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V-ets erythroblastosis virus E26 oncogene homolog (avian)/ Trefoil factor 3/high-molecular-weight cytokeratin triple immunostain: a novel tissue-based biomarker in prostate cancer with potential clinical application☆,,★

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Summary

Trefoil factor 3 (TFF3) is associated with various cancers and overexpressed in a subset of prostate cancers. Functional studies suggest that v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG) down-regulates TFF3 expression in hormone-naïve prostate cancer. To characterize this inverse relationship, we developed a triple immunostain encompassing ERG, TFF3, and high-molecular-weight cytokeratin. Triple stain was performed on 96 tumors and 52 benign cases represented in tissue microarrays. Distinct ERG and TFF3 protein was expressed in 45% (43/96) and 36% (35/96) of prostate cancers, respectively. Coexpression was observed in 5% (5/96) of tumor cases, and 24% (23/96) did not express ERG or TFF3. The inverse expression of ERG and TFF3 was significant ($P < .0001$), with 57% (30/53) of ERG-negative tumors demonstrating TFF3 expression. Sensitivity and specificity of combined ERG and TFF3 expression in detecting prostate cancer were 76% and 96%, respectively. The feasibility of triple immunostain protocol was validated in a set of 76 needle biopsies. The application of this multiplex in situ biomarker for molecular characterization of prostate cancer and as a supplemental diagnostic and prognostic tool in prostate needle biopsies should be further explored.

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★Author contribution statement: M.A.R., F.D., and J.M.M. conceived the experiments in this study. K.P. and J.M.M. performed the experiments. K.P. and Y.C. analyzed the data. K.P., M.A.R., and J.M.M. reviewed the pathology material. All authors were involved in writing the manuscript and had final approval of the submitted and published versions.

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Keywords

ERG; TFF3; Biomarker; Prostate cancer

1. Introduction

Prostate cancer is a molecularly heterogeneous disease with recently characterized cancer-specific gene aberrations [1–3]. The most common genetic alteration found in prostate cancer is a gene fusion between *transmembrane protease, serine 2* (*TMPRSS2*) and *v-ets erythroblastosis virus E26 oncogene homolog (avian)* (*ERG*), which occurs in approximately 50% of cases [4–7]. ERG overexpression caused by the androgen-regulated *TMPRSS2* gene is a proxy for the gene fusion and serves as a prostate cancer–specific biomarker [8]. The detection of overexpressed cancer-associated genes from expression profiling analyses has introduced novel potential biomarkers [9–13]. Among these, trefoil factor 3 (TFF3) has been proposed as a promising candidate gene to identify ERG-negative prostate cancer [10,14]. *TFF3* is 1 of the 3 members of the TFF gene family located near *TMPRSS2* on chromosome 21q22.3. Besides their prominent expression in mucous epithelia, these peptides are also synthesized in the central nervous system [15]. It has been reported that TFF3, overexpressed in about 50% of prostate cancers and inhibited by concurrent ERG expression and androgen receptor (AR) signaling [14,16,17], is also involved in the regulation of cell migration, invasion, and angiogenesis in other cancers. It was such reciprocal exclusion of TFF3 and ERG at the protein level that helped us envision a multiplex tissue assay to detect prostate cancer. To develop the most comprehensive in situ test, we designed a triple immunohistochemistry (IHC) stain incorporating TFF3, ERG, and high-molecular-weight cytokeratin (HMWCK). In this study, we evaluated the performance of the indicated triple immunostain in prostate cancer and benign prostate using tissue microarrays (TMAs). To assess its potential clinical application in prostate cancer diagnosis, we also confirmed the feasibility of ERG/TFF3/HMWCK triple stain in needle biopsies.

2. Materials and methods

2.1. Development of ERG/TFF3/HMWCK triple stain and IHC evaluation

ERG/TFF3/HMWCK triple IHC staining was accomplished by sequentially applying the 3 antibodies using Bond Max autostainer (Leica Microsystems, Bannockburn, IL). Heat-induced antigen retrieval was performed to all 3 stains. Bond Polymer Refine Detection was used to stain ERG (clone EPR 3864, 1:175, from Ventana Medical Systems, Inc, Tucson, AZ), and Bond Polymer Refine Red Detection was used to stain both TFF3 (clone 15C6, 1:200, from EMD Chemicals, Inc, Gibbstown, NJ) and HMWCK (clone 34BE12, 1:175, from Dako, Carpinteria, CA). Diaminobenzidine (Leica Microsystems), Refine Red (Leica Microsystems), and Vector Blue (Vector Laboratories, Inc, Burlingame, CA) chromogens were used for ERG, TFF3, and HMWCK, respectively.

Study pathologists performed semiquantitative evaluation of nuclear ERG (brown color) and cytoplasmic TFF3 (red color) expression using a 4-tier grading system: negative (0), weakly positive (1+), moderately positive (2+), and strongly positive (3+). Any staining was

considered positive for ERG expression. For TFF3, moderate and strong intensities (2+ and 3+) were considered a positive result.

2.2. ERG/TFF3/HMWCK triple-stain validation and testing

TMAs were constructed using formalin-fixed, paraffin-embedded tissue from prostatectomy specimens of 96 men who underwent radical prostatectomy at our institution, as a monotherapy for clinically localized prostate cancer. The dominant and secondary tumor nodules available from each prostatectomy specimen were represented in the TMAs. The latter also included benign tissue of 52 cases. The clinical demographics of this cohort are presented in Table 1.

After validation of ERG/TFF3/HWMCK prostate triple stain on TMAs, 76 prostate needle biopsies were subjected to IHC and evaluated by study pathologists. Forty-one of these biopsies contained different amounts of prostatic adenocarcinoma with Gleason score 3 + 3 = 6 (n = 38) and 3 + 4 = 7 (n = 3). The other 35 biopsies contained atypical glands suspicious for prostate cancer. Variable areas of high-grade prostatic intraepithelial neoplasia (HGPIN) were also present in the biopsies.

