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RFLP Report

ANALYSIS OF THE FIRST INTRON OF *TNFB* GENE BY *Nco*I RFLP IN KOREANS

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Summary The tumor necrosis factor B (*TNFB*) gene is closely liked with tumor necrosis factor A (*TNFA*) gene between the *HLA-B* and *C2* genes on chromosome 6p21.3. Several genetic variabilities at the human *TNFB* loci have been identified, which are the *Nco*I restriction fragment length polymorphism (RFLP) in the first intron, amino acid substitution at codon 26 of exon 3 and *Eco*RI RFLP in untranslated exon 4. The *Nco*I RFLP of *TNFB* gene gives two allelic fragments of 238/259 bp and 497 bp, corresponding to *TNFB*1* and *TNFB*2* alleles, respectively. To investigate the frequency of *Nco*I RFLP in the first intron of *TNFB* in Koreans and to compare to that of other ethnic population, genomic DNAs were extracted from leukocytes of 305 unrelated healthy Koreans and amplified the first intron of *TNFB* gene by PCR. The phenotype frequencies of *Nco*I RFLP such as *TNFB*1/TNFB*1*, *TNFB*1/TNFB*2* and *TNFB*2/TNFB*2* were 8.6% (n=26), 45.2% (n=138) and 46.2% (n=141), respectively. The estimated allele frequencies for *TNFB*1* and *TNFB*2* were 0.3115 and 0.6885, respectively. The observed and expected frequencies were in good agreement with the Hardy-Weinberg's equilibrium. The heterozygosity revealed 45.2% and the allele frequencies of *Nco*I RFLP of *TNFB* in Koreans were observed comparatively similar to those of other ethnic groups.

Key Words *TNFB*, first intron, *Nco*I-RFLP, Koreans

Introduction

Tumor necrosis factor β (TNF β; lymphotoxin) is a cytokine predominantly secreted by macrophage but also by activated lymphocytes (Sung *et al.*, 1988a, b). It has a high cytotoxicity to wide range of tumor cells and mediate to inflammation and graft rejection. TNF-β share 30% amino acid homology with tumor necrosis

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factor-α (TNF-α) and show similar cytostatic and cytolytic activities *in vitro* and *in vivo*.

A gene encoding TNF-β gene (*TNFB*) is located in tandem with the TNF-α gene (*TNFA*) between the *HLA-B* and *C2* genes (Nedospasov *et al.*, 1986; Spies *et al.*, 1986) on chromosome 6p21.3 (Nedwin *et al.*, 1985). The *TNFB* gene consists of four exons, which are untranslated exon 1, partially untranslated exon 4 and three introns. The cDNA revealed that a predicted length of 205 amino acids derived from 238 residue long precursor and produced a 25 kDa cytokine.

Structural and regulatory polymorphism in the human *TNFB* gene has been reported previously. The nucleotide sequence differences of a biallelic polymorphism were found in the *TNFB* gene (Abraham *et al.*, 1991). One of these differences was found to be located in exon 1, the 5′ untranslated region of the gene. A single base transition of A to G appears in the exon 1. Another nucleotide difference was found in the first intron, corresponding to a point mutation at *Nco*I site (G *vs* A). The third nucleotide difference resulted in a polymorphism of exon 3, which differed in its amino acid at position 26 of TNF-β protein (C *vs* A). And additional restriction fragment length polymorphism (RFLP) has been demonstrated for the *TNFB* gene with the use of the restriction endonuclease, *Eco*RI (Partanen and Koskimies, 1988) and *Acc*I (Webb and Chaplin, 1990), however, it was not allelic in nature.

The *Nco*I polymorphism in the first intron of *TNFB* gene allows two alleles, *TNFB*1* and *TNFB*2* (Webb and Chaplin, 1990; Messer *et al.*, 1991; Abraham *et al.*, 1991). *TNFB*1* carries the *Nco*I restriction site, lacking in *TNFB*2* due to a point mutation which segregates with the biallelic system in family study (Bettinotti *et al.*, 1993). Yamagata *et al.* (1991) have found the polymorphic *Nco*I restriction site located in the first intron of the *TNFB* gene by PCR-*Nco*I-RFLP. Messer *et al.* (1991) have showed that two *TNFB* alleles differ by one amino acid at position 26 that conserved as asparagine in the *TNFB*1* and as threonine in the *TNFB*2*.

