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Original Article

Association between interluekin-17 gene polymorphisms and the risk of cervical cancer in a Chinese population

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Abstract: We conducted a study to analyze the association of three common SNPs of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 gene polymorphisms with the risk of cervical cancer in a Chinese population. Our study included 352 cervical cancer patients and 352 controls between January 2013 and December 2014. Genotyping of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 genes was performed by multiplex PCR assays using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). By χ^2 test, there was significantly difference in the genotype distribution of IL-17A rs2275913 between cervical cancer patients and control subjects (χ^2=11.45, *P*=0.003). By conditional logistic regression analysis, we found that individuals with the GA and AA genotypes were associated with an increased risk of cervical cancer when compared with the GG genotype in codominant model, and the adjusted Ors (95% CI) were 1.57 (1.13-2.18) and 2.01 (1.15-3.49), respectively. In dominant model, we found that the GA+AA genotype of rs2275913 was correlated with a moderate increased risk of cervical cancer compared with the GG genotype (OR=1.64, 95% CI=1.20-2.24). We only found significant interaction between rs2275913 polymorphism and HPV-16 or 18 infection in the risk of cervical cancer (*P* for interaction <0.05). In conclusion, our study suggests that IL-17A rs2275913 polymorphism may affect the development of cervical cancer in codominant and dominant models, and this gene polymorphism has interaction with HPV-16 or 18 infection.

Keywords: Interluekin-17, polymorphism, cervical cancer

Introduction

Cervical cancer is the fourth most common cancers in women, and it is estimated there were 528,000 new cases and 266,000 deaths in 2012 worldwide. More than 80% of the cervical cancer occurs in the less developed regions, where it accounts for almost 12% of all female cancers [1]. It is well known that human papillomavirus (HPV) infections have been identified to be the main risk factor in the development of cervical carcinogenesis [2-4]. However, only a few HPV infected women would finally develop cervical cancer during their lifetime [5], which suggested that some genetic factors may play an important role in the development of cervical cancer. Many studies have reported that inflammation-related gene polymorphisms may be involved in the progression of cervical precancerous lesions to invasive cervical cancer, including tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), Caspase-7 and DNA repair genes [6-9].

Interleukin-17 (IL-17) is a relatively newly found cytokine which is secreted mainly from Th17 cells, a new lineage of CD4+ T helper cells [10]. Cytokine IL-17 is a member of IL-17 family that consists of six similar members, namely IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F [11]. IL-17A and IL-17F are important members of the IL-17 cytokine family preferentially produced by helper T 17 (Th17) cells, which are responsible for the pathogenic activity of the lineage of CD4+ effector cells and multiple proinflammatory mediators [12-13]. Increasing evidences have revealed that inflammation plays important role in the tumor microenvironment, which

Table 1. Baseline characteristics of cervical cancer patients and control subjects

Variables	Patients N=352	%	Controls N=352	%	χ^2 test	*P* value
Age, years						
<50	168	47.73	161	45.74		
≥50	184	52.27	191	54.26	0.28	0.60
Age at menopausal, years						
<48	189	53.69	196	55.68		
≥48	163	46.31	156	44.32	0.28	0.60
Age at primiparity, years						
<18	171	48.58	146	41.48		
≥18	181	51.42	206	58.52	4.49	0.03
Smoking status						
Non-smokers	47	13.35	32	9.09		
Smokers	305	86.65	320	90.91	3.21	0.07
Drinking status						
Non-drinkers	68	19.32	80	22.73		
Drinkers	284	80.68	272	77.27	1.23	0.27
Family history of cancer						
No	309	87.78	325	92.33		
Yes	43	12.22	27	7.67	4.06	0.04
HPV-16 or 18 infection						
Negative	65	18.47	285	80.97		
Positive	287	81.53	66	18.75	276.64	<0.001
Stage						
I-II	298	84.66				
III-IV	54	15.34				

involves in proliferation, migration and survival of tumor [14].

Only two previous studies have reported the association of IL-17A and IL-17F single nucleotide polymorphisms (SNPs) with the risk of cervical cancer [15, 16], but the results are controversial. The discrepancy may be attributed to the relatively small sample size of previous studies and the genetic heterogeneity of polymorphisms in cervical cancer among different populations. Therefore, we conducted a study to analyze the association of three common SNPs of IL-17A (rs2275913 and rs3748067) and IL-17F (rs763780) gene polymorphisms with the risk of cervical cancer in a Chinese population.