2.3. Chromogranin A IHC in benign cases

To highlight the neuroendocrine origin of TFF3-expressing cells in the nonluminal layer of benign cases [16], TMAs were stained with chromogranin A antibody (LK2H10, 1:400; Biogenex, San Ramon, CA) using Bond Max autostainer.

2.4. Assessment of ERG gene rearrangement by fluorescence in situ hybridization

Four-micrometer-thick tissue sections were used to perform dual-color break-apart interphase fluorescence in situ hybridization (FISH) assay, as previously described [7,18]. Briefly, *ERG* gene rearrangement status was assessed using centromeric (BAC clone RP11–24A11 labeled green) and telomeric (BAC clone RP11–372O17 labeled red) probes and was determined independently by the study pathologists. Detection of 2 pairs of juxtaposed red and green signals that usually form 2 yellow signals demonstrates the absence of *ERG* rearrangement. A nucleus with *ERG* rearrangement through insertion shows 1 yellow signal for the normal allele and breakup of green-red signals for the rearranged allele. Rearrangement through deletion demonstrates a yellow signal for the normal allele and a single red signal for the rearranged allele. Benign epithelial and stromal cells have 2 yellow signals per nucleus and serve as internal controls for this break-apart FISH assay.

2.5. Statistical analysis

Statistical analysis system 9.2 by SAS Institute Inc (Cary, NC) was used for data analysis. The χ^2 test or Fisher exact test was used to evaluate the association between categorical variables. One-way analysis of variance was performed to compare continuous variables (eg, age) among groups (eg, TFF3/ERG). If a continuous variable (eg, prostate-specific antigen [PSA]) was skewed, the nonparametric Kruskal-Wallis test was used to test the median among groups. For all statistical tests, a *P* value less than .05 was considered statistically significant.

3. Results

3.1. ERG and TFF3 protein expression in prostate cancer

Of the 96 tumor cases, 45% (43/96) and 36% (35/96) showed discrete ERG and TFF3 overexpression, respectively. Five percent (5/96) demonstrated coexpression, and 24% (23/96) had neither ERG nor TFF3 expression (Fig. 1). Our previous observation of inverse relationship between ERG and TFF3 expression [14] was confirmed with 57% (30/53) of ERG-negative tumors expressing TFF3 compared with 12% (5/43) of ERG-positive tumors (χ^2 test, $P < .0001$).

3.2. ERG/TFF3 staining pattern in prostate cancer

In regard to intensity, ERG IHC demonstrated homogenous nuclear staining in ERG-positive cases (Fig. 1). Based on the staining pattern of different cores taken from a given tumor nodule, the extent of ERG staining was homogenous, as well. Among 43 ERG-positive cases, 7 cases showed weak protein expression, which also harbored *ERG* gene translocation as determined by FISH (see below). Strong ERG nuclear expression in endothelial cells served as an internal control, and weak to moderate nuclear staining was also seen in lymphocytes, as previously reported [8].

TFF3 IHC revealed a heterogeneous staining pattern in terms of both intensity and percentage of positive cells within a tumor nodule. Of the total 66 tumors in which TFF3 expression was detected, 35 cases with moderate-strong protein expression were deemed TFF3 positive and were included in the final analysis. Thirty-one cases were considered negative because of weak staining intensity (Table 2). Similar to ERG, we observed TFF3 expression in isolated HGPIN, as well as in HGPIN adjacent to positive tumor glands (Fig. 2). In prostate biopsies, TFF3 had moderate to strong cytoplasmic expression in colonic mucosa, serving as an internal control (not shown).

3.3. ERG/TFF3/HMWCK staining pattern in benign tissues

No ERG protein expression was observed in benign prostate tissue. Regarding TFF3, occasional benign glands demonstrated strong-focal TFF3 positivity toward the basal cell layer. These cells were also positive for chromogranin A, confirming the presence of scattered neuroendocrine cells in benign glands (Fig. 3C), previously described as being TFF3 positive [16]. This TFF3 expression in the neuroendocrine cells of benign glands was excluded from the analysis. Mostly weak-focal TFF3 expression in luminal epithelial cells was present in 44% (23/52) of benign cases, only 2 of which had moderate-focal intensity and were considered TFF3 positive (Table 2 and Fig. 3). TFF3 protein expression correlated significantly with type of tissue ($P = .0001$, χ^2 test). Specifically, 53% (35/66 cases) of TFF3-positive tumor cases had moderate or strong intensity, but only 4% (2/52) of benign cases had moderate-focal expression of the protein.

HMWCK expression was present in the cytoplasm of basal cells in the benign glands. Some areas of partial atrophy had patchy, discontinuous staining for HMWCK (not shown).

3.4. ERG protein expression correlates with ERG gene rearrangement status

All cases represented in TMAs were interrogated for *ERG* gene rearrangement by FISH. There was 100% concordance between ERG expression by IHC, including cases with weak (1+) staining, and *ERG* gene status by FISH, as initially reported [8].

3.5. Molecular heterogeneity in prostate cancer

Fig. 4 illustrates the results of triple immunostain and *ERG* break-apart FISH assay in a full section of a prostatectomy case with 2 separate tumor foci. This particular case was reassessed, given an initial discrepancy of results on ERG and TFF3 IHC performed on TMA. Although the Gleason grade is identical and the histomorphology is very similar in 2 tumor foci, these correspond to 2 distinct prostate cancers based on their differential expression profiles detected by ERG/TFF3/HMWCK IHC and different *ERG* fusion status detected by FISH. This differential expression of ERG and TFF3 underscores the complexity and diversity of molecular events occurring in discrete tumors arising in the prostate of the same individual.

