Increased risk of prostate cancer and benign prostatic hyperplasia associated with transforming growth factor-beta 1 gene polymorphism at codon10

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Transforming growth factor-beta 1 (TGF-β1) plays a significant role in regulating the proliferation and apoptosis of prostate epithelial and stromal cells. We explored the association between the T (Leu) to C (Pro) polymorphism at codon10 of the TGF-β1 gene (TGFB1) and the risk of prostate cancer (PCa) or benign prostatic hyperplasia (BPH) in 351 PCa patients, 221 BPH patients and 303 male controls in Japan. There were significant differences in the CC versus Tc + TT genotype distribution between PCa patients and male controls (P = 0.008), and between BPH patients and male controls (P = 0.041). Males with the TC or TT genotype had a 1.62-fold increased risk of PCa (95% confidence interval (CI) = 1.14–2.30, P = 0.007) and a 1.51-fold increased risk of BPH (95% CI = 1.02–2.24, P = 0.041) compared with those with the CC genotype, therefore suggesting the dominant effect of the TGFB1 T allele on development of PCa and BPH. There were no significant differences in the TGFB1 genotype distribution between different groups of tumor grades and stages in the PCa patients and no significant differences when PCa patients were stratified by the age of onset. The results suggest that the codon10 polymorphism in TGFB1 may have a significant influence on the development of PCa and BPH, therefore underscoring the importance of the TGF pathway in the development of these prostate diseases. However, it appeared to have no impact on the disease status or age of onset of PCa.

Introduction

Transforming growth factor-beta 1 (TGF-β1) plays an important role in the cell cycle regulation and arrests the cell cycle at G1 phase, consequently inhibiting the growth of many kinds of epithelium including prostatic epithelium (1,2). In prostate cancer (PCa) cells, there are some defects in the TGF-β1 signaling pathway, and restoration of this pathway in PCa cells can suppress in vitro tumor growth by inhibiting cell proliferation (2,3). In addition, TGF-β1 inhibits the cell growth and increases the production of type I collagen, suggesting the involvement of TGF-β1 in prostatic enlargement as a modulator of the extracellular matrix (4,5). Thus, alternations in the TGF-beta 1 gene (TGFB1) might be involved in the development of PCa and benign prostatic hyperplasia (BPH).

Among several genetic polymorphisms found in TGFB1, a T to C transition at nucleotide 29 of codon10 is located in the hydrophobic core of the signal peptide, resulting in the replacement of a hydrophobic leucine with a small, neutral proline (6). Such a substitution would change the overall hydrophobicity of the core transport sequence and disrupt the alpha-helical structure of the region, therefore altering its ability to direct protein transport across the endoplasmic reticulum (7). The CC genotype was reported to be associated with hypertension, while the presence of the T allele may increase the risk of osteoporosis, male myocardial infarction or rheumatoid arthritis (8–11). Recently, Ziv et al. reported that the T allele was dominantly linked with an increased risk of the development of breast cancer in Caucasians in the US (12), while Dunning et al., on the contrary, claimed that the T allele recessively increased incidence of invasive breast cancer in an almost 100% Caucasian population in Europe (13).

In this case-control study, the codon10 polymorphism of TGFB1 was analyzed in native Japanese males in order to clarify whether the T allele is related to an increased risk of PCa or BPH.

Materials and methods

The subjects were recruited between April 1997 and November 2001 for the PCa patients, between August 1997 and November 2000 for the BPH patients, and between March 1998 and September 2001 for the male controls with informed consent. A total of 358 PCa patients and 225 BPH patients treated at Akita University Medical Center and Kyoto University Hospital were entered in this study. The male control group comprised 310 consecutive volunteers older than 60 years who attended the yearly health check-up. Of these candidate 893 subjects, the following PCR amplification was not successful in seven (2.0%) PCa samples, four (1.8%) BPH samples and seven (2.3%) control samples. Therefore, this study comprised 351 PCa, 221 BPH and 303 control subjects for final analyses. Most subjects were enrolled in our ongoing project identifying the gene polymorphisms affecting the PCA development and progression (14,15). All PCas were diagnosed histologically with transrectal biopsy or surgical specimens. All BPH patients had various degrees of lower urinary tract symptoms and prostatic enlargement screened by digital rectal examination and ultrasonography. The PSA levels were assayed in all of the BPH patients, and those with elevated PSA levels (>4.0 ng/ml; the Tandem-R assay; Hybritech, San Diego, CA) were confirmed not to have PCa by transrectal biopsies. Their serum PSA levels (the Tandum-R assay) were determined, and individuals with abnormal PSA levels (>4.0 ng/ml) were excluded from the control group. The clinical stages were categorized according to the Tumor-Node-Metastasis system (16) and PCa was classified into stage A (T1a-bN0M0), stage B (T1c-2N0M0), stage C (T3-4N0M0) and stage D (T4N1M0-I or T4N0-1M1) by the modified Whitmore–Jewett system. In cases in whom radical prostatectomy was performed, final pathological stage was applied and in other cases without radical prostatectomy, clinical stage was applied. The clinical stages of 18 patients could not be obtained because of inadequate data. Pathological grades of the PCa were determined according to the General Rule for Clinical and Pathological studies on PCa by the Japanese Urological Association and the Japanese Society of Pathology.
which is based on the WHO criteria and the Gleason score (17,18). All pathological grading was based on needle biopsy specimens in stage B–D patients and surgical specimens in stage A patients. Well, moderately and poorly differentiated carcinoma generally correspond to Gleason scores of 2–4, 5–7 and 8–10, respectively. In 32 patients, the pathological grades could not be determined because of a different or inadequate grading system used. The protocols were approved by the internal ethical boards of Akita University School of Medicine and Kyoto University Graduate School of Medicine.

