Research Article

**TMPRSS2-ERG Gene Fusion Is Not Associated with Outcome in Patients Treated by Prostatectomy**


Departments of Pathology, Molecular Cytogenetics, Epidemiology and Biostatistics, Surgery, and Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York

Abstract

A significant number of prostate cancers have been shown to have recurrent chromosomal rearrangements resulting in the fusion of the androgen-regulated TMPRSS2 promoter to a member of the ETS transcription factor family, most commonly ERG. This results in ERG overexpression, which may have a direct causal role in prostate tumorigenesis or progression. However, the clinical significance of the rearrangement is unclear, and in particular, relationship to outcome has been inconsistent in recent reports. We analyzed TMPRSS2-ERG gene rearrangement status by fluorescence in situ hybridization in 521 cases of clinically localized surgically treated prostate cancer with 93 months of median follow-up and also in 40 unmatched metastases. Forty-two percent of primary tumors and 40% of metastases had rearrangements. Eleven percent had copy number increase (CNI) of the TMPRSS2-ERG region. Rearrangement alone was associated with lower grade, but not with stage, biochemical recurrence, metastases, or death. CNI with and without rearrangement was associated with high grade and advanced stage. Further, a subgroup of cancers with CNI and rearrangement by deletion, with two or more copies of the deleted locus, tended to be more clinically aggressive. DNA index assessment revealed that the majority of tumors with CNI of TMPRSS2-ERG had generalized aneuploidy/tetraploidy in contrast to tumors without TMPRSS2-ERG CNI, which were predominantly diploid. We therefore conclude that translocation of TMPRSS2-ERG is not associated with outcome, and the aggressive clinical features associated with CNI of chromosome 21 reflect generalized aneuploidy and are not due to CNI specifically of rearranged TMPRSS2-ERG. [Cancer Res 2009;69(4):1400–6]

Introduction

Due to the clinical heterogeneity and variable natural history of prostate cancer, optimal treatment strategies rely on accurate stratification according to risk of disease progression. For patients with localized prostate cancer, those who are not likely to progress are candidates for expectant management and should be distinguished from patients who need aggressive treatment (1). The ability to tailor therapy is key to achieving optimum outcomes. Outcome prediction based on measurements of several standard preoperative and postoperative clinical and pathologic variables are helpful but have limited utility in predicting outcome differences among tumors with similar prostate-specific antigen (PSA) levels, grade, and stage (2–4). Therefore, the identification of robust, informative biomarkers, potentially adding to the value of existing parameters for reliable patient stratification, is of significant clinical interest. Identification of valid biomarkers requires the study of a large number of prostate cancer patients who have been consistently staged, treated, and monitored for disease recurrence, with long-term follow-up.

Many molecular alterations have been identified in prostate cancer and proposed as possible biomarkers of progression risk (5–10). Of recent interest has been the remarkable discovery that many prostate cancers are associated with chromosomal translocations involving members of the ETS family of transcription factors (11). The most common recurrent translocation, resulting in fusion of the androgen-regulated gene TMPRSS2 to ERG, accounts for ~90% of the prostate cancer associated translocations identified to date (12, 13). About 60% of the fusion events are due to a deletion of 3 Mb between TMPRSS2 and ERG.

Several studies have attempted to evaluate the clinical significance of TMPRSS2-ERG translocation in prostate cancer (14–18). Most were of relatively small sample size with inconsistent results. The largest study to date included 445 conservatively treated patients in which ERG translocation was detected in 30%. Interestingly, although translocation per se was not associated with outcome, cause-specific and overall survival were associated with the specific subgroup of patients having two or more copies of the TMPRSS2-ERG intergenic deletion event.

Because the relationship of the TMPRSS2-ERG rearrangement to the clinical behavior of prostate cancer is unclear, we analyzed a large retrospective cohort of men with clinically localized prostate cancer, treated by prostatectomy with long-term follow-up. This cohort is drawn from a PSA-screened population and reflects a current standard of care for this clinical state of disease in the United States. We determined the TMPRSS2-ERG gene status by fluorescence in situ hybridization (FISH) and evaluated its potential relationship to clinicopathologic variables and outcomes.