Materials and methods

Study population

Our study included 352 cervical cancer patients, who were newly diagnosed between January 2013 and December 2014. The cervical cancer patients were histopathologically confirmed independently as primary cervical cancer by two gynecologic pathologists in our hospital. Control subjects were randomly selected from individuals who underwent a routine health examination for early detection of cervical cancer during the same period. All controls subjects were confirmed to be lack of cervical lesions by cytology test in the pathology department. A total of 352 controls were collected in our study, and one control was matched with one case by age at enrollment within five years. At recruitment, each participant was scheduled for an interview once written informed consent had been obtained.

Data collection

Demographic characteristics of cervical cancer patients and controls were collected from a self-designed questionnaire. All the subjects were interviewed using a standard questionnaire regarding age at menarche, age at menopausal, age at first live birth, cigarette smoking, alcohol consumption, menopausal status and family history of cancer. Smokers were defined as having smoked at least one day to six months. Subjects who had drunk alcoholic beverages at least once a week for more than one year previously were defined as drinkers, and non-drinkers were those who had not drunk alcohol. The type 16 and 18 HPV infection and stage of cervical cancer were collected from medical records of cervical cancer patients and controls.

Genotyping

5-ml venous blood sample was collected from each participant, and the blood sample was stored in -20°C with 0.5 mg/ml EDTA. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (Beijing Bioteke Co. Ltd. Beijing, China). Genotyping of IL-17A rs2275913 and rs3748067 and IL-17F rs763780 genes were performed by multiplex PCR assays using polymerase chain reac-

Table 2. IL-17 A rs2275913 and rs3748067 and IL-17F rs763780 genotype distributions in cervical cancer patients and controls

IL-17 gene	Base change	Patients	%	Controls	%	χ^2	*P* value	*P* value for HWE	MAF	
									In database	In controls
rs2275913										
GG		135	38.40	178	50.60					
GA		173	49.10	145	41.20					
AA	G>A	44	12.50	29	8.20	11.45	0.003	0.54	0.2927	0.288
rs3748067										
CC		290	82.40	307	87.20					
CT		45	12.70	36	10.30					
TT	C>T	17	4.90	9	2.50	3.95	0.14	<0.001	0.0769	0.0765
rs763780										
TT		283	80.40	299	84.80					
TC		52	14.77	43	12.30					
CC	T>C	18	4.83	10	2.90	3.58	0.17	<0.001	0.0935	0.0905

tion-restriction fragment length polymorphism (PCR-RFLP). The following forward (F) and reverse (R) primers of each gene were used: rs2275913, 5'-ATTTCTGTTCTTCCCATTCC-3' (forward) and 5'-CCCAGGAGTCATGCTTCTTT-3' (reverse); rs3748067, 5'-AAGCAGGGAGCCTGCAGAGTG-3' (forward) and 5'-GGCACCACACAACCCAGAAAG-3' (reverse); rs763780, 5'-GCAGAGCACTCCCTAACCAG-3' (forward) and 5'-CTGCATCCCTGCTCTTGGAA-3' (reverse). The reaction was conducted at 95°C for 5 min for the initial denaturation, following 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 45 s, extension at 72°C for 30 s and final extension at 72°C for 5 mins. A 210, 188 and 217 bp amplicons represent the rs2275913, rs3748067 and rs763780 genes.

Statistical analysis

Statistically significant differences between cases and controls for demographic characteristics were assessed by χ^2 test. The association between the IL-17A rs2275913 and rs3748067 and IL-17F rs763780 gene polymorphisms and risk of cervical cancer was analyzed by calculating odds ratios (ORs), 95% confidence intervals (95% CI), and their corresponding *P*-values. While analysis for gene-environment interaction were carried out stratifying the demographic and clinical characteristics. A *P*-value of less than 0.05 was considered to be statistically significant. Departures from Hardy-Weinberg equilibrium for rs2275913, rs3748067 and rs763780 genes, which was evaluated by comparing the expected frequencies to observed genotype frequencies using χ^2 tests.

