A Gain of Function $\text{TGF}B1$ Polymorphism May Be Associated With Late Stage Prostate Cancer

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Abstract

Transforming growth factor $\beta$ (TGF$\beta$) is known to exert both positive and negative effects on different stages of tumor formation. Of the TGF$\beta$ isoforms, TGF$\beta1$ is highly expressed in prostate cancer and leads to tumor promotion and metastasis. Increased expression of TGF$\beta1$ is associated with more aggressive tumors and poor prognosis. Several polymorphisms in TGF$\beta1$ have been identified, and two variants in strong linkage disequilibrium, $\text{C} - 509T$ and $\text{T} + 29C$, show increased serum levels. Because of the potential role of TGF$\beta1$ variants in prostate cancer and progression, we hypothesized that these two TGF$\beta1$ variants would be associated with prostate cancer risk, particularly later, more aggressive stage tumors. To test this, we conducted a nested case-control study of 492 men diagnosed with prostate cancer from the Physicians Health Study and 492 age-matched controls. In this study, cases who were homozygous for the $\text{T}$ allele at position $-509$ had a 2.4-fold increased risk of more advanced stage of prostate cancer [95% confidence interval (95% CI) 1.03–5.43; $P = 0.04$]. The $\text{T}$ allele frequencies in cases and controls were 32.7% and 31.4%, respectively. The same polymorphism showed a 1.23 nonsignificant odds ratio (OR) for overall prostate cancer risk (95% CI 0.80–1.87). Cases who were homozygous for the $\text{C}$ allele at position $+29$ did not show any significant increase in risk for either total prostate cancer (OR 1.19, 95% CI 0.82–1.74) or advanced stage prostate cancer (OR 1.33, 95% CI 0.66–2.68). The $\text{C}$ allele frequency in cases and controls were 39.9% and 38.5%, respectively. Our data suggest that the $\text{TGF}B1$ C–509T variant that affects expression of TGF$\beta1$ may play a role in advanced stage prostate cancer. (Cancer Epidemiol Biomarkers Prev 2004;13(5):759–64)

Introduction

The transforming growth factor $\beta$ (TGF$\beta$) signaling pathway plays a dual role in cancer development, suppressing early stage tumor growth but promoting tumor progression and metastasis (1). In normal epithelial and hematopoietic cells, TGF$\beta$ is associated with tumor suppression by promoting differentiation and growth inhibition. In tumor cells, defects in the TGF$\beta$ signaling pathway occur in many types of cancer that lead to resistance to TGF$\beta1$-mediated growth inhibition. When the antiproliferative effect of the TGF$\beta$ signaling pathway is disrupted, increased expression of TGF$\beta1$ aids in the promotion of tumorigenesis through increased angiogenesis, immunosuppression, and ability to invade and metastasize. This biphasic nature of TGF$\beta1$ action has been demonstrated in mouse models in which the TGF$\beta$ pathway has been altered (2, 3).

Of the three TGF$\beta$ isoforms, TGF$\beta1$ is most clearly involved in tumorigenesis (1), including prostate cancer. All three isoforms of TGF$\beta$ are expressed in prostate cells, but TGF$\beta1$ is expressed at the highest level (4). Of the cells of the prostate, both the epithelium and stroma show high TGF$\beta1$ expression (5), which stimulates cell differentiation and inhibits proliferation. TGF$\beta1$ expression is up-regulated in prostate cancer cells as well. The paradox of high TGF$\beta1$ expression in both normal and tumor cells of the prostate may be explained by the observation that prostate tumor cells acquire reduced sensitivity to the growth inhibitory and apoptotic effects of TGF$\beta1$ (6). This may be due to several mechanisms including reduced expression of type I and II receptors, alterations in Smad expression, modulation of TGF$\beta1$ binding partners, and loss or reduction of downstream mediators of growth inhibition and apoptosis (7, 8). TGF$\beta1$ itself may promote changes in the cellular environment to the advantage of the tumor, including suppression of the immune system, promotion of angiogenesis and extracellular matrix formation, and increased tumor cell plasticity that enhances invasion and metastasis. Later stage tumors, particularly those that metastasize, have greater intracellular TGF$\beta1$ expression than normal prostate epithelium or prostate hyperplasia (9). High serum levels of TGF$\beta1$ are seen in men with invasive prostate cancer (10) and are associated with a poor clinical outcome (11), progression, and metastasis (12). Levels of urinary TGF$\beta1$ are increased 3.5-fold in prostate cancer cases compared with controls and are associated with advanced prostate cancer stage and grade (13).
Based on its dual role as a tumor suppressor and a tumor enhancer depending on the microenvironment, one might hypothesize that variants in TGFB1 are candidates for both prostate cancer susceptibility and cancer resistance. TGFB1 polymorphisms have been studied previously for breast cancer risk, and two TGFB1 variants have been functionally characterized. One of these polymorphisms, a T-to-C transition at the 29th nucleotide of TGFB1 changes a leucine to a proline at amino acid number 10. This change is associated with increased serum levels of TGFβ1 due to increased secretion (14–16). The T+29C polymorphism (CC) is associated with a modest 20% increase in cancer risk with a median age of breast cancer of 50 in three case-control studies of women from different populations (16). The same variant genotype showed a protective effect in women diagnosed with breast cancer at a later median age of 70 (17). A second polymorphism in the TGFB1 promoter, a transition of a C to a T at position −509, is in strong linkage disequilibrium with the T+29C polymorphism and showed a 25% increase in breast cancer risk (16, 18). The T allele is associated with higher plasma concentrations of both active and acid-activatable TGFβ1 (15).

