in contemporary needle biopsies is most likely due to extended biopsy techniques that yield higher rates of cancer detection (11).

Both HGPIN and prostate adenocarcinoma share molecular anomalies, including telomere shortening (12), RARβ2 hypermethylation (13), allelic imbalances (14), and several chromosomal anomalies and c-myc amplification (15–17). Overexpression of p16 (18), reduction of Annexin I (19), and altered proliferation and apoptosis (20) in HGPIN and prostate cancer have also been shown. Table 1 summarizes a selection of molecular alterations identified in HGPIN and prostate cancer.

Despite the association with prostate cancer, distinct subtypes of HGPIN with clinical relevance (i.e., greater risk of predicting aggressive cancer) have not been characterized. A recent rearrangement involving the androgen-regulated gene...
TMPRSS2 and members of the ETS transcription factor family has been identified (21) and confirmed by multiple other groups (22–28). In particular, the TMPRSS2-ERG gene fusion prostate cancer is associated with higher tumor stage and tumor-specific death or metastasis (25, 29–31). Two recent studies have shown the presence of TMPRSS2-ERG gene fusion in ~20% of HGPIN lesions (22, 26).

The purpose of this study was to assess the TMPRSS2-ERG gene fusion status in a large series of HGPIN lesions with paired prostate cancer. Based on the results, we postulate that TMPRSS2-ERG fusion HGPIN is a distinct molecular subtype and its identification indicates the presence of the same genetic aberration in prostate cancer if present. This may affect clinical management of isolated HGPIN in prostate needle biopsies.

Materials and Methods

Case selection. One hundred forty-three HGPIN lesions from equal number of patients were interrogated for the presence of TMPRSS2-ERG gene fusion. This study was conducted under the Institutional Review Board protocol 2006-P-000715/1 BWH at Brigham and Women’s Hospital. The HGPIN lesions were represented on 22 tissue microarrays from prostatectomy specimens (96 of 143), 34 prostate needle biopsies, and none of controls.

