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Original Article

Association between TRAIL gene polymorphisms and the susceptibility and severity of lumbar disc degeneration

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Abstract: Aim: The aim of the present study was to investigate the association between tumor necrosis factor related apoptosis-inducing ligand (TRAIL) gene polymorphisms and the susceptibility and severity of lumbar disc degeneration (LDD) in the Chinese Han population. Methods: A total of 153 patients with LDD and 131 healthy subjects were enrolled in the study. Four single-nucleotide polymorphisms (SNPs) in the 3' untranslated region (3'UTR) of TRAIL gene, including 1289 C/A, 1525 G/A, 1588 G/A and 1595 C/T, were genotyped with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Results: The genotypes and alleles frequencies of TRAIL at 1525 and 1595 positions in all subjects were the same. There was a significant association between TRAIL 1525/1595 polymorphisms and the susceptibility of LDD. The frequencies of 1525 GG /1595 CC genotype, and 1525 G/1595 C allele were higher in the patients group than that in the control group. In addition, we found patients with the 1525 AA /1595 TT genotype, as well as 1525 A/1595 T allele exhibit significantly low frequency of high grades of disc degeneration. However, there were no significant differences in the genotype or allele distribution of TRAIL 1289 C/A or 1588 G/A between the patients and the control group. Conclusion: TRAIL 1525/1595 polymorphisms were associated with the susceptibility and severity of LDD in the Chinese Han population.

Keywords: Tumor necrosis factor related apoptosis-inducing ligand, lumbar disc degeneration, polymorphism, susceptibility, severity

Introduction

Low back pain (LBP) is a prevalent, expensive and debilitating problem which affects about 80% of people worldwide at some point of their lives [1]. Lumbar disc degeneration (LDD) is a major pathological process that contributes to LBP [2]. Studies have reported that multiple factors influence disc degeneration, such as aging, gender, lifestyles and mechanical loading [3]. In addition to these environmental and behavioral factors, genetic factor has been implicated to be associated with the risk for the development of disc degeneration [4-6]. Morphological and biochemical changes occur in the disc during the process of disc degeneration. It has been demonstrated by both the in vivo and in vitro studies that intervertebral cell loss resulting from apoptosis plays vital roles in promoting the progression of intervertebral disc degeneration [7, 8].

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is a transmembrane protein that belongs to the tumor necrosis factor ligand family. It was firstly identified by Wiley et al in 1995 and was known as an important member of TRAIL-mediated biochemical signaling pathway. TRAIL induced apoptosis in transformed and cancer cells preferentially through its receptors TRAIL-R1/DR4 and TRAIL-R2/DR5 [9]. Recently, it has been revealed that TRAIL apoptotic pathway seems to be one of the pathways responsible for temporomandibular joint (TMJ) disk degeneration [10]. There was a significant positive correlation between TRAIL gene expression and the overall degenerative changes of intervertebral disc tissues [11].

TRAIL gene is located at chromosome 3q26 and encodes a 1.77 kb mRNA. It contains five exons and four introns. Several single-nucleotide polymorphisms (SNPs) have been found in

Table 1. TRAIL genotypes and alleles frequencies in the patients with LDD and the controls, and their association with the risk of LDD

Genotypes and alleles	Patients (n=153)	Controls (n=131)	χ^2	*P* value	OR (95% CI)
1525 G/A, 1595 C/T					
1525 GG/1595 CC	46 (30.07)	22 (16.79)			1
1525 GA/1595 CT	71 (46.41)	66 (50.38)	4.644	0.031	0.514 (0.280-0.946)
1525 AA/1595 TT	36 (23.53)	43 (32.82)	7.221	0.007	0.400 (0.204-0.786)
1525 G/1595 C	163 (53.27)	110 (41.98)			1
1525 A/1595 T	143 (46.73)	152 (58.02)	7.199	0.007	0.635 (0.455-0.885)
1289 C/A					
CC	115 (75.16)	102 (77.86)			1
CA	33 (21.57)	25 (19.08)	0.28	0.597	1.171 (0.653-2.100)
AA	5 (3.27)	4 (3.05)			
CA+AA	38 (24.84)	29 (22.14)	0.285	0.593	1.162 (0.669-2.018)
C	263 (85.95)	229 (87.40)			1
A	43 (14.05)	33 (12.60)	0.285	0.661	1.135 (0.697-1.846)
1588 G/A					
GG	119 (77.78)	110 (83.97)			1
GA	29 (18.95)	19 (14.50)	1.139	0.286	1.411 (0.748-2.659)
AA	5 (3.27)	2 (1.53)			
GA+AA	34 (22.22)	21 (16.03)	1.733	0.188	1.497 (0.819-2.734)
G	267 (87.25)	239 (91.22)			1
A	39 (12.75)	23 (8.78)	2.284	0.131	1.518 (0.881-2.615)

TRAIL, tumor necrosis factor related apoptosis-inducing ligand; LDD, lumbar disc degeneration; OR, odds ratio; CI, confidence interval.

