TMPRSS2-ERG Fusion Prostate Cancer: An Early Molecular Event Associated With Invasion

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Abstract: Prostate cancer (PCA) is one of the most prevalent cancers and a major leading cause of morbidity and mortality in the Western world. The *TMPRSS2-ERG* fusion was recently identified as a common recurrent chromosomal aberration in this malignancy. In our study, we interrogated a broad spectrum of benign, precursor, and malignant prostatic lesions to assess the *TMPRSS2-ERG* fusion status using a multicolor interphase fluorescence in situ hybridization assay. Samples from hospital-based cohorts consisted of 237 clinically localized PCA, 34 hormone naive metastases, 9 hormone refractory metastases, 26 high grade prostatic intraepithelial neoplasia lesions, 15 samples of benign prostatic hyperplasia, 38 of proliferative inflammatory atrophy, and 47 of benign prostatic tissue. The *TMPRSS2-ERG* fusion was present in 48.5% of clinically localized PCA, 30% of hormone naive metastases, 33% of hormone refractory metastases, and in 19% of high grade prostatic intraepithelial neoplasia lesions in intermingling to cancer foci. Almost all these fusion positive cases show a homogenous distribution of the fusion pattern. In contrast, none of the other samples harbored this genetic aberration. If we consider the high incidence of PCA and the high frequency of this gene fusion, *TMPRSS2-ERG* is the most common genetic aberration so far described in human malignancies. Furthermore, its clinical application as a biomarker and ancillary diagnostic test is promising given its high specificity.

Key Words: prostate cancer, *TMPRSS2-ERG* gene rearrangement, fluorescence in situ hybridization, PIN

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Prostate cancer (PCA) is a major leading cause of male cancer-related death, second only to lung cancer and represents 10% of all cancer deaths in men in the United States.[10,26] An estimated new 234,460 cases will be diagnosed and over 30,000 men will die of PCA in the United States in 2006. This means that 1 in 6 men will be diagnosed with PCA during their lifetime.

Until recently, carcinomas were considered to harbor only a very small fraction of known, disease-specific gene rearrangements. These had been thought to be primarily characteristic of leukemias, lymphomas, and sarcomas.[15] By applying a new bioinformatics approach and verifying by various independent techniques, a novel and common gene rearrangement in PCA was identified. The genes involved are the androgen-regulated gene *TMPRSS2* (21q22.3) and *ETS* transcription factor family members, either *ERG* (21q22.2), *ETV1* (7p21.2), or *ETV4* (17q21). Of note, the *ETS* transcription factors are also involved in translocations of the Ewing's family tumors.[5] The fusion of *TMPRSS2* with *ETS* family members explains the mechanism for over expression of the *ETS* genes in PCA. Of these, the *TMPRSS2-ERG* fusion is by far the most frequent as compared with *TMPRSS2-ETV1* or *TMPRSS2-ETV4* fusion.[23–25,27] For example, in surgical series the *TMPRSS2-ERG* gene fusion was identified in about 50% of tumors, whereas in population-based cohorts of clinically localized PCA, the frequency of *TMPRSS2-ERG* fusion was about 15%.[7] Furthermore, since our original report, using several independent techniques [fluorescence in situ hybridization (FISH), single nucleotide polymorphism arrays, and quantitative polymerase chain reaction] we discovered that approximately 65% of the tumors with the *TMPRSS2-ERG* fusion harbor a common intronic deletion site between *ERG* and *TMPRSS2* on chromosome 21q22.2-3.[17] These recurrent gene fusions represent

a new paradigm for solid tumors. Coupled with the extremely high incidence of PCA the fusion of *TMPRSS2* with *ETS* family members is likely to be the most common rearrangement yet identified in any human malignancy and the only rearrangement present in one of the most prevalent carcinomas. Similar to a previous study, here we also focus on the *TMPRSS2-ERG* fusion as the most common of the *TMPRSS2-ETS* fusions.[18] On the basis of what is known and our recent work about the *TMPRSS2-ERG* fusion, we hypothesize that *TMPRSS2-ERG* is exclusively seen in PCA and may occur as a very early event in cancer development. In a wide spectrum of prostatic tissue, we assess the frequency of the *TMPRSS2-ERG* fusion by employing a FISH assay.

