Polymorphisms of -800G/A and +915G/C in TGF-β1 gene and lung cancer susceptibility

QING-GUO DI, BAO-HUA SUN, MING-MING JIANG, JUN-FENG DU, ZHI-TAO MAI, XIN ZHANG, LI-RONG ZHOU, YU-MIN CHI and JING LV

Department of Respiration Medicine, Cangzhou Central Hospital, Cangzhou, Hebei 061000, P.R. China

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Abstract. We studied the relationship between the polymorphisms of -800G/A and +915G/C in transforming growth factor-β1 (TGF-β1) gene and lung cancer susceptibility. The sequence-specific primer polymerase chain reaction (PCR-SSP) technique was used to test 156 non-small cell lung cancer (NSCLC) patients that were selected as the observation group and 156 patients with pneumonia and tuberculosis that were selected as the control group (age and gender 1:1 proximal matching principle) and the polymorphisms of the first exon -800G/A and +915G/C TGF-β1 genes. The expression of TGF-β1 levels in peripheral blood was detected using ELISA. The proportion of -800G/A gene AA subtype and A allelic gene in the observation group was significantly higher than that in the control group, while the proportion of +915G/C gene CC subtype and C allelic gene was also significantly higher than that in the control group ($P<0.05$). The cancer risk [odds ratio (OR)] of patients with A allelic gene in -800G/A gene was 4.8 (95% CI=2.563-6.537, $P<0.05$), while the cancer risk (OR) of patients with C allelic gene in +915G/C gene was 4.7 (95% CI=2.317-5.864, $P<0.05$). The serum TGF-β1 expression levels of -800G/A gene AA subtype in the observation group was significantly higher than the GG type, GA type and the control group, while the TGF-β1 level of +915G/C gene CC subtype was significantly higher than the GG type, GC type and the control group ($P<0.05$). Therefore, the polymorphisms of -800G/A and +915G/C in TGF-β1 gene are closely related to the lung cancer susceptibility.

Introduction

Lung cancer is ranked as the highest malignant tumor that threatens human health in the world. The morbidity and mortality associated with lung cancer increases year by year with an increase in environmental pollution as well as the age of the population (1). The early detection of lung cancer is hindered due to a lack of biochemical markers with typical clinical features, and high sensitivity and specificity. In addition, the imaging examination often lags behind the occurrence and development of tumor, all of which are important factors that can lead to a poor survival prognosis (2). The transforming growth factor-β1 (TGF-β1), as a type of multifunctional cytokine which plays important role in inducing local angiogenesis, extracellular matrix secretion, immune evasion, cell heterogeneity adhesion, cell proliferation, invasion, metastasis and other aspects (3). The TGF-β1 gene, which is located on chromosome 19 of the long arm, contains seven exons and six introns. Among them, the first exon has a higher gene mutation rate and single-nucleotide polymorphism (SNP), which was confirmed to be closely related to the occurrence of a variety of malignant tumors, such as esophageal, breast, prostate, liver cancer and others (4,5). Based on this, our study analyzed the relationship between polymorphisms of -800G/A and +915G/C in the TGF-β1 gene and lung cancer susceptibility in order to provide a reference for the early diagnosis of lung cancer.

Subjects and methods

Subject information. A total of 156 patients that were admitted to Cangzhou Central Hospital and diagnosed with non-small cell lung cancer (NSCLC) from January 2013 to January 2016, were selected as part of the observation group, and 156 patients with pneumonia and tuberculosis during the same period were selected as the control group. Surgery, radiotherapy, chemotherapy and other treatments were not carried out. Tissue samples were obtained, and the tissue sections were created using the conventional method and stored at -80˚C. The ratio of age and gender of the patients in both groups was 1:1 according to the proximal matching principle. In the observation group, there were 82 males and 74 females, aged 42-77 years, with an average age of 62.3±14.5 years. Based on the tumor pathologic type, there were 95 cases with squamous carcinoma and 61 cases with adenocarcinoma. Based on the clinical TNM stage, there were 15 cases in stage I, 46 cases in stage II, 62 cases in stage III and 33 cases in stage IV, with a maximum diameter of 0.5-3.6 cm, and an average of 2.4±1.2 cm. In

Correspondence to: Dr Bao-Hua Sun, Department of Respiration Medicine, Cangzhou Central Hospital, 16 Xinhua Road, Cangzhou, Hebei 061000, P.R. China
E-mail: sunbaohua29@163.com

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Table I. Primer sequences of polymorphisms of -800G/A and +915G/C in TGF-β1 gene.

