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):6398–6403)

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. 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).

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* (exon 0)-*ERG*.
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 TMPRSS2 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.

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 tissues sections were histologically evaluated by two pathologists (T. vander Kwaast; 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 10−7 mol/L R1881 were isolated using the RNaseasy RNA extraction kit (Qiagen). RNA from clinical prostate cancer samples was isolated from frozen tissue sections using RNA-Be (Campro Scientific). Xenograft RNA was isolated according to the LCI 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 χ2 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 TMPRSS2 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). TMPRSS2-ERG fusion transcripts might also contain TMPRSS2 exon 0 (14).

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).

www.aacrjournals.org 6399 Clin Cancer Res 2009;15(20) October 15, 2009 Downloaded from clincancerres.aacrjournals.org on December 5, 2021. © 2009 American Association for Cancer Research.
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
Quantitative RT-PCR analysis for \( \text{TMPRSS2(exon 1)} \)-ERG and \( \text{TMPRSS2(exon 0)} \)-ERG fusion transcripts showed that three xenografts, PC82, PC295, and PC329, expressed \( \text{TMPRSS2(exon 0)} \)-ERG at different levels (Fig. 1D). However, the other two xenografts that expressed \( \text{TMPRSS2-ERG} \) transcripts, PCEW and PC310, did not express the \( \text{TMPRSS2(exon 0)} \)-ERG fusion transcript at all. The expression levels of \( \text{TMPRSS2(exon 1)} \)-ERG transcripts in the five xenografts did not show a large variation (Fig. 1D). The difference in \( \text{TMPRSS2(exon 0)} \)-ERG and \( \text{TMPRSS2(exon 1)} \)-ERG expression was similar to that observed for wild-type \( \text{TMPRSS2(exon 0)} \) and (exon 1) transcripts.

Next, we determined the expression of \( \text{TMPRSS2(exon 0)} \)-ERG and \( \text{TMPRSS2(exon 1)} \)-ERG transcripts in a cohort of 126 fresh-frozen clinical prostate cancer samples (81 primary tumors and 45 recurrent tumors; Fig. 2A). \( \text{TMPRSS2-ERG} \) 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 \( \text{TMPRSS2(exon 1)} \)-ERG transcripts, three samples (4%) exclusively expressed \( \text{TMPRSS2(exon 0)} \)-ERG, and 21 (26%) expressed both transcripts. Analysis of wild-type \( \text{TMPRSS2} \) in benign prostatic tissue excluded preferential expression of \( \text{TMPRSS2(exon 1)} \) or \( \text{TMPRSS2(exon 0)} \) transcripts in one of
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 ex-
pressed exclusively TMPRSS2(exon 0)-ERG and only four cases
(9%) expressed both transcripts. Expression levels of TMPRSS2
(exon 0)-ERG transcripts were significantly higher in primary tu-
mors than in recurrent tumors ($P = 0.015$), and variation in ex-
pression was much larger in the primary tumors than in the re-
currences (Fig. 2B). In contrast, the percentage of tumors ex-
pressing 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 over-
expression 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 an-
tigen recurrence after radical prostatectomy as an end point.
The patients’ demographics are summarized in Supplementary
Table S1. No differences were seen in clinical and histopatho-
logic characteristics between patients expressing TMPRSS2-ERG
and gene fusion–negative patients, although TMPRSS2-ERG–
negative patients had higher Gleason scores with borderline sig-
nificance ($P = 0.053$; Supplementary Table S3). The median
time to prostate-specific antigen progression was not signifi-
cantly different between the two groups: 73.2 versus 122.1
months [95% confidence interval (95% CI), 32.7-68.4; $P = 0.45$; Fig. 3A].

Within the TMPRSS2-ERG–positive population, the only dif-
terence 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 ≥7, pathologic stage of ≥pT3a, and the absence of TMPRSS2(exon 0)-ERG transcripts were all asso-
ciated with a worse biochemical progression-free survival. Im-
portantly, 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 pa-

tients expressing TMPRSS2(exon 0)-ERG transcripts.

It is estimated that almost half of all genes in the human ge-
nome contain more than one first exon as an important mech-
nism to regulate gene expression (19). Here, we showed that
TMPRSS2 transcripts starting at exon 0 were much more pro-
tate 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 retro-
viral 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 tran-
script 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 pro-

teins, 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 differ-
tiated phenotype. Recurrent tumors represent late-stage prostate

#### Table 1. Results of univariate and multivariate analyses

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

Abbreviations: HR, hazard ratio; psT stage, pathologic T stage; PSA, prostate-specific antigen.
cancer that normally has a less differentiated phenotype. In our study, 55% of the recurrences had a Gleason score of 2+ 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.

**Acknowledgments**

We thank Theo van der Kwaast for the pathology, Wilma Teubel for the collection of clinical samples, Wyttske van Weerden for the xenograft tissues, Anieta Siewerts for the RNA isolation, Natasha Dits for the cDNA samples of clinical prostate tumors, and Mark Wildhagen for support with the statistical analysis.

**References**

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doi:10.1158/1078-0432.CCR-09-1176

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