Materials and Methods

**Patient samples.** Samples were obtained at the time of radical prostatectomy from patients with clinically localized prostate cancer and no prior therapy. An initial study to develop laboratory methods and establish estimates of frequency was done in a sample of 70 patients (analyzable results obtained in 67), with subsequent analysis of 544 (analyzable results obtained in 521) for evaluation of clinical associations.
All studies were approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board. The samples for the gene array studies as well as the tissue microarrays were conducted from the index (largest) lesion (focus) in the majority of cases. The cores represented in the tissue microarray were three different sites within the index tumor focus in the majority of cases. A separate focus of tumor was punched only when there were small tumor foci with no dominant lesion present.

The initial sample of 70 patients had clinically localized prostate cancer treated by radical retropubic prostatectomy at our institution between 1993 and 1999. Twenty-three of these patients had been followed for more than 5 y without disease recurrence, and 35 patients had a PSA or clinically detected relapse. Tumor samples from these patients had previously been studied using Affymetrix Hu133a gene expression arrays (19). In that study, the case selection required us to enrich for outcome events to achieve better stratification based on clinical outcome. These 70 samples were used to establish laboratory methods. The presence or absence of TMPRSS2-ERG rearrangement, as obtained by FISH, was compared with ERG expression in these samples, as described below.

Once laboratory methods were established, we studied chromosomal rearrangements using tumor samples from patients treated with radical retropubic prostatectomy and pelvic lymph node dissection at our institution for localized prostate cancer between 1985 and 2003. Patients who received androgen deprivation therapy or radiation in a neoadjuvant or adjuvant setting were excluded. Five hundred twenty-one patients with a median of 95.5 mo of follow-up were evaluated. An additional group of 40 who received androgen deprivation therapy or radiation in a neoadjuvant or adjuvant setting were excluded. Five hundred twenty-one patients with a median of 95.5 mo of follow-up were evaluated. An additional group of 40 treatments consisting of two BAC clones each at 5′ ERG (RP11-35G21 and RP11-110N12) and 3′ ERG (RP11-315E22 and RP11-720N21) with an ~123.35-kb interval between the two sets, and two BAC clones each at 5′ TMPRSS2 (RP11-35C4 and RP-891L10) and 3′ TMPRSS2 (RP11-825A8 and RP11-120C17) with an ~348.9-kb interval between the two sets. DNA was labeled by nick translation using SpectrumOrange-dUTP (5′ clones) or SpectrumGreen-dUTP (5′ clones; Vysis, Abbott Molecular, Inc.). A three-color probe set was used in the second sample set by combining the 3′ ERG clones (labeled with SpectrumOrange) with 3′TMPRSS2 clones (labeled with SpectrumRed) and 5′TMPRSS2 clones (labeled with SpectrumGreen; Fig. 1, probe schematic). For each slide, 80 to 100 ng of each labeled clone were ethanol precipitated with 2 to 3 μg of Cot-1 DNA and resuspended in 15 μL of hybridization buffer (20). Hybridization, washing, and fluorescence detection were done according to standard procedures (20). Briefly, paraffin sections were dewaxed in xylene, microwaved in 10 mmol/L sodium citrate (pH 6.0) solution for 5 to 10 min, cooled to room temperature, rinsed, and then treated with pepsin-HCl for ~5 min at 37°C before being rinsed and dehydrated. The prewarmed probe mixture was applied to the slides and a coverslip sealed in place with rubber cement. The slides were then denatured at 83°C for 5 min at 37°C before being rinsed and dehydrated. The prewarmed probe mixture was applied to the slides and a coverslip sealed in place with rubber cement.

Conclusions and implications: The combination of DNA copy number analysis and FISH for detection of gene fusion status (TMPRSS2-ERG) identified approximately 50% of patients with relapse-free 5 y without disease recurrence with a minimum of 50% of patients with prostate cancer patients with a PSA >0.2 ng/mL after surgical resection with a second confirmatory PSA measurement >0.2 ng/mL.

Construction of tissue microarrays. H&E slides of the prostatectomy specimens were reviewed by two urologic pathologists, and slides containing tumor were marked and matched with corresponding paraffin blocks. Tissue cores of 0.6 mm were then punched out in triplicate from locations randomly selected within the marked tumor areas and mounted in blank recipient blocks using an automated tissue microarrayer (Beecher Instruments, Inc.).