3.6. Translational use and validation of the assay

Based on the IHC results on TMAs, we demonstrated that the sensitivity and specificity of ERG and TFF3 in staining prostate cancer was 76% and 96%, respectively. HMWCK expression in the cytoplasm of basal cells (blue chromogen) helped in the differentiation between HGPIN and tumor glands in the presence of ERG or TFF3 expression (Fig. 2). To further evaluate the potential use of the ERG/TFF3/HMWCK triple stain, we applied the IHC protocol to 76 prostate biopsies containing different extents of prostatic adenocarcinoma (n = 41), atypical glands suspicious for carcinoma (n = 35), and variable areas of HGPIN (see Materials and Methods). Robust reproducibility was seen in this set of biopsies (Table 3). Among prostate biopsies containing cancer (n = 41), tumor areas were highlighted in 78% (32/41) of them by positive staining for ERG only (n = 19), TFF3 only (n = 12), or coexpression of ERG and TFF3 (n = 1), along with the absence of basal cells (negative staining for HMWCK). Nine tumors (22%) were negative for either biomarker (Fig. 5). Forty-six percent (16/35) of foci of atypical glands suspicious for carcinoma present in the remainder of the biopsies were highlighted by positive staining for ERG only (n = 5) or TFF3 only (n = 11), accompanied by the absence of basal cells. Nineteen (54%) of these cases were negative for either biomarker (Fig. 6).

3.7. Correlation with clinicopathologic parameters

No associations between ERG/TFF3 expression in tumors (n = 96) and clinical or pathologic parameters (ie, age, PSA, Gleason score, tumor stage, and biochemical recurrence) were observed.

4. Discussion

Prostate cancer can usually be diagnosed based on morphology alone from standard hematoxylin and eosin (H&E)–stained slides. However, there is an unavoidable variability in diagnosis among pathologists when challenged with small atypical foci on prostate needle biopsies [19]. In an era where new molecular discoveries are being rapidly translated into

the clinic, patient care will be heavily dependent not only on the accurate diagnosis from prostate needle biopsy but also on further stratification using valuable tissue biomarkers that can supplement H&E staining. Based on molecular data and IHC experience from previous work [7,8,14,16,17], we selected ERG and TFF3 as potential biomarker candidates for a new tissue-based multiplex assay.

TFF3 is a member of a family of extracellular peptides produced and secreted by gastrointestinal mucosa that stimulates epithelial cell migration and mucosal restoration after injury [20]. TFF3 expression has been associated with various cancers [16,17] and found to be overexpressed in a subset of prostate cancers from gene expression profiling using complementary DNA microarray data [10,21]. In 2004, 2 studies by Garraway et al [17] and Faith et al [16] reported TFF3 protein overexpression in 10% and 19% of benign prostate tissue, 35% and 47% of HGPIN, 42% and 47% of primary prostate cancer, and 39% and 46% of metastases, with a cutoff of 50% and 20% of target cells being positive, respectively. In these studies, varying intensities of TFF3 expression were detected in tumor glands.

Using chromatin immunoprecipitation followed by DNA sequencing (CHIP-seq), our group recently elucidated a regulatory role of ERG protein on TFF3 expression by binding to ETS binding sites in the *TFF3* promoter region in the presence of AR signaling, consistent with an inverse relationship between ERG and TFF3 [14]. In the current study using ERG/TFF3/HMWCK triple immunostain, we have confirmed our previous observations with 57% (30/53) of ERG-negative cases expressing TFF3, in contrast to 12% (5/42) of ERG-positive cases ($P < .0001$). Altogether, this assay demonstrates 76% sensitivity and 96% specificity to detect prostate cancer represented on TMAs.

Coupled with HMWCK staining, the high specificity of ERG expression in prostate cancer and the overall inverse relationship of ERG and TFF3 expression could make this triple immunostain of further clinical use in diagnostically challenging prostate biopsies. In our validation set of 76 biopsies, TFF3 expression was identified in 31% of both tumors and atypical gland cases. ERG was expressed in 49% of prostate cancer cases and in 14% of atypical glands suspicious for carcinoma. The sensitivity of the ERG/TFF3/HMWCK immunostain to detect prostate cancer in needle biopsies is 78%, comparable with that observed using the TMA data. In addition to the potential diagnostic application, this assay may add prognostic information as discussed below.

ERG is involved in the most common prostate cancer–specific gene fusion [7], observed in approximately 50% of localized prostate cancer and in metastatic tumors that, originating in the prostate, share the same fusion status with their primaries [22]. We first described the use of a rabbit monoclonal ERG antibody that is highly sensitive and specific compared with FISH analysis [8]. Several subsequent IHC-based studies on independent cohorts have confirmed the efficacy of this antibody [23–25]. Consistent with the findings seen in FISH-based studies on prostatectomy tissue, both intensity and extent of ERG nuclear expression are homogenous in a given tumor nodule by IHC. In addition, a subset of HGPIN lesions also harbor *ERG* gene translocation [26], which can also be detected by IHC. Two previous studies demonstrated the use of ERG IHC on prostate needle biopsies. Tomlins et al [27] reported ERG expression in 44% of prostate cancer, 18% of HGPIN, and 11%

of atypical glands suspicious for carcinoma, in 418 biopsy cores. Gao et al [28] performed ERG IHC on 162 samples with isolated HGPIN to show that prostate cancer was detected on repeat biopsies of 95% of ERG-positive and 5% of ERG-negative HGPIN cases. This is of particular importance because most recent data highlight the potential impact of determining *ERG* status of prostate cancer, which would predict response to hormonal therapy [29] and could also provide a foundation for the development of upcoming therapeutics to prevent cancer progression [30]. As described by Karnes et al [29], adjuvant androgen deprivation therapy is more effective in ERG-positive tumors than in ERG-negative tumors. Based on the role of poly(ADP-ribose) polymerase 1 in ERG-mediated transcription and cell invasion, Brenner et al [30] tested a poly(ADP-ribose) polymerase 1 inhibitor and detected that *ERG* gene fusion–positive prostate cancer xenografts were preferentially sensitized to the inhibition compared with *ERG* fusion–negative xenografts. More recently, Shao et al [31] designed siRNAs targeting the 2 most common *ERG* fusion mRNA isoforms to be delivered in liposomal nanovectors and observed in vivo tumor growth inhibition. Therefore, assessment of *ERG* fusion status in prostate cancer (ie, use of tissue biomarker for molecular characterization) could soon translate into individualized treatment decision and management with profound clinical impact.

