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Overexpression of Prostate-Specific *TMPRSS2(exon 0)-ERG* Fusion Transcripts Corresponds with Favorable Prognosis of Prostate Cancer

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Abstract

Purpose: To gain insight in the mechanism and clinical relevance of *TMPRSS2-ERG* expression in prostate cancer, we determined the specific characteristics of fusion transcripts starting at *TMPRSS2* exon 1 and at a more upstream and less characterized exon 0.

Experimental Design: We used quantitative PCR analysis to investigate expression of wild-type *TMPRSS2(exon 0)* and *TMPRSS2(exon 1)* and of *ERG* fusion transcripts. Expression was tested in normal tissue samples, in prostate cancer cell lines and xenografts, and in fresh-frozen clinical prostate cancer samples (primary tumors and recurrences). Expression in clinical samples was correlated with disease progression.

Results: *TMPRSS2(exon 0)* and *TMPRSS2(exon 1)* transcripts were similarly androgen regulated in prostate cancer cell lines, but the expression levels of *TMPRSS2(exon 1)* were much higher. Comparison of expression in different tissues showed *TMPRSS2(exon 0)* expression to be much more prostate specific. In androgen receptor–positive prostate cancer xenografts, *TMPRSS2(exon 1)* transcripts were expressed at similar levels, but *TMPRSS2(exon 0)* transcripts were expressed at very variable levels. The same phenomenon was observed for *TMPRSS2-ERG* fusion transcripts. In clinical prostate cancers, the expression of *TMPRSS2(exon 0)-ERG* was even more variable. Expression of *TMPRSS2 (exon 0)-ERG* transcripts was detected in 55% (24 of 44) of gene fusion–positive primary tumors but only in 15% (4 of 27) of gene fusion–positive recurrences and at much lower levels. Furthermore, in primary tumors, expression of *TMPRSS2(exon 0)-ERG* transcripts was an independent predictor of biochemical progression-free survival.

Conclusion: The expression of *TMPRSS2(exon 0)-ERG* fusion transcripts in prostate cancer is associated with a less-aggressive biological behavior. (Clin Cancer Res 2009;15(20):6398–403)

Recently, recurrent fusions of prostate-specific and androgen-regulated *TMPRSS2* to the *ETS* genes *ERG*, *ETV1*, *ETV4*, and *ETV5* have been reported as the most frequent genetic alterations in clinical prostate cancer (1–7). *TMPRSS2-ERG* fusion is detected in 40% to 70% of clinical prostate cancers. Fusion of *ETV1*, *ETV4*, and *ETV5* to *TMPRSS2* are much less frequent, but *ETV1*, *ETV4*, and *ETV5* have multiple fusion partners. Expression of most of these partner genes is prostate specific and androgen regulated (1–5). Some clinical studies have shown *TMPRSS2-ERG* to be associated with a more aggressive prostate cancer phenotype (8–12). However, other studies did not find an association with outcome in patients treated by radical prostatectomy (13, 14) or even described *TMPRSS2-ERG* to be correlated with a more favorable outcome (15, 16).

TMPRSS2 has more than one first exon.[3] Not only fusion transcripts starting at the well-known *TMPRSS2* exon 1 but also transcripts that start from a more upstream and less characterized alternative first exon, here denoted exon 0, have been identified (14).[4]

In the present study, we determined the specific characteristics of *TMPRSS2* transcripts starting at exons 1 and 0 in benign prostatic tissue and in prostate cancer. Moreover, we investigated clinical prostate cancer samples (primary tumors and recurrences) for expression of *TMPRSS2(exon 0)-ERG* and *TMPRSS2*

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[3] UCSC Genome Browser (genome.ucsc.edu).

[4] K.G. Hermans, unpublished observation.

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Translational Relevance

The *TMPRSS2-ERG* gene fusion is the major genetic alteration in prostate cancer. The prognostic value of *TMPRSS2-ERG* in prostate cancer is still a subject to debate. We describe novel findings of a *TMPRSSS2* transcript starting at an alternative exon, denoted exon 0. We show that this transcript is much more prostate specific than the generally studied transcript starting at *TMPRSS2* exon 1. Furthermore, we provide important evidence that the expression of the *TMPRSS2(exon 0)-ERG* fusion transcript correlates with a good prognosis of prostate cancer, whereas *TMPRSS2(exon 1)-ERG* transcripts do not show such a correlation. Our findings urge further investigation of the heterogeneity of *TMPRSS2-ERG* in prostate cancer. More systematic identification of specific fusion transcripts like *TMPRSS2(exon 0)-ERG* or alternatively spliced mRNAs might assist in a molecular classification of prostate cancers, which can be integrated in therapeutic decision making in the future.