TRAIL gene, and TRAIL gene polymorphisms have been studied extensively in various diseases [12-15]. However, association between polymorphisms in TRAIL gene and LDD has not been reported until now.

This study for the first time investigated the potential effect of TRAIL gene polymorphisms on the susceptibility and severity of lumbar disc degeneration. It may provide a new insight into the etiology of degenerative diseases.

Materials and methods

Subjects

The study was approved by the Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University. Written informed consent was obtained from each participant before this study. A total of 153 patients with LDD and 131 healthy subjects were enrolled in the study. All subjects were Han Chinese without blood relationship. The eligible patients were proven with the degenerative discs of the lumbar spine by magnetic resonance imaging (MRI) scan (Siemens AG, Germany) between 2013 and 2015 at The First Affiliated Hospital of Xi'an Jiaotong University. Among them, 73 were males and 80 were females and their age at the first time experience of back pain ranged from 21 to 61 years (mean, 40.2±5.1). Exclusion criteria included inflammatory disease, trauma, spondylosis, tumor, heavy physical loading and previous surgical treatment in the lumbar spine. The healthy controls, including 62 males and 69 females were adults aged 25 to 58 years (mean, 38.6±5.0) with no history of back problems. There were no significant differences in gender or age ratios between the control and the patients group. The severity of disc degeneration was evaluated according to Schneiderman's classification [16]. Grade 1 indicates a normal disc; Grade 2, a slight decrease in signal intensity; Grade 3, a generalized hypointense signal; and Grade 4, a hypointense signal with disc space narrowing.

Genotyping

A total of 5 ml peripheral blood was obtained from each participant. Genomic DNA was ex-

Table 2. Grades of disc degeneration among LDD patients with different TRAIL genotypes and alleles

Genotypes and alleles	n	Grade 2	Grade 3	Grade 4	*P* value
1525 GG/1595 CC	46	14 (30.43)	12 (26.09)	20 (43.48)	
1525 GA/1595 CT	71	22 (30.99)	18 (25.35)	31 (43.66)	
1525 AA/1595 TT	36	20 (55.56)	9 (25.00)	7 (19.44)	0.012
1525 G/1595 C	163	50 (30.67)	42 (25.77)	71 (43.59)	
1525 A/1595 T	143	62 (43.36)	36 (25.17)	45 (31.47)	0.012

TRAIL, tumor necrosis factor related apoptosis-inducing ligand; LDD, lumbar disc degeneration.

tracted from peripheral blood using the phenol-chloroform extraction method, and stored at -20°C. SNP genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The primers for PCR were: TRAIL 1289 C/A, 1525 G/A and 1595 C/T forward, 5'-TGCAATGGTTAACATCTTCTGTC-3', reverse, 5'-AAACCAAGTCTCGCTCTGT-3'; TRAIL 1588 G/A forward, 5'-AGCT ACTTGAGAGG CTGCTGCA-3', reverse, 5'-AAACCAAGTCTCGCTCTGT-3'. PCR was performed in a final volume of 25 μl containing 2.5 μl 10×PCR buffer, 2 μl dNTP, 10 pM primers, 1.5 U Taq DNA polymerase and 100 ng DNA template. The DNA was initially denatured at 95°C for 5 min; followed by 35 temperature cycles of 94°C for 40 s, 58°C for 1 min and 72°C for 40 s; and a final extension at 72°C for 5 min. For 1595 C/T, the PCR products were digested by Tas I at 65°C for 3 h. For 1289 C/A, 1525 G/A and 1588 G/A, the PCR products were digested by Hin P1 I, Rsa I and Pst I at 37°C for 2 h, respectively. All these restriction enzymes were purchased from Fermentas (Vilnius, Lithuania).

Statistical analysis

All statistical procedures were conducted using SPSS 19.0 (SPSS, Inc., Chicago, IL, USA). The Hardy-Weinberg equilibrium was checked for all polymorphisms in both the control and the patients group. Differences in the frequencies of genotypes and alleles between the control and patients were analyzed using the Chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the risk for LDD. The severity of disc degeneration of different alleles and genotypes among LDD patient was determined using Mann-Whitney U and Kruskal-Wallis H test, respectively. A two-tailed *P* value less than 0.05 was considered statistically significant.

Results

The genotype and allele frequencies of TRAIL 1289 C/A, 1525 G/A, 1588 G/A and 1595 C/T polymorphisms among the controls and the patients with LDD were shown in **Table 1**.

The genotypes distributions for TRAIL 1289 C/A and 1588 G/A in both the control group and the patients group fit in with the Hardy–Weinberg equilibrium law (TRAIL 1289 C/A: χ^2=2.326 and P=0.127 for controls, χ^2=1.754 and P=0.185 for patients; TRAIL 1588 G/A: χ^2=1.168 and P=0.280 for controls, χ^2=3.342 and P=0.068 for patients). Because the AA homozygotes for the TRAIL 1289 C/A and 1588 G/A polymorphisms were rare in the present study, AA genotype was then combined with the CA genotype or GA genotype for statistical analysis. We found that the genotype and allele frequencies of TRAIL 1289 C/A and 1588 G/A in the patients group were not significantly different from that in the control group.