FISH for detection of gene fusion status. Break-apart FISH probes consisted of two BAC clones each at 5′ ERG (RP11-35G21 and RP11-110N12) and 3′ ERG (RP11-315E22 and RP11-720N21) with an ~123.35-kb gap between the two sets, and two BAC clones each at 5′ TMPRSS2 (RP11-35C4 and RP-891L10) and 3′ TMPRSS2 (RP11-825A8 and RP11-120C17) with an ~348.9-kb gap between the two sets. DNA was labeled by nick translation using SpectrumOrange-dUTP (5′ clones) or SpectrumGreen-dUTP (5′ clones; Vysis, Abbott Molecular, Inc.). A three-color probe set was used in the second sample set by combining the 3′ ERG clones (labeled with SpectrumOrange) with 3′TMPRSS2 clones (labeled with SpectrumRed) and 5′TMPRSS2 clones (labeled with SpectrumGreen; Fig. 1, probe schematic). For each slide, 80 to 100 ng of each labeled clone were ethanol precipitated with 2 to 3 μg of Cot-1 DNA and resuspended in 15 μL of hybridization buffer (20). Hybridization, washing, and fluorescence detection were done according to standard procedures (20). Briefly, paraffin sections were dewaxed in xylene, microwaved in 10 mmol/L sodium citrate (pH 6.0) solution for 5 to 10 min, cooled to room temperature, rinsed, and then treated with pepsin-HCl for ~5 min at 37°C before being rinsed and dehydrated. The prewarmed probe mixture was applied to the slides and a coverslip sealed in place with rubber cement.

Figure 1. Patterns of FISH abnormalities. A, wild-type, with two sets of triplet orange (3′ ERG), red (3′TMPRSS2), and green (5′TMPRSS2) signals in each cell. B, translocation, with one separate red signal and an orange-green doublet in one allele; the other allele is wild-type. C, deletion, with one orange-green doublet and loss of corresponding red signal in one allele and a wild-type second allele. D, CNl, with three triplet wild-type signals in each cell. E, CNl, with arrows pointing to cells with deletion of the red signal in at least two gene copies, with two wild-type alleles in the same cell. F, schematic diagram of TMPRSS2-ERG probes.
4 to 6 min on a HYBrite automated hybridizer (Vysis, Abbott Molecular) and then incubated overnight at 37 °C. After standard posthybridization washes, the slides were stained with 4′,6-diamidino-2-phenylindole and mounted in antifade (Vectashield, Vector Laboratories).

**Image analysis.** Samples were analyzed using an automated imaging system (MetaSystems) and Isis 5.0 scanning and imaging software. Slides were scanned at 5×, and the resulting composite was segmented using the Metafer microarray tool. Segmentation generated a position list corresponding to each available core, linking slide location to subsequent high-resolution FISH images. Evaluation and analysis of the cases was done by a pathologist (A.G.) and a molecular cytogeneticist (M.A.L.). A minimum of 100 cancer cells were evaluated for each case, whenever possible. Two-color probes for both TMPRSS2 and ERG were used independently in the training set to analyze both genes for rearrangement. Rearrangement status was based on agreement for the patterns with both probe sets thereby ensuring the robustness of the assay. If there was only one core that was positive, then the rearrangement status was recorded as positive for that case. For the validation cohort, we used a three-color experiment, incorporating 5′ERG, 3′TMPRSS2, and 5′TMPRSS2 clones in the probe, to facilitate the screening of a larger sample size. Examples of the various FISH abnormalities are illustrated in Fig. 1 A to E.