	Sequence	Reaction temperature (˚C)	Enzyme digestion	Length (bp)
-800G/A	F (G): 5'-ACAGTTGGCACGGGCTTTCG-3'	57	HpyCh4 IV	G: 182 and 206
	F (A): 5'-CAGACTCTAGAGACTGTCAG-3'			A: 388
	R: 5'-GTCACCAGAGAAAGAGGAC-3'			
+915G/C	F (G): 5'-GTGCTGACGCCTGGCCG-3'	58	Bsu36 I	G: 233
	F (C): 5'-GTGCTGACGCCTGGCCC-3'			C: 233
	R: 5'-GGCTCCGGTTCTGCACTC-3'			

TGF-β1, transforming growth factor-β1.

the control group, there were 80 males and 76 females, aged 40-80 years, with an average age of 63.5±15.7 years. The study was approved by the Ethics Committee at Cangzhou Central Hospital and written informed consent rights were obtained from the patients or their families.

Research methods. The sequence-specific primer polymerase chain reaction (PCR-SSP) technique was used to test the polymorphisms of the first exon -800G/A and +915G/C in the TGF-β1 gene. The expression levels of TGF-β1 in peripheral blood were detected using ELISA.

PCR-SSP technique. Tissue DNA was extracted using the kit purchased from Sigma (St. Louis, MO, USA). The main steps were conducted as follows: 20 mg tissue were taken, and after being ground, 500 µl of tissue lysate were added. The solution was soaked in the water at 50˚C for 1 h. Then, proteinase K was added to reach a final concentration of 100 µg/ml with soaking in 50˚C water for 3 h. Then, the extraction was respectively performed using an equal volume of saturated phenol, phenol-chloroform (volume ratio as 1:1) and chloroform-isoamyl alcohol each time. Sodium ethylate (1/10 volume) and ice ethanol (2-fold volume) were added to precipitate the DNA. The appropriate amount of TE solution was added to dissolve the precipitation. Subsequently, DNA concentration and purity were detected using an ultraviolet spectrophotometer (Applied Biosystems Life Technologies, Foster City, CA, USA). The primers were designed by referring to the sequence on PubMed, and the primer design was synthesized by Shanghai Bioengineering Co., Ltd. (Shanghai, China), which is shown in Table I. A total of 25 µl of the PCR amplification system included 10X buffer 2.5 µl + dNTPs (2.5 mmol/l) 2.0 µl + upstream and downstream primers (20 pmol for each) + cDNA 200 ng + Taq polymerase 1.25U and distilled water. The reaction conditions were as follows: 94˚C for 5 min, 94˚C for 40 sec, annealing for 1 min (temperature is shown in Table I), 72˚C for 1 min, 35 cycles in total, and 72˚C for 5 min. The enzyme digestion system included -800G/A and +915G/C gene expansion products (10 µl for each). The restriction enzyme (as shown in Table I; was used, after which the enzymatic digestion was performed in water at 37˚C for 3 h. The product, which was detected by 2% agarose gel electrophoresis and ethidium bromide staining method, was identified under ultraviolet light.

ELISA method. Peripheral blood (5 ml) was collected and performed by centrifugation at 3,000 x g for 20 min. The upper layer of the serum was taken and stored at -20˚C. The kits were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and the microplate reader was purchased from the Bio-Rad Laboratories, Inc. (Hercules, CA, USA). The steps were carried out strictly in accordance with the specifications.

Statistical analysis. The normal data were expressed as mean ± standard deviation, t-test was used for comparison, while the measurement data were expressed by rate. The Chi-square test was used for comparison. The exposure risk was tested using a single factor logistic model and expressed by the odds ratio (OR) value. P<0.05 indicated that the difference was statistically significant. The SPSS 20.0 software (IBM, Armonk, NY, USA) was applied for statistical analysis.

Results

Analysis of the polymorphisms of -800G/A and +915G/C gene. The proportion of -800G/A gene AA subtype and A allelic gene in the observation group was significantly higher than that in the control group, while the proportion of +915G/C gene CC subtype and C allelic gene was also significantly higher than that in the control group; differences were statistically significant (P<0.05). The cancer risk (OR) of patients with A allelic gene in -800G/A gene was 4.8 (117x96/39x60) (95% CI=2.563-6.537, P<0.05) while the cancer risk (OR) of the patients with C allelic gene in +915G/C gene was 4.7 (102x111/54x45), (95% CI=2.317-5.864, P<0.05) (Table II).

Comparison of serum TGF-β1 expression levels. The TGF-β1 expression levels of -800G/A gene AA subtype serum in the observation group were significantly higher than GG type, GA type and the control group, while the TGF-β1 levels of +915G/C gene CC subtype were significantly higher than GG type, GC type and the control group, and the differences were statistically significant (P<0.05) (Fig. 1).

Discussion

SNP is characterized in the third generation of genetic markers found currently. A previous study has shown that there are

Table II. Analysis on the polymorphisms of -800G/A and +915G/C gene [n (%)].