Results

Table 1 summarizes the characteristics of the cervical cancer patients and controls. In this present study, the mean ages of cervical cancer patients and control subjects were 46.32±11.50 and 46.10±10.75 years, respectively. Comparing with controls, cervical cancer patients were more likely to have younger age at primiparity, more family history of cancer and higher infection rate of HPV-16 or 18. Of 352 cervical cancer patients, 298 (84.66%) cervical patients were at I-II stage, and 54 (15.34%) were at III-IV stage.

The genotype distributions of IL-17 A rs2275913 genotype distribution confirmed with Hardy-Weinberg equilibrium in the control group, while IL-17A rs3748067 and IL-17F rs763780 were not (**Table 2**). By χ^2 test, there was significantly differences in the genotype distribution of IL-17A rs2275913 between cervical cancer patients and control subjects (χ^2=11.45, *P*=0.003). We found that the Minor allele frequencies of IL-17 A rs2275913 and rs3748067 and IL-17F rs763780 in controls were similar to them in NCBI (http://www.ncbi.nlm.nih.gov/pubmed/).

By conditional logistic regression analysis, we found that individuals with the GA and AA genotypes were associated with an increased risk of

Table 3. Association between rs2275913, rs3748067 and rs763780 gene polymorphisms and risk of cervical cancer

	IL-17	Cases	%	Controls	%	OR (95% CI)[1]	*P* value
Codominant model	rs2275913						
	GG	135	38.40	178	50.60	Ref.	-
	GA	173	49.10	145	41.20	1.57 (1.13-2.18)	0.005
	AA	44	12.50	29	8.20	2.01 (1.15-3.49)	0.01
Dominant model	GG	135	38.40	178	50.60	Ref.	-
	GA+AA	217	61.60	174	49.40	1.64 (1.20-2.24)	0.001
Recessive model	GG+GA	308	87.50	323	91.80	Ref.	-
	AA	44	12.50	29	8.20	1.59 (0.95-2.71)	0.06
Codominant model	rs3748067						
	CC	290	82.40	307	87.20	Ref.	-
	CT	45	12.70	36	10.30	1.32 (0.81-2.17)	0.24
	TT	17	4.90	9	2.50	2.00 (0.83-5.17)	0.09
Dominant model	CC	290	82.40	307	87.20	Ref.	-
	CT+TT	62	17.60	45	12.80	1.46 (0.94-2.27)	0.07
Recessive model	CC+CT	335	95.10	343	97.50	Ref.	-
	TT	17	4.90	9	2.50	1.93 (0.80-4.99)	0.11
	rs763780						
Codominant model	TT	283	80.40	299	84.80	Ref.	-
	TC	52	14.77	43	12.30	1.28 (0.81-2.03)	0.27
	CC	18	4.83	10	2.90	1.90 (0.82-4.69)	0.11
Dominant model	TT	283	78.20	299	84.80	Ref.	-
	TC+CC	70	19.60	53	15.20	1.39 (0.93-2.11)	0.10
Recessive model	TT+TC	335	95.17	342	97.10	Ref.	-
	CC	18	7.10	10	2.90	1.84 (0.79-4.52)	0.12

[1]Adjusted for age, age at menopausal, age at primiparity, smoking and drinking habits, family history of cancer, and HPV-16 or 18 infection.

cervical cancer when compared with the GG genotype in codominant model, and the adjusted Ors (95% CI) were 1.57 (1.13-2.18) and 2.01 (1.15-3.49), respectively (**Table 3**). In dominant model, we found that the GA+AA genotype of rs2275913 was correlated with a moderate increased risk of cervical cancer compared with the GG genotype, and the adjusted OR (95% CI) was 1.64 (1.20-2.24). However, rs3748067 and rs763780 polymorphisms had no significantly association with the risk of cervical cancer in codominant, dominant and recessive models.

By stratification analysis, we found that the GG genotype of rs2275913 was associated with an increased risk of cervical cancer in those with younger primriparity (OR=1.82, 95% CI=1.13-2.92) and positive infection of HPV-16 or 18 (OR=2.05, 95% CI=1.15-3.65) (**Table 4**). Moreover, the GA+AA genotype of rs2275913 was corrected with an elevated risk of cervical cancer in those with younger primriparity and positive infection of HPV-16 or 18 as well as with or without family history of cancer. We only found significant interaction between rs2275913 polymorphism and HPV-16 or 18 infection in the risk of cervical cancer (*P* for interaction <0.05).