**DNA index and ploidy analyses.** Flow cytometry measurement of DNA index was carried out on sections from the tissue block with the maximum tumor by Genzyme laboratories. The DNA content of 10,000 nuclei was measured with a FACScan flow cytometer (Couler XL-MCL EPICS, Beckman-Coulter). Cell cycle evaluation of the DNA histogram was done with proprietary software (Multicycle, Phoenix systems). Tumors with one identifiable G0-G1 peak were classified as DNA diploid (DNA index, 1). Tumor samples that contained a significant increase in the 4n peak (>15%) and an identifiable 8n population were classified as DNA tetraploid (DNA index, 1.9–2.1). Samples were classified as aneuploid if a separate identifiable G0-G1 peak (other than 1, 1.9–2.1) was present. All DNA histograms were analyzed without knowledge of the clinicopathologic features or patient outcomes. DNA index was acquired for 81 cases including nearly all cases showing increased copy number of the TMPRSS2-ERG locus (29 cases with copy number increase (CNI) alone and 32 cases of TMPRSS2-ERG rearrangement with CNI) and a representative subset of diploid cases (10 cases with rearrangement alone and 10 without abnormalities).

FISH for chromosome 9 was done on a tissue microarray constructed from the same 81 cases, with probes to the chromosome 9 satellite 3 repetitive region on 9qh labeled with SpectrumOrange using methods similar to those described below. The mean percentage of nuclei with two signals for chromosome 9 was 54% in benign prostatic epithelium. The mean percentage of nuclei with 0 or 1 signal was 32.5% and that with three signals was 3.5%. None of the nuclei had >3 signals. By following previously published criteria (21) as well as based on the values in benign epithelium and an inspection of the distribution of FISH signals among the carcinoma foci in our cases, the criteria we adopted for definition of FISH abnormalities were as follows: 1, diploid, if >50% of cells had ≤2 signals/nucleus; 2, aneuploid/polydiploid, if >10% of the cells had ≥3 signals/nucleus. Analyzable FISH results for chromosome 9 were obtained for 59 cases with CNI and for 19 cases without CNI of TMPRSS2-ERG.

**Statistical analysis.** Data on the following characteristics were available from each patient: (a) clinical characteristics—Gleason grade (categorized as ≤7, 7, and >7), tumor stage (categorized as ≤PT2 and ≥PT3), seminal vesicle invasion (positive or negative), and extracapsular extension (positive or negative); (b) FISH results—rearrangement (translocation/deletion) in TMPRSS2-ERG (categorized as presence or absence), CNI (presence or absence), and rearrangement with CNI. Outcomes pertaining to presence or absence of BCR, metastasis, and dead/alive status, including time at which these events occurred, were available for all patients.

Two sets of analyses were conducted. The first set focused on the initial patients used for laboratory methods evaluation. The presence or absence of TMPRSS2-ERG rearrangement was available for these patients using FISH. Gene expressions from Hu133A array were also available for these patients from our previous study. Expression values were obtained using the MAS5.0 algorithm after median normalization. Expression of the ERG probe was extracted (on the natural logarithm scale) from this array for all initial patients. The following comparisons were conducted using a two-sample t test: (a) ERG expression level in patients with versus patients without TMPRSS2-ERG rearrangement; (b) ERG expression level in patients with versus patients without BCR and metastasis, and dead versus alive patients. We compared the following using Fisher's exact test: presence or absence of rearrangement in patients with versus patients without BCR and metastasis, and dead versus alive patients. In the second set of analyses, we used Fisher's exact test to compare the clinical characteristics versus rearrangement of TMPRSS2-ERG in the second set of 521 patients. Comparisons with P < 0.05 were declared to be statistically significant. We did not adjust our analyses for multiple testing due to the exploratory nature of our investigations. All P values reported are based on two-sided tests.

**Results**

Detection of TMPRSS2-ERG rearrangement by FISH correlates with functional gene fusion in prostate cancer. The detection of TMPRSS2 and ERG structural alterations by FISH is an indirect test and, although consistent with, does not confirm fusion events. Therefore, we first evaluated the technique in an initial sample set of 70 patients that have previously been studied for gene expression using oligonucleotide arrays (19). This allowed us to correlate rearrangement status with ERG expression, the functional end-result of the gene fusion event. Tumors from 3 patients could not be evaluated, resulting in an effective sample size of 67 patients. Thirty-one of 67 evaluable prostate cancers (46%) had rearrangement of TMPRSS2-ERG detected by FISH. Deletion of 3′TMPRSS2 was detected in 21 (66%) of these. In three cases, both translocation and deletion patterns were present in different cell populations from the same tumor and these were scored as positive for rearrangement and deletion.