Table 1. Molecular evidence of association between HGPIN and prostate cancer

<table>
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<th>Focus and number of HGPIN samples</th>
<th>Technique</th>
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<td>Telomere shortening as an early somatic DNA alteration in prostate cancer: a total of 6 prostatectomies were evaluated, which included 11 HGPIN lesions, and 20 needle biopsies with HGPIN without cancer (n = 26)</td>
<td>FISH</td>
<td>Shortening seen in 93% (28/30) of HGPIN lesions is similar to what has been shown in invasive prostate cancer</td>
<td>Meeker et al. (12)</td>
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<td>Proliferation and apoptotic markers in normal and premalignant tissue associated with prostate cancer: 13 prostatectomies and 6 cystoprostatectomies were evaluated (n = 19)</td>
<td>Immunohistochemistry</td>
<td>Both preneoplastic lesions and normal-looking epithelium associated with cancer show altered proliferation and apoptosis</td>
<td>Ananthanarayanan et al. (20)</td>
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<td>TMPRSS2-ERG in HGPIN: 34 prostate cancer and 19 paired HGPIN were analyzed (n = 19); also 14 BPH and 11 normal as controls</td>
<td>Real-time PCR, sequencing, comparative genomic hybridization</td>
<td>21% of HGPIN lesions harbor the fusion, 50% of prostate cancer, and none of controls</td>
<td>Cerveira et al. (22)</td>
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<td>Quantitative methylation of RARB2: prostate cancer (118 patients), paired HGPIN lesions (n = 38), and BPH (30 patients)</td>
<td>Quantitative methylation-specific PCR</td>
<td>RARB2 hypermethylation in 97.5% prostate cancers, 94.7% HGPIN, and 23.3% BPH. RARB2 methylation levels correlated with higher pathologic stage</td>
<td>Jeronimo et al. (13)</td>
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<td>Annexin I protein expression: prostate cancer (69 prostatectomies), paired HGPIN (n = 45), and benign prostate (14 samples)</td>
<td>Immunohistochemistry, real-time PCR</td>
<td>Annexin I was significantly reduced in prostate cancer and HGPIN compared with benign prostate</td>
<td>Kang et al. (19)</td>
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<td>Overexpression of p16INK4A in HGPIN: 206 patients with clinically localized prostate cancer were screened, a subset with HGPIN (n = 154)</td>
<td>Immunohistochemistry</td>
<td>Overexpression of p16INK4A in HGPIN was independent predictor of disease relapse and increased risk of recurrence</td>
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<td>Detection of chromosomal anomalies and c-myc gene amplification in cribriform HGPIN and prostate cancer: A total of 25 prostatectomy specimens were studied, which included 48 foci of HGPIN and 71 foci of prostate cancer (n = 25)</td>
<td>FISH</td>
<td>Cribriform HGPIN and cribriform prostate cancer exhibited similar anomalies</td>
<td>Qian et al. (17)</td>
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<td>Detection of c-myc amplification and chromosomal anomalies: HGPIN (48 foci), localized prostate cancer (71 foci), and lymph node metastases (23 foci) in 25 prostatectomies (n = 25)</td>
<td>FISH</td>
<td>Gain of chromosome 8 and c-myc amplification are potential markers of prostate cancer progression; HGPIN is likely a precursor</td>
<td>Jenkins et al. (15)</td>
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<td>Chromosomal anomalies in HGPIN and prostate cancer: 40 radical prostatectomy and pelvic lymphadenectomy specimens studied, including 68 foci of HGPIN, 78 foci of prostate cancer, and 8 foci of lymph node metastases (n = 40)</td>
<td>FISH</td>
<td>HGPIN and prostate cancer have similar proportions of chromosomal abnormalities, supporting HGPIN as precursor</td>
<td>Qian et al. (16)</td>
</tr>
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<td>Assessment of allelic imbalance at 6 polymorphic microsatellite markers: 84 foci of HGPIN and 95 foci of prostate cancer from 52 completely embedded, mapped whole-mount prostates (n = 52)</td>
<td>PCR (majority of cases previously studied by FISH)</td>
<td>Rate of allelic imbalance was similar at 5 of 6 loci studied. Significant genetic heterogeneity seen, suggesting that multiple foci of HGPIN arise independently in prostate</td>
<td>Bostwick et al. (14)</td>
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NOTE: Numbers of total cases (not foci) of HGPIN per study are in bold.
and 13 full section prostatectomy samples. Of these, 87% (124 of 143) had paired prostate cancer. The remaining 19 cases showed isolated HGPIN without evidence of concurrent cancer and included two cases of HGPIN with adjacent atypical small acinar proliferation (10, 32). Clinical and pathologic demographics were available for 93 of the 143 patients. These included 70 of 124 HGPIN lesions with paired prostate cancer as follows: 40 of 96 patients represented in the tissue microarrays, all 34 patients represented in the needle biopsies, and 9 of 13 patients represented in prostatectomy samples. The mean age at presentation was 60 y with a mean preoperative prostate-specific antigen of 16.5 ng/mL. There were 30% Gleason grade 3+3, 51% Gleason grade 3+4, and 19% Gleason grade $\geq$4 prostate cancers.