Discussion

In recent years, genetic susceptibility to cancers has attracted a growing attention to investigate the gene polymorphisms in the tumourigenesis. Inflammation and related cytokines have a key role for the epithelium transformation from precancerous lesion to cervical cancer. The inflammatory state is a required step to maintain and promote cancer progression and accomplish the full malignant phenotype, such as tumor tissue rebuilding, angiogenesis,

Table 4. Association between polymorphism of rs2275913 and risk of cervical cancer stratified by demographic and clinical characteristics

Variables	rs2275913			OR (95% CI)[1]	*P*	OR (95% CI)[1]	*P*
	Cases/Controls						
	GG	GA	AA	AA vs GG		GA+AA vs GG	
Age at primiparity							
<18	65/77	76/59	30/10	3.55 (1.54-8.73	0.001	1.82 (1.13-2.92)	0.009
≥18	70/101	95/86	16/19	1.22 (0.54-2.69)	0.60	1.53 (0.98-2.34)	0.06
Family history of cancer							
No	122/166	157/134	30/25	1.71 (0.93-3.16)	0.06	1.47 (1.06-2.05)	0.01
Yes	13/12	16/11	14/4	4.71 (0.88-27.54)	0.07	4.11 (1.19-14.69)	0.01
HPV-16 or 18 infection							
Negative	29/142	30/119	6/24	1.22 (0.38-3.44)	0.69	1.23 (0.69-2.20)	0.45
Positive	106/36	143/26	38/4	3.23 (1.05-13.23)	0.03	2.05 (1.15-3.65)	0.008

[1]Adjusted for age.

metastasis and suppress the innate anticancer immune response [17]. Genetic and epigenetic mutation would trigger cell transformation and maintains the autonomous proliferation of the transformed cells to cancer proliferation.

IL-17 is an important inflammatory cytokine, which plays a role in connecting the innate and adaptive immunity [18]. Previous studies have reported that IL-17 is an essential proinflammatory cytokine to evoke many cytokines and chemokines secretion through different cell types, such as mesenchymal cells and myeloid cells to recruit monocytes and neutrophils into the microenvironment of inflammation [19]. Furthermore, IL-17 can promote the expression of antimicrobial peptides and facilitates host defense against infections [20, 21].

Currently, there are two molecular epidemiological studies have been conducted to evaluate the risk of polymorphisms of IL-17 and cervical cancer susceptibility [15, 16]. Quan et al. conducted a study in a Chinese population with 311 cervical cancer patients and 463 controls, and they reported that the AA genotype of IL-17A rs2275913 was correlated with an increased risk of cervical cancer when compared with the GG genotype [15]. Another study investigated the association between six genetic variations of IL-17A and IL-17F and risk of cervical cancer in a Chinese population, and they suggested that the AA genotype of IL-17A rs2275913 was correlated with increased risk of cervical cancer [16]. In our study, we also found that the AA genotype and GA+AA genotype of IL-17A rs2275913 were correlated with an increased risk of cervical cancer, and the results of our study were in line with the results of above-mentioned study. Further large sample size studies are greatly needed to confirm the results of our study.

Moreover, our study found that the IL-17A rs2275913 polymorphism had interaction with HPV infection in the risk of cervical cancer. Genetic factors could play an important role in the pathogenesis of cervical cancer through alteration of the inflammatory state and could interact with environmental factors. It is reported that HPV initiates local Th2 inflammation at an early stage and is involved in antibody forming cells, and fosters an immunosuppressive microenvironment to promote tumor progression [22]. Previous study has reported that increased IL-17 expression is correlated with cervical cancer cell growth along with IL-6 levels in tumor tissues, and the function of IL-17 may act through IL-6 [23]. Therefore, IL-17 gene polymorphism may have an interaction with HPV infection in the development of cervical cancer.

Several limitations should be considered in our study. First, cases and controls were selected from a single hospital, and rs3748067 and rs763780 were not in line with Hardy-Weinberg equilibrium in the control group, which suggested that the selected controls may not be representative of the general population. Second, other genetic polymorphisms may influence the development of cervical cancer in addition to

IL-17 gene. Third, the sample size of this study is relatively small, which may limit the statistical power to find differences between groups. Therefore, further large sample size studies are greatly needed to confirm the results in our study.