We acknowledge some limitations in the present study. TFF3 is not a tumor-specific marker, and therefore, its expression, especially when weak and focal, should not lead to cancer diagnosis. The latter still needs to be determined in the context of histomorphology and absence of basal cells. The main aims of this work were to confirm the inverse relationship of ERG and TFF3 at the protein level using IHC and to test the feasibility of this triple stain in clinical samples. Future studies are required to investigate TFF3 expression in benign mimickers of prostate cancer such as partial atrophy and adenosis, where basal cell markers can be patchy and even negative. Another limitation is that our TMA cohort contains a small number of Gleason grade 6 tumors (n = 12). Larger cohorts with more Gleason grade 6 prostate cancers are necessary to interrogate the frequency of ERG and TFF3 overexpression and their association with clinical parameters.

In conclusion, we have developed a multiplex in situ biomarker for prostate cancer combining IHC to detect ERG, TFF3, and HMWCK expression. ERG protein expression was present in 45% of prostate cancers, and TFF3 was overexpressed in 36%, significantly more in ERG-negative than in ERG-positive tumors. Overall, 76% of 96 prostate cancer cases in TMAs had overexpression of either ERG and/or TFF3. Relevant to the clinical setting, the feasibility of this triple immunostain in needle biopsies has also been demonstrated with comparable sensitivity (78%). Validation studies are required to assess the potential application of TFF3/ERG/HMWCK triple stain in clinical practice for diagnosis (eg, comparison with AMACR/HMWCK/p63 triple stain) and in the near future for prognostication and molecular characterization of prostate cancer.

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Conflict of interest and funding disclosures:

M.A.R. and F.D. are coinventors of the patent on the detection of gene fusions in prostate cancer, filed by The University of Michigan and the Brigham and Women's Hospital. The diagnostic field of use for *ETS* gene fusions has been licensed to Hologic Gen-Probe. This study was supported by the Early Detection Research Network (5U01 CA11275-07 and I4-A424) and DOD Synergy (W81XWH-11-1-0410).

Abbreviations:

TFF3	trefoil factor 3
ERG	v-ets erythroblastosis virus E26 oncogene homolog (avian)
HMWCK	high-molecular-weight cytokeratin
TMPRSS2	transmembrane protease, serine 2
IHC	immunohistochemistry
TMA	tissue microarray
HGPIN	high-grade prostatic intraepithelial neoplasia
FISH	fluorescence in situ hybridization
H&E	hematoxylin and eosin
siRNA	small interfering RNA

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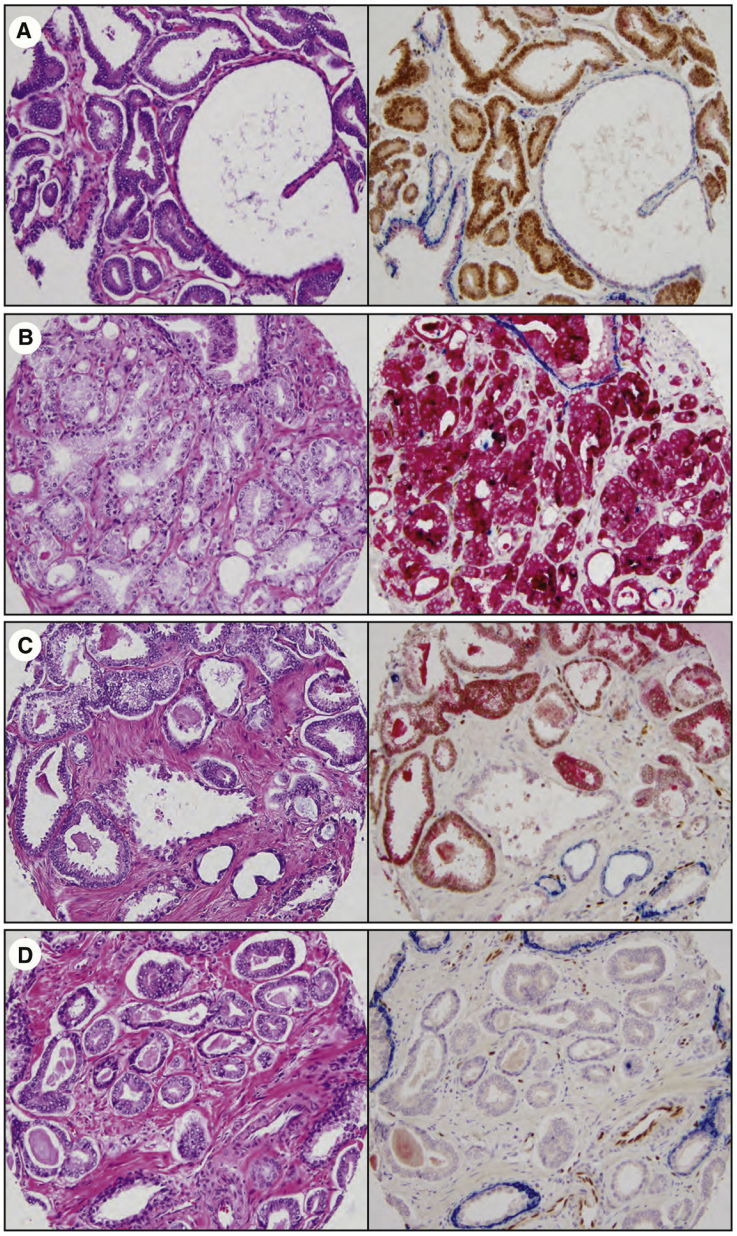

Fig. 1.