**Pathologic analysis.** The morphologic diagnosis was confirmed on H&E-stained paraffin sections by two pathologists (J-M.M. and S.P.) before assessment of gene fusion by fluorescence in situ hybridization (FISH) on a step sectioning, corresponding to one unstained section at identical level obtained at the time of initial tissue sectioning. HGPIN lesions were differentiated into four morphologic subtypes: tufting, flat, micropapillary, and cribriform (33, 34). In a subset of cases with equivocal diagnosis, immunohistochemistry for prostatic basal cells was done. These were six needle biopsy cases with atypical small acinar proliferation for which immunohistochemistry helped to confirm the diagnosis of prostate cancer. For that purpose, additional unstained slides were deparaffinized in xylene and rehydrated in graded ethanols. The tissue level of the immunohistochemical study was identical to the original H&E. Pressure cooking was applied as the antigen retrieval method. Primary antibodies against p63 (1:50 dilution of clone 4A4; NeoMarkers) and high molecular weight cytokeratin were used. These were six needle biopsy cases with atypical small acinar proliferation for which immunohistochemistry helped to confirm the diagnosis of prostate cancer. For that purpose, additional unstained slides were deparaffinized in xylene and rehydrated in graded ethanols. The tissue level of the immunohistochemical study was identical to the original H&E. Pressure cooking was applied as the antigen retrieval method. Primary antibodies against p63 (1:50 dilution of clone 4A4; NeoMarkers) and high molecular weight cytokeratin were used.

**Assessment of TMPRSS2-ERG fusion status using an interphase FISH assay.** We have previously described a dual-color interphase break-apart FISH assay to indirectly assess the fusion of TMPRSS2-ERG (25, 26, 29). Briefly, two differentially labeled probes were designed to span the telomeric and centromeric neighboring regions of the ERG locus. Using this break-apart probe system, a nucleus without ERG rearrangement shows two pairs of juxtaposed red and green signals, forming yellow fusion signals. A nucleus with an ERG break apart (reflecting a TMPRSS2-ERG fusion) shows split apart of one juxtaposed red-green signal resulting in a single red and green signal for the translocated ERG allele and a still combined (yellow) signal for the nontranslocated ERG allele in each nucleus. The samples were analyzed under a 60× oil immersion objective using an Olympus BX-51 fluorescence microscope equipped with appropriate filters, a charge-coupled device camera (Olympus), and the CytoVision FISH imaging and capturing software (Applied Imaging). Evaluation of the cases was independently done by two pathologists (J-M.M. and S.P.), both with expertise in analyzing interphase FISH experiments. For each case, we attempted to score at least 50 nuclei. Cases with significant differences between the results of both pathologists were referred by a third pathologist (M.A.R.).

**Results**

Of the 143 HGPIN cases, 16% (23 of 143) showed TMPRSS2-ERG gene fusion. All cases shared the same fusion status with the paired prostate cancer (22 of 22). There was a single case of TMPRSS2-ERG fusion HGPIN without concurrent adenocarcinoma. The follow-up biopsy of this isolated HGPIN on prostate needle biopsy had not been done at the time of preparing this article. Of 120 TMPRSS2-ERG fusion-negative HGPIN cases, 85% (102 of 120) had matching adenocarcinoma, and in 32% of these (33 of 102), the paired prostate cancer showed TMPRSS2-ERG fusion (Fig. 1).

Two cases of HGPIN also showed adjacent small atypical glands (10, 32). One was fusion positive in both areas (Fig. 2A), whereas the other one showed fusion-negative HGPIN with adjacent fusion-positive atypical glands. Neither case had follow-up rebiopsy at the time of preparing this article. Interestingly, we could identify two cases that showed presence of TMPRSS2-ERG gene fusion HGPIN and adjacent normal epithelium (with no fusion) within the same gland (Fig. 2B). Among the morphologic subtypes, 31% (44 of 143) were tufting HGPIN, 4% (6 of 143) showed flat HGPIN, 2% (3 of 143) were micropapillary HGPIN, 1% (1 of 143) of the cases had cribriform HGPIN morphology, and 62% (89 of 143) combined more than one of the above subtypes.