(exon 1)-ERG fusion transcripts. In the primary tumors, we correlated fusion gene expression with time to biochemical progression after radical prostatectomy. Our data show different expression patterns of *TMPRSS2(exon 0)* and *TMPRSS2(exon 1)* transcripts. Furthermore, our findings indicate a more favorable prognosis of tumors with *TMPRSS2(exon 0)-ERG* expression.

Materials and Methods

Prostate cancer cell lines and xenografts. Prostate cancer cell lines LNCaP and DuCaP were grown in RPMI-1640 supplemented with 5% fetal calf serum and antibiotics. Androgen receptor–positive prostate cancer xenografts PCEW, PC82, PC295, PC310, PC329, PC346, and PC374, and androgen receptor–negative xenografts PC133, PC135, PC324, and PC339 were propagated by serial transplantation on male nude mice, as described (17, 18).

Clinical samples. Primary prostate tumors were obtained by radical prostatectomy and recurrent tumors by transurethral resection of the prostate. Hematoxylin/eosin-stained tissue sections were histologically evaluated by two pathologists (T. van der Kwast; G.J.H.L. van Leenders). Although introducing a bias, only samples that contained at least 70% tumor cells were selected for analysis. The clinical and pathologic demographics of the patients with primary prostate tumors included in the statistical analysis ($n = 67$) are given in Supplementary Table S1. Tissues were snap frozen and stored in liquid nitrogen. Use of the samples for research purposes was approved by the Erasmus MC Medical Ethics Committee according to the Medical Research Involving Human Subjects Act (MEC-2004-261).

RNA isolation. RNAs from the prostate cancer cell lines LNCaP and DuCaP cultured in the absence or the presence of 10^{-9} mol/L R1881 were isolated using the RNeasy RNA extraction kit (Qiagen). RNA from clinical prostate cancer samples was isolated from frozen tissue sections using RNA-Bee (Campro Scientific). Xenograft RNA was isolated according to the LiCl protocol.

Quantitative reverse transcriptase-PCR (RT-PCR). Total RNA was reverse transcribed using M-MLV reverse transcriptase (Invitrogen) and an oligo dT12 primer. cDNAs of 16 different tissues were purchased from Clontech. Quantitative RT-PCRs were done in Power SYBR Green PCR Master Mix (25 μL), containing 0.33 μmol/L forward and reverse primer in an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Amplified products were quantified relative to porphobilinogen deaminase (*PBGD*). Primer sequences of the primers used are summarized in Supplementary Table S2.

Statistical analysis. Associations between clinical and histopathologic variables and expression of *TMPRSS2-ERG* transcripts were evaluated by the Pearson's χ^2 test, the Mann-Whitney *U* test, or Kruskal-Wallis test, where appropriate. Expression of *TMPRSS2-ERG* transcripts was correlated with the primary end point, biochemical progression-free survival, defined as time from radical prostatectomy to date of biochemical recurrence. Biochemical recurrence was defined as (*a*) a prostate-specific antigen level of >0.2 ng/mL at two consecutive measurements with a 3-month interval if the prostate-specific antigen nadir was <0.1 ng/mL or (*b*) a prostate-specific antigen nadir of ≥0.2 ng/mL. Patients that died from causes other than prostate cancer or that were lost to follow-up were censored at the date of last prostate-specific antigen test. Patients were routinely followed thrice monthly during the first year after radical prostatectomy, semiannually during the second year, and subsequently at 12-month intervals. In case of progression, patients were followed every 3 months. Kaplan-Meier curves were constructed to assess the probability of remaining free of biochemical recurrence as a function of time after surgery. The differences between the survival curves of the groups were tested using the log-rank test or Breslow method if appropriate. A Cox proportional regression analysis with forward stepwise elimination was done to assess the impact of various parameters on time to recurrence. In the multivariate analysis, the model included pathologic T stage, surgical margin status, the Gleason score of the primary tumor, and expression of indicated *TMPRSS2-ERG* fusion transcripts. Patients with unknown parameters were excluded from the analysis. Statistical analyses were done using the Statistical Package for Social Sciences, version 15.0 (SPSS), with a significance level of 0.05 (two-tailed probability).

