IL-16 rs4778889 polymorphism contribution to the development of renal cell cancer in a Chinese population

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ABSTRACT. IL-16 plays an important role in affect the secretion of tumor-related inflammatory cytokines. We aimed to assess the role of interleukin-16 (*IL-16*) rs4778889 T/C and rs11556218 T/G polymorphisms in the occurrence of renal cell cancer (RCC). This study is composed of 274 RCC patients and 274 control subjects. Genotyping of polymorphisms was performed using polymerase chain reaction combined with restriction fragment length polymorphism analysis. All statistical analysis was carried out by the SPSS statistical software package, version 16.0 (SPSS Inc., Chicago, IL, USA). Using conditional logistic regression analysis, the TC and CC genotypes of rs4778889 exhibited a higher risk of RCC, with adjusted ORs (and 95%CIs) of 1.79 (1.23-2.62) and 2.67 (1.29-5.69), respectively. Moreover, under dominant and recessive models, individuals carried the rs4778889 polymorphism was exhibited elevated RCC risk, with adjusted ORs (and 95%CI) of 1.93 (1.35-2.76) and 2.11 (1.05-4.45), respectively. No significant differences were observed in rs11556218 genotype frequencies between the study groups. In conclusion, the

results of our study reveal an association between the *IL-16* rs4778889 polymorphism and heightened risk of RCC.

Key words: *IL-16*; Polymorphism; Renal cell cancer

INTRODUCTION

The complex trait disease renal cell cancer (RCC) accounts for more than 90% of all renal malignancies (Chow et al., 2010). The incidence of RCC shows variation among different populations, which shows higher in Europeans and lower in Asians (Chow et al., 2010). The etiology of RCC is not well-understood. The pathogenesis of RCC is caused by many complex environmental and lifestyle factors, such as obesity, diabetes, hypertension, tobacco smoking, alcohol consumption, and family history (Vineis et al., 2004; Adams et al., 2008; Chow et al., 2010; Bellocco et al., 2012). Currently studies have suggested that the tumorigenesis of renal cell cancer involves complex process determined by the interactions between environmental and genetic factors (Semenza et al., 2001). It also has been suggested that many genetic elements may contribute to the occurrence of this cancer, such as DNA repair genes, cytochrome P450 family 3 subfamily A member 5 gene (CYP3A5), ATP binding cassette subfamily B member 1 gene (ABCB1), interleukin-27 (IL-27) gene, pre-miR-146a gene and vascular endothelial growth factor (VEGF) gene (Diekstra et al., 2015; Huang et al., 2015; Xian et al., 2015).

Molecular mechanisms underlying inflammation-associated cancer included DNA damage, disruption of the immune response and alternation of the tumor microenvironment, which were associated with disequilibrium of inflammatory cytokines. It is reported that IL-16 plays an important role in affect the secretion of tumor -related inflammatory cytokines (Muc-Wierzgon et al., 2006; Shanmugham et al., 2006). Two common polymorphisms in *IL-16* (rs4778889 T/C and rs11556218 T/G) have been found to be associated with inflammatory diseases, such as late-onset Alzheier's disease, knee osteoarthritis, graves' disease and endometriosis (Gan et al., 2010; Tsai et al., 2014; Anvar et al., 2015; Luo et al., 2015). Therefore, we conducted a hospital-based case-control study to evaluate the association between *IL-16* rs4778889 T/C and rs11556218 T/G polymorphisms and RCC risk.

MATERIAL AND METHODS

Subjects

A hospital-based case-control design was used in this study. The case group is composed of 274 patients with RCC who were referred to Huaihe Hospital of Henan University between May 2013 and January 2015, being newly diagnosed and confirmed as having RCC by the presence of pathological tissue. Patients with a history of malignant tumors other than RCC, serious chronic and acute infection diseases, and serious kidney and liver diseases were excluded. The mean age of patients with RCC was 52.45 ± 8.50 years, and patients were comprised of 191 (69.71%) males and 83 (30.29%) females. There were 200 (72.99%) patients at I-II TNM stage and 74 (27.01%) patients at III-IV stage.

A total of 274 cancer-free control subjects were enrolled individuals attending our hospital between May 2013 and January 2015. Each control subject was sex- and age-matched

(±5 years) with each RCC patient. The mean age of patients with RCC was 53.70 ± 9.12 years, and patients were comprised of 191 males (69.71%) and 83 females (30.29%). All the lifestyle and clinical data of patients with RCC and control subjects were included from a structure questionnaire or medical records.

Each participant signed a written informed consent forms prior to enrollment. Institute Research Ethics Committee of Huaihe Hospital of Henan University approved the performance of this study.