The *Nco*I RFLP in the first intron of *TNFB* variation is not only regarded as a genetic marker but also known for its involvement in regulation of TNF-β and TNF-α expression (Pociot *et al.*, 1993); the *TNFB*2/TNFB*2* homozygote may be associated with autoimmune-like disease (Partanen and Koskimies, 1988) and allowing better prognosis of lung or gastric cancer (Hagihara *et al.*, 1995; Shimura *et al.*, 1994, 1995).

In present paper, we have characterized the variation in the first intron of *TNFB* gene in Koreans by PCR-*Nco*I-RFLP and the frequencies have been compared to those of other ethnic groups.

Materials and Methods

Genomic DNA was extracted from peripheral blood of 305 unrelated Koreans using QIAamp Blood kit (Qiagen, USA). For *TNFB Nco*I RFLP analyses, the

following primers were used: TNFB1, 5′ GCA CAG CAG GTG AGG CTC TCC 3′ and TNFB2, 5′ GGT GGT GCC ACA CAC CCT TGG 3′ (Yamagata *et al.*, 1991). The reaction mixture contained 20 pmol of each primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.001% gelatin, 1.5 mM $MgCl_2$, 200 μM of dNTPs and 1 unit of *Taq* DNA polymerase (Poscochem, Korea). The initial denaturation time was 4 min. And amplification was performed at 95°C for 1 min/63°C for 15 sec/72°C for 20 sec during 35 cycles by Perkin/Elmer thermocycler 9600. The expected 497 bp band was amplified, directly digested with 1 unit of restriction enzyme *Nco*I (Boehringer Mannheim, Germany) for 3 hr at 37°C. The fragments obtained after amplification and digestion were analyzed by 5% of polyacrylamide gel electrophoresis and stained with ethidium bromide. The band cleaved with *Nco*I represented as the *TNFB*1* type (238/259 bp), and that not cleaved, as the *TNFB*2* type (497 bp).

Results and Discussion

*Nco*I RFLP in the *TNFB* gene was studied in 305 unrelated Koreans. The amplified 497 bp fragment was contained *Nco*I polymorphic site in the first intron of *TNFB* gene. Amplification and *Nco*I restriction of the first intron resulted in two different patterns of fragments. The *TNFB*1* allele corresponded to 259/238 bp of *Nco*I fragment with the presence of G sequence, and *TNFB*2* allele corresponded to 497 bp fragment, which lacks the *Nco*I restriction site. Individuals carrying the heterozygous *TNFB*1/TNFB*2* for *Nco*I RFLP of *TNFB* intron 1 showed three fragments, such as 497 bp, 259 bp and 238 bp bands by 5% of polyacrylamide gel electrophoresis (Fig. 1).

The first intron of *TNFB* genotype and allele frequencies were shown in Table 1. The gene frequencies were 0.3115 for *TNFB*1* allele and 0.6885 for *TNFB*2*. The heterozygosity at *TNFB* intron 1 was 45.2%. No deviation from the expectation according to the Hardy-Weinberg equilibrium was found.

Table 2 showed the genotype frequencies of *Nco*I RFLP in the first intron of *TNFB* gene in other ethnic groups. The frequencies of *TNFB*1/TNFB*1* were shown below 12.7% in most of populations. The frequency of *Nco*I RFLP in the first intron of *TNFB* in Koreans was similar to that of the other ethnic groups. Chung *et al.* (1994) had previously reported *Nco*I RELP in the first intron of *TNFB* in 129 Koreans. However, they have shown that the phenotype frequency of the rare *TNFB* allele *TNFB*1/TNFB*1* homozygote was significantly higher than our result (21.7% *vs* 8.6%, $p<0.01$). This difference might be due to less number of subjects in the experiment.