Results

TMPRSS2-ERG gene fusion is present in 40% to 70% of primary prostate tumors. *ERG* and *TMPRSS2* are located ~3 Mbp apart on chromosome 21q22.3 in the same orientation (Fig. 1A). The most common *TMPRSS2-ERG* fusion transcripts are composed of *TMPRRS2* exon 1 or exons 1 and 2 linked to exon 4 of *ERG*. Less frequently, fusion of *TMPRSS2* exon 1 or 2 to other *ERG* exons have been detected (12). Genomic databases describe that *TMPRSS2* transcripts might also contain an alternative first exon, here denoted exon 0, which maps ~4-kbp upstream of exon 1 (Fig. 1A). *TMPRSS-ERG* fusion transcripts might also contain *TMPRSS2* exon 0 (14).[3]

We determined the specific characteristics of transcripts starting at either exon 0 or exon 1 of *TMPRSS2*. First, the expression of both transcripts was investigated on a panel of cDNA from 16 different normal tissues. Although expression of *TMPRSS2 (exon 1)* mRNA was highest in the prostate, these transcripts were also detected in lung, kidney, pancreas, and colon samples. In contrast, *TMPRSS2(exon 0)* mRNA had a completely prostate-specific expression pattern (Fig. 1B). Subsequently, we did a quantitative RT-PCR analysis on RNA from prostate cancer cell lines DuCaP and LNCaP cultured in absence or presence of the synthetic androgen R1881. Both *TMPRSS2* transcripts were induced by androgens (Fig. 1C). Notably, in the cell lines, expression of *TMPRSS2(exon 0)* transcripts was much lower than expression of *TMPRSS2(exon 1)*.

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Testing of RNAs from eleven human prostate cancer xenografts for expression of *TMPRSS2* starting at either exon 0 or 1 showed that six androgen receptor–positive xenografts, PCEW, PC82, PC295, PC329, PC346, and PC374, expressed *TMPRSS2 (exon 1)* at less-variable levels (Fig. 1D). Xenograft PC310 showed no expression of *TMPRSS2* because of a deletion of the wild-type allele (17). Five xenografts expressed *TMPRSS2 (exon 0)* with a much more variable level of expression. None of the androgen receptor–negative xenografts expressed *TMPRSS2*.

Previously, we have shown that five androgen-dependent xenografts, PCEW, PC82, PC295, PC310, and PC329, contained