**Discussion**

Several suggested protocols for management of isolated HGPIN in prostate needle biopsies exist. They vary from repeat biopsy at 3 to 6 months, at 6 to 12 months, or at 3 years (35–37). The most aggressive protocol suggests repeat biopsies at 3- to 6-month intervals for 2 years, thereafter every year for life (7). Recent data suggest that the incidence of prostatic adenocarcinoma after the initial diagnosis of isolated HGPIN in needle biopsies is lower than previously reported (10, 11), and despite molecular data on HGPIN, biomarkers with direct clinical application have not been used to stratify the risk for subsequent detection of adenocarcinoma. In addition, morphologic features and extent of HGPIN show inconsistent data with their ability to predict the presence of prostate cancer on subsequent biopsies. Therefore, the clinical management of patients with isolated HGPIN is problematic, and to date, no treatment is indicated after this diagnosis is rendered.

It is valid to speculate that stratification of different subtypes of HGPIN at the molecular level (i.e., TMPRSS2-ERG fusion HGPIN) may be needed for potential prognostic implications and in view of clinical trials for chemoprevention of prostate cancer where one of the inclusion criteria is the diagnosis of isolated HGPIN (38, 39).

Our results may help in prognostication of a subset of isolated HGPIN lesions, that is, those harboring the TMPRSS2-ERG gene fusion. We have recently postulated that the TMPRSS2-ERG gene fusion is a clonal, early pathogenic event in prostate cancer (26, 40). Evidence supporting this hypothesis is that in most instances the gene fusion is homogenously present throughout the cancer within a tumor nodule, is not identified in benign prostatic tissue, and is detected only in a subset of HGPIN lesions. Another group has also confirmed the presence of TMPRSS2-ERG gene fusion in HGPIN using PCR technique (22). Interestingly, both studies show ~20% gene fusion positivity among a small series of HGPIN.

In the current study, the incidence of TMPRSS2-ERG gene fusion HGPIN is 16% in 143 cases. Given that all TMPRSS2-ERG gene fusion HGPIN lesions share the same fusion pattern with matching cancer, and no fusion-positive HGPIN lesions were associated with paired TMPRSS2-ERG fusion-negative prostate cancer, we show that the presence of TMPRSS2-ERG gene fusion HGPIN is always indicative of a prostate cancer bearing the same genetic aberration. Conversely, TMPRSS2-ERG fusion prostate cancer may present with fusion-negative...
HGPIN. Possible scenarios that could explain this finding are
that either fusion-negative HGPIN does not precede TMPRSS2-
ERG fusion prostate cancer or TMPRSS2-ERG fusion HGPIN
was not sampled if we consider the presence of gene fusion
heterogeneity in HGPIN as a possibility. In our previous
work (26, 41), we had made these observations. However,
in the series reported by Cerveira et al. (22), PCR assess-
ment yielded two cases where the fusion transcript was detected
in HGPIN but not in the concurrent cancer of the same gland.
In the present study, we have screened a significantly larger
number of HGPIN lesions using FISH, the gold standard
method to detect these molecular alterations, and we have not
observed such combination. This discrepancy could be due to
artifact in the PCR assay, or as a consequence of TMPRSS2-ERG
heterogeneity in prostate cancer, where the fusion-positive area
of tumor may have not been sampled. Although TMPRSS2-ERG
gene fusion heterogeneity in prostate cancer is out of the scope
of the current study, it is pertinent to mention that, in our
most recent study, 41% of radical prostatectomy high-stage
cases (at least pT2c) showed interfocal clonal heterogeneity
(40), also described by Mehra et al. (42) and Furusato et al.
(43). This fact may have significant clinical implications for
follow-up biopsy and treatment strategies in the context of
isolated TMPRSS2-ERG fusion HGPIN.