Based on in vitro and in vivo studies of TGFB1 in prostate and other types of cancer, we hypothesized that TGFB1 polymorphisms would play a role in prostate cancer risk. Because of the modest risks seen in the previously published breast cancer studies, the potential complexities of TGFB1 in cancer risk, and data showing an increase of TGFB1 expression in later stage prostate cancer, we decided to specifically examine the role of TGFB1 in cancer risk associated with prostate cancer stage and Gleason score. Here, we examine two TGFB1 polymorphisms in 492 men in the Physicians Health Study (PHS) who have been diagnosed with prostate cancer between 1982 and 1995 and 492 age-matched controls (19). To the best of our knowledge, this work is the first epidemiological report of TGFB1 polymorphisms and prostate cancer risk.

Materials and Methods

Study Population. The PHS was a randomized placebo-controlled trial of aspirin and β-carotene conducted among 22,071 U.S. male physicians aged 40–84 years in 1982. Men were excluded if they (a) had a history of myocardial infarction, stroke, transient ischemic attack, or cancer (except nonmelanoma skin cancer); (b) had current renal or liver disease, peptic ulcer, or gout; or (c) currently used aspirin, vitamin A, or β-carotene supplements. At enrollment and annually thereafter through 1995, men completed short, mailed questionnaires on diet, lifestyle, and medical history. Before randomization, all participants also received blood kits and instructions to have their blood drawn into Vacutainer tubes containing EDTA. The samples were centrifuged, and the plasma was sent by overnight courier, in cryopreservation vials with cold packs, to the Brigham and Women’s Hospital Channing Laboratory. The samples were then separated into aliquots and stored at −82°C. Precautions were taken to prevent thawing or warming of specimens during storage. A total of 14,916 (68%) men provided blood samples in this cohort. Through 1995, follow-up of this cohort for morbidity and mortality was more than 99% complete; additional details on this cohort can be found elsewhere (20). The Institutional Review Board of Brigham and Women’s Hospital approved the PHS, and all study participants provided informed consent. As of December 31, 1995, 786 incident cases of prostate cancer were ascertained among the 14,916 men who provided blood samples. A total of 492 case-control pairs were analyzed for TGFB1 polymorphisms and prostate cancer risk. This case-control set was 1:1 matched on age (within 5 years) and smoking status (never, past, current, or missing) for historical reasons. Because smoking status was not associated with prostate cancer risk in our study population, matching on smoking did not affect the results. On average, genotyping was performed in samples collected 9 years before the time of cancer diagnosis (minimum of 6 months and maximum of 13 years).

When a participant reported a new diagnosis of prostate cancer, we requested medical records, which were reviewed by study investigators. We defined advanced stage tumors as extraprostatic (stage C), distant metastatic (stage D), or fatal at diagnosis; early stage tumors were defined as asymptomatic or incidentally detected lesions (stage A) or palpable tumors confined to the prostate gland (stage B). We also examined high-grade (Gleason score of >7) and low-grade (Gleason score of ≤7) tumors separately. Among the 492 cases of prostate cancer in this study, 157 cases were advanced, 287 were early stage, and 48 were missing data on stage or genotype. There were 133 cases with Gleason scores of >7 and 218 with Gleason scores of ≤7; 141 cases were missing genotype or data on Gleason scores at diagnosis. 

