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Long noncoding RNA *PCA3* gene promoter region is related to the risk of prostate cancer on Chinese males

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ABSTRACT

Introduction: Long noncoding RNA *prostate cancer gene antigen 3* (*PCA3*) is one of the most prostate cancer-specific genes at present. Consequently, the prostate-specific expression and the sharp up-regulation of *PCA3* RNA in prostate cancer suggest a unique transcriptional regulation, which possibly can be attributed to promoter polymorphism. In this study, we investigated a short tandem repeat (STR) polymorphism of *TAAA* in the promoter region of PCA3 gene found in our previous study in prostate cancer (PCa) patients and benign prostatic hypertrophy (BPH) patients, aiming to evaluate the association between the STR and increased risk for PCa.
Material and methods: 120 PCa cases and 120 benign prostatic hypertrophy (BPH) cases were identified among participants. The region encompassing the *TAAA* repeat was amplified with a specific primer set we designed and screened by PCR-based cloning and sequencing in paired peripheral blood leukocytes and prostate tissues. Genotype-specific risks were estimated as odds ratios (ORs) associated with 95% confidence intervals (CIs) and adjusted for age by means of unconditional logistic regression.
Results: 5 *PCA3 TAAA* STR polymorphisms and 8 genotypes were found in both peripheral blood leukocytes and prostate tissues, the carriers with more *TAAA* repeats were associated with increased risk for PCa than individuals having less *TAAA* repeats. Interestingly, 18 (15.0%) of 120 PCa patients had more (*TAAA*)n repeats in prostate tissues than that in peripheral blood leukocytes, and 3 (2.5%) of 120 had less (*TAAA*)n repeats in prostate tissues.
Conclusions: The results of this study suggest that short tandem repeat polymorphism of *TAAA* in the promoter region of *PCA3* gene is a risk-increasing factor for prostate cancer in the Chinese population. In addition to the hereditary factor, the insertion mutation of (*TAAA*)n in a local tissue maybe another mechanism of the onset of PCa.

1. Introduction

Prostate cancer (PCa) is the most common malignancy and the second leading cause of cancer-related deaths in the Western male population (Jemal et al., 2010; Siegel et al., 2011), and its incidence is still increasing. Recently, Bussemakers et al. reported that a novel prostate-specific gene, called *DD3* (*differential display code 3*), later called *PCA3*(*prostate cancer antigen 3*) gene, which was one of the most prostate cancer-specific genes described to date, was found to be 10–100 fold over-expressed in human prostate cancer samples whereas it was not expressed in a wide range of normal human tissues and other human malignant tumors (Bussemakers et al., 1999). The prostate-specific expression and the sharp upregulation of long noncoding *PCA3* RNA in prostate cancer suggest that the *PCA3* gene promoter is a promising tool for a unique transcriptional regulation. Accordingly, the *PCA3* gene promoter attracted our attention.

In our previous study, we designed a specific primer set to screen the promoter of PCA3 gene by polymerase chain reaction (PCR)-based cloning and sequencing with the DNA extracted from peripheral blood leukocytes (PBL) of PCa cases and healthy control cases. Short tandem repeat polymorphism of TAAA was found in the promoter region of PCA3 gene. We demonstrated a significant association between the presence of the (*TAAA*)n STR polymorphisms in the PCA3 promoter region and PCa in Chinese populations (Zhou et al., 2011). These findings indicate that the PCA3 promoter STR is a genetic susceptibility factor for PCa. In present study, we screen the promoter of PCA3 gene by the same analysis with the DNA extracted from prostate tissue samples of PCa and benign prostatic hypertrophy (BPH) patients. Furthermore, we studied

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the PCA3 promoter gene in the prostate tissues and corresponding peripheral blood leukocytes of PCa patients expecting to obtain more valuable information between PCA3 promoter STR polymorphisms and PCa.

2. Material and methods

2.1. Ethics statement

The present study was approved by the ethics committee of The First Affiliated Hospital of Wenzhou Medical University, China, and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each of the participants at the time of enrollment.

2.2. Study design and patients

The study included 120 diagnosed PCa cases (aged 46–96 years) and 120 diagnosed BPH cases (aged 45–92 years) recruited from The First Affiliated Hospital of Wenzhou Medical University between August 2010 and December 2012. PCa and BPH were confirmed by histopathological evaluation in all cases. Each PCa tumor was accurately and systematically graded using the Gleason scoring system by two pathology physicians. Control subjects with BPH were screened to ensure that they had never been diagnosed with cancer or other serious disease. Each subject was interviewed for family history of prostate cancer and smoking status.

2.3. DNA extraction

Tissue genomic DNA was extracted from 10 μm-thick formalin fixed, paraffin-embedded prostate tissue samples. Briefly, tissues were deparaffinized by a serial extraction with xylene and ethanol (100%/70%/50%), and separately selected areas of tumor and normal tissue, when available, were microdissected using a sterile needle and carefully collected into a 0.6 ml PCR tube. Five milliliters of venous blood was collected in EDTA as a DNA source of peripheral blood leukocytes of PCa patients. The DNA was extracted and purified from the prostate tissue samples and corresponding peripheral blood leukocytes in PCa patients and BPH patients according to established protocols by using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

