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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 performed annually in the U.S. with up to 16% incidence of isolated high-grade prostatic intraepithelial neoplasia (HGPIN). HGPIN has low predictive value for identifying prostate cancer (PCA) on subsequent needle biopsies in PSA screened populations. In contemporary series, PCA is detected in about 20% of repeat biopsies following a diagnosis of HGPIN. Further, discrete histological subtypes of HGPIN with clinical implication in management have not been characterized. The *TMPRSS2-ERG* gene fusion that has recently been described in PCA has also been demonstrated to occur in a subset of HGPIN. This may have significant clinical implications given that *TMPRSS2-ERG* fusion PCA is associated with a more aggressive clinical course.

Experimental Design—In this study we assessed a series of HGPIN lesions and paired PCA 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. 60% of *TMPRSS2-ERG* fusion PCA had fusion negative HGPIN.

Conclusions—Given the more aggressive nature of *TMPRSS2-ERG* PCA, the findings of this study raise the possibility that gene fusion positive HGPIN lesions are harbingers of more aggressive disease. To date, pathological, molecular and clinical parameters do not help stratify

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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 PCA in subsequent biopsies.

Introduction

In the United States, approximately 1,300,000 prostate biopsies were performed 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 10–39% (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 about 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 has also been demonstrated. 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 demonstrated the presence of *TMPRSS2-ERG* gene fusion in approximately 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 impact clinical management of isolated HGPIN in prostate needle biopsies.

Materials and Methods

Case selection

143 HGPIN lesions from equal number of patients were interrogated for the presence of *TMPRSS2-ERG* gene fusion. This study was conducted under the IRB protocol 2006-P-000715/1 BWH at Brigham and Women's Hospital. The HGPIN lesions were represented on 22 tissue microarrays (TMA) from prostatectomy specimens (96/143), 34 prostate needle biopsies, and 13 full section prostatectomy samples. Of these, 87% (124/143) had paired prostate cancer. The remaining 19 cases demonstrated 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 TMAs, all 34 patients represented in the needle biopsies, and 9 of 13 patients represented in prostatectomy samples. The mean age at presentation was 60 years with a mean pre-operative PSA of 16.5 ng/ml. There were 30% Gleason grade ≤6, 51% Gleason grade 7, and 19% Gleason grade ≥8 prostate cancers.

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Pathologic analysis

The morphological diagnosis was confirmed on H&E stained paraffin sections by two pathologists (J-MM and SP) prior to assessment of gene fusion by fluorescent 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 morphological subtypes: tufting, flat, micropapillary, and cribriform[33, 34]. In a subset of cases with equivocal diagnosis, immunohistochemistry (IHC) for prostatic basal cells was performed. These were 6 needle biopsy cases with atypical small acinar proliferation (ASAP) for which IHC 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, Fremont, CA) and high molecular weight cytokeratin (1:200 dilution of clone 34βE12, DAKO, Carpinteria, CA) for the detection of basal cells were applied with over night incubation at 4°C in a humid chamber. Immunohistochemistry was performed with the avidin-biotin peroxidase technique.

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 demonstrates 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 pair resulting in a single red and green signal for the translocated *ERG* allele, and a still combined (yellow) signal for the non-translocated *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 CCD (charge-coupled device) camera (Olympus, Center Valley, PA), and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA). Evaluation of the cases was independently performed by two pathologists (J-MM and SP), 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 refereed by a third pathologist (MAR).

Results

Of the 143 HGPIN cases, 16% (23/143) demonstrated *TMPRSS2-ERG* gene fusion. All cases shared the same fusion status with the paired prostate cancer (22/22). 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 performed at the time of preparing this manuscript. Of 120 *TMPRSS2-ERG* fusion negative HGPIN cases, 85% (102/120) had matching adenocarcinoma, and in 32% of these (33/102) the paired prostate cancer demonstrated *TMPRSS2-ERG* fusion (Figure 1).