ERG transcript levels, as determined by oligonucleotide arrays, were significantly higher among the 31 patients with a translocation or deletion than in tumors without such aberrations (P < 0.0001; Fig. 2). Therefore, the presence of FISH-detected alterations was significantly associated with high ERG expression consistent with functional fusion of TMPRSS2 and ERG. In a subset of cases, this was confirmed by detection of fusion-specific transcripts by reverse transcription-PCR (data not shown). ERG expression was not significantly different between patients with and without BCR (P = 0.69), metastasis (P = 0.21), or overall survival (P = 0.96).

![Figure 2. Correlation of TMPRSS2-ERG translocation status with ERG gene expression in human prostate cancer. Bar graph of normalized ERG expression with translocated cases on the left and wild-type on the right.](image)
We also detected increased copy number (CNI) of the TMPRSS2-ERG locus in 23 of 67 (34%) prostate cancer. Seven (10%) tumors without rearrangement or deletion had three or more copies detected, and 16 (24%) prostate cancers showed rearrangement with two or more copies present. The functional relevance of CNI in the setting of TMPRSS2-ERG gene fusion is unknown. One potential effect would be increased expression of the fusion product. It is therefore of interest that we did not detect any significant difference in mean ERG expression levels between rearrangement coexisting with CNI and rearrangement alone (P = 0.37). There was also no significant difference in mean ERG expression levels in non-rearranged cases with or without CNI (P = 0.41). Finally, rearrangements in TMPRSS2-ERG were not significantly associated with clinical features of the patients (Supplementary Table S1B).

Association of TMPRSS2-ERG rearrangement with clinical and pathologic features of prostate cancer. To evaluate the relationship between alterations of TMPRSS2-ERG and pathologic and clinical features of disease, we extended our analysis to evaluate a large sample set of prostate cancer patients. We evaluated tissue microarrays of prostate cancers from 544 patients with long-term follow-up after prostatectomy. Of the 544 cancers, 23 were not evaluable due to no tissue core present on the section, no carcinoma present on the core, or a failed FISH assay, resulting in a total of 521 patients for analysis. Two hundred seventeen of 521 (42%) prostate cancers had TMPRSS2-ERG alterations (rearrangement or deletion; Table 1). One hundred twenty-eight of the 217 (59%) were deleted. CNI of TMPRSS2-ERG were present in 62 of 521 (12%) of prostate cancer; CNI was present concomitantly with rearrangement (CNI + rearrangement, which refers to CNI of a non-rearranged locus in the presence of a locus with translocation or deletion of TMPRSS2-ERG) in 32, and without rearrangement in the remaining 30. CNI of deleted TMPRSS2 (designated CNID, which refers to CNI of a deleted TMPRSS2-ERG allele, resulting in two or more copies of a deleted TMPRSS2-ERG allele in a single cell), was detected in 10 of 128 (8%) cases with deletion and 10 of 62 (16%) cases with CNI. We examined the relationship of clinical and pathologic variables with various genetic alterations of the TMPRSS2 and ERG genes, including any rearrangement, deletion alone, CNI with or without rearrangement, and CNID. Presence of rearrangement was significantly associated with a low (≤6) Gleason score (P = 0.02), but not with pathologic stage (P = 0.93; Table 2). Rearrangement was also not associated with BCR (P = 0.70), metastasis (P = 0.21), or overall survival (P = 0.38). Deletion alone was significantly associated with low Gleason score (P < 0.01) and absence of seminal vesicle invasion (P = 0.02). CNI of TMPRSS2 and ERG without rearrangement was significantly associated with high (≥7) Gleason score (P = 0.03) but not with other variables (Supplementary Table S2A). Rearrangement with CNI was significantly associated with seminal vesicle invasion (P < 0.01) and pathologic stage (P = 0.02; Supplementary Table S2B). CNID was significantly associated with seminal vesicle invasion (P = 0.01) but not with other variables (Table S2C). However, of the 10 patients with CNID, 7 (70%) had BCR, 4 (40%) developed metastasis, and 3 (30%) died, as compared with all other cases with CNI and coexistent rearrangement (n = 22), where 9 (41%) had BCR, 4 (18%) developed metastasis, and 3 (14%) died (Table 3A), and CNI without rearrangement (n = 30), where 11 (40%) had BCR, 5 (17%) developed metastasis, and 5 (17%) died. Although the low number of events precluded meaningful statistical analysis, the overrepresentation of poor outcome for tumors within the category of CNID is intriguing as discussed below.