Validation of ERG/TFF3/HMWCK triple immunostain for the detection of prostate cancer using TMAs. Four cases of prostatic adenocarcinoma with different immunoprofiles are illustrated. ERG (nuclear), TFF3 (cytoplasmic), and HMWCK (cytoplasmic) expression is highlighted by diaminobenzidine (brown), Refine Red (red), and Vector Blue (blue) chromogens, respectively. A, ERG-positive prostate cancer. B, TFF3-positive prostate cancer. The mutually exclusive expression of ERG and TFF3 is seen in these first 2 panels. C, Prostatic adenocarcinoma with ERG/TFF3 coexpression. D, Prostatic adenocarcinoma negative for both ERG and TFF3. HMWCK expression is observed in the basal cells of benign glands and HGPIN. ERG nuclear expression is present in endothelial cells, which

serves as an internal control. H&E stain in the left panels and ERG/TFF3/HMWCK triple immunostain in the right panels, ×20.

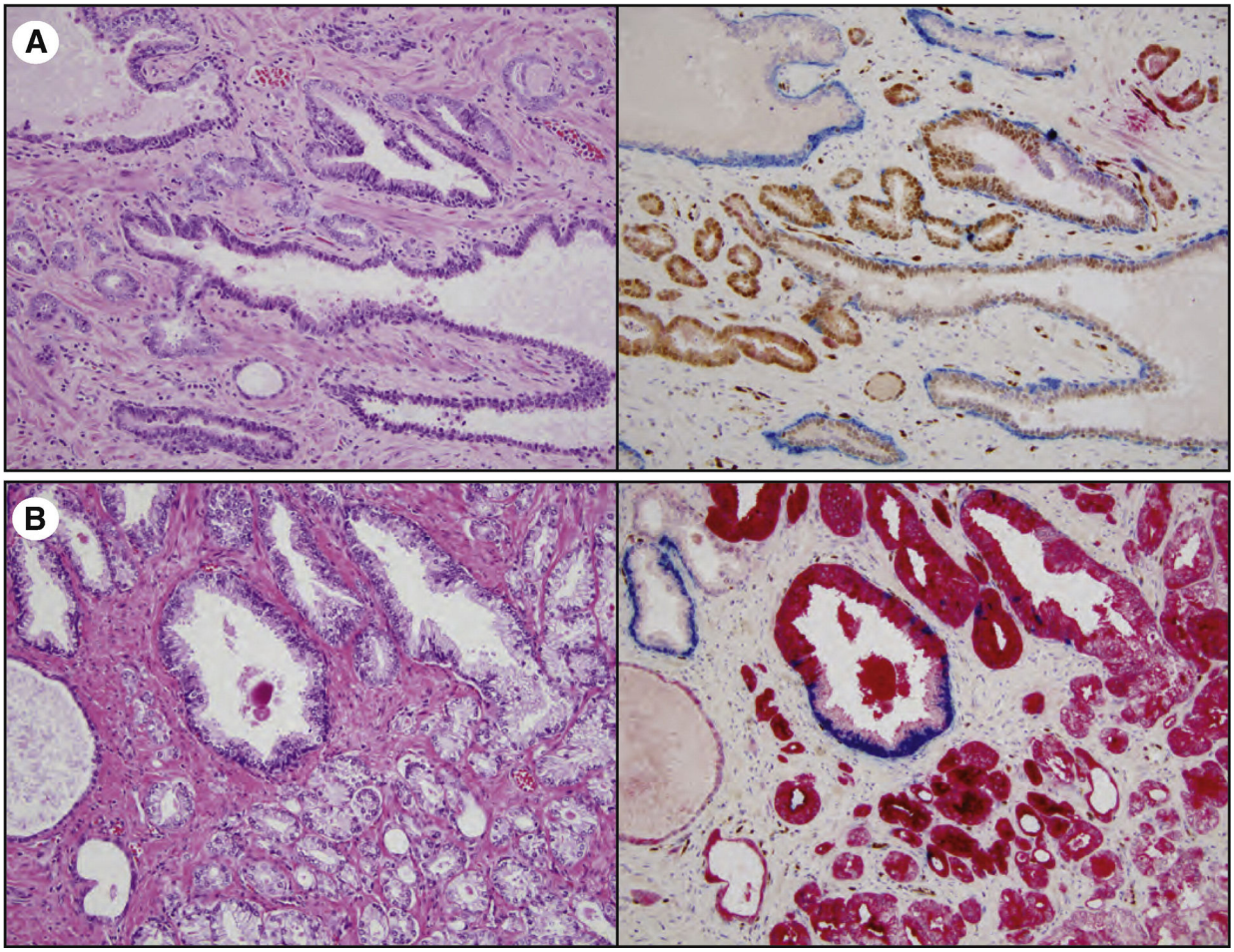

Fig. 2.

HGPIN demonstrates similar immunoprofile to adjacent prostate cancer. A, ERG-positive HGPIN and adjacent cancer glands with same biomarker expression. Note that tumor glands with perineural invasion (right upper corner) have ERG/TFF3 coexpression. B, TFF3-positive HGPIN and adjacent cancer glands with identical biomarker expression. HMWCK expression highlights the basal cells of benign glands and HGPIN. H&E stain in the left panels and ERG/TFF3/HMWCK triple immunostain in the right panels, ×20.