In conclusion, our study suggests that IL-17A rs2275913 polymorphism may affect the development of cervical cancer in codominant and dominant models, and this gene polymorphism has interaction with HPV-16 or 18 infections. However, no significant association was found between rs3748067 and rs763780 polymorphism and risk of cervical cancer. Further studies with large sample size are greatly needed to confirm our results.

Disclosure of conflict of interest

None.

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References

[1] International Agency for Research on Cancer. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.

[2] Schlecht NF, Kulaga S, Robitaille J, Ferreira S, Santos M, Miyamura RA, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, Franco EL. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001; 286: 3106-14.

[3] Woodman CB, Collins S, Winter H, ailey A, Ellis J, Prior P, Yates M, Rollason TP, Young LS. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001; 357: 1831-6.

[4] Quan Y, Zhou B, Wang Y, Duan R, Wang K, Gao Q, Shi S, Song Y, Zhang L, Xi M. Association between IL17 polymorphisms and risk of cervical cancer in Chinese women. Clin Dev Immunol 2012; 2012: 258293.

[5] Barbisan G, Perez LO, Contreras A, Golijow CD. TNF-α and IL-10 promoter polymorphisms, HPV infection, and cervical cancer risk. Tumour Biol 2012; 33: 1549-56.

[6] Roszak A, Misztal M, Sowińska A, Jagodziński PP. TNF-α-308 G/A as a risk marker of cervical cancer progression in the Polish population. Mol Diagn Ther 2015; 19: 53-7.

[7] Tuguz AR, Anokhina EN, Muzhenya DV, Rudenko KA. Polymorphisms of the anti-inflammatory IL-10 gene associated with malignancy in female reproductive system. Bull Exp Biol Med 2015; 158: 673-5.

[8] Shi TY, He J, Wang MY, Zhu ML, Yu KD, Shao ZM, Sun MH, Wu X, Cheng X, Wei Q. CASP7 variants modify susceptibility to cervical cancer in Chinese women. Sci Rep 2015; 5: 9225.

[9] Bajpai D, Banerjee A, Pathak S, Thakur B, Jain SK, Singh N. Single nucleotide polymorphisms in the DNA repair genes in HPV-positive cervical cancer. Eur J Cancer Prev 2015; [Epub ahead of print].

[10] Rutitzky LI, Lopes da Rosa JR, Stadecker MJ. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. J Immunol 2005; 175: 3920-6.

[11] Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol 2004; 114: 1265-73.

[12] Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, Fujikado N, Tanahashi Y, Akitsu A, Kotaki H, Sudo K, Nakae S, Sasakawa C, Iwakura Y. Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity 2009; 30: 108-19.

[13] Rutitzky LI, Lopes da Rosa JR, Stadecker MJ. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. J Immunol 2005; 175: 3920-6.

[14] Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. Immunity 2011; 34: 149-62.

[15] Quan Y, Zhou B, Wang Y, Duan R, Wang K, Gao Q, Shi S, Song Y, Zhang L, Xi M. Association between IL17 polymorphisms and risk of cervical cancer in Chinese women. Clin Dev Immunol 2012; 2012: 258293.

[16] Lv Q, Zhu D, Zhang J, Yi Y, Yang S, Zhang W. Association between six genetic variants of IL-17A and IL-17F and cervical cancer risk: a case-control study. Tumour Biol 2015; 36: 3979-84.

[17] Chechlinska M, Kowalewska M, Nowak R. Systemic inflammation as a confounding factor in cancer biomarker discovery and validation. Nat Rev Cancer 2010; 10: 2-3.

[18] Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity 2004; 21: 467-76.

[19] Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. Immunity 2011; 34: 149-62.

[20] Matsuzaki G, Umemura M. Interleukin-17 as an effector molecule of innate and acquired immunity against infections. Microbiol Immunol 2007; 51: 1139-47.
[21] Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, Harper RW, Wu R. Il-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via jak and nf-kappab signaling pathways. J Immunol 2004; 173: 3482-91.
[22] Feng Q, Wei H, Morihara J, Stern J, Yu M, Kiviat N, Hellstrom I, Hellstrom KE. Th2 type inflammation promotes the gradual progression of HPV-infected cervical cells to cervical carcinoma. Gynecol Oncol 2012; 127: 412-9.
[23] Paradkar PH, Joshi JV, Mertia PN, Agashe SV, Vaidya RA. Role of cytokines in genesis, progression and prognosis of cervical cancer. Asian Pac J Cancer Prev 2014; 15: 3851-64.