MATERIALS AND METHODS

Case Selection and Diagnosis Confirmation

From hospital-based cohorts, 7 tissue microarrays (TMAs) were used composed of a wide spectrum of prostatic lesions and benign prostatic tissue. One to 12 cores (median = 3) representing different areas were taken from one or more paraffin-embedded tissue blocks for each sample. We could evaluate in total 1423 cores from 397 different samples obtained from 300 patients. The samples included 237 clinically localized acinar PCA, 34 hormone naive metastases (ie, metastases to regional lymph nodes), 9 hormone refractory metastases (ie, distant metastases to distant lymph nodes, bone, soft tissue, and other sites), 9 high grade prostatic intra-epithelial neoplasia (HGPIN), 15 benign prostatic hyper-plasia, 17 simple atrophy (SA), 6 SA with cyst formation, 6 postatrophic hyperplasia, 9 unspecified atrophy, and 47 benign prostatic tissue derived from the peripheral zone of prostates containing cancer. Of the 237 clinically localized PCA samples, 17 also had high grade PIN lesion at least in one core representing PCA, adding up to a total of 26.

The demographics and clinico-pathologic for 297 cases (including most of the hormone naive cases) have been previously described as part of the Ulm University cohort[9] and PCA progression cohort.[2] For 67 cases, no clinical or pathology data were available. For the 297 cases with clinical data, the mean age at presentation was 63 years (range 48 to 76) with a mean preoperative PSA, 18.5 ng/mL (range 1.0 to 248). There were 25% Gleason grade 2 to 6, 37% Gleason grade 7, and 39% Gleason grade 8 to 10. The breakdown of pathology stage (pT) was 42% pT2, and 52% and pT3. Nineteen percent of the men exclusively from the Ulm cohort presented with positive lymph node status.

To be included in the evaluation, at least one assessable core of each sample was present in matching cores of the step section for the hematoxylin and eosin (H&E) and FISH slide. All samples were derived from IRB-approved study protocols. Morphologic diagnosis was confirmed on H&E-stained paraffin sections of each TMA before FISH assessment. In a subset of cases with equivocal diagnosis, immunohistochemistry for prostatic

basal cells was performed. For that purpose, serial paraffin sections were cut and set on coated slides. Subsequently, they were deparaffinized in xylene and rehydrated in graded ethanols. Pressure cooking was applied as 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. Immuno-staining was performed with the avidin-biotin peroxidase technique.

Assessment of *TMPRSS2-ERG* Fusion Status Using an Interphase FISH Assay Testing for ERG Break Apart