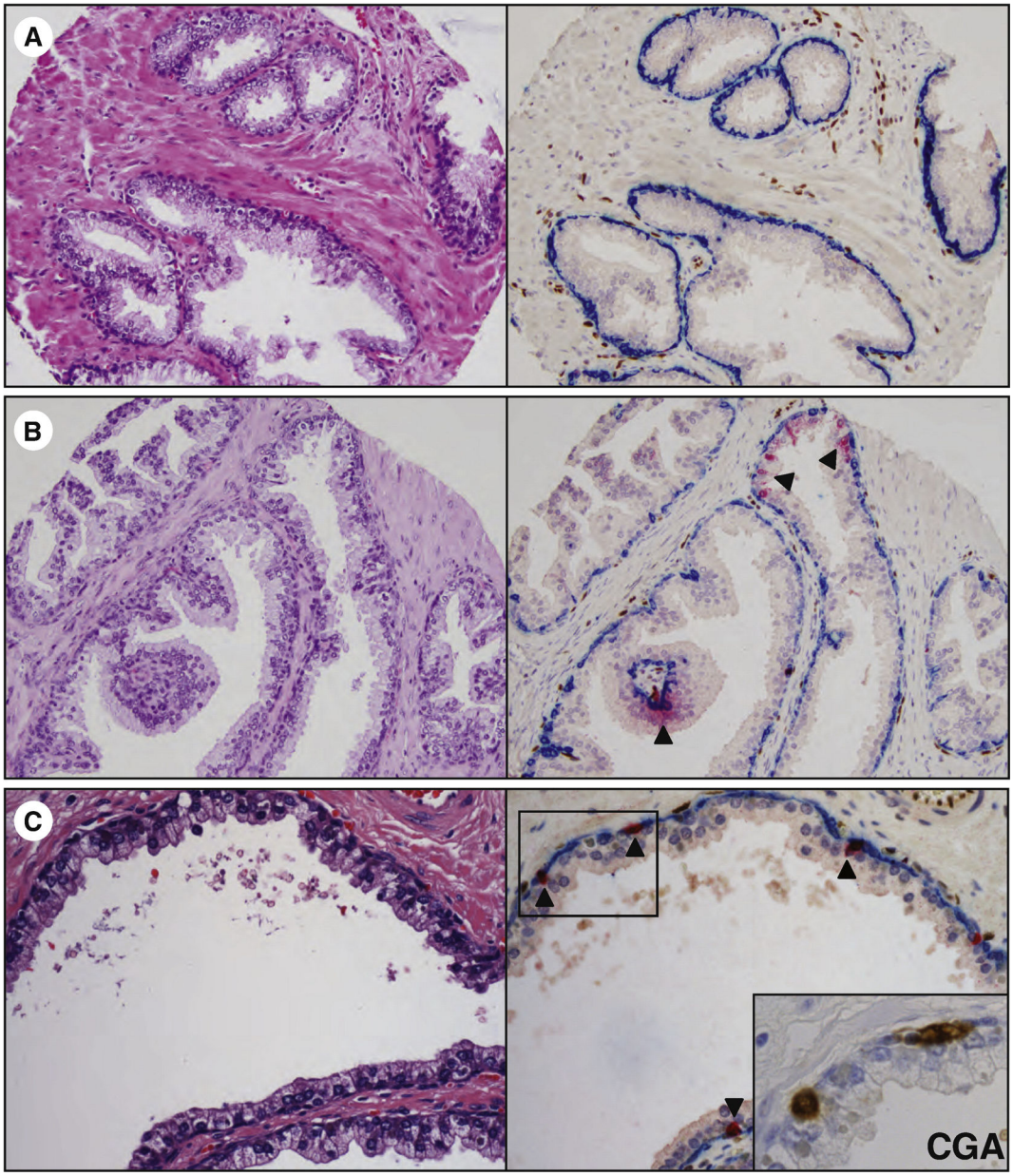

Fig. 3.

TFF3 expression in benign glands. A, TFF3-negative benign glands (×20). B, Focally TFF3-positive benign glands. Weak and focal TFF3 expression in luminal epithelial cells (arrowheads) is noted (×40). C, Focally TFF3-positive benign gland. Rare cells (arrowheads) demonstrate strong TFF3 positivity. These correspond to scattered neuroendocrine cells in benign glands, highlighted by chromogranin A (CGA) immunostain (inset; ×40). H&E stain in the left panels and ERG/TFF3/HMWCK triple immunostain in the right panels.