**Table 2.** Characteristics of patients with TMPRSS2-ERG rearrangement including those with translocation and deletion

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Patients with T/D (n = 217)</th>
<th>Patients without T/D (n = 304)</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td>Gleason score</td>
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<tr>
<td>&lt;7</td>
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<td>74</td>
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<tr>
<td>7</td>
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<tr>
<td>Stage</td>
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<td>PT2 or lower</td>
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<td>195</td>
<td>0.93</td>
</tr>
<tr>
<td>PT3 or higher</td>
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<td>109</td>
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<td>Seminal vesicle invasion</td>
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</tr>
<tr>
<td>Positive</td>
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</tr>
<tr>
<td>Extracapsular extension</td>
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<tr>
<td>Positive</td>
<td>62</td>
<td>87</td>
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*Fisher’s exact test.

**Table 1.** Frequency of TMPRSS2-ERG abnormalities in test cohort

<table>
<thead>
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<th>TMPRSS2-ERG abnormalities</th>
<th>No. of cases (%)</th>
</tr>
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<tr>
<td>T/D</td>
<td>217 of 521 (42)</td>
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<tr>
<td>CNI alone</td>
<td>29 of 521 (5)</td>
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<tr>
<td>CNI with concomitant T/D</td>
<td>32 of 521 (6)</td>
</tr>
<tr>
<td>CNI + D</td>
<td>13 of 32</td>
</tr>
<tr>
<td>CNI + T</td>
<td>19 of 32</td>
</tr>
</tbody>
</table>

Abbreviations: T, translocation; D, deletion.
Relationship of TMPRSS2-ERG CNI to tumor DNA index. The finding of a trend for CNI of TMPRSS2-ERG to be associated with high-risk features in prostate cancer patients raised two possibilities: High-risk prostate cancer could be associated with a high frequency of generalized chromosomal aberrations that included CNI of chromosome 21 or it could be due to gene CNI specifically affecting rearranged TMPRSS2-ERG. To address this issue, we measured DNA index by flow cytometry in a subset of 61 cases with CNI and 20 without CNI. One case with CNI of TMPRSS2-ERG was not evaluated by flow cytometry due to inadequate tumor in the block. We also used FISH to evaluate copy number changes of an independent chromosome (9), which is believed to be relatively neutral in prostate cancer. By flow cytometry, 41 of 61 (67%) cases with CNI of TMPRSS2-ERG were either tetraploid or aneuploid. In contrast, only 2 of 20 (10%) cases without CNI were aneuploid. CNI of chromosome 9 were detected in 38 of 59 (64%) of tumors with TMPRSS2-ERG CNI, whereas only 2 of 19 (10.5%) tumors without CNI were polyploid/aneuploid. Of the cases with CNID, 9 of 10 (90%) were tetraploid/aneuploid by flow cytometry or FISH. Therefore, the vast majority of tumors where CNI of TMPRSS2-ERG was detected by FISH seem to have generalized chromosomal aberrations resulting in an aneuploid/tetraploid DNA index. Of the aneuploid/tetraploid tumors, there was no significant difference in outcome between patients with rearrangement (23 of 43) and those without (20 of 43): In the former group, 13 of 23 had BCR, 6 of 23 had metastases, and 5 of 23 died, whereas in the latter group, 4 of 20 had BCR, 5 of 20 had metastases, and 4 of 20 died.