DNA was extracted from blood samples using a QIAamp Blood Kit (Qiagen, Hilden, Germany) or by the standard method with proteinase K digestion followed by phenol–chloroform extraction. Genotyping of the codon10 polymorphisms of TGFBI1 was performed by the amplification refractory mutation system (ARMS)–PCR method (Figure 1) (19). The 241 bp fragment containing the polymorphic site in codon10 of TGFBI1 was amplified using PCR primers as follows: the generic primer (sense), 5′-TCCTGTTGGACTAGCAC-3′; the C allele specific primer (antisense), 5′-GCAACGCTACGACAGG-3′; the T allele specific primer (antisense), AGCCGCGTACGACGACA-3′; the internal control primer 1 (P53F), 5′-TGCCCTGTCAGCTGTTG-GATT-3′; and the internal control primer 2 (P53R), 5′-GCCCGCTGCTGTTTACCATCAGTATC-3′. The internal control primers were used to check for successful PCR amplification (19) and a segment of the p53 gene located at chromosome 17p13.1 was amplified. PCR was performed in a 15 μl aliquot containing 25–50 ng of genomic DNA, 5 μM specific primer mix (consisting of generic primer and one of the two allele-specific primers), 200 μM each of deoxynucleotide triphosphates, 1 U AmpliTaq Gold DNA polymerase, 1.5 mM MgCl2 and 1 × reaction buffer supplied by the manufacturer (PE-Applied Biosystems, Foster City, CA). PCR amplification conditions were an initial denaturation at 95°C for 10 min, followed by 10 cycles of melting at 95°C for 15 s, annealing at 65°C for 50 s, and elongation at 72°C for 40 s; 25 cycles of melting at 95°C for 40 s, annealing at 59°C for 50 s and elongation at 72°C for 50 s. The TGFBI1 genotype was determined by 2.0% agarose gel electrophoresis of PCR products followed by ethidium bromide staining (Figure 1). The validity of the ARMS–PCR assay was confirmed by direct sequencing of 20 randomly selected samples using the ABI Prism BigDye™ Terminator Cycle Sequencing system (PE-Applied Biosystems).

All data were analyzed with the SPSS (Version 10.0.1, SPSS, Tokyo, Japan) software. Genotype frequency between the three groups and between the subgroups of PCa patients, were evaluated by a two-sided 2 × 2 contingency table analysis. For stratification analysis by age, the cases with tumors with higher grades or those with metastatic diagnosis was 72.2 and 70 years for 351 PCa patients, 70.6 ± 9.2 and 70 years for 221 BPH patients, and 70.7 ± 7.3 and 70 years for the 303 controls at the time of entering this study (Table I). Using the median age of PCa patients (72 years), which was similar to that of the male control group (71 years), the subjects were divided into two groups. The CC and TC + TT genotype frequencies in the PCa patients over 72 years old were 23.8 and 76.2%, and those in the PCa patients 72 years old or younger were 21.3 and 78.7%, respectively (P = 0.576). When the PCa groups were divided into three groups at the age of diagnosis (i.e. <70, ≥70 and <80, ≥80), were in Hardy–Weinberg equilibrium. There were significant differences in the genotype distribution between the PCa patients and the male controls (P = 0.030). No significant difference in the genotype distribution was observed between the BPH patients and PCa patients (P = 0.796) and the BPH patients and the male controls (P = 0.108) (χ² test, Table I).

When the TC and TT genotypes were combined and compared with the CC genotype, a significant difference was present between the PCa patients and the male controls (P = 0.008), and between the BPH patients and the male controls (P = 0.040), but not between the PCa patients and the BPH patients (P = 0.777) (all by χ² test).