		-800G/A					+915G/C				
Groups	Cases	GG	GA	AA	G	A	GG	GC	CC	G	C
Observation group	156	28	11	106	39 (25.0)	117 (75.0)	31	23	79	54 (34.6)	102 (65.4)
Control group	156	70	26	34	96 (61.5)	60 (38.5)	96	15	30	111 (71.2)	45 (28.8)
χ^2			60.471		42.423			56.794		41.793	
P-value			<0.001		<0.001			<0.001		<0.001	

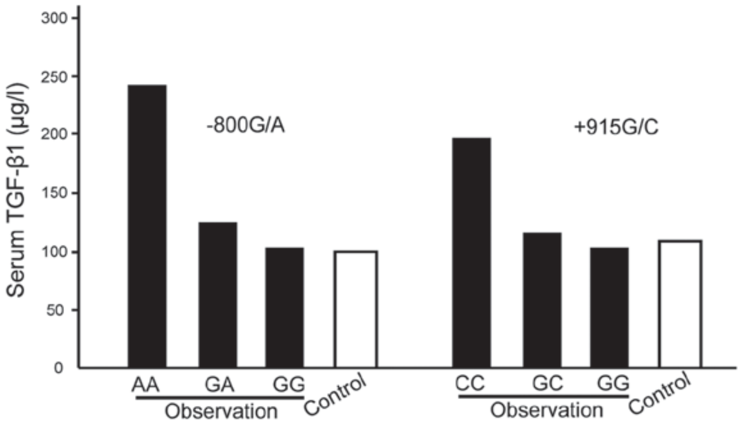

Figure 1. Comparison of serum TGF-β1 expression levels. TGF-β1, transforming growth factor-β1.

10 TGF-β1 gene SNPs at least, including -988 (C>A), +72 (insC), -509 (C>T), -800 (G>A), codon 327 (Thr>Pro), codon 10 (Leu>Pro), codon 47 (Gly>Glu), codon 263 (Thr>Ile), codon 25 (Arg>Pro) and 713-8delC (6). The detection methods of SNPs include restriction fragment length polymorphism (RFLP), single-stranded conformation polymorphism (SSCP) and allele-specific oligonucleotide (ASO) probe.

A study has demonstrated that the number of TGF-β1 SNP sites and the pattern of manifestation are specific to both the population and disease. The TGF-β1 promoter -800G/A and -509C/T polymorphisms were related to the occurrence of esophageal cancer (7), the codon 10 site was correlated with the occurrence of breast cancer (8), and -509 (C>T) and codon 10 (Leu>Pro) polymorphisms were associated with the occurrence of bladder cancer (9). This study indicates that the proportion of -800G/A gene AA subtype and A allelic gene in the observation group were significantly higher than that in the control group, while the proportion of +915G/C gene CC subtype and C allelic gene were significantly higher than that in the control group; differences were statistically significant. The cancer risk (OR) of patients with A allelic gene in -800G/A gene was 4.8, while the cancer risk (OR) of patients with C allelic gene in +915G/C gene was 4.7, which suggests that -800G/A (A>G) and +915G/C (C>G) are closely related to the lung cancer susceptibility. The serum TGF-β1 expression levels of -800G/A gene AA subtype in the observation group were significantly higher than GG type, GA type and the control group, while TGF-β1 levels of +915G/C gene CC subtype were significantly higher than GG type, GC type and the control group; differences were statistically significant. High levels of TGF-β1 play an important role in promoting the occurrence and development of lung cancer (10). Through the reconstruction of the eukaryotic expression recombinant of TGF-β1 promoter codon 10 *in vitro* and the transfection of HeLa cell line, it was found that the mutation of single SNP site could lead to abnormally high expression of circulating TGF-β1 levels, the pathogenesis of which is closely related to the processing, modification, transport and release of proteins after translation rather than the transcriptional activity of the gene. In addition, the different expression of amino acids caused by an adjustment of polymorphism of relative sites would form different primary peptide chain structures, which would eventually decide the biological behavior of the translated protein (11).

The study on the mechanism of polymorphic sites and tumorigenesis has considered that activator protein 1 (AP1) and hypoxia-inducible factor-1 (HIF-1) are important factors in the regulation of the expression of TGF-β1 (12). When -800G/A (A>G) exist, the binding activity of AP1 with this site is increased, which would promote the transcription and translation of the TGF-β1 gene (12). For HIF-1, the presence of either A or G is not relevant and can be combined, however, for G, the competitive binding with AP1 would be formed (13). TGF-β1 gene polymorphism is also associated with self-autoimmune diseases, repair in trauma, inflammatory reaction, organ fibrosis and other diseases (14,15). After HBV infection, secondary cirrhosis may be related to the SNP of codon 10, and the possibility of codon 10 (Leu>Pro) developed to liver cirrhosis would be increased, while its severity may be associated with the SNP of -509 site, and the disease condition at -509 (C>T) may become more serious (16).

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