Discussion

Since the discovery of the recurrent translocation involving TMPRSS2 and ERG on chromosome 21 in prostate cancer (11), many reports have confirmed and extended these findings (22–28). However, controversy continues to exist about the relationship of this gene fusion event to a prognostically distinct subset of prostate cancer patients. We showed that rearrangement of TMPRSS2 and ERG detected by FISH was associated with strong overexpression of ERG as expected of a functional rearrangement. TMPRSS2-ERG gene rearrangement was detected in 42% of 521 patients representative of a PSA-screened population treated with prostatectomy. Intergenic deletion of chromosome 21 occurred in 63% of the cases with rearrangement. This frequency of rearrangement is comparable to that determined by Perner and colleagues (58 of 118, 49%), but higher than that reported by Attard and colleagues (134 of 445, 30%) and much higher than that found by Demichelis and colleagues (17 of 111,15%). It should be noted that the patients studied by Attard and Demichelis were conservatively managed, European cohorts that have different clinical and demographic characteristics.

Table 3. Comparison of clinicopathologic variables and outcome for patients with CNID versus all other CNI + rearrangement except CNID

(A) Clinicopathologic variables

<table>
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<th>Variable</th>
<th>Categories</th>
<th>Patients with CNID</th>
<th>Other CNI + rearrangement</th>
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<td>Pathologic Stage</td>
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<tr>
<td></td>
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(B) Status of BCR, metastases, and overall survival

<table>
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<tr>
<th>End points</th>
<th>Status level</th>
<th>CNID (n = 10)</th>
<th>Other CNI + rearrangement (n = 22)</th>
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<td>Overall survival</td>
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<tr>
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<tr>
<td>BCR</td>
<td>No and Alive</td>
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<tr>
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<td>Yes</td>
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<td></td>
<td>Dead before BCR</td>
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<td>1</td>
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<tr>
<td>Metastases</td>
<td>No and alive</td>
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<td>Yes</td>
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</tr>
<tr>
<td></td>
<td>Dead before metastases</td>
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</table>

*Fisher's exact test.
features from the PSA-screened, U.S. population that we evaluated. A significant strength of our analysis is the large sample size of the cohort and long-term follow-up (14, 16). To our knowledge, this is the largest study to date looking at a PSA-screened population treated by prostatectomy for clinically localized prostate cancer. In this group, 50% of patients are alive without progression, 28% have had BCR, 11% have had metastatic disease, 4% died of disease, and 7% died of other causes in the follow-up period. In contrast, the study of Attard and colleagues included a very different patient population with 445 patients who presented with obstructive symptoms and underwent transurethral resection of the prostate but were otherwise treated conservatively. Only 27% of those patients were alive without progression, 17% died from prostate cancer, and 33% died of other causes (29).

Prior studies have variously reported the clinical significance of TMPRSS2-ERG gene fusion events in prostate cancer. The largest study to date found significant associations for the presence of the deletion event (and not other rearrangements) with higher Gleason score, worse disease-specific and overall survival using univariate and multivariate analyses (18). Perner and colleagues (30) found that prostate cancers with TMPRSS2-ERG deletion were of higher stage and frequently had pelvic lymph node metastases. In the population-based watchful waiting cohort of Demichelis and colleagues (17), rearrangement was associated with metastasis or prostate cancer–specific death, but the association was not significant after adjusting for tumor grade. Others have reported that only specific alternative TMPRSS2-ERG mRNA fusion isoforms were associated with features of aggressive prostate cancer (31). On the other hand, Petrovics and colleagues (32) observed that higher ERG mRNA levels were significantly associated with longer PSA recurrence-free survival, low Gleason grade, and lower pathologic stage. Tu and colleagues (14), in a study of 82 cases, found that fusion positive cancers tended to have lower Gleason score, but this was not statistically significant. Saramaki and colleagues (33) found that the presence of the rearrangement was an independent predictor of favorable outcome on multivariate analysis in patients treated by radical prostatectomy. In the present study of more than 500 patients, we found that rearrangement or deletion was significantly associated with lower-grade cancers and that deletion was significantly associated with absence of seminal vesicle invasion. These alterations were not associated with worse outcome measures. Our findings argue that the presence of the TMPRSS2-ERG rearrangement does not predict for biologically aggressive cancers in this patient population. Recent studies have brought to light heterogeneity in TMPRSS2-ERG gene fusion status in different foci of carcinoma within the same gland (34, 35). However, we did not attempt to address this issue in this study. This phenomenon would be a likely explanation for the few cases where there was discordance between the ERG expression levels and TMPRSS2-ERG rearrangement status.