We have previously described the FISH analysis for the fusion of *TMPRSS2-ERG*. Both genes are located so close together that a fusion cannot be observed directly (data not shown). Therefore, we developed a break-apart assay to identify when the telomeric and centromeric components of *ERG* split apart.[24] However, we pre-viously demonstrated by quantitative polymerase chain reaction that cases showing *ERG* break apart with the FISH assay had a *TMPRSS2-ERG* fusion. This *ERG* break-apart FISH assay for indirect evidence of the *TMPRSS2-ERG* fusion is explained in Figure 1. The *ERG* break-apart probe system was applied, consisting of the Biotin-14-dCTP labeled BAC clone RP11-24A11 (eventually conjugated to produce a red signal) and the Digoxigenin-dUTP labeled BAC clone RP11-137J13 (eventually conjugated to produce a green signal), spanning the neighboring centromeric and telomeric region of the *ERG* locus, respectively. All BAC clones were obtained from the BACPAC Resource Center, Children's Hospital Oakland Research Institute (CHORI), Oakland, CA. Using this break-apart probe system, a nucleus without *ERG* rearrangement demon-strates 2 pairs of juxtaposed red and green signals. Juxtaposed red-green signals generally form a yellow fusion signal. A nucleus with an *ERG* break apart (which is indicative of 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 nontranslocated *ERG* allele in each cell. This FISH assay was modified from the assay originally described by Tomlins et al.[24] The new FISH assay moved the 5' telomeric probe about 600 kb in a telomeric direction (closer to *TMPRSS2*) with the intention of developing a more specific assay.[17] Before tissue analysis, the integrity and purity of all probes were verified by hybridization to normal peripheral lymphocyte metaphase spreads. Tissue hybridization, washing, and fluorescence detection were performed as described previously.[8,20] At least one TMA core could be evaluated on 397 samples (equals 300 patients) from 7 different TMAs, adding up to a total of 1423 assessable cores. The technical difficulties with this assay included the absence of diagnostic material to
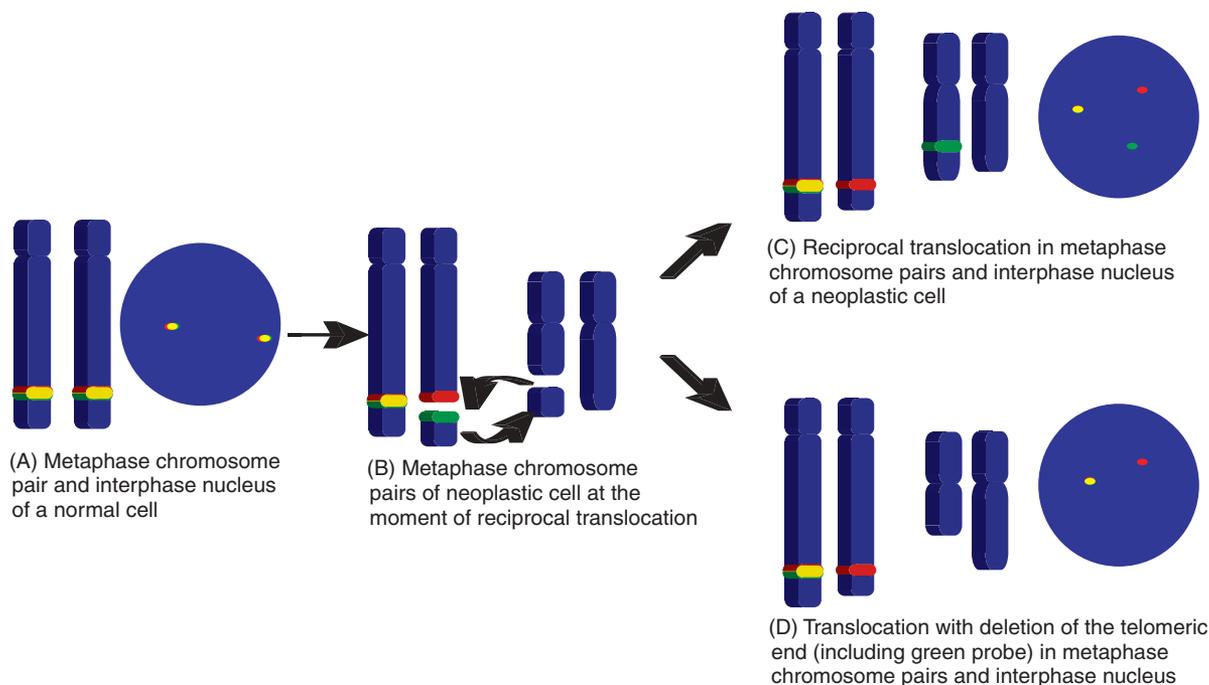
FIGURE 1. Schematic representation of the characteristics of a dual color probe FISH break-apart assay. A, Metaphase chromosome pair and corresponding interphase nucleus of a normal cell. Two colored probes span the centromeric and telomeric sequences (red and green, respectively), neighboring the region of interest. Depending on probe design (eg, the distance between the hybridized sequences) and state of the secondary structure of the genomic DNA at the time of fixation, a break-apart signal pair may appear either as a juxtaposed red and green signal or as a single yellow signal. B, Two metaphase chromosome pairs of a neoplastic cell at the time of a reciprocal translocation. The break apart occurs between the 2 differentially colored break-apart probes. C, Metaphase chromosome pair and corresponding interphase nucleus of a neoplastic cell carrying the reciprocal translocation. One of the yellow signals splits in separate red and green signals of the derivative chromosomes. The nonderivative chromosome keeps the yellow signal. D, Metaphase chromosome pairs and corresponding interphase nucleus of a neoplastic cell carrying the translocation with a deletion of the telomeric probe (green) at the time of the break apart. One of the yellow signals splits and the telomeric segment (green probe) is deleted. This results in a single red signal of one of the derivative chromosomes, and a yellow signal of the nonderivative chromosome.

evaluate, weak probe signals, and overlapping cells preventing an accurate assessment in a total of 741 cores from 144 samples (equals 133 patients).