Recently, there have been many reports of which the polymorphism of the *TNFB* RFLPs might be involved in differential TNF-α or -β secretion. Individuals carrying the *TNFB*2* of *Nco*I RFLP in intron 1 had a higher TNF-α secretory capacity than those carrying the *TNFB*1* (Pociot *et al.*, 1993). Messer *et al.* (1991) have characterized the *TNFB*1* which presents significantly higher

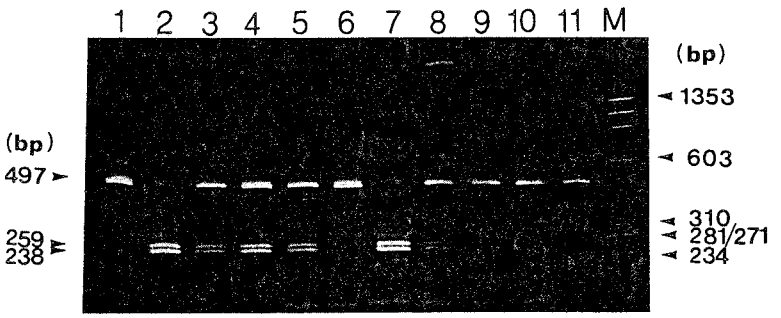

Fig. 1. Patterns of polymorphic restriction fragments with restriction endonuclease, *Nco*I. *Nco*I digestion of amplified *TNFB* gene were given on 238/259 bp for the *TNFB*1* allele and the 497 bp for the *TNFB*2* allele, Lane M: the size marker ϕX174/*Hae*III, lane 1: PCR amplified fragment without *Nco*I, lanes 2, 7: *TNFB*1/TNFB*1*, lanes 3, 4, 5, 8: *TNFB*1/TNFB*2*, lanes 6, 9, 10, 11: *TNFB*2/TNFB*2*.

Table 1. Distribution of genotype of the first intron region of *TNFB* and allele frequencies by *Nco*I RFLP in Koreans.

Genotype	Observed (%)	Expected (%)	Allele frequencies±SE
*TNFB*1/TNFB*1*	26 (8.6)	29.6 (9.7)	*TNFB*1*=0.3115±0.0187
*TNFB*1/TNFB*2*	138 (45.2)	130.8 (42.9)	*TNFB*2*=0.6885±0.0187
*TNFB*2/TNFB*2*	141 (46.2)	144.6 (47.4)	
Total	305 (100.0)	305.0 (100.0)	

χ^2=0.93.

Table 2. Comparison of *Nco*I RFLP of the first intron of *TNFB* in other ethnic groups.

Population	N	*TNFB*1/TNFB*1* n (%)	*TNFB*1/TNFB*2* n (%)	*TNFB*2/TNFB*2* n (%)	References
Korean	305	26 (8.6%)	138 (45.2%)	141 (46.2%)	This study
〃	129	28 (21.7%)	61 (47.3%)	40 (31.0%)	Chung *et al.*, 1994
Japanese	32	2 (6.2%)	14 (43.8%)	16 (50.0%)	Yamagata *et al.*, 1991
〃	75	8 (10.7%)	27 (36.0%)	40 (53.3%)	Mizuki *et al.*, 1992
〃	141	14 (9.9%)	69 (48.9%)	58 (41.1%)	Shimura *et al.*, 1995
(Honshu)	165	21 (12.7%)	56 (34.0%)	88 (53.3%)	Shimura *et al.*, 1994
(Okinawa)	74	4 (5.4%)	35 (47.3%)	35 (47.3%)	Hagihara *et al.*, 1995
Danes	131	8 (6.1%)	60 (45.8%)	63 (48.1%)	Fugger *et al.*, 1989
〃	262	16 (6.0%)	97 (37.0%)	149 (57.0%)	Laitinen *et al.*, 1992
Europeans	173	17 (10.0%)	72 (41.5%)	84 (48.5%)	Badenhoop *et al.*, 1992
German	191	21 (11.0%)	69 (36.1%)	101 (52.9%)	Bettinotti *et al.*, 1993
〃	179	20 (11.2%)	78 (43.6%)	81 (45.2%)	Messer *et al.*, 1991
Canadian	91	10 (11.0%)	39 (42.8%)	42 (46.2%)	Goldstein and Sengar, 1993
Finns	242	29 (12.0%)	110 (45.3%)	103 (42.4%)	Laitinen *et al.*, 1992

TNF-β mRNA and protein synthesis upon PHA stimulation of peripheral blood mononuclear cell and T lymphocytes than the *TNFB*2* allele does. TNF-α secretion by monocytes from individuals depends on different *TNFB* genotypes. Because the TNF-α potentiates the cytostatic/cytotoxic effects, although further studies are necessary, we suggest that *TNFB*2/TNFB*2* associated with increased amounts of TNF-α secretion may be correlated with a resistance to cancer and lead to a better prognosis.

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