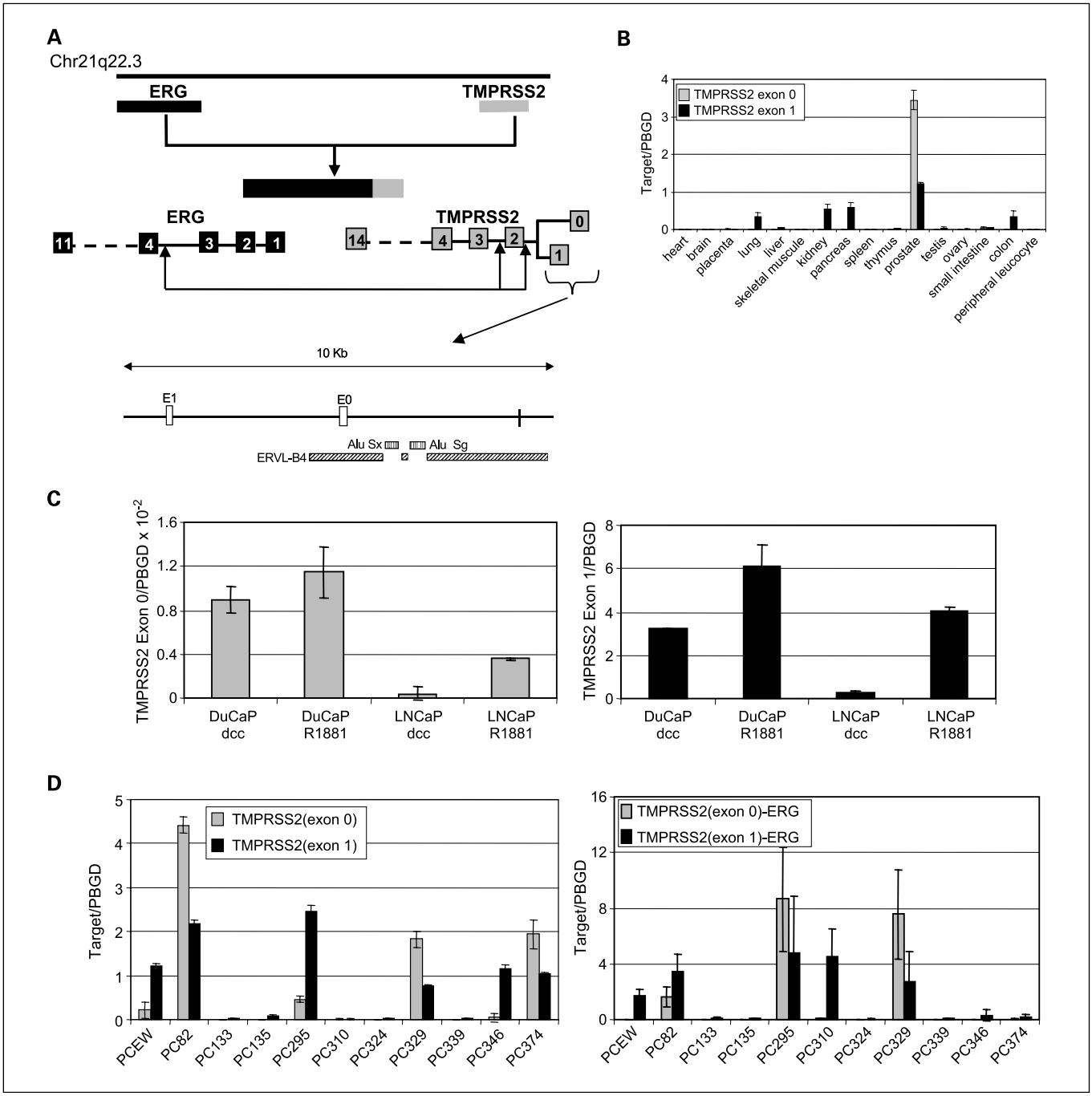

Fig. 1. Characterization of *TMPRSS2* and *TMPRSS2-ERG* transcripts starting at exon 0 or 1. *A,* schematic representation of the *TMPRSS2-ERG* locus on chromosome band 21q22.3. The most frequent gene fusion events are indicated. The enlarged genomic region containing *TMPRSS2* shows exons 0 and 1 and repeat sequences. *B,* tissue-specific expression of *TMPRSS2(exon 0)* and *TMPRSS2(exon 1)* mRNA assessed by quantitative RT-PCR analysis on a cDNA panel from 16 different normal tissues. Mean of duplicate experiment relative to *PBGD* with SD. *C,* androgen-regulated expression of *TMPRSS2(exon 0)* (*left*) and *TMPRSS2(exon 1)* (*right*) mRNA in androgen receptor–positive prostate cancer cell lines LNCaP and DuCaP. LNCaP and DuCaP cells were grown in the absence and presence of synthetic androgen R1881 (10^{-9} mol/L) for 24 h. Mean of duplicate experiments relative to *PBGD* with SD are depicted. Note that the level of *TMPRSS2(exon 0)* expression is much lower in the cell lines than in the normal prostatic tissue (*B*). *D,* quantitative RT-PCR analysis of *TMPRSS2(exon 0)* and *TMPRSS2(exon 1)* (*left*) and *TMPRSS2(exon 0)-ERG* and *TMPRSS2(exon 1)-ERG* (*right*) transcripts in 11 human prostate cancer xenografts. Mean of duplicate experiments relative to *PBGD* with SD.