The samples were analyzed under a $100\times$ oil immersion objective using an Olympus BX-51 fluorescence microscope equipped with appropriate filters, a CCD (charge-coupled device) camera and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA). Evaluation of the tests was independently performed by 2 pathologists (S.P. and J-M.M.) both with experience in analyzing interphase FISH experiments. For each case, we attempted to score at least 100 nuclei. Cases with significant differences between the 2 independent evaluations were refereed by a third pathologist (M.A.R.).[7,17]

Statistics

The Gleason score was explored for association with rearrangement status. χ^2 test and Fisher exact test were used appropriately. The statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc, Chicago, IL) with a significance level of 0.05.

RESULTS

Of the clinically localized PCA samples, 48.5% (115/237) demonstrated the *TMPRSS2-ERG* fusion. Thirty percent (71/237) demonstrated the *TMPRSS2-ERG* fusion through the deletion of the telomeric break-apart probe (Figs. 2A, B, E, and F), and 18.5% (44/237) showed *TMPRSS2-ERG* fusion through translocation (Figs. 3C, D). Thirty percent (10/34) of the hormone naive metastases demonstrated the *TMPRSS2-ERG* fusion. In the group of the hormone naive metastases, 12% (4/34) had the *TMPRSS2-ERG* fusion through the deletion of the telomeric break-apart probe and 18% (6/34) showed *TMPRSS2-ERG* fusion through translocation. A third group of hormone refractory metastases consisted of 9 samples. Of these, 33% (3/9) demonstrated the *TMPRSS2-ERG* fusion, all through deletion (Table 1).