Genotyping Analysis. To preserve DNA, 2–5 ng of each sample underwent whole genomic amplification prior to genotyping (21). TGFB1 polymorphisms were genotyped using the ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA). PCR reactions contained 5 μl whole genomic amplification DNA, 1× TaqMan universal PCR master mix, forward and reverse primers (900 nM), 200 nM VIC labeled probe, and 200 nM FAM labeled probe. Sequences of the primers and probes are as described (16). Amplification conditions on a MJ Tetrad thermal cycler (GRI, Waltham, MA) were as follows: 1 cycle of 50°C for 2 min followed by 1 cycle of 95°C for 10 min and 40 cycles of 95°C for 15 s and 62°C for 2 min. Completed PCRs were read on an ABI PRISM 7700 sequence detector and analyzed using the Allelic Discrimination Sequence Detection Software (Applied Biosystems). Double-stranded artificial template controls were constructed using a long forward primer specific for each single nucleotide polymorphism with a long common reverse primer that overlapped the forward primer. Primers were filled-in using standard methods. Genotyping calls were conducted independently by two researchers (A. Ewart-Toland and J. Yuan) who were blinded to case-control status. Any discrepancies were noted and analyses were repeated.

Statistical Analyses. For analysis of the prostate cancer cases and controls, we used conditional logistic regression models to estimate the odds ratios (OR) and 95% confidence intervals (CI) for different genotypes and risk
of prostate cancer. The cases and controls were matched on age and smoking status, and we further adjusted for age as a continuous variable within the conditional logistic regression models to address any potential residual confounding. We also conducted an unmatched analysis using unconditional logistic regression, adjusting for age and smoking in the models. The results were essentially the same as those observed for the conditional logistic regression models, and we present the matched analysis results throughout this report, unless otherwise noted. All statistics were calculated using SAS, version 6.12 (SAS Institute Inc., Cary, NC). A 0.05% significance level was used for all tests. We also conducted analyses focused on early and late stage prostate cancer and low and high Gleason score prostate cancer as the primary outcomes.

Deviation of the genotype frequencies from those expected under Hardy-Weinburg equilibrium was assessed by \( \chi^2 \) tests as described (16). \( D' \) values for linkage disequilibrium were calculated as described (22).

### Results

To determine the risk of TGF\( \beta \)1 polymorphisms for prostate cancer, 492 prostate cancer cases and 492 controls from the PHS (19) were genotyped for the TGF\( \beta \)1 C\( \rightarrow \)509T and the T+29C (L10P) polymorphisms (Tables 1 and 2). There was no significant difference in genotype distribution between cases and controls for either polymorphism, although there was a nonsignificant trend for the rare homozygote TT at position \( 509 (TT) \) and +29 (CC) to increase risk (\( 509 TT \) versus CC: OR 1.23, 95% CI 0.80–1.87; +29 CC versus TT: OR 1.19, 95% CI 0.82–1.74; Table 1). In the cases and controls, there was no deviation of genotype frequencies for either the C\( \rightarrow \)509T or the T+29C variants from Hardy-Weinburg equilibrium. As with previous studies (16, 18), we found strong linkage disequilibrium between the two polymorphisms in our data set (\( D' = 0.9 \)).

Because in vivo and in vitro data suggested that TGF\( \beta \)1 overexpression is associated with tumor progression, we tested the hypothesis that these functional polymorphisms would be associated with later stage prostate tumors. There was no difference in genotype frequencies in individuals with early stage cancer. Among individuals with later stage prostate cancer, there was a 2.36 OR (95% CI 1.03–5.43; \( P = 0.04 \)) associated with the rare homozygote TT at position \( 509 (TT) \) (Table 3). However, in this subgroup analysis, men in the control group also had a lower frequency of the TT genotype (10 of 160, 6.8%) compared with the overall controls (49 of 492, 10%). When using an unmatched analysis (unconditional logistic regression adjusted for age and smoking in the model) of the C\( \rightarrow \)509T TT genotype, the OR for advanced stage prostate cancer is 1.35 (95% CI 0.75–2.44; \( P = 0.32 \)). The T+29C polymorphism showed a nonsignificant trend in the same direction (Table 4). To further examine whether genotype is associated with more aggressive phenotype, we analyzed the association with low and high Gleason score tumors. There was no statistically significant difference in genotype frequencies by the different Gleason score categories (Tables 5 and 6); however, we were missing Gleason score data on 141 cases, which could not be included in the analysis.

The observed association between the C\( \rightarrow \)509T polymorphism and the risk of advanced stage prostate cancer was unchanged when exercise and body mass index were included in the multivariate model. In addition, the association was not likely confounded by other previously identified risk factors in this nested case-control population because there was no correlation between this polymorphism and circulating levels of lycopene, testosterone, sex hormone binding globulin, estrogen, insulin-like growth factor-1, insulin-like growth factor binding protein-3 or vitamin D, or the androgen CAG polymorphism among the subset of controls who had these measurements.