Taking these results together, we consider that TMPRSS2-ERG
fusion HGPIN is a true precursor of a subset of TMPRSS2-ERG
prostate cancer, and the presence of the former is always
indicative of the latter. Remarkably, we identified two cases
where TMPRSS-2-ERG fusion showed either early invasion
(see Fig. 2A) or coexistence with normal epithelium in the same
gland (see Fig. 2B). This morphologic/gene fusion status
correlation further supports our statement as well as the
hypothesis of HGPIN to cancer progression (in this case,
of those lesions harboring the TMPRSS2-ERG fusion). These
observations are clinically relevant because there is emerging
data supporting that TMPRSS2-ERG fusion prostate cancer is
associated with worse prognosis, namely, higher tumor stage
and tumor-specific death or metastasis (24, 25, 29, 31, 44, 45).
Hence, the finding of isolated TMPRSS2-ERG fusion HGPIN
in needle biopsies may have the highest predictive value for

![Fig. 1. H&E stains and corresponding FISH images of TMPRSS2-ERG fusion assay. A, TMPRSS2-ERG fusion prostate cancer, Gleason grade 3 + 4 = 7. Inset, a nucleus with one yellow and one red signal, showing the presence of TMPRSS2-ERG fusion through deletion. B, paired HGPIN lesion of prostate cancer in A. The HGPIN features tufting morphology. Inset, a nucleus with two yellow signals, showing absence of genetic aberration. C, TMPRSS2-ERG fusion prostate cancer, Gleason grade 4 + 4 = 8. The prostatectomy on this case showed predominant cribriform morphology. Inset, a nucleus with one yellow and one red signal, showing the presence of TMPRSS2-ERG fusion through deletion. D, paired HGPIN lesion of prostate cancer in C. The HGPIN features tufting and micropapillary morphology. Inset, a nucleus with the same pattern as the matching prostate cancer, showing the presence of TMPRSS2-ERG fusion. Original magnification of H&E images, 20× objective. Original magnification of FISH images, 60× objective.](https://www.aacrjournals.org/clinicanimages/14/11/3383/fig1.png)
further detection of fusion-positive prostate cancer with the significant clinical implication noted above.

Based on the results of our recent work on morphologic features associated with \textit{TMPRSS2-ERG} fusion prostate cancer (46), we also considered a potential correlation between the morphology of HGPIN and the \textit{TMPRSS2-ERG} fusion status. However, 62% of HGPIN cases combined two or more of the morphologic subtypes, and a significant association was not seen.

Although prospective studies with follow-up of isolated \textit{TMPRSS2-ERG} gene fusion HGPIN are needed to modify the current approach of management of isolated HGPIN, our results show convincing evidence that fusion-positive HGPIN lesions are consistently associated with \textit{TMPRSS2-ERG} prostate cancer. To further support our findings, studies with follow-up of patients with isolated \textit{TMPRSS2-ERG} fusion HGPIN or \textit{TMPRSS2-ERG} fusion HGPIN with adjacent small atypical glands like one or our cases are under way as part of an Early Detection Research Network protocol. Further, evaluation of the status of \textit{TMPRSS2-ERG} fusion could also modify inclusion criteria in the aforementioned clinical trials. Moreover, the development of noninvasive (i.e., urine based) diagnostic tests for fusion transcripts could also help in these protocols (47).

In summary, we have assessed the largest series of HGPIN lesions for \textit{TMPRSS2-ERG} fusion status to date and confirmed a prevalence of 16%, similar to previously reported series. In all instances, fusion-positive HGPIN is associated with concurrent \textit{TMPRSS2-ERG} prostate cancer. Given the worse prognosis linked to the latter, detection of isolated \textit{TMPRSS2-ERG} fusion HGPIN may help us stratify patients into a discrete risk group.

\textbf{Disclosure of Potential Conflicts of Interest}

The University of Michigan and the Brigham and Women’s Hospital have filed a patent on ETS gene rearrangements. Drs. Perner and Rubin are among the co-inventors. The diagnostic field of use has been licensed to Gen-Probe Incorporated.

\textbf{Acknowledgments}

We thank Chungdak Namgyal (Dana-Farber/Harvard Cancer Center Tissue Microarray Core Facility), Christopher LaFargue for technical support critical to this study.

\textbf{References}


Characterization of TMPRSS2-ERG Fusion High-Grade Prostatic Intraepithelial Neoplasia and Potential Clinical Implications

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