Noteworthy, 19% (5/26) of high grade PIN lesions revealed the *TMPRSS2-ERG* fusion through the deletion of the telomeric break-apart probe. None of the high grade PIN lesions showed *TMPRSS2-ERG* gene fusion through translocation. Thus, the majority of high grade
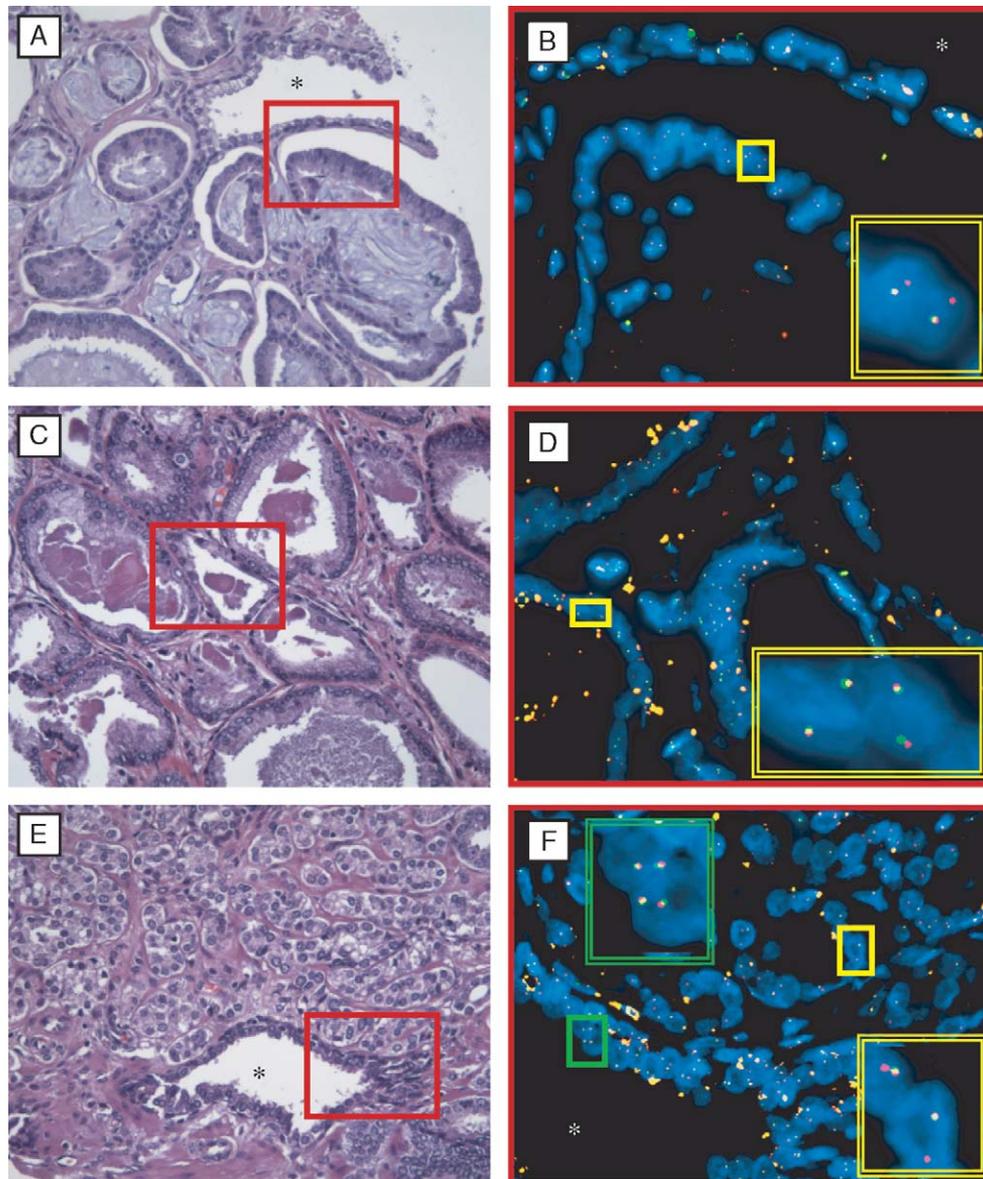
FIGURE 2. H&E stains and corresponding FISH images of *TMPRSS2-ERG* fusion assay. A, Prostatic tissue with PCA glands surrounding a benign prostatic gland (lumen of the benign prostatic gland marked with asterisk). B, FISH image of the red-boxed area in (A), displaying part of the benign gland on top and part of the PCA gland below. Each nucleus of the benign gland shows 2 yellow signals indicating absence of *TMPRSS2-ERG* fusion. In contrast, the nuclei of the PCA glands show 1 yellow and 1 red signal, demonstrating *TMPRSS2-ERG* fusion through deletion. The double-framed yellow inset is a magnification of the yellow-boxed area showing 2 representative nuclei of a PCA gland. C, Prostatic tissue with PCA glands (Gleason score 3+3 = 6). D, FISH image of the red-boxed area in (C), displaying absence of *TMPRSS2-ERG* fusion. Two yellow signals per nucleus are present. The double-framed yellow inset is a magnification of the yellow-boxed area showing representative nuclei of the PCA gland. E, Prostatic tissue with PCA glands (Gleason score 4+3 = 7) surrounding an atrophic prostatic gland (lumen of the atrophic prostatic gland marked with asterisk). F, FISH image of the red-boxed area in (E), displaying part of the atrophic gland on lower left, and PCA glands on upper right. Each nucleus of the atrophic gland shows 2 yellow signals indicating absence of *TMPRSS2-ERG* fusion. The double-framed green inset is a magnification of the green-boxed area showing representative nuclei of the atrophic gland. In contrast, the nuclei of the PCA glands show 1 yellow and 1 red signal, demonstrating *TMPRSS2-ERG* fusion through deletion. The double-framed yellow inset is a magnification of the yellow-boxed area showing representative nuclei of PCA gland. Original magnification of H&E images, 20 × objective. Original magnification of FISH images, 60 × objective.