An intriguing finding in our study was that tumors with CNI of the TMPRSS2-ERG locus trended toward poor outcome. This finding is similar to that of Attard, the only other study that has analyzed the potential biological significance of increased copy number of the rearrangement (18), although other authors have noted the presence of this event (14, 30). The number of cases with CNID in our study (10 of 544, or 1.8% of prostate cancers overall) was much less than that detected by Attard and colleagues (6.6% of prostate cancers overall) probably reflecting the different patient populations. The small number of deleted cases with CNI in our study precludes any meaningful statistical conclusions; however, a greater proportion of patients with this FISH abnormality had BCR, metastasis, and death as compared with patients with rearrangement and CNI overall. In addition, the incidence of this abnormality was much higher in metastases (12.5%) than in primary tumors. This finding raises the question of whether CNI specifically of the chromosome 21 region is associated with more aggressive disease or may simply reflect the fact that aneuploid cancers, known to be associated with aggressive disease, also have coincidental CNI of TMPRSS2 and ERG gene rearrangements. To investigate this further, we analyzed the DNA content of the tumors by flow cytometry and also performed FISH for an independent chromosome.

DNA ploidy has been shown in several studies to be an independent prognostic marker of survival, especially in non–organ-confined prostate carcinoma, and to be significantly associated with Gleason grade (36–42). Diploid tumors have been shown to be of lower grade and to have a better prognosis and response to therapy than DNA nondiploid or aneuploid tumors (39, 43, 44). FISH can be used to evaluate chromosomal ploidy and has been reported to be more sensitive than flow cytometry for detecting tetraploidy and aneuploidy (21). About two thirds (67%) of our cases with concomitant rearrangement and CNI of the TMPRSS2-ERG loci and nearly all the cases of the CNID subset were nondiploid by flow cytometry. In further support of the finding that the CNI were not specific to the TMPRSS2-ERG loci on chromosome 21, FISH for 9qh showed nondiploid status in most (64%) of these same cases, indicating that copy number changes were not limited to chromosome 21. In contrast, the overwhelming majority of cases without CNI of TMPRSS2-ERG were diploid by flow cytometry and by FISH, which strengthens the hypothesis that generalized chromosomal instability may significantly contribute to the unfavorable biological features associated with the CNI subgroup originally detected using TMPRSS2-ERG FISH. Although the overall numbers of cases with CNI were relatively small, the proportion of patients with BCR, metastases, and deaths was higher in the aneuploid/tetraploid tumors than in the diploid tumors, consistent with what has been reported.

Recently, Tomlins and colleagues (45) described the mutual exclusivity of ERG and ETV1 fusion status and expression of SPINK-1 (at the transcript and protein levels) in ~10% of prostate cancers. SPINK-1 encodes a 56-amino-acid peptide known as TATI and has been shown to play roles in modulating activity of cancer-related proteases and in DNA synthesis. These authors found that SPINK-1 outlier status identified a more aggressive subset of prostate cancer, albeit this did not hold in a nomogram model. Through functional experiments, they postulated that SPINK-1 overexpression may occur late in prostate cancer in the presence of coexisting genetic lesions, consistent with its association with aggressive prostate cancer. Thus, molecular alterations driving ETS gene fusion–negative prostate cancer may play key roles in disease progression and have to be explored further.

In summary, we conclude that rearrangements of the TMPRSS2 and ERG genes alone are not associated with outcome and, in fact, are significantly associated with cancers of lower grade in this PSA-screened U.S. population with surgically treated, clinically localized prostate cancer. Furthermore, prostate cancers with CNI of the TMPRSS2-ERG loci predominantly have aneuploid/tetraploid DNA indices and additional chromosomal abnormalities. Therefore, we believe that the aggressive features associated with CNI of chromosome 21 and, in particular, the subset of the latter with two or more copies of the deleted locus (CNID) reflect generalized
aneuploidy and are not independently related to CNI of rearranged TMPRSS2:ERG. However, given the relatively modest number of cases with CNI and concomitant rearrangement as well as carcinoma specific deaths, confirmation of our findings is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Anuradha Gopalan, Margaret A. Leversha, Jaya M. Satagopan, et al.


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