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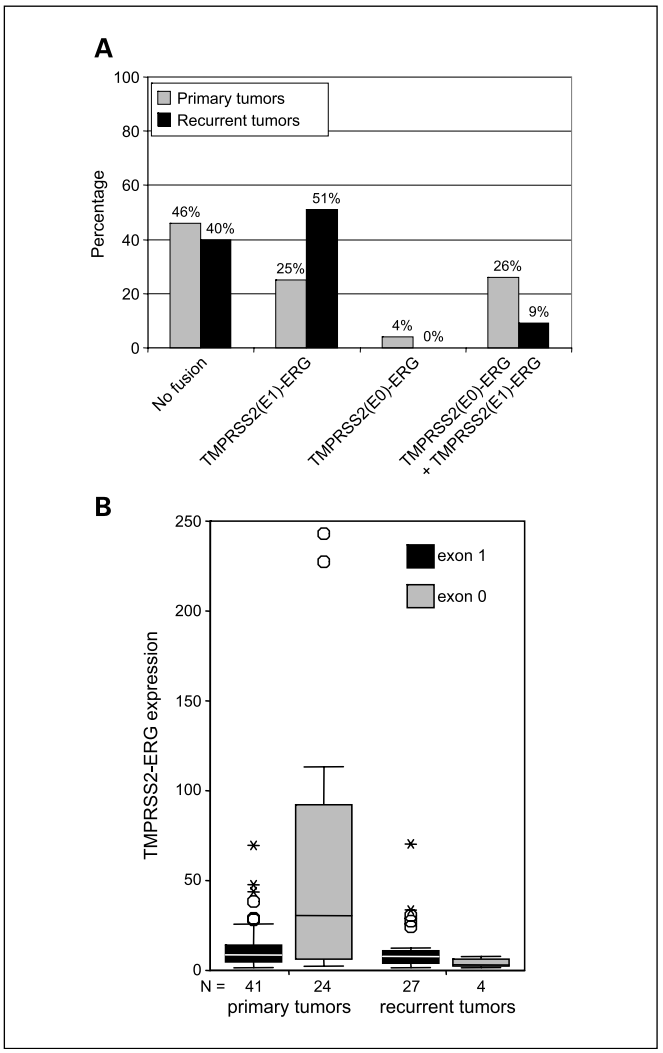

Fig. 2. Expression of *TMPRSS2(exon 0)-ERG* and *TMPRSS2(exon 1)-ERG* transcripts in clinical prostate cancer samples. *A,* distribution of both *TMPRSS2-ERG* fusion transcripts in primary and recurrent tumors. Primary tumors, $n = 81$; recurrent tumors, $n = 45$. *B,* box plot of *TMPRSS2 (exon 0)-ERG* and *TMPRSS2(exon 1)-ERG* mRNA expression levels in primary tumors and recurrences. Open circle, outliers; asterisk, extremes.

TMPRSS2(exon 1)-ERG mRNA (17). Quantitative RT-PCR analysis for [*TMPRSS2(exon 1)-ERG* and *TMPRSS2(exon 0)-ERG*] fusion transcripts showed that three xenografts, PC82, PC295, and PC329, expressed *TMPRSS2(exon 0)-ERG* at different levels (Fig. 1D). However, the other two xenografts that expressed *TMPRSS2-ERG* transcripts, PCEW and PC310, did not express the *TMPRSS2(exon 0)-ERG* fusion transcript at all. The expression levels of *TMPRSS2(exon 1)-ERG* transcripts in the five xenografts did not show a large variation (Fig. 1D). The difference in *TMPRSS2(exon 0)-ERG* and *TMPRSS2(exon 1)-ERG* expression was similar to that observed for wild-type *TMPRSS2(exon 0)* and *(exon 1)* transcripts.

Next, we determined the expression of *TMPRSS2(exon 0)-ERG* and *TMPRSS2(exon 1)-ERG* transcripts in a cohort of 126 fresh-frozen clinical prostate cancer samples (81 primary tumors and 45 recurrent tumors; Fig. 2A). *TMPRSS2-ERG* transcripts were detected in 54% (44 of 81) of the primary tumors and in 60% (27 of 45) of the recurrences. In the primary tumors, 20 (25%) of 81 of the cases exclusively expressed *TMPRSS2(exon 1)-ERG* transcripts, three samples (4%) exclusively expressed *TMPRSS2(exon 0)-ERG*, and 21 (26%) expressed both transcripts. Analysis of wild-type *TMPRSS2* in benign prostatic tissue excluded preferential expression of *TMPRSS2(exon 1)* or *TMPRSS2(exon 0)* transcripts in one of