The genotypes distributions for TRAIL 1525 G/A and 1595 C/T in the control group and the patients group were in line with the Hardy–Weinberg equilibrium law (χ^2=0.153, P=0.695 for controls; χ^2=0.706, P=0.401 for patients). The results showed that 1525 G/A and 1595 C/T were in complete linkage disequilibrium. The genotypes and alleles frequencies of TRAIL at the two sites in all subjects were the same. The SNPs of TRAIL gene at 1525 G/A and 1595 C/T were associated with the susceptibility of LDD. GG genotype and G allele of 1525 G/A, CC genotype and C allele of 1595 C/T were more frequent in the patients group than in the control group. Subjects carrying at least one variant A allele at site 1525, and T allele at site 1595 had decreased risk for lumbar disc degeneration compared with the subjects with the GG and CC genotype.

Subsequently, we examined the association between the TRAIL 1525 G/A and 1595 C/T polymorphisms, and the severity of lumbar disc degeneration in the patients group. It was shown in **Table 2** that patients with the 1525

AA/1595 TT genotype, as well as 1525 A/1595 T allele exhibit significantly lower frequency of high grades of disc degeneration compared with those with the 1525 GG/1595 CC or 1525 GA/1595 CT genotypes, and the 1525 G/1595 C allele.

Discussion

Accumulating evidences indicate that many mutations may alter the balance between cell proliferation and apoptosis, thus leading to the development of LDD [17-20]. TRAIL is a potent apoptosis inducer in cancer cells. It has been found that TRAIL also exists in the three distinct regions, including the vertebral endplates (EPs), nucleus pulposus (NP) and annulus fibrosus (AF), of normal intervertebral disc tissues [21]. The percentage of TRAIL-positive cells was higher in the intervertebral disc tissues of patients with cervical spondylosis compared with the control [22]. Leonardi et al found that TRAIL expressed in the cytoplasm of the TMJ disk cells, and the immunostaining score of TRAIL was correlated to the histopathologic grading of the disk degeneration [10]. It was reported by Bertram et al that TRAIL could be detected in intervertebral disc cells by both the quantitative PCR and immunohistology analysis. There was a significant positive correlation between TRAIL gene expression and the overall degenerative changes of intervertebral disc tissues. Furthermore, TRAIL expression correlated with cellularity, changes in mucoid matrix and occurrence of tears and clefts [11]. In the present study, we investigated the effect of TRAIL on LDD via the aspect of TRAIL gene polymorphism.

It is widely reported that SNPs in the human genes encoding TRAIL and TRAIL receptors are associated with multiple sclerosis susceptibility [12]. Verim et al reported that there was a significant association between TRAIL and TRAIL-DR4 polymorphisms and laryngeal cancer by haplotype analysis [13]. In addition, polymorphisms in the TRAIL gene were associated with chronic lymphocytic leukemia, Waldenström's (WM) [14], and lymphoma [15]. 3' untranslated region (3'UTR) has a significant role in gene regulation [23, 24]. In the present study, we investigated the association between the 4 polymorphisms in the 3'UTR of TRAIL gene, including 1289 C/A, 1525 G/A, 1588 G/A and 1595 C/T, and LDD susceptibility, in a Chinese Han population. We found that the A allele at SNPs 1289 C/A and 1588 G/A of TRAIL gene was not common in the Chinese Han population. In addition, there was no significant association between the 1289 C/A or 1588 G/A polymorphism and LDD.

Consistent with the reports by Yan et al and Yu et al, 1525 G/A and TRAIL 1595 C/T are common polymorphisms in Chinese population, and the two sites were in complete linkage disequilibrium [25, 26]. TRAIL gene polymorphism in the 3'UTR at nucleotide 1595 of exon 5 has been reported to be associated with the progression of breast cancer in Turkish population [27]. In addition, the TRAIL SNP at site 1595 C/T was associated with type 2 diabetes mellitus (T2DM) susceptibility in a Han Chinese population. The CC genotype of this SNP increased the susceptibility to patients with T2DM [26]. In the present study, we firstly found a significant association between TRAIL 1525/1595 polymorphisms and the susceptibility of LDD. The A/T alleles of TRAIL gene at 1525/1595 position engendered a lower risk of LDD. Furthermore, we found that TRAIL 1525/1595 polymorphisms are associated with the severity of disc degeneration.

This is the first study reporting the potential role of TRAIL gene polymorphisms in LDD susceptibility and severity in a Chinese population. Further studies of other relevant polymorphisms in larger Chinese populations may contribute to the improved understanding about the potential role of TRAIL gene in degenerative diseases.

Disclosure of conflict of interest

None.

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