Age-adjusted logistic analysis indicated that males with the TC and TT genotype had a 1.60- and 1.67-fold increased risk of PCa, respectively, thus suggesting a dominant effect of the T allele on the development of PCa (Table II). The risk of BPH in males with the TC and TT genotype against the CC genotype was 1.56 and 1.40, respectively (Table II). Taken together, males with the TC or TT genotype had a 1.62-fold increased risk of PCa or a 1.51-fold risk of BPH compared with males with the CC genotype (Table II). When the BPH patients were taken as the controls, no increased risk of PCa was observed in males with the TC or TT genotype compared with those with the CC genotype (aOR = 1.03; 95% CI = 0.69–1.54, P = 0.877, Table II).

The relation between TGF-β1 genotypes and the pathological grade and stage of PCa was examined. There were no significant differences in the genotype distribution between different groups of tumor grades and stages in the PCa patients (P = 0.835 and 0.183, respectively). The patients with the TC and TT genotypes showed no significantly increased risk of having tumors with higher grades or those with metastatic disease compared with those having the CC genotype.

Then, stratified analyses were conducted according to the age at diagnosis. The mean ± SD and median age at the diagnosis was 72.2 ± 8.2 and 72 years for 351 PCa patients, 70.6 ± 9.2 and 70 years for 221 BPH patients, and 70.7 ± 7.3 and 70 years for the 303 controls at the time of entering this study (Table I). Using the median age of PCa patients (72 years), which was similar to that of the male control group (71 years), the subjects were divided into two groups. The CC and TC + TT genotype frequencies in the PCa patients over 72 years old were 23.8 and 76.2%, and those in the PCa patients 72 years old or younger were 21.3 and 78.7%, respectively (P = 0.576). When the PCa groups were divided into three groups at the age of diagnosis (i.e. <70, ≥70 and <80, ≥80),
there was also no significant difference in the genotype distribution \( (P = 0.698, \text{data not shown}) \). Furthermore, there was no significant difference at the mean age of diagnosis of PCa according to the three genotypes \( (P = 0.325, \text{by one-way ANOVA}) \).

**Discussion**

The \textit{TGFB1} genotype frequency in the controls of this study appeared to be consistent with those of previous studies conducted in Japanese populations \( \text{(9,10)} \). A Japanese study about \( \text{PCa} \) appeared to be consistent with those of previous studies conducted in Japanese populations \( \text{(9,10)} \). A Japanese study about \( \text{PCa} \) compared with those having the \( \text{TGFB1} \) genotype had a significantly higher serum level of TGF-\( \beta_1 \) compared with those having the \( \text{TC or TT} \) genotype \( \text{(9)} \). Although it is not clear whether these findings generally apply to any populations, the increased serum levels of TGF-\( \beta_1 \) in the presence of the \( \text{C} \) allele might contribute to a long-term suppression of prostate epithelial proliferation, leading to a decrease in the risk of \( \text{PCa} \). Furthermore, \text{Ziv et al.} \text{(12)} reported that Caucasian women aged 65 years and older with the \text{CC} genotype had a significantly lower risk of developing breast cancer compared with those with the \text{TC or TT} \) genotype. Their results are consistent with those of the present study on \text{PCa}, suggesting a dominant influence of the \text{TGFB1 T} \) allele on the development of some cancers, probably through reducing the serum and/or tissue level of TGF-\( \beta_1 \). On the other hand, \text{Dunning et al.} \text{claimed that the TGFB1 C} allele was recessively associated with increased incidence of invasive breast cancer, accompanied by increased, secretion rates of TGF-\( \beta_1 \) \( \text{(13)} \). These contradictory results might be caused by a case selection bias because the cases in the cohort of \text{Ziv et al.} \text{seemed to be relatively older than those of Dunning, and the breast cancer cases in the former study contain substantial (=13%) non-invasive cancers (12,13). In \text{PCa}, Shariat et al. reported that plasma TGF-\( \beta_1 \) levels were elevated in men with metastatic lesions compared with healthy men (20). It remains to be elucidated whether the high plasma TGF-\( \beta_1 \) levels might play any causative role in \text{PCa} progression or merely reflect the systemic reaction as the results of advanced disease status in breast cancer and \text{PCa}. In addition, we do not know the exact mechanism why the \text{T} \) allele had a dominant effect on \text{PCa} and \text{BPH} susceptibility in this study and a breast cancer risk in \text{Ziv’s study} \text{(12). There may be a certain threshold affecting the susceptibility for carcinogenesis by the altered level of TGB-\( \beta_1 \) caused by this polymorphism. Otherwise, there might be an intermediate risk status with a gene-dosage effect in the \text{T} \) heterozygotes, which could not be discernible in the relatively small scale of these studies. Furthermore, there was no association between the disease status in \text{PCa} and the \text{TGFB1 polymorphism}. Because the prostatic epithelial cells may change their response to TGF-\( \beta_1 \) and TGF-\( \beta_1 \) may stimulate growth of transformed epithelial cells instead of inhibiting, the \text{TGFB1 polymorphism} might have a contradictory effect on \text{PCa} onset and its progression. Therefore, it would be important to know the relation between the \text{TGFB1 genotype} and plasma TGF-\( \beta_1 \) levels in both \text{PCa} patients with various disease status and matching normal subjects.