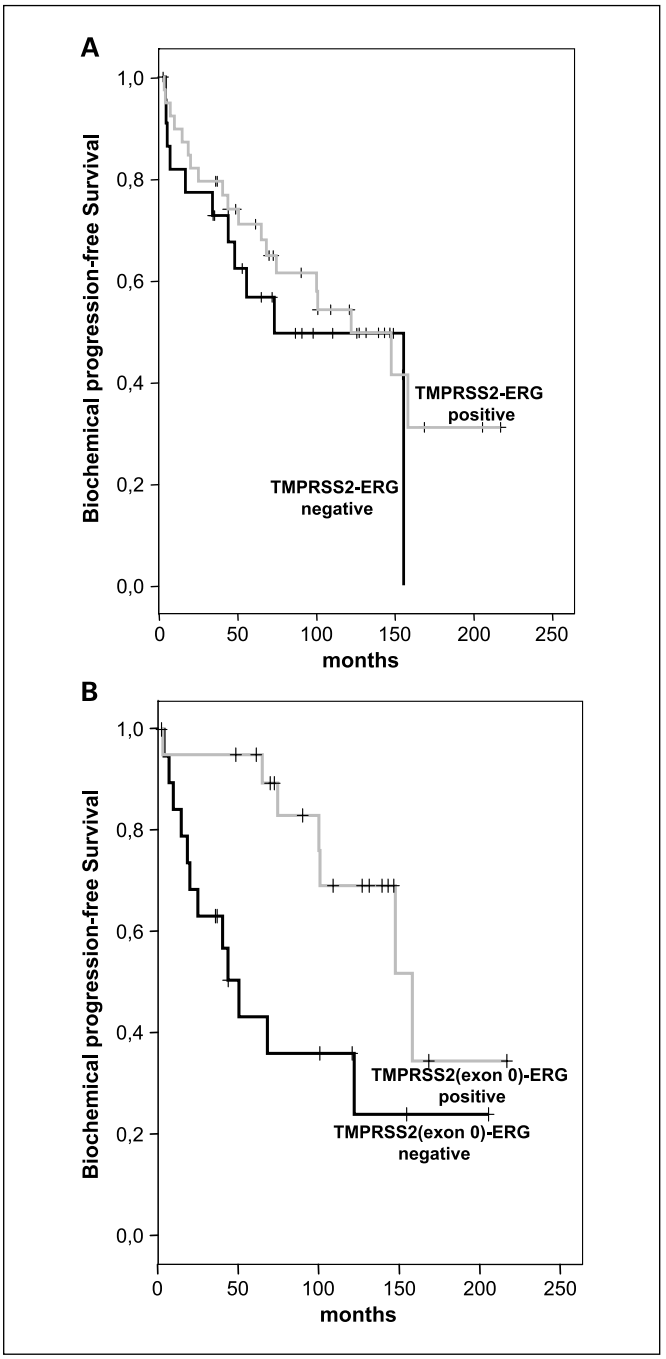

Fig. 3. Kaplan-Meier curves for time to prostate-specific antigen recurrence after radical prostatectomy defined by *TMPRSS2-ERG* fusion transcript status. *A,* biochemical progression-free survival curves for prostate cancer patients with or without expression of *TMPRSS2-ERG* transcripts. *B,* biochemical progression-free survival curves for the *TMPRSS2-ERG*–positive group, which was stratified in patients with and without expression of *TMPRSS2(exon 0)-ERG* transcripts.

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the different prostate zones (data not shown). In the recurrent tumors, exclusive expression of *TMPRSS2(exon 1)-ERG* was detected in 23 (51%) of 45 of the cases, whereas none expressed exclusively *TMPRRS2(exon 0)-ERG* and only four cases (9%) expressed both transcripts. Expression levels of *TMPRSS2 (exon 0)-ERG* transcripts were significantly higher in primary tumors than in recurrent tumors ($P = 0.015$), and variation in expression was much larger in the primary tumors than in the recurrences (Fig. 2B). In contrast, the percentage of tumors expressing *TMPRSS2(exon 1)-ERG* transcripts was in the same range for primary and recurrent tumors, and the expression levels of these transcripts did not differ between both tumor types ($P = 0.74$).