Genotyping

Genomic DNA was isolated from 5 mL of peripheral blood lymphocytes using a Qiagen Blood Mini Kit (QIAGEN, Hilden, Germany). Genotyping determination of *IL-16* rs4778889 T/C and rs11556218 T/G polymorphisms was done by polymerase chain reaction (PCR) combined with restriction fragment length polymorphism assay. The forward and reverse primers used to amplify rs4778889 T/C were 5'-CTCCACACTCAAAGCCTTGTCCTATG-3' and 5'-ATACGCTGGTTCCACTTCT-3', while those for rs11556218 T/G were 5'-GCTCAGGTTCACAGAGTGTTTCCATA-3' and 5'-TGTGACAATCACAGCTTGCCTG-3', respectively. The PCR product sizes for *IL-16* rs4778889 T/C and rs11556218 T/G were 171 and 280 bp, respectively. The restriction enzymes for *IL-16* rs4778889 T/C and rs11556218 T/G were *Nde*I and *Ahd*I, respectively. PCR amplification was set as follows: one cycle of DNA denaturation at 95°C for 5 min, then 30 cycles of 94°C for 60 s, 55°C for 60 s, and 72°C for 2 min, followed by a final extension step of 72°C for 5 min.

Statistical analysis

Differences in the demographic and clinical data were compared between the two groups by the chi-square test. A goodness of fit chi-square test with one degree of freedom was taken to assess the Hardy-Weinberg equilibrium (HWE) in the control group was assessed using. The relationship between *IL-16* rs4778889 T/C and rs11556218 T/G polymorphisms and RCC risk was determined using conditional logistic regression analysis, and the results are expressed as odds ratios (ORs) and their 95% confidence intervals (95%CI). All statistical analysis was carried out by the SPSS statistical software package, version 16.0 (SPSS Inc., Chicago, IL, USA). Two-sided P-values less than 0.05 were considered statistically significant.

RESULTS

All the demographic and lifestyle characteristics of RCC patients and control subjects were collected prior to any treatment and were shown in Table 1. The chi-square test revealed that a significant different was found between participants with RCC and controls in terms of hypertension (chi-square = 10.79, $P < 0.001$) and diabetes (chi-square = 7.91, $P = 0.005$). The RCC patients are comparable with the control subjects with respect to age, sex, cigarette smoking, alcohol consumption, or family history of cancer. In the RCC patients, 200 (72.99%) exhibited TNM stages I-II, while 74 (27.01%) were at stages III-IV. Clear cell RCC was seen in 229 cases (83.58%).

Table 1. Demographic and clinical characteristics of patients with RCC and control subjects.

Characteristics	Cases	%	Controls	%	χ^2 test	P value
Age, years						
<60	176	64.23	180	65.69		
≥60	98	35.77	94	34.31	0.13	0.72
Sex						
Male	191	69.71	191	69.71		
Female	83	30.29	83	30.29	0.00	1.00
Cigarette smoking						
Never	162	59.12	174	63.50		
Ever	112	40.88	100	36.50	1.11	0.29
Alcohol drinking						
Never	145	52.92	155	56.57		
Ever	129	47.08	119	43.43	0.74	0.39
Hypertension						
No	184	67.15	218	79.56		
Yes	90	32.85	56	20.44	10.79	0.001
Diabetes						
No	232	84.67	253	92.34		
Yes	42	15.33	21	7.66	7.91	0.005
Family history of cancer						
Never	251	91.61	257	93.80		
Ever	23	8.39	17	6.20	0.97	0.32
Stage						
I-II	200	72.99				
III-IV	74	27.01				
Histology						
Clear cell	229	83.58				
Papillary	10	3.65				
Chromophobe	22	8.03				
Others	13	4.74				

The analysis revealed that the genotype frequencies of rs4778889 T/C and rs11556218 T/G did not deviate from HWE, with P values of 0.34 and 0.22, respectively (Table 2). We observed significant differences in rs4778889 genotype frequencies between the study groups (chi-square = 15.22, $P < 0.001$). Compared to the TT genotype, the TC and CC genotypes of rs4778889 exhibited a higher risk of RCC, with adjusted ORs (and 95%CIs) of 1.79 (1.23-2.62) and 2.67 (1.29-5.69), respectively. In addition, under both dominant and recessive models, individuals carried the rs4778889 polymorphism was exhibited elevated RCC risk, the adjusted ORs (and 95%CIs) being 1.93 (1.35-2.76) and 2.11 (1.05-4.45), respectively. However, no significant differences were observed in rs11556218 genotype frequencies between the study groups.

The gene-environmental analysis was carried out to assess the effects of sex, age, hypertension, and diabetes on the relationship between rs4778889 and RCC risk. We found that the rs4778889 was correlated with the susceptibility to the risk of this disease regardless of these factors. Therefore, this SNP demonstrated no interactions with sex, age, hypertension, or diabetes in influencing RCC risk (Table 3).

DISCUSSION

The incidence of RCC showed highly discrepancies in different ethnicities, which suggested that the genetic factors may contribute to the susceptibility to this disease. We carried out a study to evaluate the role of two important SNPs in the *IL-16* gene, and their roles

Table 2. Genotype frequencies of *IL-16* rs4778889 T/C and rs11556218 T/G among the cases with RCC and controls and their association with risk of RCC.