PIN lesions (81%) did not have *TMPRSS2-ERG* fusion (Figs. 3A, B), although the corresponding PCA could harbor the fusion (Figs. 3C, D). Of the high grade PIN lesions harboring the gene fusion, all but one were in tight proximity of PCA and showed the same fusion as the corresponding PCA (data not shown).
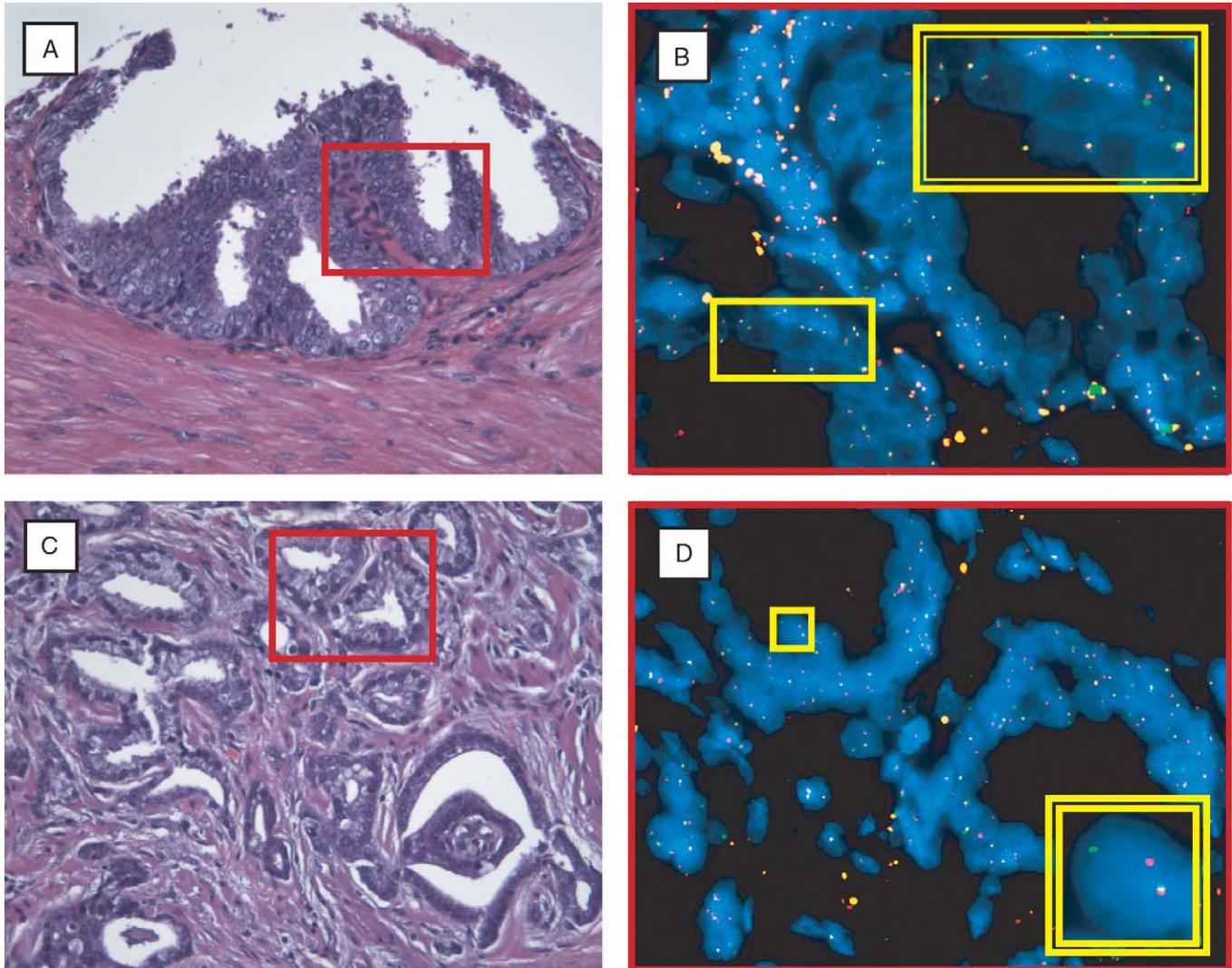
FIGURE 3. H&E stains and corresponding FISH images of *TMPRSS2-ERG* fusion assay. A, Prostatic tissue with focus of high grade PIN. B, FISH image of the red-boxed area in (A), displaying 2 yellow signals per nucleus indicating absence of *TMPRSS2-ERG* fusion in all nuclei of high grade PIN. The double-framed yellow inset is a magnification of the yellow-boxed area showing representative nuclei of the high grade PIN lesion. C, Prostatic tissue with PCA (Gleason score 3+4 = 7) from the corresponding case illustrated in (A). D, FISH image of the red-boxed area in C, displaying *TMPRSS2-ERG* fusion through translocation. The double-framed inset illustrates the magnified view of the yellow-boxed area. One yellow, 1 green, and 1 red signal are present demonstrating *TMPRSS2-ERG* fusion through translocation. In contrast, note the signal pattern for nonfusion in the high grade PIN area (see B above). Original magnification of H&E images, 20 × objective. Original magnification of FISH images, 60 × objective.