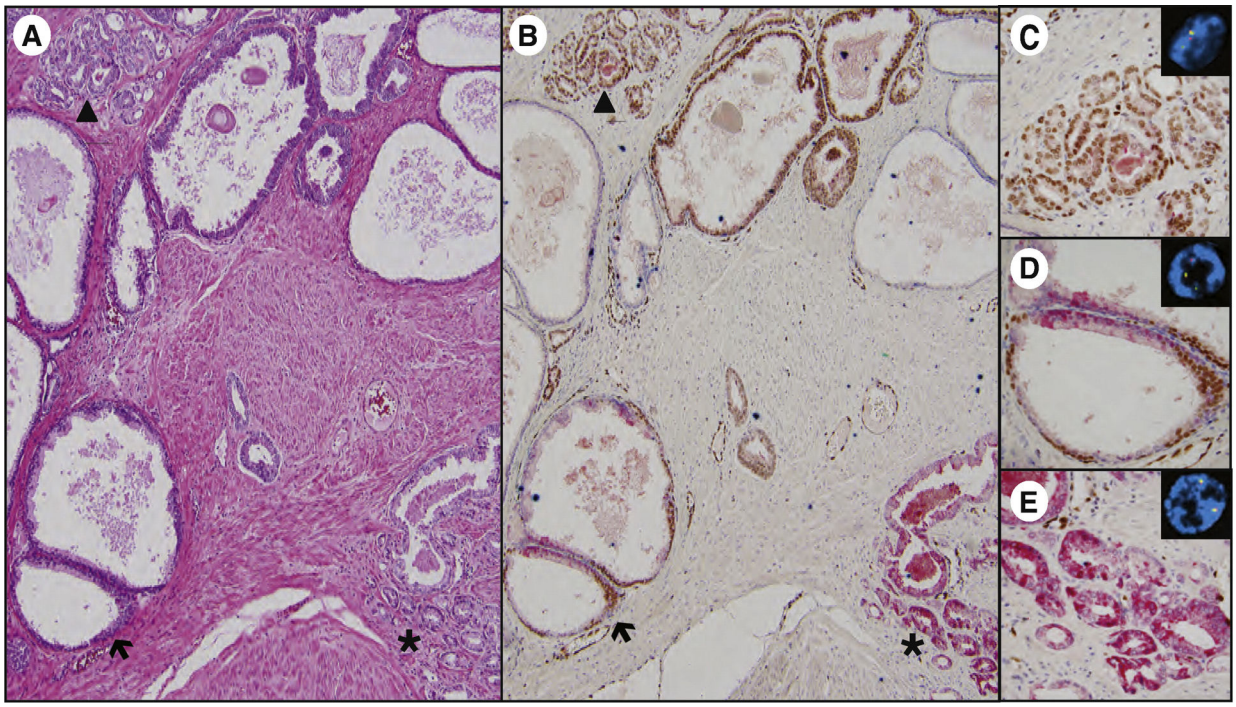

Fig. 4.

Heterogeneous ERG and TFF3 expression in prostate cancer. A, Two separate foci (arrowhead and asterisk) of prostatic adenocarcinoma Gleason score 3 + 3 = 6 and multiple HGPIN lesions (arrow) are seen on H&E (×10). B, Distinct areas are noted with ERG/TFF3/HWMCK triple immunostain: ERG-positive prostate cancer adjacent to ERG-positive HGPIN (arrowhead), TFF3-positive prostate cancer adjacent to TFF3-positive HGPIN (asterisk), and ERG/TFF3-positive HGPIN (arrow; ×10). C, ERG-positive tumor demonstrates *ERG* translocation through insertion by FISH break-apart assay (inset; ×40). D, ERG/TFF3-positive HGPIN demonstrates *ERG* translocation through insertion by FISH break-apart assay (inset; ×40). E, TFF3-positive tumor demonstrates no *ERG* translocation by FISH break-apart assay (inset; ×40).

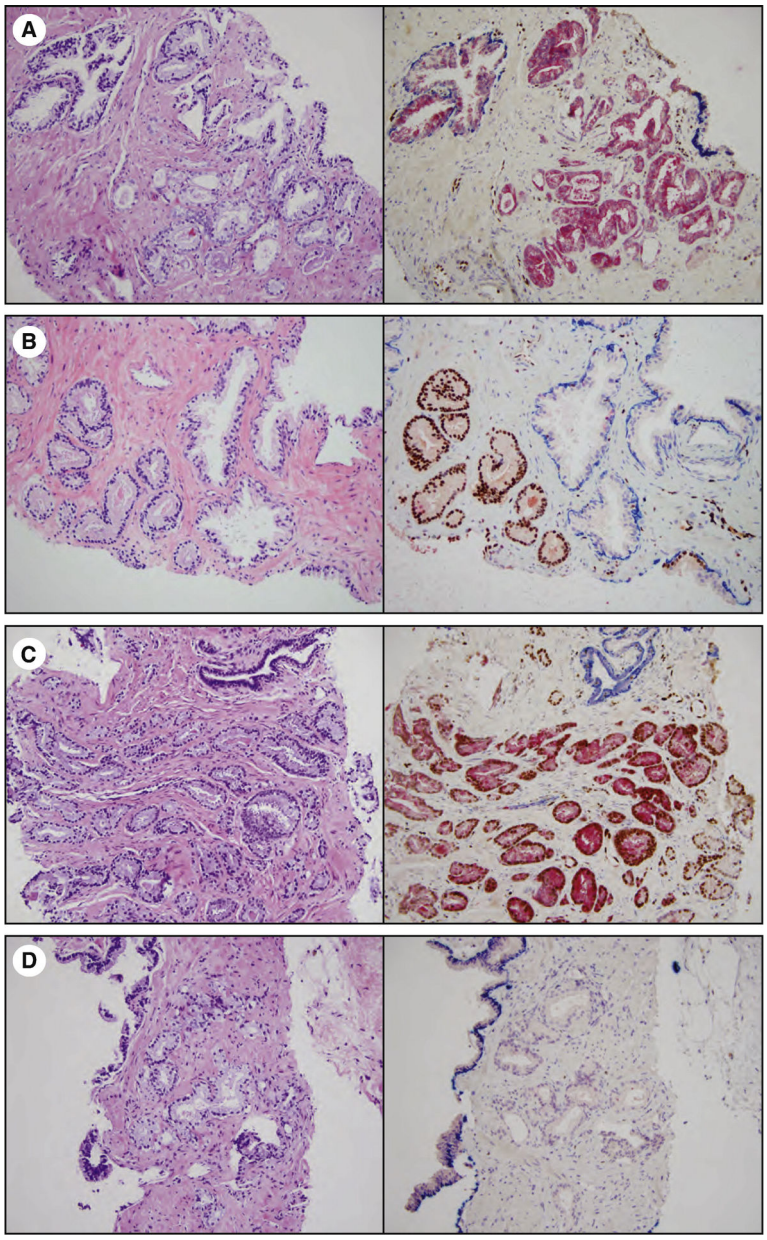

Fig. 5.