The present study revealed that the \text{T} \) allele might also confer an increased risk of \text{BPH}. In addition to acting as a proliferation inhibitor of human prostate epithelium, TGF-\( \beta_1 \) is an important modulator of the extracellular matrix. As indicated above, the increased risk of \text{BPH} conferred by the \text{T} \) allele might be mediated through the reduced inhibitory effect on the growth of both prostate epithelium and stromal cells due to the decreased production of TGF-\( \beta_1 \) \text{(4,5). Although there is no direct biological relation between BPH and \text{PCa}, it is well conceivable that genetic polymorphisms affecting levels of growth factors including TGF-\( \beta_1 \) may have a significant impact on both \text{BPH} and \text{PCa} because both disease conditions are under the influence of common growth factors.}

In conclusion, the codon10 polymorphism in \text{TGFB1} may have a significant influence on the development of \text{PCa} and \text{BPH}, therefore underscoring the importance of the TGF pathway in the development of these prostatic diseases. However, it appeared to have no impact on the disease status or age of onset of \text{PCa}.

\begin{table}[h]
\centering
\caption{\textit{TGFB1} genotype frequencies (%) in controls, \text{BPH} subjects and \text{PCa} subjects}
\begin{tabular}{|l|c|c|c|c|}
\hline
Group & Genotype frequency (%) & \\
\hline
 & \text{N} & \text{CC} & \text{TC} & \text{TT} \\
\hline
Control & 303 & 96 (31.7) & 137 (45.2) & 70 (23.1) \\
(Mean age ± SD) & 70.7 ± 7.3 & 70.9 ± 7.1 & 70.5 ± 7.2 & 70.8 ± 7.9 \\
BPH & 221 & 52 (23.5) & 116 (52.5) & 53 (24.0) \\
(Mean age ± SD) & 70.6 ± 9.2 & 69.8 ± 9.0 & 70.8 ± 9.3 & 70.6 ± 9.1 \\
PCa & 351 & 79 (22.5) & 179 (51.0) & 93 (26.5) \\
(Mean age ± SD) & 72.2 ± 8.2 & 71.2 ± 8.7 & 72.0 ± 7.8 & 72.8 ± 8.2 \\
\hline
Tumor grade\textsuperscript{a} & & & & \\
Well & 42 & 8 (19.0) & 23 (54.8) & 11 (26.2) \\
Moderately & 144 & 29 (20.1) & 79 (54.9) & 36 (25.0) \\
Poorly & 133 & 31 (23.3) & 64 (48.1) & 38 (28.6) \\
\hline
Tumor stage\textsuperscript{b} & & & & \\
A & 22 & 6 (27.3) & 13 (59.1) & 3 (13.6) \\
B & 116 & 28 (24.1) & 56 (48.3) & 32 (27.6) \\
C & 68 & 17 (25) & 34 (50) & 38 (55.9) \\
\hline
Localized \textsuperscript{a} & 206 & 41 (19.7) & 115 (55.8) & 50 (24.5) \\
Metastatic \textsuperscript{b} & 127 & 33 (26.0) & 58 (45.7) & 36 (28.3) \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}The tumor grade was determined according to the General Rule for Clinical and Pathological Studies on Prostate Cancer by Japanese Urological Association and the Japanese Society of Pathology \text{(17,18). By the Whitmore–Jewett system.}

\begin{table}[h]
\centering
\caption{\textit{TGFB1} genotype and risk of \text{PCa} and \text{BPH}}
\begin{tabular}{|l|c|c|c|}
\hline
Group & \text{aOR (95% CI, \text{P})} & \text{TT} & \text{TC + TT} \\
\hline
PCa against control & 1 & 1.67 (1.08–2.57, 0.020) & 1.62 (1.14–2.30, 0.007) \\
PCa against \text{BPH} & 1 & 1.16 (0.71–1.89, 0.568) & 1.03 (0.69–1.54, 0.877) \\
BPH against control & 1 & 1.56 (1.03–2.38, 0.036) & 1.51 (1.02–2.24, 0.041) \\
\hline
\end{tabular}
\end{table}
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References


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