Two cases of HGPIN also demonstrated adjacent small atypical glands[10, 32]. One was fusion positive in both areas (Figure 2A), whereas the other one showed fusion negative HGPIN with adjacent fusion positive atypical glands. Neither case had follow-up re-biopsy at the time of preparing this manuscript. 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 (Figure 2B). Among the morphological subtypes, 31% (44/143) were tufting HGPIN, 4% (6/143) showed flat HGPIN, 2% (3/143) were

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micropapillary HGPIN, 1% (1/143) cases had cribriform HGPIN morphology, and 62% (89/143) 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 three to six months, at six to twelve months, or at three years[35–37]. The most aggressive protocol suggests repeat biopsies at three to six-month intervals for two 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 to predict risk of consecutive prostate cancer. 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 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 polymerase chain reaction (PCR) technique[22]. Interestingly, both studies show approximately 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 demonstrate 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 fusion negative HGPIN does either not precede *TMPRSS2-ERG* fusion prostate cancer, or that *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) demonstrated interfocal clonal heterogeneity[40], also described by Mehra *et al*[42] and Furusato *et al*[43]. This fact may have

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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 HGPIN was showing either early invasion (see Figure 2A) or coexistence with normal epithelium in the same gland (see Figure 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 since 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 morphological 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, hampering a significant association.

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 underway as part of an Early Detection Research Network (EDRN) protocol. Further, evaluation of the status of *TMPRSS2-ERG* fusion could also modify inclusion criteria in the aforementioned clinical trials. Moreover, the development of non-invasive (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.

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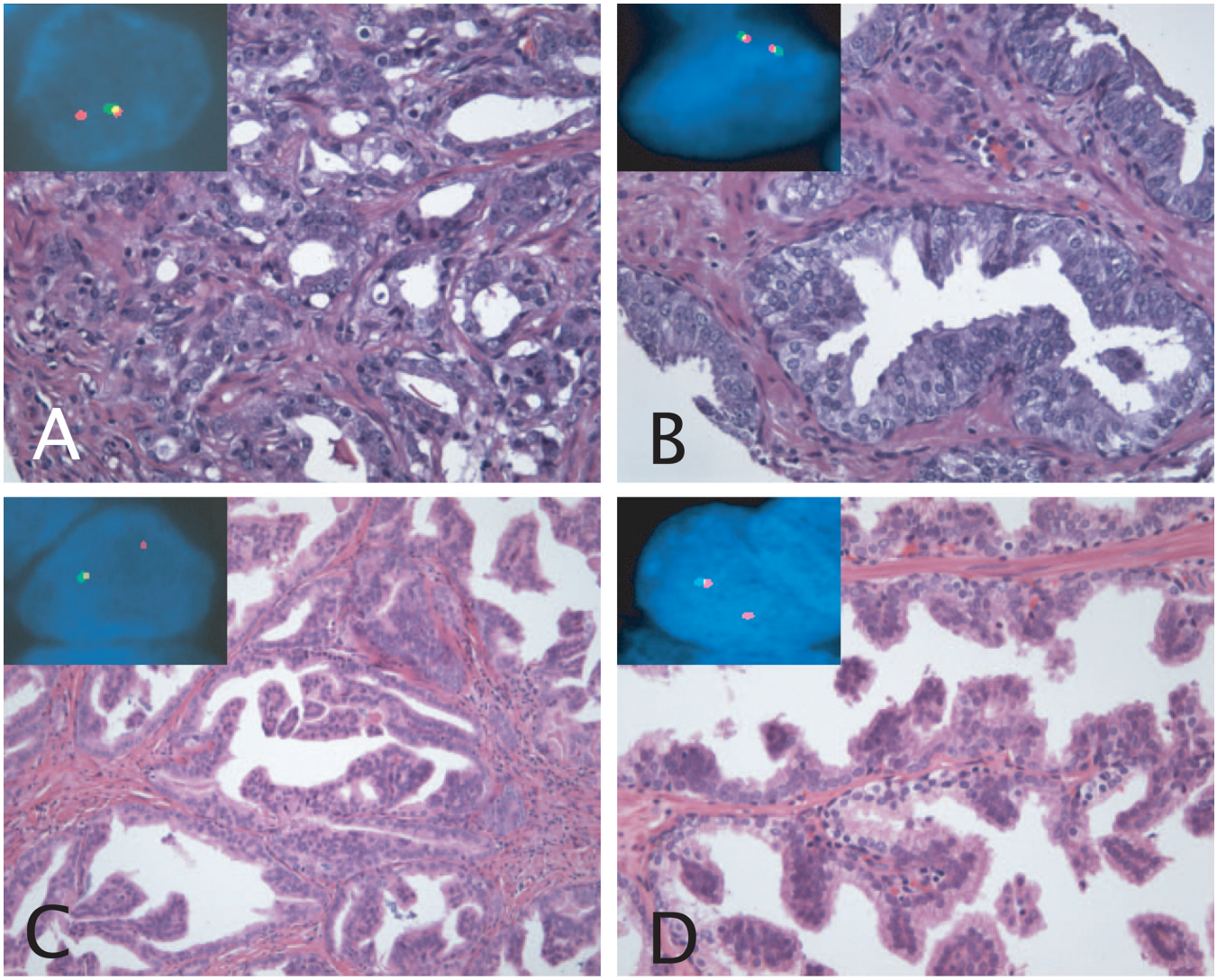