We correlated the expression of *TMPRSS2(exon 0)-ERG* with clinical outcome in the primary prostate cancer cohort ($n = 81$) to see whether it was of prognostic value. We excluded from the analysis 10 patients that were known to harbor fusion or overexpression of other *ETS* genes and four patients whose primary treatment was not a radical prostatectomy. Despite the very long follow-up available (median, >10 years), only 11 of the remaining 67 patients died from prostate cancer, precluding statistical analysis. Instead, we used time to prostate-specific antigen recurrence after radical prostatectomy as an end point. The patients' demographics are summarized in Supplementary Table S1. No differences were seen in clinical and histopathologic characteristics between patients expressing *TMPRSS2-ERG* and gene fusion–negative patients, although *TMPRSS2-ERG*–negative patients had higher Gleason scores with borderline significance ($P = 0.053$; Supplementary Table S3). The median time to prostate-specific antigen progression was not significantly different between the two groups: 73.2 versus 122.1 months [95% confidence interval (95% CI), 32.7-113.7 versus 70.6-173.6; $P = 0.45$; Fig. 3A].

Within the *TMPRSS2-ERG*–positive population, the only difference between patients that exclusively expressed *TMPRSS2(exon 1)-ERG* transcripts and patients that expressed the *TMPRSS2 (exon 0)-ERG* subtype was that the former had higher pathologic stages than the latter ($P = 0.009$; Supplementary Table S4). The median time to prostate-specific antigen progression for patients expressing *TMPRSS2(exon 0)-ERG* transcripts was significantly longer than for patients that exclusively expressed *TMPRSS2(exon 1)-ERG* transcripts: 158.2 versus 50.5 months (95% CI, 98.9-217.5 versus 32.6-68.4; $P = 0.012$; Fig. 3B).

Using a Cox proportional hazards model, positive surgical margins, Gleason score of ≥7, pathologic stage of ≥pT3a, and the absence of *TMPRSS2(exon 0)-ERG* transcripts were all associated with a worse biochemical progression-free survival. Importantly, multivariate analysis with forward stepwise selection showed expression of *TMPRSS2(exon 0)-ERG* fusion transcripts to be an independent predictor of progression-free survival (hazard ratio, 0.34; 95% CI, 0.14-0.84; $P = 0.019$; Table 1).

Discussion

This study addresses two important aspects of *TMPRSS2-ERG* expression in prostate cancer. First of all, a remarkable difference in expression characteristics was detected between *TMPRSS2(exon 1)* and *TMPRSS2(exon 1)-ERG* transcripts on the one hand and *TMPRSS2(exon 0)* and *TMPRSS2(exon 0)-ERG* transcripts on the other hand. Secondly, the clinical data indicated a more favorable prognosis for prostate cancer patients expressing *TMPRSS2(exon 0)-ERG* transcripts.

It is estimated that almost half of all genes in the human genome contain more than one first exon as an important mechanism to regulate gene expression (19). Here, we showed that *TMPRSS2* transcripts starting at exon 0 were much more prostate specific than those starting at exon 1 (Fig. 1B) and that the expression level of transcripts containing exon 0 was much more variable (Fig. 1D). *TMPRSS2* exon 0 is located in a retroviral repeat element, ERVL-B4 (Fig. 1A). This repeat does not contain a standard long terminal repeat promoter element; however, other retroviral repeat sequences might function as cryptic promoters (19, 20). Within the same retroviral repeat, the *TMPRSS2* sequence present in a *TMPRSS2-ETV4* fusion transcript is located (6). Although a different 5′-untranslated region might affect translation efficacy, the major proteins translated from the fusion transcripts seem identical N-truncated ERG proteins, which are translated from an ATG codon in the *ERG* exon 4 part of the fusion transcripts.