### Discussion

Here, we add to the growing body of evidence that TGF\( \beta \)1 polymorphisms contribute to cancer susceptibility. Given the proposed role of TGF\( \beta \) in prostate cancer,
we tested TGFB1 polymorphisms for overall prostate cancer risk and for risk of more advanced tumor stage in cases and matched controls from the PHS. To our knowledge, this is the first case-control study of TGFB1 polymorphisms and prostate cancer risk. We observed that the C–509T polymorphism in the TGFB1 promoter was associated with a greater than 2-fold increase in risk for later stage prostate cancers. This significant result may not be due to the increased number of high-risk patients with the TT genotype but due to a decrease in the controls with the TT genotype because an unmatched analysis was not statistically significant. A closely linked polymorphism resulting in a proline at amino acid 10 (T+29C) showed a positive but nonsignificant trend. Although, apparently, in a larger study series, the T+29C polymorphism would have shown a significant risk for later stage prostate cancers as well, we cannot exclude the possibility of a chance finding for C–509T because of the decreased numbers of matched controls with the TT genotype. In the breast cancer study by Dunning et al. (16), they found a greater OR (1.25) associated with the C–509T polymorphism than the T+29C polymorphism (1.21), although they propose that the functional single nucleotide polymorphism is the T+29C polymorphism. We did not observe any differences in association by Gleason score category; this may partially be explained by more measurement error in Gleason score versus stage classification due to interpathologist variability.

Several case-control studies of TGFB1 polymorphisms with noncancer diseases have been reported. TGFB1 is implicated in risk for systemic sclerosis (23, 24), obesity-related phenotypes (lower abdominal sagittal diameter and fasting glucose levels; Ref. 25), asthma (26), end stage heart disease caused by dilated cardiomyopathy (27), and myocardial infarction (14) as well as showing both positive and inverse correlations for bone mineral loss (15). Based on in vivo and in vitro work, TGFB1 polymorphisms may be important risk factors for many types of cancer including prostate cancer. Most of the published work on TGFB1 polymorphisms in cancer risk has been in breast cancer, which serves to illustrate some of the complexities with association studies in cancer. TGFB1 has been studied as a cancer susceptibility risk factor in five breast cancer populations with seemingly contradicting results (16, 17, 30). In one study, in which women from three different populations were diagnosed with breast cancer between 22 and 92 years of age and a median age of 50, individuals homozygous for the rare allele at position +29 (CC) showed a modest increase in cancer risk of 1.21 (95% CI 1.05–1.37; Ref. 16). In the same study, the rare homozygote at position −509 (TT) showed an increase in cancer risk of 1.25 (95% CI 1.06–1.48). In a second study of women who were diagnosed with breast cancer at a median age of 70, there was an inverse effect of the C allele at position +29 (OR 0.36, 95% CI 0.17–0.75; Ref. 17). No other TGFB1 polymorphisms were tested in this study. This is the opposite effect from that seen by Dunning et al., although not entirely surprising given the potentially differing roles of TGFβ. One might hypothesize that in women with other predisposing risk factors, the +29C allele (or T at position −509) is a susceptibility allele that helps initiate tumorigenesis, in agreement with the documented role of TGFβ1 in tumor progression. In women without such other risk factors for the early initiation of cancer, the +29C allele (or T at position −509) is inversely associated with risk by virtue of its functions in cell differentiation and inhibition of proliferation. Alternatively, these results could be the result of chance due to the small number of breast cancers studied by Ziv et al. (17). These two studies demonstrate the potential complexities of association studies when one variant may both protect from and contribute to cancer risk depending on the environment, cellular microenvironment, and other interacting genetic factors.

A third study looked at the risk of the TGFB1 T+29C (Leu10Pro) alleles in breast cancer in premenopausal and postmenopausal women in the Japanese population (30). In this study, no effect of the TGFB1 polymorphism (+29C) was seen in postmenopausal women (OR 1.40, 95% CI 0.64–3.08), but a inverse effect of the +29C allele (Pro10) was seen in the premenopausal women (OR 0.45, 95% CI 0.20–0.98). This, again, is the opposite effect from that seen by Dunning et al. (16) in the early onset breast cancers of women of northern European ancestry. It is similar to the study by Ziv et al. (17) in which the +29C allele (Pro10) was protective; however, in the Japanese study, the protective effect was observed only in premenopausal women, whereas the study by Ziv et al. found the protective effect in postmenopausal women.