Figure 1. H&E stains and corresponding FISH images of *TMPRSS2-ERG* fusion assay

A: *TMPRSS2-ERG* fusion prostate cancer, Gleason grade 3+4=7. The inset picture shows a nucleus with one yellow and one red signal, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. B: Paired HGPIN lesion of prostate cancer in A. The HGPIN features tufting morphology. The inset picture shows a nucleus with two yellow signals, demonstrating absence of genetic aberration. C: *TMPRSS2-ERG* fusion prostate cancer, Gleason grade 4+4=8. The prostatectomy on this case showed predominant cribriform morphology. The inset picture shows a nucleus with one yellow and one red signal, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. D: Paired HGPIN lesion of prostate cancer in C. The HGPIN features tufting and micropapillary morphology. The inset picture shows a nucleus with the same pattern as the matching prostate cancer, demonstrating the presence of *TMPRSS2-ERG* fusion.

Original magnification of H&E images, 20× objective. Original magnification of FISH images, 60× objective.

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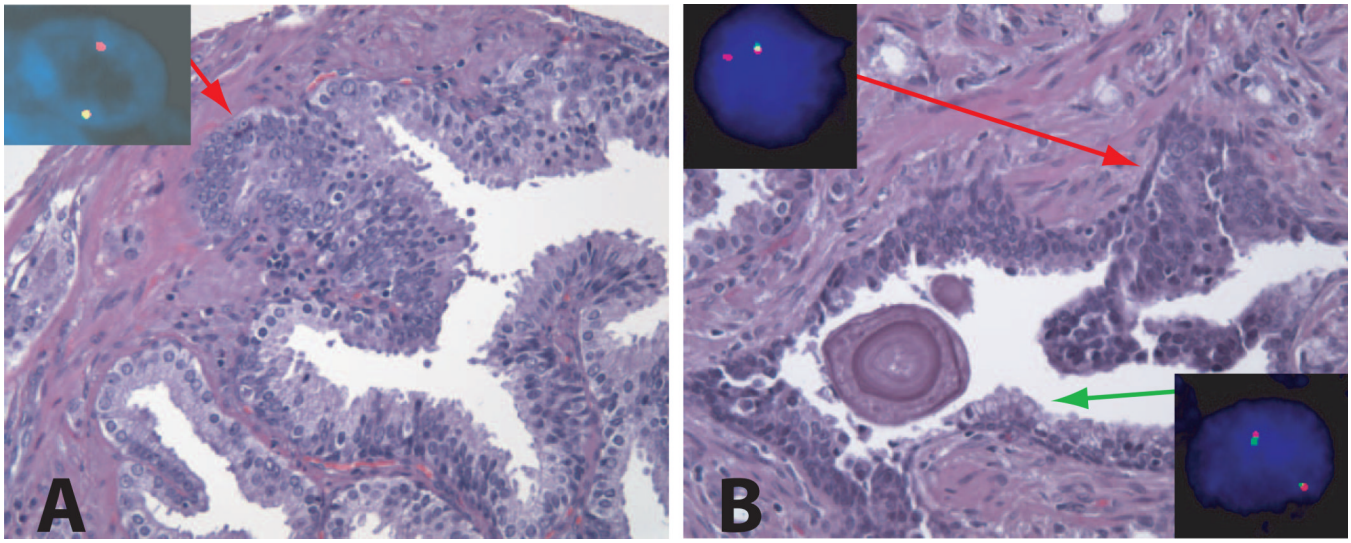

Figure 2. H&E stain and corresponding FISH image of *TMPRSS2-ERG* fusion assay

A: HGPIN lesion with adjacent atypical small acinar proliferation. This may represent either outpouching area or tangential section of HGPIN, or true early invasive adenocarcinoma. The red arrow points this area. The inset picture shows a nucleus with one yellow and one red signal, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. Original magnification of H&E images, 20× objective. Original magnification of FISH images, 60× objective.

