

CHARACTERIZATION OF *TMPRSS2-ERG* FUSION HIGH-GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA AND POTENTIAL CLINICAL IMPLICATIONS



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Introduction

More than 1,300,000 prostate needle biopsies are performed annually in the United States with an approximate 15% incidence of isolated high-grade prostatic intraepithelial neoplasia (HGPIN). HGPIN has a low predictive value for identifying prostate cancer on subsequent needle biopsies in PSA screened populations. Further, discrete histological subtypes of HGPIN with clinical implication in management have not been characterized.

Both HGPIN and prostate adenocarcinoma share molecular anomalies (Table 1).

Despite the association distinct subtypes of HGPIN with clinical relevance (i.e. greater risk of predicting aggressive cancer) have not been characterized.

The *TMPRSS2-ERG* gene fusion that has recently been described in prostate cancer has also been demonstrated to occur in a subset of HGPIN.

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).
<i>TMPRSS2-ERG</i> 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).

Materials and Methods

143 HGPIN lesions from equal number of patients were interrogated for the presence of *TMPRSS2-ERG* gene fusion using a break apart FISH assay. The HGPIN lesions were represented on 22 tissue microarrays (96/143), 34 prostate needle biopsies, and 13 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.

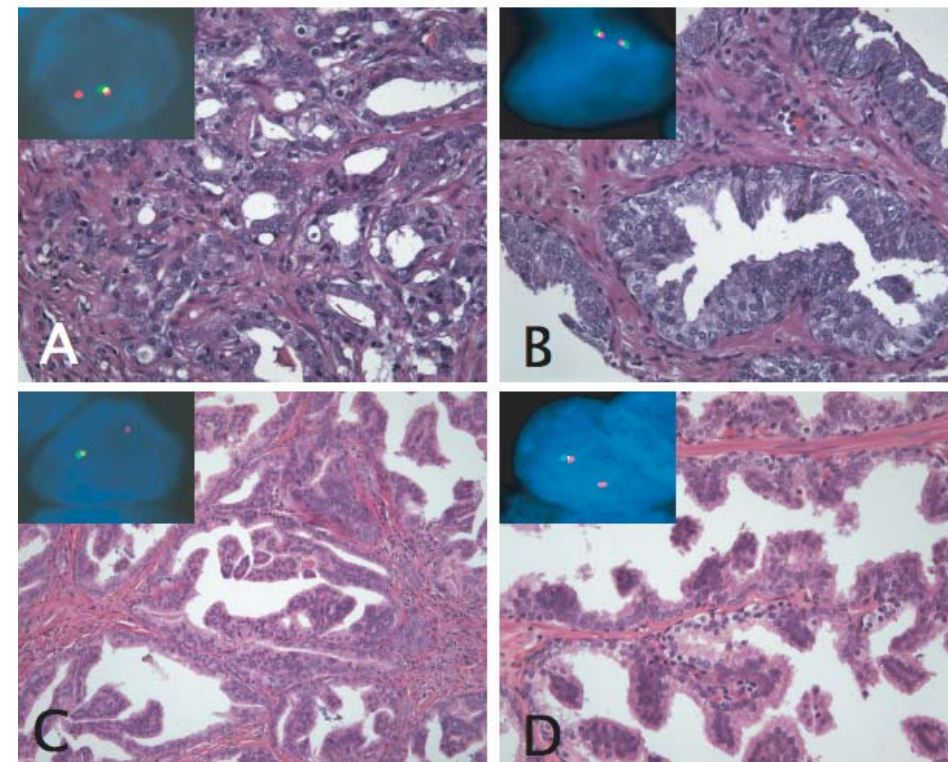


Figure 1. H&E stains and corresponding FISH images of *TMPRSS2-ERG* fusion assay. A: *TMPRSS2-ERG* fusion prostate cancer, Gleason grade 3+4=7. The inset picture shows a nucleus with one yellow and one red signal, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. B: Paired HGPIN lesion 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 with 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, 20x objective. Original magnification of FISH images, 60x objective.

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). 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. One was fusion positive in both areas (Figure 2A), whereas the other one showed fusion negative HGPIN with adjacent fusion positive atypical glands. Interestingly, we could identify two cases that showed the presence of *TMPRSS2-ERG* gene fusion HGPIN and adjacent normal epithelium (with no fusion), within the same gland (Figure 2B).

No association of *TMPRSS2-ERG* fusion status and morphologic subtypes of HGPIN was found.

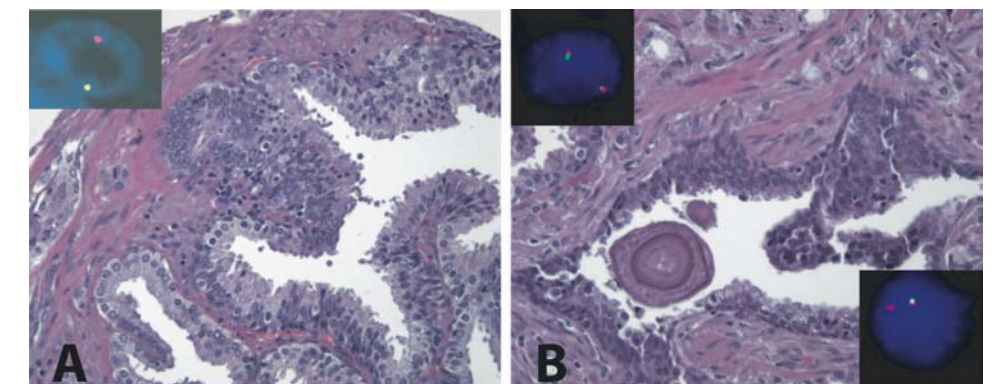


Figure 2. H&E stain and corresponding FISH image of *TMPRSS2-ERG* fusion assay. A: HGPIN lesion with adjacent atypical small acinar proliferation. This may represent either outpouching area or tangential section of HGPIN, or true early invasive adenocarcinoma. The inset picture shows a nucleus with one yellow and one red signal, demonstrating the presence of *TMPRSS2-ERG* fusion through deletion. B: HGPIN and normal prostatic epithelium in the same gland. 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.

Conclusions

All *TMPRSS2-ERG* gene fusion HGPIN lesions in our study shared the same fusion pattern with matching cancer, and no fusion positive HGPIN lesions were associated with paired fusion negative prostate cancer

We postulate that *TMPRSS2-ERG* fusion HGPIN is a distinct molecular subtype and its identification always indicates the presence of *TMPRSS2-ERG* prostate cancer.

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.

The detection of isolated *TMPRSS2-ERG* HGPIN could improve the positive predictive value of finding (fusion positive) prostate cancer in subsequent biopsies. This may impact clinical management of isolated HGPIN in prostate needle biopsies.