Feasibility of ERG/TFF3/HMWCK triple immunostain in prostate needle biopsies. A, TFF3-positive prostate cancer and adjacent TFF3-positive HGPIN. B, ERG-positive prostate cancer close to ERG-positive HGPIN. C, ERG/TFF3-positive prostatic adenocarcinoma. D, One of the cases of prostatic adenocarcinoma that is negative for ERG or TFF3 expression. HMWCK expression is observed in the basal cells of benign glands and HGPIN, and ERG expression in endothelial cells serves as an internal control. H&E stain in the left panels and ERG/TFF3/HMWCK triple immunostain in the right panels, ×20.

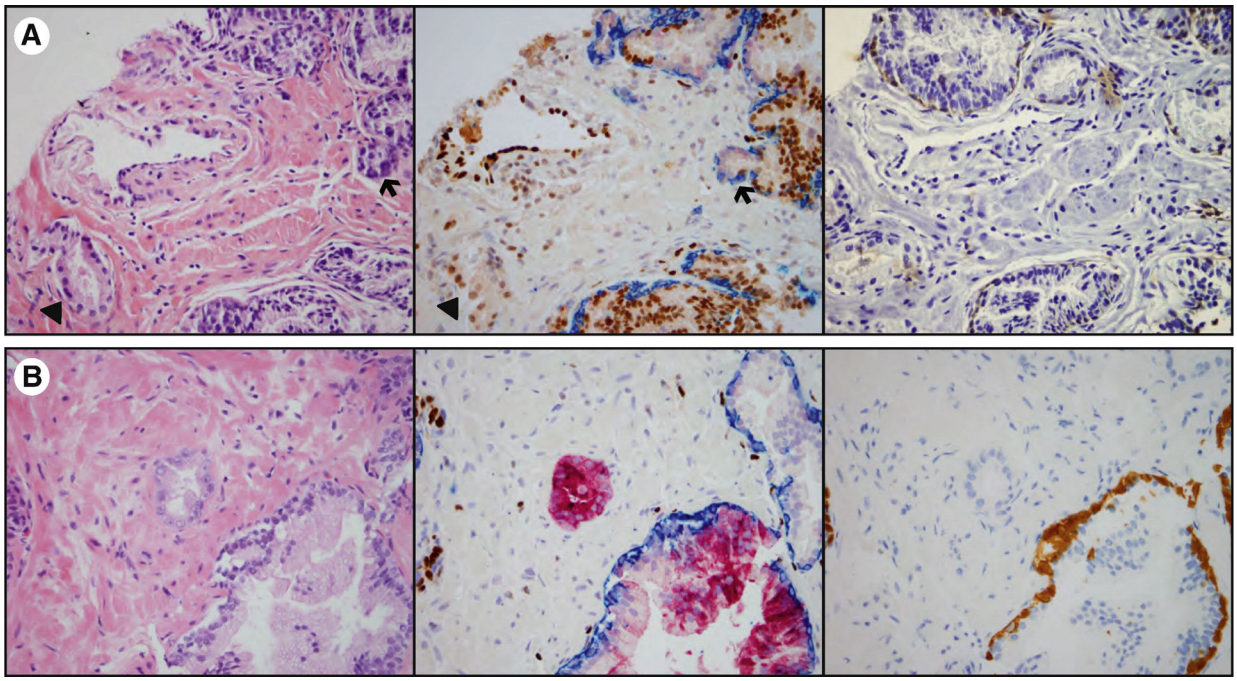

Fig. 6.

Potential application of ERG/TFF3/HMWCK triple immunostain in prostate needle biopsies. Strong expression of ERG or TFF3 (midpanel) in atypical glands suspicious for carcinoma compared with H&E (left panel) and p63/HMWCK stain (right panel). A, ERG-positive atypical gland suspicious for carcinoma (arrowhead) close to ERG-positive HGPIN. Arrow indicates outpouching of HGPIN. B, TFF3-positive atypical gland suspicious for carcinoma and adjacent TFF3-positive HGPIN. HMWCK expression is observed in the basal cells of benign glands and HGPIN, and ERG expression in endothelial cells serves as an internal control. H&E stain in the left panels, ERG/TFF3/HMWCK triple immunostain in the midpanels, and p63 and HMWCK stain in the right panels, ×40.

Table 1

Clinical demographics of the prostate cancer cohort (n = 96)

Age (y)	
Maximum	75
Minimum	42
Median	62
PSA (ng/mL)	
Maximum	24.23
Minimum	1.9
Median	5.1
PSA <10	83
PSA 10-<20	10
PSA≥20	3
Gleason score	
<7	12
7	71
>7	13
Tumor stage	
pT2	62
pT3	32
pT4	2
Surgical margin	
Negative	80
Positive	16
PSA biochemical recurrence[a]	
No	86
Yes	9

[a]Clinical follow-up of 1 patient was not available.

Table 2

Intensity of TFF3 protein expression in tumor and benign glands on TMAs

Intensity	Benign	Tumor
Negative (0)	29	30
Weak (1+)	21	31
Moderate-Strong (2+ and 3+)	2	35
Total	52	96

Table 3

ERG/TFF3/HMWCK immunostain on prostate needle biopsies (n = 76)

Empty Cell	PCA	Atypical glands suspicious for carcinoma
ERG+/TFF3−	19 (46.3%)	5 (14.3%)
ERG−/TFF3+	12 (29.3%)	11 (31.4%)
ERG+/TFF3+	1 (2.4%)	0 (0%)
ERG−/TFF3−	9 (22%)	19 (54.3%)
Total	41[a]	35

[a]Three of 41 PCA cases were Gleason grade 7 (3+4). Two were ERG+/TFF3−, and 1 was negative for both ERG and TFF3.