2.4. Sequencing and analysis of polymorphisms

The *PCA3* promoter was amplified from extracted genomic DNA by PCR on the basis of the sequence of the *PCA3* gene presented in GenBank accession numbers AL359314.14 and AF279290.1 with Taq polymerase (Qiagen, Hilden, Germany) with the following primers: 5′-GATGGGAA CTCACATTTGG-3′ (forward) and 5′-CTGATGCCAGCTTCTCG-3′ (reverse). Each PCR reaction was performed in a GeneAmp PCR System 2720 thermo cycler (Applied Biosystems, Foster, USA) at a final volume of 50 μl (containing 2 μl forward and reverse primer (5 μmol/l), respectively; 1 μl of extracted DNA, 5 μl 10× PCR Buffer (100 mmol/l Tris–HCl (pH 9.2)); 3 μl $MgCl_2$(25 mmol/l), 5 μl dNTPs (2 mmol/l), 31.6 μl distilled water and 0.4 μl Taq-polymerase. PCR cycles used were as follows: 95 °C for 4 min, 40 cycles of denaturing at 95 °C for 1 min, annealing at the indicated temperature for 30 s by 60 °C, extension at 72 °C for 1 min, and a single final extension at 72 °C for 10 min. After electrophoresed on a 1.5% agarose gel and purified by QIAquick Spin PCR Purification Kit (Qiagen, Hilden, Germany), all amplified products were sent to Invitrogen Corporation in Shanghai for cloning and sequencing assay with no information on the specimens or knowledge of the study.

2.5. Statistical analysis

The Statistical Package for Social Sciences for Windows (version 13.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The values for age were reported as mean ± SD. Statistical analysis of age was performed by the independent-samples t-test. Genotype-specific risks were estimated as odds ratios (ORs) associated with 95% confidence intervals (CIs) and adjusted for age and sex by means of unconditional logistic regression. Possible deviation of the genotype frequencies from PCa and BPH expected under Hardy–Weinberg equilibrium was assessed by the chi-square test. Significance statements refer to P values of 2-tailed tests that were less than 0.05.

3. Results

A total of 240 subjects, including 120 patients with PCa and 120 patients with BPH as control group from The First Affiliated Hospital of Wenzhou Medical University, were analyzed for polymorphisms in promoter of the *PCA3* gene. DNA samples from the prostate tissue from individual patients exhibited a single band after PCR amplification, and the corresponding normal DNA from peripheral blood leukocytes of the same patients was also PCR amplified at *PCA3* gene promoter loci and their patterns were compared with the DNA samples from the prostate tissues.

The cloning and sequencing assay revealed a (*TAAA*)n STR in the promoter region of the *PCA3* gene. The numbers of (*TAAA*)n repeats showed a distribution of 4–8 in the individuals studied (Fig. 1), and eight genotypes were founded. The values for age are reported as mean ± SD. Statistical analysis of age was performed by the independent-samples t-test (Table 1). The *PCA3* promoter genotype distributions were in the Hardy–Weinberg equilibrium for patients ($\chi^2 = 1.44$, df = 3, P = 0.70) and control subjects ($\chi^2 = 0.14$, df = 1, P = 0.71).

Verhaegh et al. have shown that no known initiator motif, no *TATA-box*, no *CAAT-box*, and no GC-rich regions were found at consensus positions within the *PCA3* promoter. Therefore, novel and tissue-specific cis-acting elements and trans-acting transcription factors might define the specific and characteristic expression of *PCA3* gene. Mutation of a A + T-rich region, within the *PCA3* promoter, resulted in a decreased transcription rate, suggesting a positive role for this element in *PCA3* transcription (Verhaegh et al., 2000). We hypothesized that one *TAAA* was one unit of the transcriptional initiation positions of *PCA3* gene. *TAAA* repeats up-regulate *PCA3* transcription, the same repeat numbers of *TAAA* might be associated with the same transcription rate and the same increased expression of *PCA3* gene. So the increase of (*TAAA*)n repeats in the promoter of *PCA3* would increase the transcriptional initiation positions of *PCA3* and up-regulate the expression of *PCA3* RNA in PCa patients. This was in concordance with our earlier published data (Chen et al., 2008; Tao et al., 2010). In usual cases, a blood sample has two different sizes of *TAAA* repeats from the two different alleles. Each repeat number was calculated of these cloned alleles used as size markers. So corresponded to total numbers of *TAAA* repeat in the alleles, eight genotypes were classified into three groups: ≤10*TAAA*, 11*TAAA*, and ≥12*TAAA* (Zhou et al., 2011) (e.g. the total *TAAA* repeat numbers of genotype (*TAAA*)n/(*TAAA*)m is x [x = n + m],which will be put into the "x" *TAAA* group).

In the control subjects, distributions of 120 patients were 94 (78.33%) for class ≦10*TAAA*, 23 (19.17%) for 11*TAAA* and 3 (2.6%) for class ≧12*TAAA*, respectively. Likewise, in patients with PCa, distributions of 120 patients were 63 (52.50%) for class ≦10*TAAA*, 39 (32.50%) for class 11*TAAA* and 18 (15.00%) for class ≧12*TAAA*, respectively. All subjects were Chinese males and they represented an ethnic isolation. Therefore, statistical artifacts caused by population stratification could be ruled out, as described by Pritchard and Rosenberg (1999), and ages of subjects were matched in the present study (Table 1). Chi-square test was performed and showed statistically significant difference in the construction of PCa and controls between the three groups ($\chi^2 = 20.96$, df = 2, P < 0.001). A significant association was observed between the pooled genotypes and PCa risk. To explore the correlation between the risk of PCa and size of (*TAAA*)n repeats, odds ratios and 95% confidence intervals were calculated, to assess the relative disease risk conferred by genotype. In this study, the 11*TAAA* carriers were