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

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**Abstract**  
A recent rearrangement involving the androgen-regulated gene ***TMPRSS2-ERG*** has been described in prostate cancer. TMPRSS2-ERG fusion prostate cancer (TMPRSS2-ERG fusion HGPIN) has low predictive value for identifying prostate cancer on subsequent needle biopsies in prostate-specific antigen (PSA)-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 has recently been described in prostate cancer and 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.
studies have shown the presence of tumor-specific death or metastasis (25, 29–31). Two recent prostate cancer is associated with higher tumor stage and groups (22–28). In particular, the gene fusion has been identified (21) and confirmed by multiple other transcription factor family.

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

<table>
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<tr>
<th>Focus and number of HGPIN samples</th>
<th>Technique</th>
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<td>Quantitative methylation of RARB2: prostate cancer (118 patients), paired HGPIN lesions (n = 38), and BPH (30 patients)</td>
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<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>
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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>
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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>
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NOTE: Numbers of total cases (not foci) of HGPIN per study are in bold.

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 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 ≤6, 51% Gleason grade ≥7, and 19% Gleason grade ≥8 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 section, 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 confirmed 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.).

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 assessment 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 TMPRSS2-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
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 TMPRSS2-ERG fusion prostate cancer (46), we also considered a potential correlation between the morphology of HGPIN and the 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 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 TMPRSS2-ERG prostate cancer. To further support our findings, studies with follow-up of patients with isolated TMPRSS2-ERG fusion HGPIN or 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 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 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 TMPRSS2-ERG prostate cancer. Given the worse prognosis linked to the latter, detection of isolated TMPRSS2-ERG fusion HGPIN may help us stratify patients into a discrete risk group.

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.

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References
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