In contrast, none of the 100 total samples from benign prostatic epithelium, benign prostatic hyperplasia, unspecified atrophy, SA, SA with cyst formation, and postatrophic hyperplasia demonstrated the aforementioned genetic aberration (see atrophic gland in Figs. 2E, F). There was no statistical significance for association between the *TMPRSS2-ERG* fusion status and Gleason score (data not shown). Association between tumor stage and fusion status was not assessed. However, results of our previous studies showed that positive fusion status was directly associated with higher tumor stage.[17]

With the exception of 3 fusion positive PCA cases (2 clinically localized PCA and 1 hormone naive metastatic tumor), the fusion status was homogeneously present throughout all cells of a given tumor, regardless of Gleason score. In the hormone naive metastatic case, we observed the *TMPRSS2-ERG* fusion through deletion in about 90% of the tumor cells. The remaining 10% of randomly intermixed tumor cells displayed the fusion through translocation of the genes.[17] In the 2 localized PCA cases, the rearrangement status was different in 2 separate tumor areas: the tumor cells of one area

TABLE 1. Summary of the *TMPRSS2-ERG* Fusion Status

Tissue Type	No Fusion % (n)	Fusion Through Deletion % (n)	Fusion Through Translocation % (n)
Normal prostatic tissue	100 (47)	0 (0)	0 (0)
Benign prostatic hyperplasia	100 (15)	0 (0)	0 (0)
Atrophy (unspecified)	100 (9)	0 (0)	0 (0)
SA	100 (17)	0 (0)	0 (0)
SA with cyst formation	100 (6)	0 (0)	0 (0)
Post atrophic hyperplasia	100 (6)	0 (0)	0 (0)
High grade PIN*	81 (21)	19 (5)	0 (0)
Localized PCA*	51.5 (122)	30 (71)	18.5 (44)
Hormone naive metastases	70 (24)	12 (4)	18 (6)
Hormone refractory metastases	67 (6)	33 (3)	0 (0)

*Of the 237 localized PCA samples, 17 also included high grade PIN lesions.

displayed a signal pattern for fusion, and the tumor cells of the other area did not reveal the gene fusion.

DISCUSSION

Recent work identified a novel genetic rearrangement in PCA involving the androgen-regulated *TMPRSS2* and members of the *ETS* family of transcription factors (eg, *ERG*, *ETV1*, and *ETV4*).[24,25] In the current study, we evaluated a wide spectrum of benign prostatic lesions, precursors of PCA, and PCA samples including clinically localized PCA, hormone naive metastases, and hormone refractory metastases for the most common gene fusion, *TMPRSS2-ERG*, using a multicolor interphase FISH assay. Approximately half of the clinically localized PCAs (48.5%) harbored the *TMPRSS2-ERG* gene fusion. The hormone naive metastases showed the fusion in a lower percentage (30%) as compared with the clinically localized PCA cases. This is consistent with our previous observations of the fusion frequency in hospital-based cohorts where the majority of PCA samples are derived from men who have undergone a screening process making them eligible for surgery.[17,24] We have also examined PCA samples from a well-characterized cohort of men with early stage disease (T1a-b, Nx, M0) diagnosed between 1977 and 1991, before PSA screening was available.[1,11–13,21] In this cohort where less than half of the men died from PCA with over 20 years of clinical follow-up, we observed 15% *TMPRSS2-ERG* gene fusion PCAs.[7] This difference in fusion status of PCA may be explained by the lower percentage of high risk cases in the population-based Watchful Waiting cohort and argues in support that the current selection process for radical surgery identifies men with a higher risk of dying from PCA. These later

observations also highlight the importance of describing the manner a disease population is identified for study. The frequencies reported in the current study, therefore, may best reflect surgically treated PCA cases due to selection bias and not the entire population of men diagnosed with PCA. Additional work using larger population-based cohorts should give us a more precise understanding of the frequency of the *TMPRSS2-ETS* gene fusion in PCA.