It could be speculated that the prostate-specific *TMPRSS2 (exon 0)* transcripts are expressed in tumors with a more differentiated phenotype. Recurrent tumors represent late-stage prostate

Table 1. Results of univariate and multivariate analyses

Variable	*n*	Univariate			HR (95% CI)	*P*	Multivariate	
		Median time to PSA recurrence (mo)	95% CI	*P*			HR (95% CI)	*P*
Surgical margins								
Positive	30	43.9	31.0-56.8	<0.001	7.5 (3.0-18.5)	<0.001	7.7 (3.0-19.4)	0.001
Negative	31	122.1	49.5-194.7		1.0			
Gleason score								
≥7	26	68.2	41.5-94.9	0.037	2.1 (1.0-4.5)	0.041		
<7	35	155.4	117.7-192.2		1.0			
pT stage								
Extraprostatic	43	65.0	37.4-92.5	<0.001	5.7 (1.9-16.8)	0.002		
Organ confined	18	158.2	146.7-169.7		1.0			
TMPRSS2(exon 0)-ERG expression								
Yes	21	158.2	98.9-217.5	0.015	0.36 (0.15-0.85)	0.02	0.34 (0.14-0.84)	0.019
No	40	68.2	36.8-99.6		1.0			

Abbreviations: HR, hazard ratio; pT stage, pathologic T stage; PSA, prostate-specific antigen.

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cancer that normally has a less differentiated phenotype. In our study, 55% of the recurrences had a Gleason score of ≥8 compared with 15% of the primary tumors. An alternative explanation is that expression from exon 0 is stimulated by the stromal compartment, which will be different in primary tumors and recurrences. Obviously, there are no stromal cells present in *in vitro* cultures of prostate cell lines, which showed very low expression levels of *TMPRSS2(exon 0)* (Fig. 1).

The prognostic significance of *TMPRSS2-ERG* gene fusion remains subject of debate, although a growing number of studies has been published on this matter (8–13, 16). Because technology used to investigate *TMPRSS2-ERG* varies (FISH or quantitative RT-PCR) and compositions of patient cohorts differ considerably, it is difficult to draw general conclusions from available data. In the present study on a selected and rather limited group of well-defined patients with a very long median follow-up, there was no difference in time to prostate-specific antigen recurrence after radical prostatectomy between patients that expressed *TMPRSS2-ERG* and patients without expression of the fusion gene.

In two studies on watchful waiting cohorts, it was shown that patients having *TMPRSS2-ERG* fusion had a higher incidence of metastases or cancer-specific death than gene fusion–negative patients (8, 9); however, this was not confirmed in a large surgically treated cohort (13). Attard et al. (8) showed by FISH analysis that patients with an interstitial deletion of genomic sequences between *TMPRSS2* and *ERG* (so called "class Edel") had poorer cancer-specific and overall survival than gene fusion–negative patients or than patients with *TMPRSS2-ERG* fusion without loss of the genomic region between the two genes. Other studies have correlated *TMPRSS2-ERG* with biochemical progression after radical prostatectomy, like in the present study. Before the identification of *TMPRSS2-ERG*, Petrovics et al. (15) found that patients with high expression levels of *ERG* had longer prostate-specific antigen recurrence-free survival than patients without *ERG* overexpression. Recently, similar results were reported by Saramaki et al. (16), using FISH analysis of *TMPRSS2-ERG*. However, other studies claimed a negative correlation between *TMPRSS2-ERG* and prostate-specific antigen recurrence (10–12). Perner et al. indicated that patients with *TMPRSS2-ERG* rearrangement through deletion showed a trend for higher prostate-specific antigen recurrence rate than patients without fusion (11). Wang et al. (12) provided evidence that specific *TMPRSS2-ERG* splice variants were associated with early prostate-specific antigen recurrence.

Information on *TMPRSS2(exon 0)-ERG* transcripts in prostate cancer is still scarce. Thus far, this transcript was only identified by Lapointe et al. (14). However, the clinical implications of this specific fusion transcript subtype were not investigated. The low expression frequency of *TMPRSS2 (exon 0)-ERG* transcripts in late-stage prostate cancer and the favorable prognosis for patients expressing this fusion transcript in primary tumors, as shown in our study, urges further investigation of the heterogeneity of *TMPRSS2-ERG* in prostate cancer. More systematic identification of specific fusion transcripts like *TMPRSS2(exon 0)-ERG* or alternatively spliced mRNAs (12) might assist in a molecular classification of prostate cancers, which can be integrated in therapeutic decision making in the future.

Disclosure of Potential Conflicts of Interest

The authors declare no conflict of interest.

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