Table 4. Risk of low and high stage prostate cancer by TGFB1 T+29C polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Low stage cases/controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
<th>High stage cases/controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>99/106</td>
<td>1.00</td>
<td></td>
<td></td>
<td>60/63</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>139/138</td>
<td>1.05</td>
<td>0.73–1.52</td>
<td>0.79</td>
<td>76/78</td>
<td>1.04</td>
<td>0.64–1.68</td>
<td>0.88</td>
</tr>
<tr>
<td>CC</td>
<td>48/42</td>
<td>1.25</td>
<td>0.76–2.06</td>
<td>0.38</td>
<td>24/19</td>
<td>1.33</td>
<td>0.66–2.68</td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td>286/286</td>
<td></td>
<td></td>
<td></td>
<td>160/160</td>
<td></td>
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</table>

Table 5. TGFB1 C–509T polymorphism and risk of low and high prostate cancer Gleason score

<table>
<thead>
<tr>
<th></th>
<th>Low Gleason cases/controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
<th>High Gleason cases/controls</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>106/100</td>
<td>1.00</td>
<td></td>
<td></td>
<td>60/64</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>89/95</td>
<td>0.87</td>
<td>0.58–1.30</td>
<td>0.50</td>
<td>57/55</td>
<td>1.04</td>
<td>0.64–1.69</td>
<td>0.88</td>
</tr>
<tr>
<td>TT</td>
<td>23/23</td>
<td>1.04</td>
<td>0.55–1.98</td>
<td>0.91</td>
<td>16/14</td>
<td>1.34</td>
<td>0.60–3.00</td>
<td>0.48</td>
</tr>
<tr>
<td>Total</td>
<td>218/218</td>
<td></td>
<td></td>
<td></td>
<td>133/133</td>
<td></td>
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</table>
The somewhat contradictory findings in these studies may be explained by the small number of premenopausal breast cancer cases used in the Japanese study or by ethnic nature of the study groups. However, because there are only modest differences in allele frequencies of the T+29C variant in control populations in United Kingdom and Japan, ethnic differences in TGFβ1 allele frequencies are unlikely to be the explanation (16, 30). This is not the first time that the T+29C TGFβ1 polymorphism conferring risk in a population of northern European/Caucasian ethnicity showed inverse effects in a population of Japanese ancestry. Two studies of bone mineral density loss show opposite effects where the homozygote +29C allele in a German population showed a positive correlation for risk, but in a Japanese population, the homozygote +29C allele showed an inverse effect for risk (28, 29). Serum concentrations of TGFβ1 also differed in these studies in opposite directions. Other association studies in the Japanese population have found an inverse effect with the +29C allele. A study looking at myocardial infarction risk in Japanese males found that the +29T genotype (Leu) increased risk (OR 3.5, 95% CI 2.0–6.3; Ref. 14). The +29T allele has also been shown to be a positive risk factor for rheumatoid arthritis in individuals from central Japan (31). It may be that individuals of Japanese ancestry have variants in other genes that interact with the TGFβ pathway differently than variants in the northern European population.

It is very probable that TGFβ1 polymorphisms play a role in general prostate cancer risk but that our study size did not have the power to detect this increase in risk. Association studies are very infrequently replicated. Nonreplication may be due to inadequate follow-up study design and population heterogeneity. However, the inability to replicate a study may also be because genetic variations, like those in TGFβ1, play a role in both risk and protection depending on other inherited genetic variants, the microenvironment, the external environment, and other factors. Careful refinement of phenotypes to be studied and identification of other members in a genetic network may help to overcome some of the problems with current association studies. Results of these studies are important for design of therapeutic strategies, because drugs that stimulate the tumor suppressive effects of TGFβ signaling at early stages of tumor development may also increase the risk of late stage progression.

TGFβ1 is not the only gene to act both positively and negatively in tumorigenesis. Many genes, including ras and c-myc, and the pathways in which they operate, can also act both positively and negatively in cancer (32). Ras induces cell growth arrest at early stages but later stimulates growth and survival. c-Myc induces cell growth and proliferation as well as apoptosis depending on the conditions and the cell type (33). Association studies using variants in these types of genes will need to be designed carefully to test very specific phenotypic questions to allow for the possibility that a variant could act in both a protective and a susceptible manner.

**Acknowledgments**

We thank Drs. Bruce A. J. Ponder and Alison Dunning for prepublication information on TGFβ1 breast cancer risks and Taqman allelic discrimination probe and primer sequences, Dr. Meir Stampfer for facilitating the collaboration with the PHS, and Dr. Rosemary Akhurst for her thoughtful comments on the manuscript. The UCSF Prostate Spore and a UCSF Prostate Cancer Center Award supported this work.

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