B: HGPIN and normal prostatic epithelium in the same gland. Red and green arrows point representative areas of HGPIN and normal prostatic epithelium, respectively. The inset pictures show a nucleus of normal epithelium with juxtaposed red-green signal pair (upper left), and a nucleus of HGPIN with one yellow and one red signal, demonstrating *TMPRSS2-ERG* fusion through deletion (lower right). The surrounding prostatic cancer, mostly Gleason pattern 4, also shared the same gene fusion pattern.

Original magnification of H&E images, 20× objective. Original magnification of FISH images, 60× objective.

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Table 1

Molecular evidence of association between HGPIN and prostate cancer (PCA). Numbers of total cases (not foci) of HGPIN per study are in **bold**.

Focus and number of HGPIN samples	Technique	Main conclusions	Reference
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**)	FISH	Shortening seen in 93% (28/30) of HGPIN lesions is similar to what has been shown in invasive PCA.	Meeker AK et al, Cancer Res 2002 (11).
Proliferation and apoptotic markers in normal and premalignant tissue associated with PCA: 13 prostatectomies and 6 cystoprostatectomies were evaluated (**n=19**)	IHC	Both preneoplastic lesions and normal looking epithelium associated with cancer show altered proliferation and apoptosis	Ananthanarayanan V et al, BMC Cancer 2006 (19).
TMPRSS2-ERG in HGPIN: 34 PCA and 19 paired HGPIN were analyzed (**n=19**). Also 14 BPH and 11 normal as controls.	Real time PCR, sequencing, CGH	21% of HGPIN lesions harbor the fusion, 50% of PCA, and none of controls	Cerveira N et al, Neoplasia 2006 (21).
Quantitative methylation of RARB2: PCA (118 patients), paired HGPIN lesions (**n=38**), and BPH (30 patients)	Quantitative methylation specific PCR	RARB2 hypermethylation in 97.5% PCA, 94.7% HGPIN, and 23.3% BPH. RARB2 methylation levels correlated with higher pathological stage	Jeronimo C et al, Clin Cancer Res 2004 (12).
Annexin I protein expression: PCA (69 prostatectomies), paired HGPIN (**n=45**), and benign prostate (14 samples)	IHC, real-time PCR	Annexin I was significantly reduced in PCA and HGPIN compared to benign prostate	Kang JS et al, Clin Cancer Res 2002 (18).
Overexpression of $p16^{INK4A}$ in HGPIN: 206 patients with clinically localized PCA were screened, a subset with HGPIN (**n=154**)	IHC	Overexpression of $p16^{INK4A}$ in HGPIN was independent predictor of disease relapse and increased risk of recurrence	Henshall SM et al, Clin Cancer Res 2001 (17).
Detection of chromosomal anomalies and c-myc gene amplification in cribriform HGPIN and PCA: A total of 25 prostatectomy specimens were studied, which included 48 foci of HGPIN and 71 foci of PCA (**n=25**)	FISH	Cribriform HGPIN and cribriform PCA exhibited similar anomalies	Qian J, Jenkins RB and Bostwick DG, Mod Pathol 1997 (16).
Detection of c-myc amplification and chromosomal anomalies: HGPIN (48 foci), localized PCA (71 foci), and lymph node metastases (23 foci) in 25 prostatectomies (**n=25**)	FISH	Gain of chromosome 8 and c-myc amplification are potential markers of PCA progression, HGPIN is likely a precursor	Jenkins RB et al, Cancer Res 1997 (14).
Chromosomal anomalies in HGPIN and PCA: 40 radical prostatectomy and pelvic lymphadenectomy specimens studied including 68 foci of HGPIN, 78 foci of PCA, and 8 foci of lymph node metastases (**n=40**)	FISH	HGPIN and PCA have similar proportions of chromosomal abnormalities, supporting HGPIN as precursor.	Qian J et al, Cancer Res 1995 (15).
Assessment of allelic imbalance at 6 polymorphic microsatellite markers: 84 foci of HGPIN 95 foci of PCA from 52 completely embedded, mapped whole mount prostates (**n=52**)	PCR (majority of cases previously studied by FISH)	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	Bostwick DG et al, Cancer 1998 (13).