Genotypes	Patients	%	Controls	%	χ^2 test	P value	HWE	OR (95%CI)[1]	P value
rs4778889									
Codminant									
TT	132	48.18	176	64.23				1.0 (Ref.)	-
TC	113	41.24	84	30.66				1.79 (1.23-2.62)	0.002
CC	28	10.22	14	5.11	15.22	<0.001	0.34	2.67 (1.29-5.69)	0.004
Dominant									
TT	132	48.18	176	64.23				1.0 (Ref.)	-
TC+CC	142	51.82	98	35.77	14.35	<0.001		1.93 (1.35-2.76)	<0.001
Recessive									
TT+TC	246	89.78	260	94.89				1.0 (Ref.)	-
CC	28	10.22	14	5.11	5.05	0.03		2.11 (1.05-4.45)	0.02
rs11556218									
Codminant									
TT	149	54.38	155	56.57				1.0 (Ref.)	-
TG	110	40.15	107	39.05				1.07 (0.74-1.54)	0.71
GG	15	5.47	12	4.38	0.49	0.78	0.22	1.30 (0.55-3.15)	0.51
Dominant									
TT	149	54.38	155	56.57				1.0 (Ref.)	-
TG+GG	125	45.62	119	43.43	0.27	0.61		1.09 (0.77-1.55)	0.61
Recessive									
TT+TG	259	94.53	262	95.62				1.0 (Ref.)	-
GG	15	5.47	12	4.38	0.17	0.68		1.26 (0.54-3.02)	0.55

[1]Adjusted for gender, age, hypertension, and diabetes.

Table 3. Stratification analysis between *IL16* rs4778889 T/C and risk of RCC by gender, age, hypertension, and diabetes.

Characteristics	Patients		Controls		OR (95%CI)	P value
	TT	TC+CC	TT	TC+CC		
Age, years						
<60	84	92	114	66	1.89 (1.21-2.96)	<0.001
≥60	48	50	62	32	2.02 (1.08-3.77)	0.02
Sex						
Male	90	101	118	73	1.81 (1.18-2.78)	0.004
Female	42	41	58	25	2.26 (1.14-4.51)	0.01
Hypertension						
No	88	96	137	81	1.85 (1.21-2.80)	0.003
Yes	44	46	39	17	2.40 (1.13-5.19)	0.01
Diabetes						
No	118	114	162	91	1.72 (1.18-2.52)	<0.001
Yes	14	28	14	7	4.00 (1.16-14.30)	<0.001

in the development of RCC. The results of this study suggest that the TC and CC genotypes of rs4778889 were associated with increased risk of RCC compared to the TT genotype, and revealed that this polymorphism was connected to elevated risk under dominant and recessive genetic models, even after adjusting for age, sex and other confounding factors. *IL-16* is a multifunctional cytokine in pathophysiology of inflammatory diseases, as well as in tumor growth and progression. Previous studies have reported that genetic variation of *IL-16* are associated with susceptibility to various inflammatory diseases, such as late-onset Alzheimer's disease, graves' disease, coronary artery disease and asthma and allergic rhinitis (Reich et al., 2003; Afifi et al., 2004; Hosseini-Farahabadi et al., 2007; Wu et al., 2011; Tsai et al., 2014; Anvar et al., 2015). Afifi et al. (2004) carried out a case-control study in An Egyptian population, finding that the IL-16 may be considered as a marker of severity of airway inflammation. Wu et al. (2011) conducted a study of 157 patients with CAD and 202 healthy controls, and reported that IL-16 genetic variation may be used as a genetic marker for CAD

susceptibility. Tsai et al. (2014) conducted a further investigation involving 474 CAD patients with graves' disease, and reported that polymorphisms in IL-16 may be as genetic markers for the diagnosis of graves' disease. Anvar et al. (2015) carried out a study consisted of 144 patients with late-onset Alzheimer's disease and 173 healthy individuals, suggesting that IL-16 genetic polymorphisms may play a protective role in the progression of Alzeimer's disease. Previous studies have reported that IL-16 genetic variation could influence the development of several kinds of cancer, such as nasopharyngeal cancer, renal cell cancer, hepatocellular cancer, colorectal cancer, glioma cancer and gastric cancer (Gao et al., 2009a,b; Obara, 2010; Li et al., 2011; Azimzadeh et al., 2011; Zhang and Wang, 2013; Luo et al., 2014; Qin et al., 2014). The abovementioned studies reveal that *IL-16* gene polymorphisms are associated with cancer development.

SNPs in the IL-16 genes could alter gene expression, structure, as well as the level of proteins produced, which in turn affects gene function. Currently, only one previous study reported the role of *IL-16* polymorphism in RCC susceptibility in a Chinese population (Zhu et al., 2010). This study conducted a case-control study involving 335 RCC patients and 340 cancer-free controls, and reported that IL-16 -295T>C polymorphism is significantly associated with a higher risk of developing RCC in Chinese population. However, none have documented the relationship between the rs4778889 T/C and rs11556218 T/G SNPs and RCC occurrence. In our study, we firstly reported that *IL-16* rs4778889 gene polymorphism was correlated with the RCC risk. Therefore, further studies are greatly required to verify our findings.

In summary, we reveal that the *IL-16*rs4778889 polymorphism was correlated with elevated risk of RCC. Our investigation offers insights into the influence of *IL-16* on the occurrence of this disease.

Conflicts of interest

The authors declare no conflict of interest.

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