Even though this is not a longitudinal study, we believe that the *TMPRSS2-ERG* fusion is an early event in the development of invasive PCA. Evidence supporting this hypothesis includes observations from previous work and from the current study. First, the *TMPRSS2-ERG* fusion is a homogenous event. If it occurred late in the development of PCA progression, we would anticipate that it would have heterogeneous distribution within the population of tumor cells. Second, we have not identified *TMPRSS2-ERG* fusion in benign prostate tissue or proliferative inflammatory atrophy (PIA) (also commonly referred to as "focal prostate atrophy" or just "prostate atrophy").[6] Some PIA lesions demonstrate early molecular alterations such as low level gain in chromosome 8 centromere and hypermethylation of CpG dinucleotids in the promoter region of the *GSTP1* gene. This suggests that some atrophy lesions may become PCA precursors, but the capability for invasion may arise later.[4,16,22] Third, the *TMPRSS2-ERG* fusion was observed in a subset of high grade PIN lesions intermingling with PCA with the same fusion pattern. We did not observe the *TMPRSS2-ERG* fusion in high grade PIN lesions geographically distant to PCA, even if the PCA from the same individual demonstrated the *TMPRSS2-ERG* fusion. Hence, we believe these high grade PIN lesions are a subset of true precursors for *TMPRSS2-ERG* positive PCA. A significant clinical implication for this finding is the assessment of *TMPRSS2-ERG* fusion status in problematic prostate needle core biopsies with high grade PIN and adjacent small atypical glands. Another possible explanation for the presence of the gene fusion in high grade PIN is the presence of intraductal tumor spread with PIN-like morphology. The observation that PIA never demonstrated gene fusion and high-grade PIN only in about a fifth of cases would be consistent with these 2 putative precursor lesions being sequential events in PCA progression or on 2 separate pathways leading to PCA.

To further investigate fusion status in high grade PIN and potential precursor lesions, a larger number of these lesions including those from prostates lacking PCA should be assessed.

In the current study, we did not evaluate other ETS genes therefore the total percentage of PCAs driven by the *TMPRSS2-ETS* fusions still needs to be determined. However, on the basis of the unpublished data, we believe that the *TMPRSS2-ERG* fusion will account for most of the gene fusions and the other *TMPRSS2-ETS* fusions are less common.

Although the majority of *TMPRSS2-ERG* fusion PCA cases demonstrate homogenous gene fusion

throughout the tumor cell population, we did identify rare cases (3/128) with a heterogeneous fusion status. One PCA case predominantly showed *TMPRSS2-ERG* fusion through deletion and only a subset of randomly intermixed tumor cells featured *TMPRSS2-ERG* through translocation. The other 2 cases had physically separated tumor foci exhibiting fusion in one area and absence of the genetic aberration in the other area. These may demonstrate truly independent tumors. The remaining tumors from this study were homogenous regarding the fusion status, independent of different Gleason patterns and morphologic features. Nineteen cases of matched clinically localized PCA and corresponding lymph node metastases demonstrated similar *TMPRSS2-ERG* fusion status. Therefore, multifocal PCA may more commonly arise from the same lesion than represent truly independent lesions, and thus the *TMPRSS2-ERG* fusion assay could serve as a test for clonality.

The detection of *TMPRSS2-ERG* by FISH is a very specific and sensitive assay for fusion positive PCA. This may be an extremely useful ancillary test, especially because the number of prostate needle biopsies for which a definitive diagnosis cannot be established is on the rise. Basal cell specific immunohistochemical markers (ie, 34βE12 and p63) and AMACR are used as an adjunct in establishing a definitive diagnosis.[3,14,19] When used in combination, these immunostains can increase the likelihood to render a definitive diagnosis in almost 70% of cases. The implementation of the FISH assay may increase this percentage even further.

In summary, we confirm that *TMPRSS2-ERG* gene fusion PCA is common and occurs early in the development of invasive adenocarcinoma. Considering the high incidence of PCA and the high frequency of the fusion, *TMPRSS2-ERG* is the most frequent gene rearrangement described, although the exact frequency needs to be determined in population-based studies. The clinical utility as a tissue biomarker and diagnostic tool is promising given the high specificity.

ACKNOWLEDGMENTS

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